Appendix C to the final report on: Plant health surveys for the EU territory: an analysis of data quality and methodologies and the resulting uncertainties for pest risk assessment (PERSEUS) CFP/EFSA/PLH/2010/01

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A Review of the Literature Relevant to the Monitoring of Regulated Plant Health Pests in Europe

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Contents

Acleris spp. (non-European)

Common name(s): Eastern/Western blackheaded budworms
Taxa: Insecta: Lepidoptera: Totricidae
EPPO A1 list: No. 32
EU Annex designation: I/A1

Organism

Acleris gloverana (the western blackheaded budworm) and *A. variana* (the eastern blackheaded budworm) feed on conifer species such as *Abies* and *Picea* spp. in regions of North America. The two species are present in different geographical areas. Defoliation occurs in particular at the tops of the trees. The adults are capable of flying long distances, but introduction is most likely to be via infested host plants.

1. Detection

Sex pheromones have been identified for both *A. gloverana* and *A. variana*. The main pheromone component for both species is (E)-11, 13- tetradecadienal (Gries *et al*. 1994, Gray *et al*. 1996). The addition of (Z)-11, 13- tetradecadienal doubled the number of *A. gloverana* males caught in traps (Gray *et al*. 1996), but had no effect on the number of *A. variana* males caught (Gries *et al*. 1994).

2. Delimitation

No relevant references found.

3. Monitoring

Monitoring surveys have been conducted to facilitate predictions of outbreaks. It is recommended that *A. gloverana* is monitored using egg or larval sampling (Randall, 2004). Larvae are dislodged by beating branches above a ground sheet. Each plot consists of three trees and sampling is undertaken annually (Randall 2004). Eggs are sampled from a 45 cm branch tip per tree from each of the north and south aspects, from the mid-crown of 10 trees per sample location. The average number of healthy eggs per branch provides an estimate the defoliation levels expected in the next year. Visual assessments or the hot water method are used to count the number of eggs per sample (Randall, 2004).

The effectiveness of tree beating as a method to examine populations of forest insect pests including *A. gloverana* has been examined (Harris *et al*. 1972). This study demonstrated that a minimum of three tree samples should be taken at a sampling point and that rain, or wet foliage, reduced the number of *Acleris* larvae found when sampling (Harris *et al*. 1972).

Adult insects can be monitored using pheromone traps. Location of permanent sentinel pheromone monitoring traps in areas that are repeatedly defoliated has been recommended (Otvos 2001).

4. Commodities

EPPO recommends that importation of plants for planting (except seeds and tissue cultures) and cut branches of *Abies* and *Picea* from North America are prohibited (OEPP/EPPO 1990).

References: 12 retained out of 70 retrieved, 3 added, 6 used for summary

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Aculops fuchsiae Keifer

Common name(s): Fuschia gall mite
Taxa: Arachnida: Acarina: Prostigmata
EPPO list: No. 185
EU Annex designation: II/A1

Organism

Fuchsia gall mite, *Aculops fuchsiae* Keifer (Prostigmata: Eriophyidae), originates from South America, has been found in France (Streito et al. 2005).

1. Detection

The first report of this pest in Europe was in November 2003, in France (Bretagne) (Streito et al. 2004).

2. Delimitation

No information available.

3. Monitoring

No information available.

4. Commodities

No information available.

References: 3 retained out of 8 references retrieved, none added, 2 used for summary

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Agrilus planipennis Fairmaire

Common name(s): Emerald ash borer
Taxa: Insecta: Coleoptera: Buprestidae
EPPO: A2 action list No. 322
EU Annex designation: II/A1

Organism

Native hosts of *A. planipennis* include several Asian *Fraxinus* species*, Juglans mandshurica*, *Pterocarya rhoifolia*, *Ulmus davidiana* and *Ulmus propinqua*. In North America, *Fraxinus americana*, *Fraxinus nigra* and *Fraxinus pensylvanica* have also been attacked. Except from the easterly regions of Russia *A. planipennis* is neither recorded within the EPPO region nor in the EU. The species is distributed in North America (Canada and USA) and Asia (north-eastern China, Japan, Korea Republic, Mongolia and Taiwan).

The larvae typically feed in the cambium of trees or in the stems of vines and small woody plants. The adults are attractive insects with striking metallic colours and are often referred to as jewel beetles. In China, *A. planipennis* typically has one generation per year although some individuals may require two years to complete a generation. Adults are active between mid-May and July. After emergence, they walk to the crown of their host tree and feed on the foliage.

Eggs are laid individually on the bark surface, inside bark cracks and crevasses. Each female can lay 68–90 eggs. Larvae tunnel through the bark to the cambium, where they feed from mid-June to mid-October. Larvae make long serpentine galleries (up to 26–32 mm long) into the sapwood which enlarge as they grow and which are filled with brownish sawdust and frass. Pupation occurs in April and May at the end of a tunnel near the surface (Anonymous, 2005). The typical symptoms on the host trees caused by *A. planipennis* consist of: larval galleries in the cambium filled with frass and sawdust, adult exit holes, yellowing and thinning of foliage, dying of branches, dieback and mortality of the tree.

Trees attacked by *A. planipennis* are ultimately killed.

1. Detection

Traps have been developed incorporating attractive visual and olfactory cues. Odours from the leaves of stressed ash trees (Rodriguez-Saona *et al*. 2006), ash leaf volatiles (deGroot *et al*. 2008, Grant *et al*. 2010) and volatiles from ash bark (Crook *et al*. 2008) elicit antennal responses and are attractive to EAB. The beetles are also attracted to certain colours including purple as well as to different trap placement and design (Francese *et al*., 2008, Francese *et al*., 2010, Marshall *et al*., 2009). Also, a bright shade of light green (Crook *et al*. 2009) is also attractive.

A. planipennis is attracted to the colour purple, to blends of host volatiles from ash bark and foliage, and to girdled ash trees. The attraction of *A. planipennis* is evaluated to triangular purple panel traps baited with various combinations of ash volatiles that elicit antennal responses by *A. planipennis* as well as volatiles from ash that have behavioural activity in other bark or wood-boring insects. Multicomponent traps included triangular purple panels mounted at 1.5 and 2.5 m on a purple pole. The upper and lower panels were baited with foliar and bark volatiles, respectively. Panels were also coated with a rough “bark” texture. (Poland et al. in Hodges & Morse, 2009).

Girdled ash trees, especially open grown trees, are significantly more attractive to EAB than healthy ash trees and can be used for survey and detection (McCullough *et al*. 2009a, 2009b).

2. Delimitation

For the SLAM pilot study, fairly detailed ash inventory data are being collected. Suppression treatments include a) removal of infested trees, b) insecticide treatments, c) clusters of 3-4 girdled trees, and d) ash utilization or selected ash removal to reduce brood material (Poland, T. M. and D. G. McCullough, 2010).

3. Monitoring

With the pilot study SLAM, consisting of a consortium of national institutions working in plant health, agriculture, forestry research and the Michigan State University, Michigan Technological University the goal is to reduce EAB population growth, which in turn, slows the progression of widespread ash mortality within and beyond the project area. The SLAM program incorporates 1) surveys to determine EAB distribution and density, 2) inventories or surveys to assess ash abundance and distribution, 3) activities to suppress EAB populations, 4) regulatory measures, and 5) public information and outreach campaigns.

In 2008, an extensive systematic grid-based survey was conducted to more accurately determine the extent of the infestation as well as to determine EAB spatial distribution and density. Surveys were conducted using girdled trap trees (ash trees with a band of bark and phloem removed around the circumference of the tree) and artificial sticky traps (Poland, T. M. and D. G. McCullough, 2010).

The 2008 EAB survey was based on systematic grids of girdled trees. Artificial traps were used when an ash tree was not available for girdling. Traps used in the SLAM pilot study consisted of purple prism panels coated with Pestick to capture beetles. Traps were hung in the canopy of ash trees and baited with Manuka oil or an 80:20 mixture of Manuka and Phoebe oil. These natural oils contain several attractive volatiles found in ash bark. Surveys to assess EAB distribution were repeated in 2009 (Poland, T. M. and D. G. McCullough, 2010).

In a survey the density of *A. planipennis* and canopy condition on green ash (*F. pennsylvanica*) and white ash (*F. americana*) street trees in four neighbourhoods and on white and blue ash (*F. quadrangulata*) trees in two woodlots in southeast Michigan was investigated. Therefore, visual estimates of canopy dieback were made annually in late summer, when damage from the current year larvae had become apparent. D-shaped exit holes made by emerging *A. planipennis* adults and woodpecker attacks were counted in the fall, after adult beetle emergence was complete (Anulewicz, *et al*., 2007).

4. Commodities

After the discovery of *A. planipennis* in south-eastern Michigan in 2002, quarantines were enacted including prohibition of transporting firewood across the Mackinac Bridge between Michigan´s Lower and Upper peninsulas. Concern over this pest has led to “don´t move firewood” campaigns and restrictions on U.S. imports of fuel wood (Haack *et al*., 2010).

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Aleurocanthus spp.

Common name(s): Citrus blackfly (*A. woglumi*) Orange spiny whitefly, spiny blackfly (*A. spiniferus*)
Taxa: Insecta: Hemiptera: Aleyrodidae
EPPO A1 list: No. 103 (*Aleurocantus woglumi* Ashby)
EPPO A2 list: No. 186 (*Aleurocantus spiniferus* (Quaintance))
EU Annex designation: II/A1, under "*Aleurocanthus* spp."

Organism

Aleurocanthus is a palaearctic genus belonging to the family Aleyrodidae, with over 70 described species, 65 of them from South-East Asia and Africa. Many species of *Aleurocanthus* are pests of economically important crops, especially the well-known *Aleurocanthus spiniferus* and *Aleurocanthus woglumi.* These two species have as main hosts *Citrus* spp*.,* but have been recorded on other crops, for example grapes, pears, and roses. Both species originated from South-East Asia and have spread widely in tropical and subtropical regions. *A. spiniferus* has been introduced into Europe (Italy) whereas *A. woglumi* into the American continent. A female may lay 35-100 eggs in her lifetime. The emerging crawlers (nymphs) are black, flattened and disperse for a short time, then begin feeding upon the phloem sap. They then moult, losing their legs in the process, to become minute, flattened insects attached to the leaf by their mouthparts. After two more moults the adults emerge. In the adults, both sexes are winged and feed upon phloem sap.

1. Detection

A high number of studies evaluated the more efficient trap to capture in particular *A. woglumi*. Translucent fluorescent yellow traps were significantly more attractive than opaque yellow (Summy *et al*. 1986). Moreover, fluorescent yellow with a reflectance of 500-555 nm proved to be the most attractive shade of yellow (Dowell and Cherry 1981), or fluorescent yellow traps with a reflectance in the range of 550-553 nm were more attractive than ordinary yellow ones with a similar reflectance (Meyerdirk *et al*. 1979a). The trap shape (square, rectangle, triangle or circle) had no influence on efficiency, but the total number of adults caught per trap had a positive correlation with its surface area (Meyerdirk *et al*. 1979a). Optimum trap height within a citrus grove was determined as 2-3 m above ground level, which was at the maximum girth of the tree crown (Hart *et al*. 1978) or 1.5 m above ground level (Meyerdirk *et al*. 1979b). However, the visual surveys proved efficiency superior to sticky traps for detecting *A. woglumi* at low densities (<5% leaves infested) on citrus trees in an urban setting (Dowell and Cherry 1981). The method of visual survey is used in generic monitoring in the case of first record as for *A. spiniferus* reported for the first time in Italy (Porcelli 2008) or in specific monitoring to assess the distribution and population density of *A. spiniferus* in areas of new introduction in the Republic of Palau (Muniappan *et al*. 2006). Morphological identification of whitefly is limited by small size and the high degree of similarity and polymorphism. Molecular techniques should be useful for detection; for *A. spiniferus* a method of DNA markers for rapid detection was developed based on sequence characterized amplified region (SCAR) derived from a randomly amplified polymorphic DNA (RAPD) band (Liu *et al*. 2009).

2. Delimitation

No information available

3. Monitoring

Sticky traps are used to monitoring the population density and the phenology of *A. woglumi*

(Nguyen *et al*. 1983, Kuchanwar *et al*. 1989). The application of airborne video data with global positioning system (GPS) or airborne colour-infrared (ACIR) and geographical information system (GIS) technologies were used for mapping citrus blackfly outbreaks (Everitt *et al*. 1994, Fletcher *et al*. 2004). A non-destructive counting techniques based on 35-mm black-and-white photography were developed for counting *A. woglumi* on citrus (Summy *et al*. 1984).

4. Commodities

A review is presented of the arthropods detected in 36 samples of curry leaves imported into England and Wales for culinary purpose from Africa, Asia and the Caribbean; among the twenty-eight arthropod *taxa* identified, there is also *A. woglumi* (Malumphy 2011).

References: 24 retained out of 584 retrieved, none added, 14 used for the summary

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Alternaria alternata (Fr.) Keissler

(=*Alternaria mali* Roberts)
Common name(s): Alternaria blotch of apples (*A. mali*)
Taxa: Fungi: Ascomycota: Pleosporomycetidae
EPPO A1 list: No. 277
EU Annex designation: II/A1

Organism

Alternaria alternata is a ubiquitous fungal pathogen of a wide range of crops. In the EU/EPPO region the disease of concern is *Alternaria mali*, a species belonging to the *A. alternata* group. The following review takes into consideration all surveying methodologies for *A. alternata* as the literature specific to *A. mali* is quite limited. *Alternaria mali* has been detected in Europe, but predominantly occurs in Asia and North America where the hosts attacked include *Malus* although *Prunus*, *Pyrus* and *Cedonia* are also known to be affected. By contrast, *Alternaria alternata* is has a virtually global distribution and its host range is vast.

1. Detection

Alternaria alternata has a wide range of detection techniques available, with traditional methods based on incubation, plating-out or histopathology (Jasbir *et al*. 1990, Ali *et al*. 1991, Andrews 1992). In tomato, for example, visual observation of fungal filaments were the standard diagnostic approach (the "Howard" mould count method") for the detection of this disease (Zur *et al*. 1999). The "blotter" test has been, and continues to be, widely used, involving the incubation of seeds (for example) on filter paper, freezing and viewing the subsequent growth with UV light using a microscope (Kumar and Singh 1996, Teixeira *et al*. 2002). Because of this group of fungi have a propensity to occur in foodstuffs or cause allergic responses in humans, many detection methods that are described in the literature are largely irrelevant from the perspective of plant health although some are probably applicable to both contexts. For example, a lectin-binding based assay for the detection of fungal chitin has been employed by the food industry although its power to differentiate species is doubtful (Potts *et al*. 2000). A number of ELISA assays have also been used in the food/health arena (Lin *et al*. 1986, Harvey *et al*. 1987, Lin and Cousin 1987). The toxins produced by *Alternaria* can be used for diagnosis in wheat (Suchita and Kang 1989) and other crops.

In common with many diseases of plant health importance, molecular approaches have become more prominent as diagnostic tools. For example, PCR techniques have been developed and described since the late 1990s for a number of crops or commodities (Konstantinova *et al*. 2002) (Zur *et al*. 1999, Barnett and Beck 2001, Konstantinova *et al*. 2001). Many of the methodologies described are optimised for detection in seeds (Zur *et al*. 2002). In particular, a method for the diagnosis *Alternaria mali* is described by Johnson *et al*. (2000) that brought a higher degree of accuracy over previous methodologies. In recent times the use of RAPD-PCR has been widely employed for the identification / differentiation of different isolates of *Alternaria* (Tigano *et al*. 2003, Ma and Michailides 2004a, Ma and Michailides 2004b, Dongo *et al*. 2005). Real-time PCR methods are also now becoming common diagnostic tools for this group of diseases (Sarlin *et al*. 2006). However, despite the development of new methodologies, it appears that the diagnosis of *A. alternata* is still most commonly achieved using the more traditional methods as there would seem to be little trouble in ascertaining the identity of this pathogen.

A number of surveys described in the literature have been used to detect the presence of *A. alternata* for the first time within a given geographic zone. These include the surveying of

sunflower in Greece (Thanassoulopoulos 1987) and banana in India (Roy *et al*. 1989). A survey of mandarin in Spain, following the observation of disease symptoms, identified *A. alternata* in citrus for the first time in that country, the diagnosis being made using selective media culturing (Vicent *et al*. 2000). The incidence of *A. alternata* in sugar beet was first detected in the Czech Republic using a detection survey in 2000 (Rohacik and Hudec 2002), whilst the disease was also first recorded in *Impatiens* spp. (balsams) in Japan in 2002 following collection of symptomatic material (Kubo *et al*. 2005). A survey of rubber trees in India has, more recently, detected the presence of the disease with diagnosis conducted using RAPD analysis (Roy *et al*. 2006), whilst the fungus has been also found to occur in *Hydrangea* in Italy (Garibaldi *et al*. 2007). In the latter case, samples were collected from symptomatic plants from retail premises and diagnosis made through morphological assessments of the fungi.

2. Delimitation
No information

3. Monitoring
Monitoring-type surveys are described in the literature for a wide range of circumstances. These include forestry (Tarocinski and Zielinski 1978, Huang and Kuhlman 1990), wheat (Mehrotra and Dwivedi 1980), groundnut (Subrahmanyam *et al*. 1981), maize (Caretta *et al*. 1985), poppy (Kishore *et al*. 1985), mango (Om and Raoof 1985), soil (Abdulla and El-Gindy 1987), tomato (Mikhajlova and Nguyen Kim 1988), dates (Barkai-Golan *et al*. 1989, Abdulsalam *et al*. 1992), cabbage (Raju and Sivaprakasam 1989) and a range of other crops (Chiang *et al*. 1990, Visconti *et al*. 1992).
Many of the surveying efforts have been described were undertaken to determine the broad range of pathogens infecting crops and are not specific for *Alternaria alternata*. For example, monitoring of lentil in Turkey revealed widespread infection with a number of diseases, including *A. alternata* (Karahan and Katrcoglu 1993). A similar survey was conducted for olive in Italy (Nicoletti and Rinaldi 1993). In-field infection of rice has been surveyed for in India (Usha *et al*. 1993), for wheat and chilli in Bangladesh (Ahmed *et al*. 1994, Basak *et al*. 1994), mulberry in India (Gunasekhar *et al*. 1994), pepper and sesame in Korea (Lee and Yu 1995), amongst a range of similar surveys.
Sampling is variable with crop. For example, in growing wheat, foliar samples (e.g. flag leaves) have been taken for examination (Cromey *et al*. 1994, Shazia and Iftikhar 2005). The latter paper describes a sampling method based on collections made along linear transects. Similarly, the disease in mango has been surveyed for through sampling tree material (terminal branches) and subsequently assaying the sampled tissue via the use of selective media (Ploetz *et al*. 1997). Mango was also surveyed by Nizamani *et al*. (2005) specifically for *A. alternata*. Cactus pear (*Opuntia*) has also been surveyed for using ostensibly the same techniques (Granata and Sidoti 1997).
Growing apples (i.e. non-commodity, on the tree) have been surveyed through random bud and fruit collections and assessed for infection using selective media followed by diagnosis based on sporulation patterns (Serdani *et al*. 1998) whilst hops were surveyed in Argentina through visual assessment of infected material (Perez *et al*. 2002). A wide-ranging survey of tomato in Turkey allowed the prevalence of *A. alternata* to be determined relative to a number of other diseases (Ozan and Maden 2004, 2005) whilst pomegranate has also been examined in this country (Pala *et al*. 2009). Service trees in Slovakia were surveyed in the autumn of 2003 through tissue sampling and, subsequently, selective media growth, that demonstrated the widespread occurrence of *A. alternata* and other fungal pathogens (Labuda *et al*. 2005). A survey of Poplar in Spain ascertained the prevalence of fungal disease through collecting twigs and bark (960 samples) and incubating

them for the development of fruit bodies (when kept in high humidity) or using selective media techniques for diagnosis (Santamaria and Diez 2005). An extensive survey of glasshouse and field tomato in Syria was able to identify the widespread occurrence of the disease in open-field situation (Atik *et al*. 2007).
Hazelnut in Iran was assessed through the random sampling of nuts from groves whereby 20 trees in each stand, and 400 leaves per tree, were collected and assessed for disease (Moghaddam and Taherzadeh 2007). Similarly, hazelnut was monitored for four years in Italy with visual assessments and lower/fruit collections (n=100) made for laboratory diagnosis via selective media (Osiru *et al*. 2007). A very well articulated description of a survey in Uganda for *A. alternata* combined visual assessments, tissue sampling, questionnaires to growers, and historic data to provide an extensive picture of the disease's prevalence within sweet-potato growing regions (Osiru *et al*. 2007). An excellent description of the diagnostic procedures used for tissue samples is also provided here. In a nice Chinese example, surveying of jujube (*Ziziphus jujuba*) over three years was conducted whereby 10 plants per orchard were surveyed, with 100 fruits and 5 branches per plant examined for symptoms (Zhen *et al*. 2009).

4. Commodities
As might be expected for a seed-borne disease, there is a considerable weight of literature concerning the surveying of commodities for *A. alternata*, much deriving from Asia and South America. In particular, there is information on a wide range of crops at the post harvest stage, particularly seeds of the major staples. For example, selective media assessments have been used to detect *A. alternata* in a small samples of broad beans ((Khan *et al*. 1984), pigeonpea (Kumar and Patnaik 1985), wheat and barley (Hysek *et al*. 1986, Abramson *et al*. 1987), pomegranate(Sonawane *et al*. 1986), rice (Abdel-Hafez *et al*. 1987), sorghum (Abdullah and Kadhum 1987), lotus (Madia de Chaluat 1994) amongst others. In most of the aforementioned cases, however, the surveys dwelt on very specific cases, were largely scientific exercises, and little useful information was to be gained.
More detailed examples of the surveying of commodities are, however, available. For instance, a survey of birdsfoot trefoil seed in Uruguay (Rubio and Altier 1995) provides a good description of how seed lots were sampled. A medium-scale survey of sorghum mycoflora in Argentina indicates that lots of 2 kg of seed are appropriate quantities for this commodity for the determination of presence/absence of the disease (Gonzalez *et al*. 1997). A similar, although slightly more extensive, survey of wheat has also been conducted in Argentina (Gonzalez *et al*. 1995). A better description of sampling, this time for flint corn, in Argentina is also available although the focus on this survey was *Fusarium* and not *Alternaria* (Gonzalez *et al*. 2002). Stored sorghum has also been surveyed for mycoflora in India, with the example of Navi (2005) providing details of sampling and diagnosis. Although the focus of the study by Medina *et al*. (2006) was mycotoxins determination, there are good details of sampling, sample weight taken and allied procedures for malting barley mycoflora determinations. Details of sample processing are also extensively described in this paper.
A survey of cereal and soybeans in Argentina provides a very good description of sampling, with material taken at six positions per lot (truckload), pooled and 1 kg subsamples taken for 162 different sampling localities (Broggi *et al*. 2007). The down-stream processing of the samples is also well described in this paper. Useful, though less extensive details are given for sampling sorghum in a seed-borne disease survey carried out in Bangladesh (Islam *et al*. 2009) and sunflower seed in Iraq

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Amauromyza maculosa (Malloch)

Common name(s): Chrysanthemum / burdock leaf miner
Taxa: Insecta: Diptera: Agromyzidae
EPPO A1 list: No. 152
EU Annex designation: I/A1

Organism

Amauromyza maculosa is known to be polyphagous within the Asteraceae. In the EPPO region ornamental plants and vegetable crops, particularly those grown in controlled environments are regarded as potential hosts, e.g. *Aster* spp., chrysanthemums, *Dahlia* spp. and lettuce.

The species is widely distributed in North Central and South America, but is absent within the EPPO region as well as in the EU.

The biology of *A. maculosa* is not as well known as other leaf mining diptera species. In order to oviposit, the female flies puncture the leaves of their host plants causing small wounds. These punctures also serve as feeding sites. Feeding punctures cause the destruction of a larger number of cells and are more clearly visible to the naked eye. Considerable damage to the leaves is caused by the larvae. Mines are usually white with dampened black and dried brown areas. *A. maculosa* produces a blotch mine.

Larval development is generally completed within a span of 4-7 days (at mean temperatures above 24°C). It varies according to temperature and host plant. Eggs are inserted below the leaf surface. The number of eggs laid varies according to temperature and host plant. Eggs hatch in 2-5 days according to temperature. Pupation takes place either on the foliage or in the soil just beneath the surface. Adult emergence occurs 7-14 days after pupariation, at temperatures between 20 and 30°C. At low temperatures emergence is delayed.

Amauromyza maculosa completes several generations in one year. A heavy attack with up to 80 mines per leaf reduces the photosynthetic area and consequently affects plant growth. Severe leaf fall is not uncommon. There is a high risk of entry and establishment of these pests as the vase life of chrysanthemum is sufficient to allow completion of the life cycle. Dispersal over long distance takes place by means of planting material of host species. Cut flowers can also present a danger. Large numbers can breed in a single leaf and the host range is wide (Anonymous, 1984).

1. Detection

No information available.

2. Delimitation

No information available.

3. Monitoring

No information available.

4. Commodities

No information available.

References: 3 retained out of 14 retrieved, none added, 1 used for summary

Anonymus (1984). Leafminers of chrysanthemum (Diptera: Agromyzidae). Plant Quarantine Leaflet, Commonwealth Department of Health, Australia: 4.

Anastrepha fracterculus (Wiedemann)

Common name(s): South American fruit fly
Taxa: Insecta: Diptera: Tephritidae
EPPO A1 list: No. 229
EU Annex designation: I/A1

Organism

This tephritid fruit fly attacks a range of fruits, including guava (*Psidium guajava*), mangoes (*Mangifera indica*) and apples (*Malus pumila*), coffee and citrus amongst a range of other fruit hosts (Herrera A and Vinas V 1977, Briceno Vergara 1996, Salles 1999) (Schmid and Santos 1988). Larvae feed on the growing fruit and the pest is widespread in the Americas and the Caribbean but is absent from Europe. Within its range it can be one of the most damaging pests of fruit (Chavarria et al. 2009)

1. Detection

The identification of the *A. fracterculus* is generally made through taxonomical features although a RAPD-PCR assay has been developed to differentiate this species from other closely related species (Cenis and Beitia 1994). Detection surveys in fruit-growing regions have been carried out for this species in China using a range of collection methods, indicating its absence (Chen and Tseng 1993). Insects are generally detected using the trapping methods described below.

2. Delimitation

No information

3. Monitoring

Monitoring in South a Central America for *A. fracterculus* is commonly achieved through the use of traps. These include McPhail and Valencia ("Valenciano" type) traps that will trap a range of species within the genus (Herrera A and Vinas V 1977, Fehn 1981, Malo et al. 1987). Typically the traps are baited with attractive materials such as yeast hydrolysate, ammonium sulphate, vegamine, papaya juice, orange juice/sugar syrup (Harper et al. 1989, Martins et al. 1993, Raga et al. 1996, Dias and Arthur 2000, Malavasi et al. 1990). A comparison of the merits of various fruit juices for trapping *Anastrepha* species indicated differential preferences of male and female flies for certain baits (Salles 1997). Some baits appear to become more attractive as they decompose and that allowing for this is important when using them for trapping this fruit fly (Malo 1992) and, as a result, trap turnaround should allow the bait to age. Diluted human urine has been evaluated as a low-cost and readily available attractant for *Anastrepha* species and compares well with other bait types (Aluja and Pinero 2004). It has been noted that there is no "universal" attractant for *Anastrepha* species and a range of substrates are roughly equal in their capacity to attract the fly, although the proportions of females and males captured vary significantly (Aluja and Pinero 2004). Multi-lure traps have been indicated, however, to be more effective than those using only single materials (Toledo et al. 2005). Commercially available materials based on some of the substrates mentioned here, particularly yeast hydrolysates, are available (Raga et al. 2006)

Trap type comparisons have been made and, for example, have demonstrated that plastic McPhail traps are superior to glass variants (Barros et al. 1991). Light traps (UV) are also used, often in concert with baits, and are effective (Fehn and Bertels 1979, Mendonca et al. 2003). Trapping throughout the seasons has shown that fly activity is greater in the wetter periods of the year in some studies (Aguiar-Menezes et al. 2008) but not in others (Raga et al. 1996)

Although adult trapping is by far the most common method for monitoring for *A. fracterculus*

activity, the physical monitoring of fruit for the activity of the larvae has also been described for the purposes of determining population levels. (Olarte Espinosa 1980)

4. Commodities

Given the biology of this pest, there is the potential it to travel in infested fruit. A survey of fruit entering Japan (>37,000 cases examined) was conducted through incubating material and subsequently dissecting–out the insects present (Mastumoto et al. 1992). This approach confirmed the presence of several fruit flies, including *A. fracterculus*, in a number of cases of produce.

References: 71 retained out of 220 retrieved, none added, 24 used for summary

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Anastrepha ludens (Loew)

Common name(s): Mexican fruit fly
Taxa: Insecta: Diptera: Tephritidae
EPPO A1 list: No. 230
EU Annex designation: I/A1

Organism

Anastrepha ludens is a tephritid fruit fly whose larvae attack a range of fruit crops, including orange (*Citrus sinensis*), mangoes (*Mangifera indica*), guava (*Psidium guajava*), peaches (*Prunus persicae*) and apples (*Malus pumila*) (Jimenez et al. 1992). The fly occurs in North, Central and South America, and the Caribbean, but is absent in the EU/EPPO region. Larvae tunnel within fruit and can cause considerable damage before the infestation is externally apparent and, where it is present, very significant economic losses can and do occur (Robacker 1992).

1. Detection

The detection of *A. ludens* is typically achieved using traps (detailed below) followed by taxonomical diagnosis. In California, the insect was eradicated in the early 1980s and trapping following this enabled the occasional re-emergence of examples of this insect (Anonymous 1986b). In response to concerns that the 1984 Olympic Games may increase the risk of reintroduction of *A. ludens*, the McPhail trapping effort around the city was increased to detect this pest (Anonymous 1984a). A Chinese detection survey using multiple capture methods, however, indicated the absence of the pest (Chen and Tseng 1993).

2. Delimitation

An attempt to delimit the presence of *A. ludens* has been described for California where McPhail traps were deployed around the original outbreak site at 25 distinct locations to indicate that the insect had spread beyond the original outbreak zone. This information was used to create an eradication zone where malathion-containing baits were deployed (Anonymous 1984b).

3. Monitoring

Most information referring to monitoring deals with the development of trapping technologies for use within the pest's known range. The presence and size of *A. ludens* populations are monitored for almost exclusively using traps. In common with other *Anastrepha* species, the McPhail trap is most frequently used (Gonzalez Hernandez and Tejada 1980, Houston 1981, Aluja et al. 1987) and shown to be superior to most other designs (Sanchez Salas 1982). The traps are frequently baited with a number of attractant materials, including hydrolysed maize protein (Sanchez Salas and Padron Chavez 1981) and may include a toxicant to kill the insects captured, such as dichlorvos, or a fluid such as water of ethyl glycol. However, the use of insecticidal materials can deter insects entering the trap (Rhode and Sanchez R 1982). A number of alternative trapping methods, including various coloured and transparent sticky traps have been evaluated and, although often inferior in performance to the McPhail trap, offer significant cost benefits (Blanco Montero and Sanchez Salas 1990; Robacker and Heath 2001). Trap colour and shape has been investigated and appears have a significant effect on catches although the presence / absence of baits appears to be

a more important consideration (Robacker et al. 1990b, Robacker 1992) (Epsky et al. 1995) (Robacker and Heath 2001).

McPhail traps are not species specific and an alternative cylindrical plastic “dry” trap has been developed that has similar performance when used with a lure but catches very few non tephritid flies (Heath et al. 1995). A number of trap colours have been examined of which green appears to be the most effective. A number of other studies have investigated the merits of different traps and discussed the cost-effectiveness of the different types for surveillance of *A. ludens* (Thomas et al. 2001, Montoya et al. 2002, Toledo et al. 2005). Similarly, time of day affects capture with more insects typically caught in the afternoon and early evening (Malo and Zapien 1994).

Extraction of some bait materials has identified a number of molecules that elicit strong attraction responses in the fly, such as hexanal, ethyl hexanoate and 1,8-cineol, that have the potential be used in traps as a multi-component lure (Robacker et al. 1990a, Robacker et al. 1992). A series of studies have evaluated attractants deriving from a range of fruits (Robacker et al. 1997) whilst human urine is also effective (Aluja and Pinero 2004). However, despite the success of these food baits, attractants based on the chemical communications of *A. ludens* have also been developed (Landolt and Heath 1996). A male produced pheromone has been identified that elicits attraction response in virgin females (Robacker and Moreno 1988). As has recently been indicated, trapping fruit flies as part of surveillance programmes is affected by multiple components and no one trap type or bait is likely to be ideal in all circumstances (Diaz-Fleischer et al. 2009).

A number of studies describe the practical use of the surveillance methods detailed here. In a well described example, a five year study in Mexico monitored the populations of *Anastrepha* flies using McPhail traps. Here traps were baited with hydrolyzed protein (+ borax as a preservative) and placed at ¾ of mango tree height with a total of 80 traps per orchard deployed, which were examined weekly (Aluja et al. 1996). A total of five orchards were monitored to give a good picture of seasonal abundance and how the insects tended to be captured more frequently at the edges, as opposed to the centre, of orchards.

4. Commodities

The ability of the pest to travel with infested fruit necessitates the inspection of traded material. For example, the pest was discovered in mangoes imported from Mexico to the USA, a finding that led to new quarantine regulations being instigated (Anonymous 1985). Further finds of the pest led to a temporary cessation in imports of fruit from Mexico into California (Anonymous 1986a).

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Anastrepha obliqua (Macquart)

Common name(s): West Indian / Antillian fruit fly
Taxa: Insecta: Diptera: Tephritidae
EPPO A1 list: No. 231
EU Annex designation: I/A1

Organism

Anastrepha obliqua is a tephritid fruit fly whose larvae attack several fruit crops of which the most Important is mango (*Mangifera indica*), where it can be the predominant species, although other species are affected to a lesser extent (Aluja et al. 1987, Martinelli et al. 1997). In common with other *Anastrepha* species, the fly lays its eggs under the skin of fruit, the larvae subsequently feeding within the fruit making detection somewhat difficult until the later stages of infestation. The fly occurs throughout the Americas but is absent from the EU/EPPO region.

1. Detection

The detection of *Anastrepha* species is primarily through the use of traps, detailed below. However, accurate diagnosis of *A. oblique* and differentiation from other related species is typically made on the basis of morphological characteristics (Aluja et al. 1987), including puparial characteristics (Barbosa et al. 2005). The potential for molecular differentiation has also been explored and a Heteroduplex Mobility Assay (HMA) has been shown to be effective in detecting variability of the D2 domain of the 28S rRNA gene and gives rise to the potential for the rapid determination of the identity of *A. obliqua* (Barbosa et al. 2005). A detection survey targeting several invasive species conducted in China, using a range of trapping methods, confirmed the absence of *A. obliqua* within the country (Chen and Tseng 1993).

2. Delimitation

Although not specifically designed as a delimiting survey, the extensive monitoring of *Anastrepha* species in Mexico using several hundred McPhail traps within mango, citrus and guava orchards (up to 80 per site) was effective in determining the ranges of several species from the genus (Aluja and Birke 1993).

3. Monitoring

In common with other *Anastrepha* species, significant effort has been expended on evaluating the best traps and lures for monitoring purposes. The McPhail trap has been, and continues to be, widely used in conjunction with a number of baits in order to monitor populations of *A. obliqua* (Aluja et al. 1987, Soto-Manitiu and Jiron 1989). However, as Aluja (1999) notes, this trap design is costly, non-selective and inefficient but has continued to be used due to an absence of effective commercially available alternatives. This notwithstanding, the McPhail trap frequently performs well in comparison with other designs (Gonzalez et al. 1994, Montoya et al. 2002) although some alternatives are easier to implement (Vasquez and Jimenez 2004). A number of good examples of monitoring exercises have been produced including several from Mexico that describe various schemes for McPhail trap placement (Aluja et al. 1987, Aluja et al. 1990, Celedonio-Hurtado et al. 1995, Rodriguez-Navarro et al. 2001) and Costa Rica (Jiron and Hedstrom 1991, Hedstrom 1993).

Other monitoring exercises have used the “Valenciano” and “Melpan” type traps (Zahler 1991b, a) as well as bottles hung within trees (Martins et al. 1993). It has been noted that traps placed at the periphery of orchards are typically more effective in catching *Anastrepha* than those centrally placed (Aluja et al. 1996). Light traps have also been evaluated for trapping fruit flies, including *A. obliqua* (Mendonca et al. 2003).

A range of attractants have been used, including Torula yeast, protein hydrolysate, molasses, human urine, fruit juices, ammonium acetate and borax amongst others (Hedstrom and Jiron 1985, Hedstrom 1988, Hedstrom and Jimenez 1988, Aluja et al. 1989, Jiron and Soto-Manitiu 1989, Lemos et al. 2002). In particular, human urine has been shown to out-perform many other types of bait for *A. obliqua* (Aluja and Pinero 2004). Several workers have evaluated naturally occurring or waste materials for use as baits as well as extracts of host fruits (Fragenas et al. 1996, Ortega Zaleta and Cabrera Mireles 1996) (DeMilo et al. 1997). Many have been proven to be highly attractive and differences between them are probably due, in part, to the context they are used in and trap design (Diaz-Fleischer et al. 2009). It has been noted that baits often perform best after a period of around ten days of decomposition, a fact that suggests that trap turnaround times should reflect this (Malo 1992).The quantity (dose) of bait placed within traps has been shown to be an important consideration for trapping (Cabrera Mireles et al. 1995).

4. Commodities

No specific information found although procedures are likely to be similar to those detailed for other *Anastrepha* species.

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Anastrepha suspensa (Loew)

Common name(s): Caribbean fruit fly
Taxa: Insecta: Diptera: Tephritidae
EPPO A1 list: No. 200
EU Annex designation: I/A1

Organism

Anastrepha suspensa is a tephritid fruit fly that primarily attacks guava (*Psidium guajava*) and Surinam cherry (*Eugenia uniflora*) although it known from other economically important fruit crops, including citrus (Calkins and Webb 1988, Nguyen et al. 1992). The pest is of particular importance in the Greater Antilles and southern Florida (Boykin et al. 2006).

1. Detection

Trapping is the primary method for detection of this pest (detailed below) and a number of trap designs have been evaluated for this purpose (Burditt 1982). The efficacy of traps as a detection tool (probability of detection) has been examined using McPhail traps and detailed information on the likelihood of catching a fly for a given trap density (from 1-32 traps per hectare) and population size has been produced (Calkins et al. 1984). The probability of detecting infested fruit through dissection has also been addressed and the differences in the likelihood of detecting the insect from different fruit determined (17-83.5%) (Gould 1995). It was also noted that large differences occurred in the capacity of different inspectors to detect the pest through dissection due to differing approaches of workers to implementing the protocol. As an alternative to dissection acoustic methods for the detection of the larvae at very low levels (one per fruit) have been developed and successfully tested (Anonymous 1985, Pyrah 1985, Calkins and Webb 1988).

2. Delimitation

Certification of fly free-zones is achieved using traps in the United States (Mason and Baranowski 1989).

3. Monitoring

Monitoring for *A. suspensa* is carried out using traps, of which the design and features that promote capture have been extensively examined. Whilst the McPhail trap is often used (pictured by Davis et al,(1984)) alternatives such as sticky traps of various designs and colours (with and without bait) have also been evaluated as cost-effective alternatives (Davis et al. 1984). Various modifications to the McPhail trap have been examined with varying degrees of success (Mason and Baranowski 1989). Jackson traps (a triangular tubular sticky trap) has also been examined as a cheap alternative to the McPhail trap (Greany et al. 1982). Sticky traps have also been baited with males as an attractant (see below). Colour of sticky traps has been shown to be an important consideration and the fruit fly appears to prefer orange and trap efficacy was correlated with the proportion of light reflected at 580-590 nm (Greany et al. 1977). Furthermore, when fluorescent orange traps were examined they improved catches over non-fluorescent traps (Greany et al. 1978). A variety of spherical traps of different diameters and colours performed comparably with McPhail traps when bait was used (Sivinski 1990). A number of other investigations concerning traps design and/or bait efficacy are available (Thomas et al. 2001, Hall et al. 2005, Pingel et al.

2006).

Anastrepha suspensa females have been demonstrated to be attracted to volatiles released from males in the laboratory (Perdomo et al. 1975) and under field conditions (Perdomo et al. 1976). Interestingly, males appear to also be attracted to volatiles released by other males and traps can be at least as effective as food-baited McPhail traps. Pheromone-based trapping programmes have been suggested that take advantage of the knowledge of the chemical communications of this species (Landolt and Heath 1996) and the sex pheromone blend has been characterized (Lu and Teal 2001) although such methods are yet to supplant traditional trapping methodologies.

Food baits have been widely evaluated and, in common with other *Anastrepha* species, protein hydrolysates perform well as do fruit juices, molasses and host extracts (Sharp and Chambers 1983, Murguido et al. 1987) and some commercially available/synthetic materials (e.g. NuLure, BioLure, putrescine)(Epsky et al. 1993, Heath et al. 1993, Epsky et al. 1994, Holler et al. 2009). Various combinations of attractant compounds have also been evaluated in attempts to optimize trap catches (Sharp 1987). The effective range of various baits has been investigated as a means of determining optimal trapping strategies (Kendra et al. 2010).

Monitoring efforts for *A. suspensa* have been described in several situations, a good example provided by a study in Florida where *A. suspensa* was monitored in urban, semi-urban and agricultural areas in a highly systematic and very well detailed study (Nguyen et al. 1992). This survey used approximately 2000 McPhail traps at a rate of 10-15 per square mile (2.56 km^2) baited with Nulure and/or Torula yeast. This study also dissected fruit and gives the infestation rates for grapefruit, orange, tangerine, calamondin and kumquat, indicating them to be minor hosts. Loquat, Surinam cherry and guava were much more heavily infested, confirming them to be preferred hosts for this generalist pest. A survey in Puerto Rico used McPhail traps to monitor populations of *A. suspensa* in 14 citrus cultivars (Pantoja et al. 2007) that indicated the strong influence of the season on abundance of the pest.

4. Commodities

Various methods for detection of the *A. suspensa* within fruit have been evaluated for quarantine proposes. These methods, include dissection and acoustic techniques, are detailed in section 1 (above)

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Andean potato latent tymovirus

Common name(s): APLV
Taxa: Viruses: Tymoviridae: Tymovirus
EPPO A1 list: No 244
EU Annex designation: I/ A1

Organism
The principal host of APLV is potatoes (*Solanum tuberosum*). The virus can also be transmitted mechanically to species of Amaranthaceae, Chenopodiaceae, Curcurbitaceae and Solanaceae. The virus is widespread in Andean countries (Bolivia, Colombia, Ecuador and Peru) especially at high altitudes. APLV belongs to the tymovirus group, members of which are typically beetle-transmitted. *Epitrix* spp. (flea beetles) could act as natural vectors, but only when beetle populations are high. The virus is also transmitted by contact between plants and through true potato seed. Transmission to tubers is erratic. A wide daily fluctuation in temperature, particularly cold conditions, favours symptom expression in infected plants growing at high altitude. Severe symptoms are also induced by mixed infections with other potato viruses. The isolates of APLV can be combined into three major strain groups recognized by spur formation tests (Koenig *et al*., 1979).

1. Detection
Symptoms of the virus vary depending on virus strain, potato cultivar and growing conditions, and range from mild to severe mosaic with necrotic flecking, curling and leaf-tip necrosis. *Nicotiana bigelovii* is the best indicator host plant for detecting APLV. Local lesions develop in inoculated leaves followed by systemic and characteristic systemic netting of minor veins. *Nicotiana clevelandii* shows necrotic or chlorotic spots on inoculated leaves.
The latex agglutination test detects all known isolates with the same antiserum, but is less sensitive than ELISA (Koening and Bode, 1978; Fribourg and Nakashima, 1984). ELISA is rather strain specific (Koening *et al*., 1979) but this can be overcome by using a mixture of antisera for each of the three virus strain groups (Schroeder and Weidemann, 1990). Abdullahi *et al*. (2005) demonstrated the potential of microarray-based hybridisation for the identification of a range of viral pathogens of potato, including APLV.

2. Delimitation
No information.

3. Monitoring
No information.

4. Commodities
There are strict quarantine measures in the EPPO region against the introduction of APLV. Potato seedlings and plants are inspected once a week for virus symptoms. Leaf tissue is serologically tested using the latex test (or other sensitive serological test with broad specificity). All material is rejected where infection is detected by serological tests. Remaining seedlings and plants are tested by inoculating combined leaf samples into indicator plants of *Nicotiana clevelandii*. Indicator plant hosts are maintained under cool conditions for at least 5 weeks. Tissue cultures, tubers and cuttings are also tested serologically for APLV (Anon, 1984).

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Andean potato mottle comovirus

Common name(s): APMoV
Taxa: Viruses: Secoviridae: Comovirus
EPPO A1 list: No. 245
EU Annex designation: I/ A1

Organism
Andean potato mottle virus (APMoV) primarily infects potato and other solanaceous hosts. It is currently absent from the EPPO region and its current range is restricted to South America and the Caribbean (Anonymous 1999). Symptoms in potatoes include mosaic and mottle symptoms, necrosis and stunting.

1. Detection
A number of methods exist for the detection of APMoV. Earlier methods were based on a latex agglutination assay (Koenig and Bode 1978, Fribourg and Nakashima 1984). However, these methods have been superseded by immunosorbent and molecular techniques. ELISA and DAS-ELISA procedures have been developed with high specificity and sensitivity (Dusi and De Avila 1988, Schroeder and Weidemann 1988, 1990). The use of RT-PCR has also been demonstrated to effectively detect this virus (Brioso 1999) as has, more recently, microarray hybridization which allows the diagnosis of several different target viruses simultaneously (Abdullahi *et al*. 2005).
In addition to the assay methods described above, the use of mechanically inoculation of indicator plants has been used as an effective means of detecting APMoV and other seed-borne potato diseases (Verhoeven and Roenhorst 2000, 2003) (plant species and methods are detailed).

2. Delimitation
No information available

3. Monitoring
Little information on surveying for this virus was available. Descriptions of *ad hoc* monitoring exercises are provided by Clausen *et al*. (2005) for a survey in Argentina. A survey for a series of potato viral pathogens from Costa Rica provides more details of sampling but was, again, not a well defined survey (Vasquez *et al*. 2006). General EPPO phytosanitary procedures for potatoes post-entry are also applicable to this pathogen (Anonymous 2004).

4. Commodities
A small number of studies describe the surveying of imported batches of seed potato for APMoV. These include the examination of imports to India (Dwivedi 1986) and the EU (Anonymous 2004).

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Anisogramma anomala (Peck) E. Muller

Common name(s): Eastern filbert blight
Taxa: Fungi: Ascomycetes: Diapothales
EPPO A1 list: No. 201
EU Annex designation: II/A1

Organism

Anisogramma anomala is a biotrophic fungal parasite of *Corylus* spp., which is grown in culture only with difficulty. It is indigenous and considered an endemic pest on the native American filbert (*Corylus americana)*, a common understorey shrub of forests in the eastern USA. However on the European cultivated hazelnut (*Corylus avellana)* it causes the severe disease eastern filbert blight (EFB). It has also been reported on other *Corylus* spp.

1. Detection

The stroma is the main diagnostic character of the fungus (EPPO, 2009). Stromata usually occur in single or double rows in 1 year old cankers (also known as pustules) with 3-5 rows of stromata in 2 or 3 year old perennial cankers. On small diameter wood (ca. 1cm or less) pustules occupy sufficient area to be quite noticeable, whereas in wood of a greater diameter perennial cankers are often only visible from the side or a branch. Death of the cambium in the area of the canker results in a sunken appearance as the surrounding cambium continues to grow. Visual estimations of the percentage of the total number of tree limbs with cankers were estimated to determine disease severity. Infection can also be detected by examination of subperidermal sections for distinctive intracellular hyphae.

Dispersal of spores is determined by spore discharge, analysis of spore trap slides and spore dispersal patterns. Spore trapping studies in orchards have shown that there are distinct episodes of spore release that correspond to intense periods of rain. Aspirated spore samplers placed on towers in an orchard showed that spores dispersed vertically and horizontally away from canopy during methods of extended branch dampness. Spore traps can consist of gutter traps, funnel traps or Burkhard volumetric traps that collect rain water or sample the air (Johnson *et al*., 1993; Pinkerton *et al*., 1993a). Traps drain into reservoirs with 5% copper sulphate as a preservative. Presence of spores in rain or irrigation water is examined following filtration through a 0.8um cellulose nitrate membrane and are changed monthly (Johnson *et al*., 1993; Pinkerton *et al*., 1993a).

Identification is generally based on morphological characters *in vivo*, which are very specific and unlikely to be confused with other fungi (EPPO, 2009). ELISA rapid screening was developed to identify and screen for resistance to EFB, but antisera are no longer available and this test is not recommended (EPPO, 2009). However real-time PCR is currently being developed at Rutgers University (Molnar and Zhang, 2011). Detection and identification of *A. anomala* in nursery stock and breeding populations is challenging and time-consuming using conventional methods, because disease symptoms show only after 16 months from infection, and the fungus can only be cultured from sporulating perithecia (Molnar and Zhang, 2011). Molnar and Zhang (2011) developed a culture-independent TaqMan real-time PCR assay that enabled pathogen detection from field samples within a few hours. The assay was validated with the target pathogen, closely related fungal species, and a number of other microorganisms that inhabit *C. avellana.* The detection limit of the assay is 0.1 pg *A. anomala* genomic DNA, which enables EFB diagnosis many months before disease symptoms develop. Compared with traditional diagnostic methods, the TaqMan real-time PCR assay was more sensitive, efficient, and rapid.

2. Delimitation
No information.

3. Monitoring
Regular scouting is key to early detection of this disease. Disease scouting is primarily done in winter when leaves do not obstruct cankers and in late summer when cankers can be detected by the presence of dead leaves attached to dead branches. Hazelnut orchards were surveyed by walking along rows of trees and closely checking for branch dieback or flagging. Closer inspection of flagged branches was made for evidence of perennial cankers and the distinctive large (3 x 6 mm) black oval shaped perithecia that protrude in rows along the canker surface (Buonassisi, 1990).
Surveys of orchards carried out by Pinkerton *et al*. (1992, 1993b) involved inspection of susceptible trees after leaf fall until branches were obscured by new leaves in April. In orchards with highly susceptible cultivars main crop trees in every third row were inspected. Each tree was inspected for about 5 minutes. When EFB was detected, surrounding trees were also inspected. Twigs and branches were visually examined for dead branches, sunken cankers, stromata and unabscised dead leaves. Binoculars were used to inspect branches in the upper canopy. Criteria used to verify EFB infection included canker morphology and the presence of stromata of *A. anomala*. Trees were rated for disease severity: 0= no detectable cankers, 1= one single canker, 2= multiple cankers on a single branch, 3= multiple branches with cankers, 4= >75% of branches with cankers, 5= canopy dead except for basal suckers. Age of the oldest canker was estimated by counting the row of stromata within the perennial cankers.
Five funnel spore traps were placed at different places within an orchard and examined at weekly intervals (Johnson *et al*., 1993). Burkhard volumetric spore traps were positioned between two tree rows, 2.5m from surrounding tree with trap orifices positioned 1.5m above ground (Pinkerton *et al*. 1993b). Traps were changed and processed weekly.

4. Commodities
No information.

References 39 retained out of 129 retrieved, 2 added, 7 used for summary

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Anomala orientalis (Waterhouse)

(=*Blitopertha orientalis* (Waterhouse))
Common name(s): Oriental beetle
Taxa: Insecta: Coleoptera: Scarabidae
EPPO A1 list: No. 33
EU Annex designation: I/A1 as *Anomala orientalis*

Organism
Anomala orientalis is a polyphagous pest whose larvae feed on the roots of grass and many vegetable crops and have been recorded damaging maize, pineapples and sugarcane. The adults feed on flowers (*Alcea rosea, Dahlia* spp., *Iris* spp., *Phlox* spp. and roses).
The lifecycle is usually completed in 1 year (latitude New York) although individuals may spend 2 winters as larvae. Adults emerge towards the end of June and are present for about 2 months. Swift, low, short flights (1m) are characteristic during the day from 08:00 to 16:00. From early July to September, females burrow into the soil to lay eggs beneath the surface. Eggs hatch in a few days and larvae burrow to 10-20cm from the soil surface and feed on roots. There are 3 larval instars. From mid-October larvae descend to 20-42cm in soil where they overwinter. Towards the end of April they return to the surface and feed until early June when they pupate. The insect is found in the Far East and North America.

1. Detection
The identification and synthesis of the female sex pheromone of the oriental beetle has led to the development of an effective synthetic pheromone trap which has facilitated the monitoring and detection of oriental beetle populations.
Alm *et al*., (1999) describe the use of traps baited with the pheromone, (Z)-7-tetradecen-2-one, to determine the current distribution of oriental beetles on golf courses in the United States. They evaluated various pheromone concentrations, release devices (rubber septa and polyethylene pellets), trap designs (Trece and Fuji trap) and heights for beetle captures. There was no significant difference in beetle captures where 100 or 1000 µg of pheromone was released from rubber versus polyethylene septa. There was no significant difference in trap captures between Trece and Fuji traps where 3000 µg of pheromone was released from polyethylene pellets in 7 out of 8 comparisons. There was a significant increase in captures where the funnel rims of traps were placed at ground level versus 30cm above the ground. Oriental beetle flight is arrested up to a meter or more from the trap, but they will continue to walk towards the pheromone source. Oriental beetles generally fly at night and are only seen flying in large numbers during the day when the pheromone causes them to emerge from the turf. Rubber septa loaded with 10, 100 and 300 µg per septum provided a steady rate of pheromone release over 4 weeks in laboratory studies.
Larval densities have been assessed in turf by using a golf course cup cutter and removing approx. 0.3m^2 of turf to a depth of 6-8cm and hand digging to a depth of 12cm; and by taking 20 soil cores using a golf hole cutter (10.8 cm in diameter by 10cm in depth) from the centre of each plot. Larvae were collected by shifting and shaking from the soil and identified to species by examining the raster with a hand lens.
Emergence cones, turf and soil sections, 5 and 15 min random walks, vegetation visits, pheromone trapping, transects and pupal sampling have been used to assess the distribution of *A. orientalis* on golf courses,

2. Delimitation
No information.

3. Monitoring
Alm *et al*. (1999) placed traps baited with pheromone, 10m apart and checked weekly for male beetle captures to assess the current distribution of *A. orientalis* in the USA. The recommendation was to use at least 1000µg of pheromone and to place funnel trap rims at ground level (Alm *et al*., 1999).
Different formulations of pheromone on mating disruption have been assessed by monitoring male beetle captures from early June until the end of the flight period around mid August in the USA. Trece Japanese beetle traps were baited with a rubber septa containing 30 or 300 µg of *A. orientalis* pheromone. One trap was placed in the centre of a 0.093 ha plot. The traps were fitted into a hole in the ground so that only the funnel portion remained above ground. Septa were replaced with fresh septa lures every 3 weeks. The traps were emptied twice per week (Behle *et al*., 2008; Koppenhoefer *et al*., 2008a; Rodriguez-Saona *et al*., 2010).

4. Commodities
No information

References: 23 retained out of 140 retrieved, none added, 4 used for the summary
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Anoplophora chinensis (Forster)

There is some overlap in the synonymy of the two species *Anoplophora chinensis* and *Anoplophora malasiaca* (Thomson), which are very closely related. It has been suggested that they are simply forms of a single species. For example, Sorauer (1954) treats the forms in China and Japan as the single species *Melanauster chinensis* Forster, as does Duffy (1968) under the generic name *Anoplophora*.

Common name(s): Citrus long-horn beetle
Taxa: Insecta: Coleoptera: Cerambycidae
EPPO A1 list: No. 187
EU Annex designation: I/A1

Organism

Anoplophora chinensis feeds on a wide range of woody host plants. These include species of: *Acer*, *Citrus*, *Cryptomeria japonica*, *Malus*, *Populus*, *Salix*, *Ficus*, *Hibiscus*, *Mallotus*, *Platanus*, *Pyrus* and *Rosa*. The species is widely distributed in Asia, e.g. China (subtropical areas, including Fujian, Jiangsu), Hong Kong, Korea Republic, Malaysia, Myanmar, Vietnam. It is recorded in the USA (Hawaii; interceptions on mainland, but is absent in the EPPO region and EU.

The female beetle lays about 70 single eggs directly under the bark of the trunk. The egg laying zone may vary from right above the soil surface to 60 cm higher. The larva feed inside of branches and trunk under the bark, but in the progress of development enter the woody tissues of the lowest portions of the trunk and roots. Pupation takes place in the wood, often in the upper part of the feeding area. It is reported from tropical and subtropical regions, that one single generation per year develops. Depending on climatic and feeding conditions the life cycle occasionally takes within two years. The adults, living about a month between May and August are feeding on leaves, petioles and young bark of the various host trees. The beetles are able to fly and have been found in unheated glasshouses and even outdoors, damaging trees and shrubs in the summer.

Infested trees are weakened by larval attack and are more readily susceptible to diseases and wind damage. Eventually, most infested trees will die, although this may take a few years. Damage to small, young trees is most serious (Scholte *et al*., 2007).

In international trade, *Anoplophora* spp. are most likely to move as eggs, larvae or pupae in woody plants intended for planting, including bonsai plants, and possibly in packing material. Individuals (larvae and adults) have entered Germany and Netherlands on bonsai plants of *Acer buergeranum*, *A. palmatum*, *Celastrus*, *Cydonia sinensis*, *Malus micromalus* and *Sageretia* from China and Japan.

1. Detection

Early detection of invasive species of wood boring Coleoptera is often very difficult because of their hidden living spaces. Two new approaches are described in the literature.

As visual inspection and manual destruction of samples is time consuming and expensive, two devices for the acoustic detection of wood boring Coleoptera were developed at the University of York, Department of Electronics. Addressing the difficulty of detection arising from little or no evidence of the presence of larvae inside the imported material, acoustic methods are employed were used to detect movement and feeding activity and, what's more, species can be identified where there are distinctive feeding characteristics. The devices aim to provide plant health inspectors with practical tools capable of detecting Larvae *in situ*. According to Chesmore and Schofield (2010) a hand held instrument, which will be used for the rapid checking of material, and a data logging system which will have applications in longer term monitoring of outbreak sites,

sentinel trees and ecological studies are under development.
As an interesting second methodology, sniffer dogs, known from their valuable work, e.g. for the police, are trained especially to detect the scent sources associated with infestation. Therefore, dog races bred for many generations for their sniffer-dog performance are especially useful. According to Hoyer-Tomiczek and Sauseng (2009) the training aims to precisely detect all developmental stages of ALB and CLB both indoors as well as in the open field. Outdoors the dogs are capable of detection up to 2.5 meters above ground, in the trunk base, stumps, roots and sprouts. Indoors the dogs can find the pest in both imported host plants and in imported wood packaging material. The combination of the usual visual controls and the dogs' olfactory search is more effective than the formerly used method. These sniffer dogs are successfully employed in Germany.
A first detection report is available from Croatia in a nursery in September 2007. According to the EPPO quarantine procedures, a ban on plant relocation was immediately issued, together with tracking and isolation of the small number (less than 50) of plants that were transported to two nurseries inside Croatia (Zagreb and Split). Both suspicious, sickening and clearly infested plants were burned, while all other plants in the nursery of first CLB record were closely monitored and banned from relocation for the following two years (Vukadin and Hrasovec, 2008).

2. Monitoring
The type of monitoring carried out mainly aims at the delimitation of the plant health pest.
The distribution of the pest in Italy was determined during an intensive survey program covering the previously known infested sites and their surroundings within a radius of two kilometres around every infested tree. The monitoring was based on visual inspection of susceptible trees to find symptoms of CLB presence. From summer 2005 onwards, around 60 000 trees were checked in the public areas of more than 60 municipalities (Maspero *et al*., 2008).
Monitoring of citrus longhorn beetle was done by checking for the presence of holes, sawdust and oviposition scars in host trees, and by collecting adults and other stages. Such monitoring commenced at the core of the infestation, and was then extended out from this zone over increasing distances. All infested trees were located on a map, by means of GPS; a database, with information concerning these trees, was then created (Maspero *et al*., 2005).

3. Delimitation
An extended summary of the current situation in Europe that discusses eradication approaches of several papers in detail is provided in van der Gaag *et al*., 2010. The authors describe eradication measures conducted in France, Croatia, Italy and the Netherland in detail. Most measures are varying in the size of radius around the initially infested tree or shrub and the further demarcated area to be surveyed. Also, the kind of dissecting and insect removal procedures, or the wire mesh covering procedures in infested trees varies widely between countries. On this basis the paper allows a systematic comparison of measures to be made.
The EU Commission Decision 2008 / 840 /EC requires, among other things, that when an infestation is discovered a buffer zone of at least 2 km should be demarcated around the infested area. Following a delimiting survey, the buffer zone may be reduced to 1 km on the 1st detection of the pest. Measures for import and trade apply to: *Acer* spp., *Aesculus hippocastanum*, *Alnus* spp., *Betula* spp., *Carpinus* spp., *Citrus* spp., *Corylus* spp., *Cotoneaster* spp., *Fagus* spp., *Lagerstroemia* spp., *Malus* spp., *Platanus* spp., *Populus* spp., *Prunus* spp., *Pyrus* spp., *Salix* spp., and *Ulmus* spp. (van der Gaag *et al*., 2010).
In France infested trees were removed and stumps were partially dissected resulting in the finding

of more larvae and eggs. Surveys were conducted within a radius of 1 km around the infested site in years 2003–2005. Since 2006 *A. chinensis* was officially declared eradicated (van der Gaag *et al*., 2010).

In Croatia monthly surveys were performed at a nursery from May to October in 2008 and 2009. During these surveys frass was found on pots of another 22 and 38 *Acer palmatum* plants in 2008 and 2009, respectively. Cutting the stem revealed the presence of larvae inside the stem. Intensive surveys were performed at the nursery and surroundings in 2008 and 2009, but no other signs of the pest were found. Surveys were continued at the nursery and surroundings and movement of plants from the nursery is prohibited until the end of 2010. The status of *A. chinensis* in Croatia should be considered as transient under eradication (van der Gaag, 2010).

In Italy an area with a radius of 1 km has been demarcated around the centre of an infestation and a buffer zone of 2 km (1–3 km zone) around the 1 km zone. Surveys within the 1- and 1–3-km zone will be performed at least until 2012. Trees were removed and checked for presence of *A. chinensis* as follows: After a visual inspection of the whole tree that aimed to detect exit holes and / or larval frass, the trunk of each tree was cut at 2 m height. A few trees were cut higher, approximately at 6 m height, because exit holes were detected near the major tree branches insertions. All the upper parts of trees were destroyed by chipping or burning in accordance to the decree in force (MiPAAF 2008, Decreto 9 Novembre 2007 – Disposizioni sulla lotta obbligatoria contro il cerambicide asiatico *Anoplophora chinensis* (Thomson). The 2 m trunks (or larger in a few cases) were cut in portions of 20 cm in thickness and inspected for any signs of larval holes. The non-infested sections were chipped while the infested portions were stored in a walk-in insect cage for 1 year, until March 2010, to study the development of the pest after which they were chipped. The stumps and superficial roots were neutralized by mechanical removal, chipping, chemical treatment to kill the stumps (using a mixture of glyphosate and picloram) or by covering with wire mesh (mesh size: 6 mm) and soil or concrete. The wire mesh and concrete will stay for at least 2 years and are regularly inspected by the Plant Protection Service for integrity and effectiveness. No signs of *A. chinensis* on any the 59 trees that had been removed in a precautionary way were found (van der Gaag *et al*., 2010).

In the Netherlands a survey was performed within a radius of 600 m around an infested tree. In a radius of 100 m around plants showing exit holes, all plants belonging to the host plant genera *Betula* (7 trees), Salix (5), *Populus* (2), *Corylus* (5), *Carpinus* (3) and *Fagus* (4) were removed. In a radius of 200–300 m all Acer spp. (88) were removed of which approximately 60–70 were within the 1st 100 m. A radius of 300 m was chosen in those directions where no *Acer* spp. were present in the 100–200 m zone. In total, 114 trees and shrubs were removed, of which 51 had a diameter of 30 cm or more at breast height. Every tree or shrub that was removed was checked for presence of *A. chinensis* as follows: Each trunk was cut at different heights above soil level: 5, 40 and 100 cm and each cut edge was observed for any signs of the pest (larval holes). Shrubs and small trees were pulled out the soil and cut at about 0, 40 and 100 cm height;

When symptoms were observed the trunk part was transported to the Plant Protection Service in Wageningen for further examination; when symptoms were observed on the 100 cm cutting edge the higher part of the trunk (above 100 cm) was further examined. When symptoms were observed on the 5 cm cutting edge, the tree or shrub was uprooted and transported to the laboratory for further examination. Where no symptoms were observed on the 5 cm cutting edge, the stump was removed by grinding to a depth of about 5 cm below soil level. When symptoms were observed at the 5 cm cutting edge the rest of the plant was uprooted for further examination. If no symptoms were observed the roots were ground away to a depth of about 30–40 cm. The 5–40 cm part of each trunk was always transported to the Plant Protection Service in

Wageningen. At the Plant Protection Service, the bark was peeled off to further check for presence of any larvae or symptoms (van der Gaag *et al*., 2010). More details regarding the Italian eradication measures can be found in Maspero, M., C. Jucker, *et al*. (2005) and (Maspero *et al*., 2008).
Similar methods to delimitate and eradicate *A. chinensis* are reported for the USA and Canada in Haack *et al*. (2010). Also, the authors compile eradication program costs and discuss mechanical, chemical and biological pest management issues along with the prevention of introduction.

4. Commodities
Since 2005, in international trading the IPPC (International Plant Protection Convention) standard ISPM (International Standard for Phytosanitary Measures) No. 15 has to be observed. In most cases this implies a heat treatment or fumigation, e.g. with methyl bromide, for wood packaging material. Spraying of pesticides is an alternative but is rarely done. From time to time larvae, pupae or beetles in wooden material survive the treatment and there remains the challenge to find ways of improving this situation (Benker, 2008).

References: 28 retained out of 99 retrieved, none added, 9 used for summary

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Anoplophora glabripennis (Motschulsky)

Common name(s): Asian long-horn beetle
Taxa: Insecta: Coleoptera: Cerambycidae
EPPO A1 list: No. 296
EU Annex designation I/A1

Organism

The host range of *A. glabripennis* is split according to the developmental stages of larvae (development to maturity) and adults (maturation feeding). The major hosts of *A. glabripennis* in China are species and hybrids of the Aegeiros section of the genus *Populus*: *P. nigra*, *P. deltoides*, *P. x canadensis* and the Chinese hybrid *P. dakhuanensis*. Some poplars of the other sections of the genus (*Alba* and *Tacamahaca*) are also attacked. *Salix* spp. (*S. babylonica*, *S. matsudana*) are also major hosts. Various other woody plants have also been recorded as hosts in China: *Acer*, *Alnus*, *Malus*, *Morus*, *Platanus*, *Prunus*, *Pyrus*, *Robinia*, *Rosa*, *Sophora* and *Ulmus*. Within the urban outbreak areas in North America, *A. glabripennis* has mainly been found on Acer spp. (*A. negundo*, *A. platanoides*, *A. pseudoplatanus*, *A. rubrum*, *A. saccharinum* and *A. saccharum*) and on *Aesculus hippocastanum*. However, it has also been found on a range of other hardwood species: *Betula*, *Fraxinus*, *Liriodendron tulipifera*, *Morus alba*, *Populus*, *Robinia pseudacacia*, *Salix* and *Ulmus*. The species is indigenous to China, but also reported from Korea Democratic People's Republic, Korea Republic and Taiwan as well as from North America: USA (New York City and Illinois).

According to climate and feeding conditions, the development of a generation takes between one and two years. Thus, there can be one or two overlapping generations per year. Adults emerge between May and October and live for about a month. The adults usually remain on the tree from which they emerged, or fly short distances to nearby trees, and feed there on leaves, petioles and young bark. The eggs are laid one by one under the bark, in oviposition slits chewed out by the female. The larva feeds in the cambial layer of bark in the branches and trunk and later enters the woody tissues. Pupation takes place in chambers in the heartwood, accompanied by presence of characteristic wood “shavings” that are packed into the chamber. Adults emerge from circular holes, 10 mm across, above the sites where the eggs were laid (EPPO data sheets on quarantine pests).

1. Detection

As the detection is commonly done together with *A. chinensis* methods used are similar basing on the typical symptoms of the species. Nevertheless, as visual inspection and manual destruction of samples is time consuming and expensive, two new approaches, also used in *A. chinensis* detection and different from the destruction of trees, by olfactory and acoustic means are introduced. The first to notice is the employment of sniffer dogs reported by Hoyer-Tomiczek and Sauseng (2009). A more detailed description of the method is provided in the summary for *A. chinensis*.

The second method is an acoustic technology, which has potential for reducing costs and hazards of tree inspection. The development of practical methods for acoustic detection requires the solution of technical problems involving transmission of resonant frequencies in wood and high background noise levels in the urban environments where most infestations have occurred. A study was conducted to characterize sounds from larvae of different ages in cambium, sapwood, and heartwood of bolts from three host tree species (Mankin *et al*., 2008).

In China field trapping experiments with baited *A. glabripennis* male-produced pheromone in the summers of 2007 and 2008 were conducted according to Nehme *et al*. (2010).

To confirm the assumption of larvae belonging to the species *A. glabripennis* they were sent to laboratories for DNA-analysis.

DNA markers were identified for the molecular detection of the Asian long-horned beetle (ALB), *Anoplophora glabripennis*, based on sequence characterized amplified regions (SCARs) derived from random amplified polymorphic DNA (RAPD) fragments (Kethidi *et al*., 2003).

2. Monitoring

The type of monitoring carried out mainly aims at the delimitation of the plant health pest.

Mostly visual inspections for the presence of holes, sawdust and oviposition scars in host trees, and by collecting adults and other stages is done (see *A. chinensis* summary.

In the USA a model of spatial establishment patterns was developed. Delimiting hot spots for invasions (i.e., areas where establishment is likely) within urban areas would facilitate monitoring efforts. A propagule-pressure framework has been used to delimit establishment hot spots of a hypothetical generalist EFI in six U.S. urban areas: Chicago, Detroit, Houston, Los Angeles-Long Beach-Santa Ana, New York-Newark, and Seattle. Using a lattice of 5-km-diameter cells for each urban area, the input data was used (urban tree cover and propagule pressure) to model establishment and Moran's I to delimit hot spots. Urban population size and the area of commercial-industrial land use as indicators of propagule pressure in the model (Colunga-Garcia *et al*., 2010).

3. Delimitation

Eradication programs were conducted in Italy, France, Austria and Germany shortly after detection of *A.glabripennis* (Hoyer-Tomiczek, 2008; Krehan, 2008; Smith *et al*., 2009). Most eradication exercises comprise of thorough removal, chipping and incineration of the infested wood.

All infested trees were cut; the complete organic material was chopped into small pieces and then burned. Also, a monitoring program of all potential host trees was initiated; first in the vicinity of infested trees and later on also in other parts of the town and the surrounding forest. During the monitoring activities, carried out so far twice a year … several hot spots of infestation have been found (Krehan, 2008).

According to Herard et al., for EPPO (2009) in the Italian eradication program at Corbetta, the four *A. glabripennis*-infested trees were removed by ERSAF (Regional Institution for Agriculture & Forest Services) under the supervision of the Plant Protection Service of Lombardia. The trunks and branches were cut in 50 cm-long logs that were collected and appropriately transported, under official authorization

The eradication campaign consisted of destroying the infested trees and the uninfested susceptible trees belonging to the genera *Acer*, *Betula*, Salix and *Populus* located in a radius of 500 m around the yard containing the 4 infested trees. Further, a map was drawn covering a radius of 500 m around the initial point of infestation. Within this radius 309 susceptible trees were cut, 36 trees in the public domain and 273 trees on private property. The plant material was initially stored in a secured area then chipped and burned. Additionally, a campaign aiming to improve public awareness was implemented to help people understand the necessity of some tree felling.

Benker and Boegel (2006) reported about the situation in Bavaria. According to them a safety zone of 2 km was established around the infestation centre. In this safety zone monitoring for damaged deciduous trees, which were assumed to be host plants of the beetle, were carried out. The citizens of the affected area were informed of the situation and a general ordinance (disposition) was published committing the inhabitants for assistance in eradication measures. Altogether 16 trees were cut down, chaffed and burned. All the trees were within a radius of about 500 m of the centre of infestation. The monitoring has to be continued in the following years.

4. Commodities

No explicit information is provided for *A. glabripennis*.

References: 64 retained out of 127 retrieved, 1 added, 10 used for summary

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Franck HERARD, M. M., Nathalie Ramualde, Costanza Jucker, and M. C. Mario Colombo, Beniamino Cavagna Anoplophora glabripennis - Eradication programme in Italy

Anoplophora malasiaca (Thomson)

There is some overlap in the synonymy of the two species, which are very closely related. It has been suggested that they are simply forms of a single species. For example, Sorauer (1954) treats the forms in China and Japan as the single species *Melanauster chinensis* Forster, as does Duffy (1968) under the generic name *Anoplophora*.

Common name(s): White-spotted longicorn beetle
Taxa: Insecta: Coleoptera: Cerambycidae
EPPO A1 list: No. 188
EU Annex designation: I/A1

Organism
A. malasiaca is polyphagous on woody hosts, having been recorded on at least 68 species of host trees belonging to 19 families. These include *Alnus*, *Casuarina*, *Citrus*, *Litchi*, *Melia*, *Morus* and *Salix*. The species is widely distributed in Asia including Japan (Honshu, Kyushu, Shikoku, less common in Hokkaido), Korea Democratic People's Republic, Korea Republic (including Cheju Island), Taiwan. In the EPPO region and EU it is still absent (Data Sheets on Quarantine Pests).
The female beetle lays about 70 single eggs directly under the bark of the trunk. The egg laying zone may vary from right above the soil surface to 60 cm higher. The larva feed inside of branches and trunk under the bark, but in the progress of development enter the woody tissues of the lowest portions of the trunk and roots. Pupation takes place in the wood, often in the upper part of the feeding area. It is reported from tropical and subtropical regions, that one single generation per year develops. Depending on climatic and feeding conditions the life cycle occasionally takes within two years. The adults, living about a month between May and August are feeding on leaves, petioles and young bark of the various host trees. The beetles are able to fly and have been found in unheated glasshouses and even outdoors, damaging trees and shrubs in the summer.
In international trade, *Anoplophora* spp. are most likely to move as eggs, larvae or pupae in woody plants intended for planting, including bonsai plants, and possibly in packing material. Individuals (larvae and adults) have entered Germany and Netherlands on bonsai plants of *Acer buergeranum*, *A. palmatum*, *Celastrus*, *Cydonia sinensis*, *Malus micromalus* and *Sageretia* from China and Japan.

1. Detection
According to Colombo and Limonta (2001) *A. malasiaca* is recorded for the first time in Italy, being a quarantine pest for Europe. Detection of *A. malasiaca* during a survey of roadside trees in Fukuoka City (Japan) is reported by Ohga *et al*. (1995).

2. Monitoring
No information available.

3. Delimitation
Field studies were conducted in Shizuoka, Japan, in 1988 to determine the effectiveness of applying fine-meshed wire netting (25 m in width with 6 holes/cm) to prevent *Anoplophora malasiaca* from ovipositing on Citrus trees. Three treatments were assessed: an untreated control wrapping netting around the tree trunk without soil piled at the trunk base and wrapping netting around the tree trunk with soil piled at the trunk base. It is concluded that wire netting and protecting the base of the trunk with soil could be used as a basic control tactic in the integrated

control of *A. malasiaca* (Adachi, 1990).
The results of experiments using several oviposition-preventing methods located at the base of Citrus tree trunks are discussed. Oviposition by *A. malasiaca* was most effectively prevented by covering the bottom of trunks with netting (6 holes/cm). Covering the trunk with sticky cardboard or 2 cm fishing net treatment was effective at capturing adults as well as preventing oviposition (Adachi and Korenaga, 1989).

4. Commodities
No information available.

References: 8 retained out of 90 retrieved, none added, 4 used for summary

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Anthonomus bisignifer Schenkling

Common name(s): Strawberry weevil
Taxa: Insecta: Coleoptera: Curculionidae
EPPO A1 list: No. 189
EU Annex designation: II/A1

Organism
Strawberries (*Fragaria* spp.) are the main economic host crop, but *A. bisignifer* (strawberry weevil) has also been recorded on *Rubus* and wild roses (Anonymous, 2008). In Sendai, Japan adults emerge from hibernation in late April, mate and begin oviposition, mostly during the day, reaching a peak in mid to late May. Eggs are laid in holes excavated in the flower buds of strawberries which the female then bites off at the stalk a few millimetres below the bud. Most obvious signs of damage are partially severed buds hanging from plants and severed buds on the ground. The number of eggs laid is highest when temperature is above 20^{o}C and there is 12 hours of sunshine, but eggs are also laid at 12^{o}C. Weevils are usually inactive during the night or under cool cloudy conditions by day, but start crawling in the field at an air temperature of 7.2^{o}C. Flight begins at about 18^{o}C in the field.

1. Detection
Adults of a closely related species (*A. rubi*) are thought to be attracted to strawberries by chemicals released by the plants. Males also release aggregation pheromones which have been used as lures in baited traps (Innocenzi *et al*., 2001). Significant numbers of males and females were caught in the traps, with females more predominant in mid season and more males caught late season (Innocenzi *et al*., 2001).

2. Delimitation
No information.

3. Monitoring
No information.

4. Commodities
EPPO has drafted a commodity-specific phytosanitary consignment inspection procedure of *Fragaria* plants for planting (Anon, 2008). *Anthonomus bisignifer* is only likely to be detected at import on green plants. Partially severed buds can be seen hanging from infected plants. Severed buds may contain larvae and pupae which should be sent to laboratory for identification. Species has to be distinguished from the commonplace and widespread European *A. rubi* which is very similar in appearance and habits. There is no EPPO diagnostic protocol available.

References: 2 retained out of 37 retrieved, 4 added, 2 used for the summary
Anonymous (2008). Draft commodity-specific phytosanitary procedure consignment inspection of *Fragaria* plants for planting. Bulletin OEPP 38(3): 396-406.
Innocenzi, P.J., Hall, D.R. and Cross, J.V. (2001) Components of male aggregation pheromone of strawberry blossom weevil, *Anthomonus rubi*, Herbst. (Coleoptera: Curculionidae). J. Chem. Ecol., 27, 1203–1218.

Anthonomus grandis Boheman

Common name(s): South-eastern boll worm, Thuberia boll weevil, Mexican boll weevil.
Taxa: Insecta: Coleoptera: Curculionidae
EPPO A1 list: No. 34
EU Annex designation: II/B

Organism

The species is segregated by several adult characteristics into *A. grandis grandis* (South-eastern boll weevil), *A grandis thurberiae* (Thurberia boll weevil) and intermediates (Mexican boll weevil) (EPPO datasheet). *A. grandis* is indigenous to Central America and has since spread to all cotton growing areas in the USA. The principal host of *A. grandis grandis* is cotton including *Gossypium barbadense*, *G. hirsutum* and wild *Gossypium* spp. There is also significant reproduction in nature on a number of wild malvaceous hosts including weeds. *Anthonomus grandis thurberiae* mainly feeds on wild *G. thurberi* but also on cultivated cotton. In the EPPO region, cotton is the only host to be considered.

Especially in arid areas thermal convection may disperse flying adult weevils for long distances; up to 72km has been recorded. In Texas the greatest dispersal occurs from mid-August to September. In international trade boll weevils may be carried with cotton seeds, or bolls with raw cotton and various cotton products. During the spring, flight from hibernation areas to cotton appears to be confined mostly to low levels whereas during the late-summer and autumn migratory period, a significant increase in the height of flight was recorded (Rummel *et al*., 1977).

Under favourable conditions the lifecycle of *A. grandis* is completed in 17-21 days (30^{o}C) with as many as 7 generations a year. There is peak emergence of overwintering adults emerge in mid-May in Texas. Adults feed on developing cotton foliage and eggs are laid singly in cotton flower buds. Late in the season eggs are laid in flower buds and young bolls. Larvae feed inside the flower or boll then pupate. The emerging adults cut their way out of the flowers or bolls then mate. The females are attracted to the male pheromone. *A. grandis grandis* diapauses in forest litter or on various malvaceous host. *A .g thurberiae* diapauses as an unfed adult in the larval cell until the summer rains release it. The Mexican boll weevil survives in the larval cell in cotton bolls with adults also found overwintering in litter. High temperatures in the USA in June-August suppressed boll weevil populations. There is very high weevil mortality with about 95% of overwintering adults dying due to heat, dry weather, parasites and predators. The tropical origin of the boll weevil and high overwinter mortality in temperate regions suggest that it did not evolve to endure cold winters and thus may not have developed a true diapause response (Showler 2010).

It is commonly believed that colonization of early-season cotton by overwintered boll weevils is concentrated within field margins. However, Reardon and Spurgeon (2003) found that boll weevils do not consistently exhibit a strong edge-oriented colonization pattern.

1. Detection

The symptoms of an early attack are recognizable by a small puncture (egg or feeding puncture) at the side of the flower bud. The bracteoles spread out and buds turn brown and fall off. In later attacks, the flowers turn yellow and fall to the ground, as do small bolls. Punctured large bolls usually remain on the plant but are of poor quality (EPPO datasheet).

Trapping of adult weevils is used as a monitoring technique and as a method of eradication in US boll weevil eradication programs (USDA, Animal and Plant Inspection Service, http://www.aphis.usda.gov/plant_health/plant_pest_info/cotton_pests/index.shtml). The boll weevil trap has three parts: a body, a moulded screen cone and a collection chamber. The trap is

yellow-green in colour to mimic the food plant of the weevil. Traps are placed on support poles 1m above the ground and baited with pheromone dispensers. An artificial pheromone is placed in the collection chamber to attract weevils along with an insecticide strip to kill weevils that enter the trap. Weevils attracted to the trap land on the outside of the body and crawl to the inside of the cone. An opening in the top of the cone allows the weevils to enter the collection chamber and be killed. However, Suh *et al*. (2009) found that the use of kill strips is likely to be unnecessary in eradication programs, but may be a consideration in situations when the numbers of deployed traps are reduced and chronic problems with weevil predation or trap obstruction exist.

Boll weevil traps are baited with grandlure, the male sex pheromone, and are the exclusive monitoring device used to determine area wide distributions and presence of *A. grandis grandis* (Tumlinson *et al*. 1969). Recent dispensers have increased the grandlure component to 25mg and added 30 mg eugenol (Armstrong, 2010). Increasing the amount of grandlure extended the field life of the lure and eugenol was reported to increase trap catch, although Armstrong (2010) found that eugenol did not reserve or encourage the release of grandlure and did not increase trap catch when boll weevil densities were low. The addition of ethephon to grandlure can also significantly improve trap attractancy (Parajulee and Slosser (2001). Showler *et al*., (2006) found that traps collected more boll weevils under field and laboratory conditions as the amount of grandlure in laminated plastic strips was increased from 0 to 10, 30, and 60 mg. The underlying factor in boll weevil attraction to grandlure strips was dosage, the amount of volatilized pheromone available for interacting with an adult boll weevil.

Spurgeon and Raulston (2006) indicated that the immediate trap surroundings strongly influenced the effectiveness of the boll weevil pheromone trap. These results also suggest that effectiveness of current trapping programs may be improved through purposeful association of traps with selected vegetation features. The response of both early- and late-season weevils to the traps increased as the intensity of reflected light was increased and also when the pigment more closely approached 500-525nm (Cross *et al*., 1976).

The design configuration, operation, and field performance of an electro-mechanical capture detection and time-logging system for boll weevil pheromone traps is described (Beerwinkle 2001). The system uses an infrared light blockage detector in conjunction with an air-pressure capture assist mechanism, a one-way insect gating valve, and a solid-state, self-contained event logger to automatically record the precise times of insect captures (Beerwinkle 2001).

By counting the number of one-third grown or larger squares that flared or fell from plants as a result of weevil oviposition and by monitoring the fruiting load of plants, an estimate of the number of developing larvae per acre can be obtained. (Herzog and Lambert, 1984)

2. Delimitation

No information.

3. Monitoring

Pheromone traps have been employed as a means of detection of boll weevils particularly in boll weevil eradication campaigns in the US (USDA, Animal and Plant Inspection Service, Texas Boll Weevil Eradication Foundation, http://www.txbollweevil.org/trapping; USDS, Animal and plant health inspection service http://www.aphis.usda.gov/plant_health/plant_pest_info/cotton_pests/index). Traps are placed around cotton fields in the spring as the cotton is planted. Control operations are based on the results of the trap catches. Traps are placed around fields at a rate of one trap every tenth of a mile (1.61km). The density may be doubled to two traps alongside any overwintering habitat such as grass, shrubs, weeds, etc. Traps between two adjacent cotton

fields are space at one trap per one-tenth of a mile by alternating trap positions and assignments between the two fields. Traps are placed around the perimeter of all cotton fields on four foot long wooden stakes driven into the ground. Each trap is set on a nail halfway into the top of the stake. Traps are placed as near to the cotton fields as possible but in a position that avoids the regular path of field equipment to prevent damage. The trap is positioned in as open an area as possible free from weeds or other obstacles that may obstruct trap visibility or interfere with free airflow. Judicious placement of traps in locations protected from prevailing winds should improve detection efficiency in areas where early warning of weevil presence is critical, such as in eradication and post-eradication zones (Sappington and Spurgeon (2000). Each trap is identified by a unique number. Traps are inspected once a week. The lure is replaced every two weeks the date of the lure replacement is indicated on the lure. Insecticide strips are replaced once a month. Quality control consists of visual inspection by a supervisor and spiking in a percentage of the fields selected for quality control, with a known number of weevils or tokens.

4. Commodities
No information.

References: 293 retained out of 597 retrieved, 3 added, 13 used for the summary

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Showler, A. T., D. Robacker and E. Salgado (2006). Grandlure dosage and attraction of boll weevils (Coleoptera : Curculionidae). Journal of Economic Entomology 99(5): 1675-1681.

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USDA, Animal and Plant Inspection Service, http://www.aphis.usda.gov/plant_health/plant_pest_info/cotton_pests/index.shtml

Anthonomus quadrigibbus Say

(=*Tachypterellus quadrigibbus* Dietz*)*
Common name(s): Apple curculio, Western Curculio, large apple curculio
Taxa: Insecta: Coleoptera: Curculionidae
EU Annex designation: II/A1 – as *Tachypterellus quadrigibbus*

Organism
Anthonomus quadrigibbus is associated with plants of the family Rosaceae and also with *Cornus* spp. Larval development takes place in fruit (Burke and Anderson 1989). This species is univoltine, the period of emergence varies with the climatic condition from mid August to mid-September, the adult weevils overwinter on the ground beneath the trees. Its range includes North America and Mexico (Anonymous 2004).

1. Detection
Information not available.

2. Delimitation
Information not available.

3. Monitoring
Information not available.

4. Commodities
Information not available.

References: 2 retained out of 10 retrieved, none added, 2 used for the summary

Anonymous. 2004. Anthonomus quadrigibbus. Page Map 653 Distribution Maps of Plant Pests. CAB International, Wallingford.

Burke, H. R. and R. R. S. Anderson. 1989. Systematics of Species of Anthonomus Germar Previously Assigned to Tachypterellus Fall and Cockerell Coleoptera Curculionidae. Annals of the Entomological Society of America 82:426-437.

Anthonomus signatus Say

Common name(s): Strawberry / strawberry bud weevil
Taxa: Insecta: Coleoptera: Cuculionidae
EPPO A1 list: No. 164
EU Annex designation: II/A1

Organism

This beetle is primarily a pest of *Fragaria* (strawberry) and *Rubus* species (blackberry etc), with the larvae causing damage to the plant through loss of buds (English-Loeb *et al*. 1999), particularly in North America (Anonymous 1989, 2004). The severed buds of plants can contain larvae that can be spread in consignments of fruit. Temperature studies have indicated that the pest has a low developmental threshold that gives it the potential to survive and develop across most of the EPPO region (Clarke and Howitt 1975).

1. Detection

Detection of the adult on emergence and the signs of oviposition are the critical elements of detection of this pest. The observation of "clipped" or cut-off buds before blooming are symptomatic of this pest (Randell 1984) and adults can be observed from May to June, depending on the region (Rivard *et al*. 1979). Adult collections can be facilitated using D-Vac suction sampling or sweep netting (Bostanian *et al*. 1999).

2. Delimitation

No specific information although Kovach *et al*. (Kovach *et al*. 1999) describe procedures that are applicable to this area.

3. Monitoring

Monitoring for this pest takes the form of implementing the detection procedure of searching-out adults or observing clipped buds. The emergence of the adult population can be predicted using models based on accumulated degree-days (Mailloux and Bostanian 1993, Bostanian *et al*. 1999). Ongoing monitoring of *A. signatus* is described for the USA whereby strawberry crops are assessed for the presence of clipped buds using 0.5 m lengths of plants at 10 locations within a field that is continued 1-2 times weekly until secondary flower bloom (Handley and Dill 2009). An extremely detailed account of a monitoring exercise is also provided by Kovach *et al*. (1999) that is based on the observation of clipped buds and floral injury.

4. Commodities

The importation of *Fragaria* spp., typically as young plants, is detailed by EPPO (Anonymous 2008). The procedures detailed include the visual assessment of an "adequate proportion" of the consignment based on the EPPO Standard PM 3/65 Sampling of consignments for visual phytosanitary inspection. In the case of *A. signatus*, assessment is based on the presence of clipped buds on green plants

References: 22 retained out of 45 retrieved, none added, 11 used for summary

Anonymous 2004. Anthonomus signatus. Page Map 654 Distribution Maps of Plant Pests. CAB International, Wallingford.

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Clarke, R. G. and A. J. Howitt. 1975. Development of the strawberry weevil under laboratory and field conditions. Annals of the Entomological Society of America 68:715-718.

English-Loeb, G., M. Pritts, J. Kovach, R. Rieckenberg, and M. J. Kelly. 1999. Compensatory ability of strawberries to bud and flower removal: Implications for managing the strawberry bud weevil (Coleoptera: Curculionidae). Journal of Economic Entomology 92:915-921.

Handley, D. T. and J. F. Dill. 2009. A strawberry integrated pest management program for a cold climate, direct-marketing region. Acta Horticulturae 842:645-648.

Kovach, J., R. Rieckenberg, G. English-Loeb, and M. Pritts. 1999. Oviposition patterns of the strawberry bud weevil (Coleoptera: Curculionidae) at two spatial scales and implications for management. Journal of Economic Entomology 92:1358-1363.

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Rivard, I., G. Mailloux, R. O. Paradis, and G. Boivin. 1979. The times of appearance of adults of the strawberry weevil, Anthonomus signatus Say, on strawberry and raspberry in Quebec. Phytoprotection 60:41-46.

Aonidiella citrina (Coquillett)

Common name(s): Yellow scale
Taxa: Insecta: Hemiptera: Diaspidae
EU Annex designation: II/A1

Organism

Anonidiella citrina is a polyphagous armoured scale insect attacking plant species belonging to more than 50 genera in 32 families. The scale of the adult female is circular, 1.5-2mm in diameter, flat, yellow-brown, composed of waxy secretions and exuviae of previous instars. The male scale is oval elongate and smaller. The main hosts of economic importance are *Citrus* spp. especially oranges, but the insect is also recorded incidentally on a wide range of ornamentals and some fruit crops. In the EPPO region the main potential hosts are *Citrus* spp. growing in the southern part of the region, around the Mediterranean.

Anonidiella citrina can be easily confused with *A. aurantii*, which is one of the most commonly intercepted scale insect on imported citrus fruits. *A. citrina* usually attacks the leaves and fruit but rarely the bark (*A. aurantii* occurs on all aerial parts of the plant). Heavy infestations may result in leaf drop, dieback of apical twigs and discoloured, stunted and pitted fruits which fall prematurely or are unmarketable. The main dispersal stage is the first instar which may be naturally dispersed by the wind and animals. Once a feeding site has been selected the insect becomes sessile and is not naturally dispersed. It is readily carried on consignments of plant material and fruits.

1. Detection

The small size, pale colour and sessile nature make *A. citrina* difficult to detect unless present in large numbers. *Anonidiella citrina* can be detected by visual examination of fruits and leaves, but careful examination is required when the population is very low. No reliable identification of *A. citrina* can be achieved by macroscopic field examination and authoritative identification can only be based by studying slide mounted specimens. Important diagnostic characters include the wax-producing ducts, pores, plates and setae and internal apophyses.

Sex pheromones have been used to enhance the trapping of male *A. citrina*. A method is described for handling sticky trap cards and evaluating catches, using the sex pheromones of *A. citrina* (Fargerlund and Moreno, 1974). Traps have consisted of 7.6 x 12.7cm cards coated with glue to which was attached a pheromone-treated rubber septa (100µg/lure) at the top using a paper clip. More males were collected on baited traps with mature virgin females when the traps were 2.4 m above ground on Citrus trees.

2. Delimitation

No information.

3. Monitoring

Surveys of citrus orchards used sticky traps baited with sex pheromone lures (100µg/lure) which were hung for 4-6 weeks in citrus groves in California during the September-October flight period (Grafton-Cardwell *et al*., 2000). One trap was placed in each orchard and total number of males per trap were recorded (Grafton-Cardwell *et al*., 2000). In lure longevity experiments pheromones were changed monthly and trap cards were changed weekly over a 4 month sampling period (Grafton-Cardwell *et al*., 2000). Differences between new and aged lures appeared most pronounced during periods of low population density (August to mid-September). Replacement of lures in baited traps at monthly intervals or just prior to the estimated onset of male emergence,

should provide consistent trap captures for monitoring purposes (Grafton-Cardwell *et al*., 2000). The traps can be used effectively with low doses of pheromones (5 or 1µg per lure) (Grafton-Cardwell *et al*., 2000). The traps monitor flight phenology and should be re-baited before each emergence of males from March to November (Grafton-Cardwell *et al*., 2000).

4. Commodities
No information.

References: 20 retained out of 114 retrieved, none added, 2 used for the summary

Fargerlund, J. and D. S. Moreno (1974). A method of handling card traps in mealybug, scale surveys. Citrograph 60: 26-28.

Grafton-Cardwell, E. E., Millar J. G., O'Connell, N. V., Hanks, L. M. (2000). Sex pheromone of yellow scale, Aonidiella citrina (Homoptera: Diaspididae): Evaluation as an IPM tactic. Journal of Agricultural & Urban Entomology 17: 75-88

Aphelenchoides besseyi Christie

Common name(s): Rice white tip nematode, crimp disease nematode
Taxa: Nematoda: Aphelenchida: Aphelenochoididae
EPPO A2 list: No. 122
EU Annex designation: II/A1 on rice, II/A2 on strawberry

Organism

The main host plants are strawberries and rice but this species has also been found on *Boehmeria nivea,* numerous ornamental plants (including chrysanthemums, *Ficus elastic, Hibiscus, Polianthes tuberosa* and *Saintpaulia ionantha*) and grasses (*Panicum, Pennisetum, Setaria, Sporobolus*). *A. besseyi* is not found beyond latitude 43^{o}N on rice or beyond 40^{o}N on strawberries.
On rice, infested seed is the primary inoculum source. When the crop is grown the nematodes become active and move to the growing points of leaves and stems where they feed ectoparasitically. *Aphelenchoides besseyi* may reproduce parthenogenetically. Optimum temperature for development is 21-25^{o}C, with the lifecycle taking 8-10 days and there are several generations in season. The grain itself is never invaded. *Aphelenchoides besseyi* can withstand desiccation, retaining viability for 2-3 years on dry grain but dies within 4 months on grain left in the field; the nematode is not thought to survive long periods in the soil between crops. On strawberries, the nematode is also ectoparastic, feeding on young tissue. In the USA it has been reported that the strawberry form of the parasite can survive in the soil over winter and that larval stages can withstand moderate desiccation in plant material.

1. Detection

On rice there is a characteristic whitening of the top 3-5cm of the leaf tips, which later become necrotic, and a crinkling and distortion of the flag leaf enclosing the panicle; the latter is reduced in size, as are the grains. Symptoms may be confused with calcium and magnesium deficiency. On strawberries there is leaf crinkling and distortion, and dwarfing of the plant with an associated reduction in flowering.
Details of the identification of *A. besseyi* are included in the EPPO standard 'PM7/39 (1) Diagnostic protocol for regulated pests: *Aphelenchoides besseyi* (OEPP/EPPO, 2004). Diagnostic protocols include brief general pest information and a detailed description of the detection and identification methods. The protocols do not include specific information on inspection and sampling (e.g. statistical aspects) but may include specific advice on how to take appropriate samples, for example, the plant parts capable of harbouring a pest. The protocols also include specific guidance on record keeping (for example, the methods, including controls, used in the diagnosis and the results obtained).
All developmental stages (females, males and juveniles) can be isolated from infested plant tissues or soil, examined under a stereomicroscope and identified measuring the features characteristic of the species (OEPP/EPPO, 2004). The techniques used for extraction and detection of the nematodes (including *A. besseyi*) include the Baermann funnel, tray technique, use of mistifier apparatus, sieving and blending and cyst detection by Fenwick can. Baermann funnel and mistifier apparatus should be run for 48 hours to detect low levels of infestation. In rice the use of undehulled seeds placed in Baermann funnels or sieves fitted in a dish was found to be practical for detecting *A. besseyi*. A considerable number of nematodes were detected from seeds after two days immersion in water. Nematode recovery in undehulled seeds, especially for dry seed, was increased by extending extraction time to more than 2 days. A further development describes a method for the mass extraction of *A. besseyi* from rice seeds by splitting individual rice seeds

longitudinally and placing on a stainless wire netting tray. The tray containing the split seeds was placed in a container with water for 4 h at 25°C. The mass extraction method is applicable for determining low nematode densities per seed (Hoshino and Togashi, 2002).
Morphological criteria and ribosomal RNA gene sequencing have been used to identify an *Aphelenchoides* spp. (most likely *A. besseyi*) nematode parasitic of *Polianthes tuberose* (Cuc and Pilon, 2007). Polymerase chain reactions (PCR) have been used as a rapid detection method to detect nematode species of the same genus (*A. ritzemabosi* and *A. fragariae*) by amplifying the ribosomal DNA, indicating the potential for this method to detect other nematode species. (Cui *et al*., 2010; McCuiston *et al*. 2007).

2. Delimitation

In Italy, visual inspections of rice crops were carried out initially as part of the phytosanitary measures against *A. besseyi*, but they were not found to be efficient because of the low infestation levels (Bergamo *et al*., 2007). Subsequent measures were based on the laboratory testing of all lots of rice seeds and on the elimination of infested lots. After 10 years, the number of infested seed lots has been reduced from 17% in 1996 to 2 % in 2006 and the nematode has been eliminated from many rice varieties undergoing the certification procedure (Bergamo *et al*. 2007).

3. Monitoring

In Australia, growers producing their own runners are advised to inspect them regularly, and treat or destroy plants with symptoms. The distribution of *A. besseyi* on paddy seeds was studied to determine the optimum sample size for seed certification and for quarantine processing of paddy germplasm imported for research purpose (Rajan and Lal, 2005). Based on detection levels and statistical analysis for reliability of results, the optimum sample size for certification purpose was found to be 1000 seeds and for quarantine processing, observation of at least 50 seeds is necessary (Rajan and Lal, 2005). Systematic monitoring of Italian-produced rice seed was initiated in 1997 and control measures had reduced the incidence of nematode infested seed lots by 65% in 2000.

4. Commodities

EPPO has drafted a commodity-specific phytosanitary procedure Consignment inspection of *Fragaria* plants for planting (Anon, 2008). Isolation and identification of *A. besseyi* can be done from infected plant tissues (apex, leaves or stems). Details of the identification of *A. besseyi* are included in the EPPO standard 'PM7/39 (1) Diagnostic protocol for regulated pests: *Aphelenchoides besseyi*'. Plants used for planting of strawberries from infested countries where the nematode occurs should come from an area where the pest does not occur or should have been treated (Anon, 1994).
The presence of *A. besseyi* on rice seed can be determined following EPPO standard PM3/38 (1) (OEPP/EPPO, 1998): Soak small samples of rice seed (e.g. 100 seeds) in shallow dishes of water, supported on a small nylon sieve, for up to 4-5 days at 25-30°C. The nematodes leave the seeds and crawl through the pores of the mesh to be collected in the water below. Each day, temporarily remove the sieve carrying the seeds, collect the water for microscopic examination and add fresh water to the dish, replacing the sieve. Allow the water sample to stand for at least 1 h to allow any nematodes to settle to the bottom of the container, then examine the sample with a stereoscopic microscope. Because many harmless microbivorous nematodes commonly occur in seeds, it is necessary to confirm the identification, at higher magnification, of any nematodes suspected of being *A. besseyi.* With 5-fold replication, infection can be guaranteed to be less than 1% with 99%

confidence if no *A. besseyi* are found. With 50 replicates, this figure falls to less than 0.1%. This method uses whole seeds but Moretti *et al*. (1999) recommends the use of rice chaff or hull as an alternative testing material.

ISTA testing method no 7-025: Detection of *Aphelenchoides besseyi* on *Oryza sativa* uses dehulled seeds for the extraction of nematodes and has found and an increased number of nematodes compared to the EPPO method (ISTA, 2009). The sample (total number of seeds tested) and subsample size to be tested depends on the desired tolerance standard (maximum acceptable percentage of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected). Maximum sub-sample size should not exceed 100 seeds (ISTA, 2009).

References: 34 retained out of 143 retrieved, 8 added, 11 used for the summary

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Apiosporina morbosa (Schweinitz) von Arx

Common name(s): Black knot
Taxa: Fungi: Ascomycota: Pleosporomycetidae
EPPO A1 list: No. 10
EU Annex designation: II/A1

Organism
The principal host is plums (*Prunus domestica*) (American, European and Japanese cultivars are attacked); but also reported on apricots, cherries, damsons and peaches and various wild North American *Prunus* spp.Wild species of *Prunus* occur widely and could be a potential reservoir of the pest.
The spores germinate and enter through wounds or directly at the bases of small twigs of the current season's growth. Infection takes place usually immediately after bud break. Where infections are on older wood, it is always in conjunction with fruit or leaf buds. Minimum temperature at which infection will occur is considered to be 11^{o}C. After infection, a swelling develops late that same year or the following spring. Ascospores are the primary sources of inoculum with peak production taking place before or after petal fall in the USA. Following wetting in rain the ascospores are forcibly ejected and carried in air currents. Prunings containing excised knots can continue to produce viable spores for several weeks. Knots once formed continue to produce ascospores each year although productivity decreases with age.
Under natural conditions, *A. morbosa* spreads readily by ascosphore dispersal within orchards. Movement by human agency (pruning, transport of plants) has not been especially noted. In international trade it is liable to be carried on infected plants for planting of *Prunus*.

1. Detection
Plants for planting of *Prunus* should be inspected for the presence of knots or cankers. Infected material should be excised at least 10cm below the visible swelling. Commercial orchards use routine inspections to detect the presence of the pest.
A. morbosum produces conidia on media, but not ascospores. Genetic characterization of strains isolated from black knots used amplified fragment length polymorphism markers (Chen *et al*., 2002a). A ribosomal region on the genome was amplified with PCR using ITS universal primers (Chen *et al*., 2002b). ITS fragment could be used as a cDNA probe to identify *A. morbosum* genome extracted from diseased tissue using southern blot (Chen *et al*., 2002b). The sequences of the ITS region had > 99% similarity among 30 isolates of *A. morbosa*, and a total of eight unique genotypes were found, based on ITS sequence alignment (Fernando *et al*., 2005). The most common genotype was manifested in 21 of 30 *A. morbosa* isolates, based on the ITS sequences. Three *A. morbosa* isolates had an identical sequence to that of an isolate of *Cladosporium. herbarum* (Fernando *et al*., 2005).
Zhang *et al*. (2004, 2005) developed a specific and sensitive polymerase chain reaction (PCR) assay to detect *Apiosporina morbosa*. A pair of *A. morbosa*-specific forward and reverse primers (AMF and AMR) was designed from the internal transcribed spacer (ITS) regions of *A. morbosa*, preamplified by universal ITS primers ITS1 and ITS4, and compared with the ITS region sequences of *Fusarium*, *Alternaria, Phoma*, and *Cladosporium* species associated with black knots. No product was amplified from DNA of other fungal species, confirming the specificity of the newly designed primers. Within plant tissues, the pathogen was detected at further distances from the edges of knots on thicker branches bearing larger knots compared with thinner branches bearing smaller knots. The PCR assay showed high sensitivity, needing only 100 fg of the *A. morbosa* DNA for a

reliable PCR amplification with the AMF and AMR primers Zhang *et al*. (2004, 2005).

2. Delimitation
No information.

3. Monitoring
In Alberta, Canada, surveyors visited native and cultivated stands of *Prunus* spp. in the spring before the trees were in full leaf and assessed disease incidence and severity (Howard *et al*., 2004). Knot samples were collected for genetic analysis using PCR technology (Howard *et al*., 2004). In Utah USA, the incidence of black knot was measured by determining the ratio of total black knot gall length to total stem length of plants and then expressing that value as a percentage of diseased stems in the sample plot.

4. Commodities
No information.

References: 11 retained out of 59 retrieved, none added, 6 used for the summary

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Apple proliferation phytoplasma (*Phytoplasma mali* Seemüller & Schneider**)**

Common name(s): Apple proliferation, witches' broom
Taxa: Bacteria: Tenericutes: Acholeplasmataceae
EPPO A2 lists: No. 87
EU Annex designation: I/A2

Organism
Apple proliferation is a phytoplasma that infects *Malus* species, causing characteristic "witches' broom" type symptoms. Leaves on infected trees appear early and are small and irregularly serrated. Chlorosis, premature reddening and defoliation often occur. Leaf stalks are abnormally short and have up to four unusually long stipules. Leaves on infected trees are particularly susceptible to powdery mildew. Present throughout mainland EU.

1. Detection
The method for the detection of apple proliferation (AP) as set-out by EPPO standard 57 indicates using woody indicators for root grafting with prolonged incubation period (2 years) (Anonymous 1994). This method has been largely superseded by chemical staining and immunosorbent assay techniques and, more lately, a range of molecular detection techniques. The confirmation of the presence of phytoplasmas can be undertaken using staining using DAPI (4'-6 diamidino-2-phenylindol) (Davies *et al*. 1985, Medina and Llacer 1988) although this method is considered unreliable at low MLO concentrations. ELISA methods have also been used (Cank and Ertunc 2007). The use of polymerase chain reaction amplification of 16s rDNA followed by restriction fragment length polymorphism analysis confirms the presence of phytoplasmas in fruit trees and allows phytoplasmas from different fruit trees to be differentiated (Avinent and Llacer 1994, Hurtt *et al*. 1995). Procedures using 16S rRNA have been widely validated. An alternative to restriction digestion was developed using an ELISA system for the detection of PCR products for a range of phytoplasmas (Pollini *et al*. 1997). More recently a nested PCR system has been employed that allows the easier interpretation of electrophoresed products (Bertaccini *et al*. 2001). The detection of AP has been further improved by the use of real-time PCR and AP-specific primers that increases both the sensitivity and specificity of detection (Baric and Dalla-Via 2004, Baric *et al*. 2005). TaqMan real-time PCR assays have further increased sensitivity and lowered the limit of detection for AP (Baric *et al*. 2006). Several variants of real-time PCR assays, differing slightly in some aspects have been validated and represent the most advanced, accurate, and reliable means of AP detection.
Detection surveys require the sampling of material for subjection to the various assays described above. Detection can be achieved through sampling symptomatic trees in the late summer/early autumn (Cank and Ertunc 2007). Detection surveys have successfully identified AP to be to have spread to Slovenia, Albania, Bosnia, Kosovo and Greece (Myrta *et al*. 2003, Delic *et al*. 2005, Myrta *et al*. 2006) (Rumbou *et al*. 2008) (Brzin *et al*. 2001) in recent years. A survey in Belgium in 2011 has, for the first time confirmed the presence of AP there, with the MLO found in both roots samples from asymptomatic trees and in the psyllid vector (Anonymous 2011).

2. Delimitation
No specific information was identified in the literature although extensive surveying of apple

orchards in Trentino, North Italy, in 2001 was used to indicate that the disease was spreading (Ferrari 2003) and therefore effectively delimited the disease within an area.

3. Monitoring

Monitoring surveys can be undertaken through the collection of material from orchards and the extraction of AP DNA from the midribs of fresh apple leaves and/or phloem using chloroform/phenol (Bertaccini *et al*. 2001). Roots can also be collected (Anonymous 2011) from symptomatic/asymptomatic trees. Surveys of large numbers of symptomatic trees (>200) in late summer/early autumn has recently been carried out in Turkey (Cank and Ertunc 2007). Similar surveys (symptomatic and asymptomatic trees) have recently been undertaken in Serbia (Duduk *et al*. 2008); recent monitoring surveys employed PCR-based detection methodologies, in most cases exclusively. A number of surveys in the Czech Republic conducted over recent years have similarly used molecular detection methods to assess the extent of the disease's prevalence and spread within the country (Bertaccini *et al*. 1998, Bertaccini *et al*. 2001, Navratil *et al*. 2001, Blazek *et al*. 2005, Franova 2005, Franova *et al*. 2008). Subtypes of AP have also recently been monitored for in Italy through sampling a range of apple trees and rootstock, as well as the vector plant hopper *Metcalfa pruinosa* (many thousands sampled)(Cainelli *et al*. 2004). The surveying of *M. pruinosa* for the presence of AP has also been conducted without sampling trees (Danielli *et al*. 1996) as part of an assessment of the risk they pose in the spread of AP.

4. Commodities

No information available.

References: 173 retained out of 238 retrieved, 2 added, 26 used for summary

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Apricot chlorotic leafroll phytoplasma

(=*Phytoplasma prunorum* Seemüller & Schneider)
Common name(s): Apricot chlorotic leafroll, apricot dieback, European stone-fruit yellows
Taxa: Bacteria: Tenericutes: Acholeplasmataceae
EPPO A2 list: No. 146
EU Annex designation: I/A2

Organism
The main hosts this organism, also known as European stone fruit yellows phytoplasma (ESFY), are apricot, peaches and *Prunus salicina*. Plums can be symptomless carriers of the disease. Most *Prunus* spp. can be experimentally infected and some show severe symptoms. Some weeds can be naturally infected. The pathogen has not yet been cultured on synthetic medium. Found locally across Europe.

1. Detection
Disease symptoms can be detected throughout the year, since one effect is stimulation of new growth during winter dormancy, but this is blocked by frost (Anon, 2004). Symptoms are best observed before flowering and at the end of summer. In spring infected trees bear leaves before the flower buds open. If winter temperatures fall below -5°C, infected trees show browning of the middle layer of the bark, darker and thicker depending on severity of winter. The cambium may be affected but in spring the outside bark appears normal. One to two months later the external part of the bark dries out. Leafroll symptoms develop through the summer and are most visible in September. The lamina rolls up along lines running from the petiole to the tip, giving a cone or polygonal outline. Irregular interveinal chlorosis is also seen. There is a proliferation of rudimentary buds at the end of short shoots and a tendency for buds to open on old wood. Fruits are typically smaller, fewer and ripen late, if at all; they may drop prematurely. Trees decline progressively and may die quickly or over several years; occasionally some infected trees may recover spontaneously (Anon, 2004). Diagnosis requires laboratory examination as some of these symptoms can be caused by other factors. It is extremely unlikely to detect phytoplasmas in plants showing no symptoms (Anon, 1994).
Positive identification can by done by a graft-transmission test: a bud from the apricot tree under test is double budded in June with a healthy bud onto a plum rootstock. Detection of fluorescence of phytoplasmas in the sieve tubes of leaf veins provides a rapid test. The EPPO phytosanitary procedure describes the use of visual inspection, microscopy and the use of woody indicators (*Prunus armeniaca, P. persica*) to inspect for Apricot chlorotic leafroll phytoplasma (Anon, 1994).
Medina and Llacer (1988) assessed Seemuller's fluorochromate technique with DAPI (4'-6 diamidino-2-phenylindol) to detect mycoplasma-like organisms on apricot infected with apricot chlorotic leaf roll. Results showed that the test was unreliable for the early diagnosis of MLOs due to the low concentration and irregular distribution of MLOs in the infected trees.
PCR, nested PCR, PCR-ELISA, multiplex real-time PCR and RFLP analyses has been used to detect phytoplasmas in fruit trees (Avinent, and Llacer, 1995; Bertaccini *et al*., 1997; Bissani, *et al*. 2002; Carraro, *et al*., 1998; Dudek *et al*., 2008, Jarausch, *et al*. 2001; Navratil *et al*. 2004; Pignatta *et al*., 2008). Detection of ESFY is possible throughout the year using PCR methods; however, the accuracy of this diagnostic tool varies with the seasons and also depends on which plant tissue is examined. It is generally more accurate in the growing season, and although it can be used in the dormant season, this is not appropriate for the plant health inspections under the certification scheme (Necas, *et al*., 2008).

2. Delimitation
No information.

3. Monitoring
Surveys of apricot growing areas involve the visual inspection of typical symptoms of ESFY, such as early bud break in late winter and chlorotic leafroll in the summer. Branch samples with typical symptoms and randomly collected samples from trees with other symptoms are collected in the summer and analysed by PCR (Jarausch *et al*., 2008). Analysis of leaf samples from suspected trees, or in the absence of symptoms, apparently healthy trees, is also done by PCR (Genini and Ramel, 2004). The optimal time for the diagnosis of ESFY is June for phloem samples and September for leaf-stalk samples (Necas, *et al*., 2008).

4. Commodities
No information

References: 74 retained out of 202 retrieved, 3 added, 14 used for the summary

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Arabis mosaic nepovirus

Common name(s): ArMV, Arabis mosaic, bare ine of hop, mosaic of rhubarb (and others)
Taxa: Viruses: Secoviridae: Nepovirus
EU Annex designation: II/A2

Organism
This virus has been detected in a very wide range of hosts, although the crops of interest are principally fruits. Commonly affected species include *Vitis*, *Rubus*, *Rheum* as well as hops, ash, several flowering plants, a range of wild hosts and field crops (Vuittenez *et al*. 1972, Shukla *et al*. 1974, Cooper 1975, Shukla and Schmelzer 1975, Asjes 1976, 1978, Eppler 1992). The virus is found across Europe and most of the rest of the world. A number of nematode species are suspected in vectoring the virus, although there is only definitive evidence for *Xiphinema diversicaudatum.*

1. Detection
A large array of methodologies have been assessed to diagnose infection by AMV. Early methods include the use of indicator plants/visible symptoms (Kleinhempel 1970, Cooper and Sweet 1976) and the gel-diffusion method used to detect the virus in plant sap (Schmelzer and Stahl 1972, Thresh *et al*. 1977). However, by the mid-1970s ELISA techniques had been developed and successfully used for detection of AMV(Voller *et al*. 1976) and demonstrated to be much more sensitive than previous techniques (Kaniewski and Skotland 1979). Notably, Walter *et al*. (Walter *et al*. 1984) noted that ELISA can become unreliable at certain times of the year for grapevine, whilst the appropriate timing and tissue sampling is required for accurate detection in a number of crops (Hill and Blunt 1985, Gangl *et al*. 2006). This notwithstanding, ELISA / DAS-ELISA methods have been routinely used for detection of the virus in a range of plant species (Polak *et al*. 1989, Borgo 1990, Etienne *et al*. 1991, Tobias 1995, Felix *et al*. 2002).
Dot blot hybridisation has also been proven to be a highly sensitive means of detection of ArMV (Adams *et al*. 1980, Steinkellner *et al*. 1989) whilst, more recently, methods have been employed (Ipach *et al*. 1992). PCR and RT-PCR methods have been demonstrated to give at least a tenfold increase in sensitivity over ELISA (Brandt and Himmler 1995, Yu *et al*. 2008)and have been used widely (Pantaleo *et al*. 2001, Kominek *et al*. 2003, Felix and Clara 2008). Mutiplex type RT-PCR assays have also been developed that facilitates the simultaneous detection of a suite of viruses (Nassuth *et al*. 2000, Bertolini *et al*. 2001, Bertolini *et al*. 2003, Gambino and Gribaudo 2006, Zitikaite *et al*. 2006a), including AMV. An AmpliDet system has also been developed for ArMV (Klerks *et al*. 2001), and the technology underpinning this isothermal RNA detection method is well described. Still more recently, microarray techniques have begun to be employed although whether these techniques have advantages over other methods is not clear from the literature (Abdullahi *et al*. 2007, Abdullahi and Rott 2009)
ELISA has been used to detect AMV for the first time, such as in grapevine in New Zealand (Kearns and Mossop 1984). RT-PCR was used to provide the first evidence of the virus in tomato in Lithuania. (Zitikaite *et al*. 2006b). The first detection survey for viruses of olive in Turkey used DAS-ELISA indicated that ArMV was present in about 10% of trees sampled (Caglayan *et al*. 2004). In Slovenia however, the first occurrence of the nematode vector prompted investigations into the presence of AMV, using indicator plants, which found the virus to be absent (Sirca *et al*. 2007). The virus was first detected in grapevine in Spain in 2007 although the authors fail to describe the context of its discovery (Abelleira *et al*. 2010)

2. Delimitation
No information specific to ArMV

3. Monitoring

There are a wide range of reports of monitoring exercises for AMV. Earlier monitoring efforts frequently involved visual assessments for symptoms (Forster and Milne 1975) whilst later ones have employed the range of serological and molecular techniques described above. As the presence of the nematode vector is a good indicator that plants may be at risk of ArMV, *Xiphinema diversicaudatum* has also been extensively monitored for, with good examples deriving from the UK (Brown *et al*. 1990), Switzerland (Klingler *et al*. 1990) and New Zealand (Hay and Close 1992). A Spanish survey for the nematode examined over a 1000 samples taken across NW-SE transect through the whole Iberian Peninsula.

Details of an extensive survey of hops in Germany, including sampling and locations, have been produced where diagnosis was made using DAS-ELISA (Eppler 1992). A second survey of hops in New Zealand, whereby 60 plants per field were sampled, used ELISA demonstrated >70% infection (by field) (Hay *et al*. 1992). A survey of *Alstroemeria* spp. cut flowers in Italy considered visibly symptomatic material, which is pictured, and diagnosed the disease through a number of techniques, including the use of indicator plants (Bellardi *et al*. 1994). Brief details of a survey of raspberries in the Czech Republic evaluated symptomatic leaves from 150 plants which were analysed using DAS-ELISA, with about 20% infected (Spak 1995). A national survey in Canada evaluated the prevalence of four viral pathogens of grapevine, including ArMV (MacKenzie *et al*. 1996). In this case, all known vineyards and nurseries were sampled, with sampling designed to provide a detection limit of <0.05% with 95% confidence, resulting in over 11,000 samples taken and subjected to DAS-ELISA analysis. Sampling to the same tolerances was also undertaken by Martin *et al*. in the United Sates when surveying grapevine, who provide excellent details of the surveying methodology, data recording, interaction with growers, timing (usually autumn) and analytical procedures used (Martin *et al*. 2005). Grapevine surveys in Syria have also been well described using procedures generally in accord with those mentioned in the North American studies (Mslmanieh *et al*. 2006). Field surveys of grapevine in Turkey were conducted through taking 4-6 cuttings from individual vines from several plants in 98 vineyards which were subsequently analysed using DAS-ELISA (Cigsar *et al*. 2002). The results showed a very low incidence of ArMV in grapevine within the surveyed areas. However, the virus was found to be widely distributed in grapevine in the Czech Republic (Kominek and Holleinova 2003). Grapevine surveys are also described to some extent in a number of other papers (Zorloni *et al*. 2007, Fiore *et al*. 2008, Kontic *et al*. 2009)

Strawberry has been surveyed in the Czech Republic, whereby ca 1500 samples were taken from a range of situations (gardens, nurseries, plantations) and analysed by DAS-ELISA to show that the virus is exceedingly rare (Franova *et al*. 2001). Stone fruit orchards (>100) have been extensively surveyed in Palestine with the disease found to be absent (Jarrar *et al*. 2001) whilst brief details of a survey of berry fruit in Hungary are also available (Nyerges *et al*. 2001). Berry producing fruit production sites in Chile have been the subject of a relatively recent survey where the upper leaves from 12 plants per hectare were sampled and stored at -70°C prior to analysis by DAS-ELISA (Fiore *et al*. 2008).

Olive surveyed throughout the Mediterranean basin has been demonstrated to be frequently infected with ArMV (Saponari *et al*. 2002). Detailed reports of surveys of olives in Syria and Lebanon describes the sampling of material that was subjected to RT-PCR analysis (Al Abdullah *et al*. 2005, Fadel *et al*. 2005) to indicate >1.0% infection rates in both countries. A similar approach examined olives in Italy has also been reported (Faggioli *et al*. 2005) A multination survey of strawberries, taking over 5000 samples demonstrated the virus to be largely absent from the participant countries (Germany, Poland, Czech Republic, Lithuania and Italy) (Babini *et al*. 2004).

On some occasions water courses have been sampled for the presence of viruses. In a Hungarian example, rivers and lakes were sampled and ELISA detection ArMV for the first time in this context (Horvath *et al*. 1999).

4. Commodities

ArMV has been detected from a range of traded commodities, including strawberry (Fujiwara *et al*. 1993), Iris (Tanaka *et al*. 1997), carnation (Silva *et al*. 2005), *Gladiolus* (Qin *et al*. 2008) and tulip (Li *et al*. 2010) using the testing procedures outlined above. The treatment of commodities with respect to ArMV and other viruses is covered by detailed phytosanitary procedures outlined by EPPO (Anonymous 2008b, a).

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Arceuthobium spp. (non-European)

Common name(s): Dwarf mistletoes
Taxa: Angiosperms: Santalacea
EPPO A2 list: No. 24
EU Annex designation: I/A1

Organism

Arceuthobium spp. are dwarf mistletoes which parasitize trees in the Pinaceae family e.g *Abies*, *Larix, Picea* and *Pinus* spp. Most dwarf mistletoes occur principally on one or several species of the same genus. A few are host specific and others have a broad host range but in general *Arceuthobium* spp. do not frequently occur on secondary hosts in the absence of principal hosts. *Arceuthobium* spp. may also attack extra limital hosts, i.e. hosts that do not naturally occur within their range. It is thought to have originated in eastern Asia and is now known to be distributed throughout North America.

Infected host plants are the only likely means of international spread. All countries should prohibit the importation of plants for planting (except seeds and tissue cultures) and cut branches of *Abies*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga* and *Tsuga* from Canada and USA and other countries where non-European *Arceuthobium* spp. occur.

1. Detection

Some dwarf mistletoes are conspicuous large parasites while others never grow larger than a few cm. All species initially grow within the host tissue and cannot be detected at this stage by visual inspection. A PCR technique has been developed to allow early and rapid detection the endophyte phase of the parasite (Marler et al., 1999). The first external symptom is usually swelling of the host tissues at the infection site. In the absence of mistletoe shoots, a cross section of infected bark will reveal yellowish, wedge-shaped sinkers. Profuse dense masses of distorted host branches may develop (witches' broom). Once the lower half of the tree's crown is parasitized the growth rate declines rapidly, foliage yellows and becomes sparse, with a subsequent top dieback and eventual death.

Aerial surveying using visual observations, GPS technology and remote sensing has been used to locate dwarf mistletoe infected residuals which are then mapped from the air (Baker et al., 1992, 2001; Heller and Berger, 1973; Zavala and Zavala, 1993).

2. Delimitation

No information

3. Monitoring

Monitoring surveys can range from simple stand examinations to a thorough investigation with large-scale maps and can include aerial surveys, aerial photography, forest inventory plots, road and plot surveys and transects and grids (Dooling, 1979; Muir and Moody, 2002).

Surveys of seven western hemlock (*Tsuga heterophylla*) stands in south coastal British Columbia used fixed-radius plots to assess dwarf mistletoe (*Arceuthobium tsugense*) infection intensity level and spatial distribution patterns (Alfaro et al., 1985).

Aerial surveys conducted in Manitoba during the winter on stands of mature jack pines (140,000 ha) used the conspicuous witches brooms as indicators of infection, with 8.7% infection detected

(Baker et al., 1992). Flight lines were 1.6 km apart and flying heights were 150 to 500 m above ground. This allowed observers to see the edge of the swath and was close enough to accurately detect and rate the severity. The 6-class system for rating dwarf mistletoe intensity is widely used but is dependent on the observer (Dooling, 1979; Shaw et al., 2000).
Roadside surveys can be reliable for estimating dwarf mistletoe infection as long as the road network provides a representative sample of the stands being surveyed (Merrill et al, 1985).

4. Commodities
No Information.

References: 72 retained out of 194 retrieved, none added, 10 used for the summary

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Arracacha virus B oca strain

Common name(s): AVB-O
Taxa: Viruses: Secovitidae: Cheravirus
EU Annex designation: I/ A1

Organism
Two strains of this virus exist. The T strain, which is found in *Arracacia xanthorrhiza,* does not infect potatoes. Strain O (oca strain) was originally discovered in oca (*Oxalis tuberosa*) and potatoes. Experimental host range includes 30 species of the families Aizoaceae, Amaranthaceae, Apiaceae, Chenopodiaceae, Cucurbitaceae, Fabaciae, Portulacaceae and Solanaceae. The virus is found in the Andean highlands of Peru. Strain O differs from strain T in host range and in symptomatology in indicator plants. The two strains are serologically only distantly related. (Jones and Kenten, 1981). AVB-O could be regarded, among the group of South American potato pathogens, as of relatively minor importance. Little is known about the biology of AVB-O. No vectors are known. The virus is easily transmitted by mechanical inoculation and through true seed and pollen of potato. It enters most tubers produced by infected plants (Jones, 1982). In international trade it could be carried by potato tubers or by true seed of potato.

1. Detection
The presence of the AVB-O can be determined by symptoms on indicator hosts, properties in sap, particle morphology and serologic reactions (Jones, 1981). AVB-O forms isometric particles about 26 nm in diameter (Jones and Kenten, 1978).
AVB-O causes symptomless infections in experimentally inoculated potato plants. Naturally infected plants show calico symptoms, but other viruses have always been found in mixed infections (EPPO datasheet). On indicator plants (*Chenopodium murale*) AVB-O gives occasional expanding necrotic spots and/or ringspots in inoculated leaves, systemic chlorotic mottle and twisting of young leaves followed by necrosis of the tip and upper leaves. On *Chenopodium amaranticolor*, small chlorotic or necrotic spots or rings in inoculated leaves, followed by systemic mild mosaic. On *Cucumeris sativus*, systemic mild mosaic, leaves produced later are symptomless.
Gel diffusion and ELISA are suitable for use in routine detection tests (Jones and Kenten, 1985; Schroeder and Weidemann, 1990). A nested reverse transcription polymerase chain reaction (RT-PCR) method is described that allows detection and identification of virus species including AVB (Maliogka *et al*., 2004). RNA-dependent RNA polymerase (RdRp) of Arracacha virus B (AVB) was amplified and sequenced (Maliogka *et al*., 2004).

2. Delimitation
No information.

3. Monitoring
No information.

4. Commodities
Measures taken against other non-European potato viruses will protect against the introduction of ABV-O. Potato seedlings and plants are inspected once a week for virus symptoms. Leaf tissue is serologically tested for ABV-O (if antiserum is available). All material is rejected where infection is detected by serological tests. Remaining seedlings and plants are tested by inoculating combined leaf samples into indicator plants of *Chenopodium murale.* Indicator plant hosts are maintained

under cool conditions for at least 5 weeks. Tissue cultures, tubers and cuttings are also tested serologically for ABV-O (if antiserum is available) (Anon, 1984).

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Arrhenodes minutes (Drury)

Common name(s): Oak timberworm
Taxa: Insecta: Coleoptera: Brentidae
EU Annex designation: I/ A1

Organism
Arrhenodes minutus is a member of the family Brentidae, a small group of Coleoptera; in Florida, it is a pest of economic importance of oaks and some other forest trees (Anonymous, 2006, Thomas 1996). *Arrhenodes minutus* has been hypothesized as potential vector of oak wilt fungus (*Ceratocystis fagacearum*) (Thomas 1996), however there is no confirmation. In particular, the duration of the larval stage (2-4 years) is too long for an effective link with *C. fagacearum* in a dead tree.

1. Detection
No information available.

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
No information available.

References: 2 retained out of 4 retrieved, 1 added, 2 used for the summary

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Thomas, M. C. 1996. The primitive weevils of Florida (Coleoptera: Brentidae: Brentinae). Page 3 Entomology Circular (Gainesville). Division of Plant Industry, Gainesville; Florida Department of Agriculture and Consumer Services.

Aschistonyx eppoi Inouye

Common name: Juniper gall midge
Taxonomy: Insecta, Diptera, Cecidomyiidae
EPPO A1 list: No. 150
EU Annex designation: II/A1

Organism
The adult midges emerge from the ground from mid-May to early-June in Japan and lay eggs on juniper apical buds. The larva develops in the bud, inducing a very small quadrangular pyramid-gall. It overwinters within the bud gall where it stays until next May. It then drops to the ground for pupation (Inouye 1964). Absent from the EU/EPPO region.

1. Detection
The swollen apical buds, very obvious on bonsais, can be easily detected in spring (Baker 1995) but later the twigs become dry and brown (Inouye 1964). Morphological details of males, females, larvae and pupae have been supplied by Inouye (1964).

3. Delimitation
No traps available.

3 Monitoring
No semiochemical attractant is available.

4. Commodities
The midge is likely to be introduced through plant trade of junipers (Baker 1995). Interceptions have been made in UK on consignments of bonsai trees (*Juniperus chinensis*) (Bowman and Bartlett 1978)

References: 1 retained out of 1 retrieved, 2 added, 3 used for the summary

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Bowman, C. E. and P. W. Bartlett. 1978. Pentamerismus oregonensis McGregor (Acari: Tenuipalpidae) infesting imported Juniperus chinensis L. in England. Plant Pathology 27:200-201.

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Atropellis spp.

Common name(s): Branch and trunk canker of pine, twig blight
Taxa: Fungi: Ascomycota: Helotiales
EPPO A1 list: No. 5
EU Annex designation: II/ A1

Organism

Two species are of economic importance, *Atropellis piniphila* (Weir) Lohman & Cash and *A. pinicola* Zeller and Gooding. The main host of *A. piniphila* is *Pinus contorta* but it will also attack *P. albicaulis, P. banksiana, P. jeffreyi, P. monticola, P. ponderosa*, *P. taeda* and *P. virginiana*. On *P. contorta, A. pinicola* can cause important branch and trunk cankers. *A. pinicola* causes minor cankers on *P. lambertiana*, *P. monticola* and *P. strobus* and minor twig blights on P. nigra and *P. sylvestris*.

Atropellis piniphila is sporadic in western states of the USA and widespread in Alberta and British Colombia in Canada. *A. pinicola* is present in Canada (British Colombia) and USA (Idaho, Montana, Oregon, Washington. The primary source of inoculum is ascospores, released in wet weather. Ascospores may be primarily air-disseminated, although rain may play a secondary role. Under natural conditions, *Atropellis* spp. spread by ascospore dispersal within pine stands. In international trade, logs with bark attached may contain ascospores or traces of mycelium as may cankers on younger branches and twigs of growing material.

1. Detection

Incipient cankers give no external sign of underlying infection. Dark-brown necrotic spots 5mm in diameter occur within the bark. The first sign of external symptom is a drop of resin on the bark surface. Copious fresh resin is found during summer at the margin of the cankers. Cankers are elongated and flattened but deep and covered with bark which is cracked. They occur particularly at the branch whorls on young branches. Multiple stem cankers can be found. Needles on attacked trees may become chlorotic in summer (Hopkins, 1963).

With *A. piniphila* there is a characteristic blue-black staining of the wood beneath cankers. A red or brown discoloration is usually present in xylem at the edge of the blue-black zone. Apothecia erumpent, brownish black, irregularly disc-shaped with a short central stalk, 2-5 mm in diameter. Ascospores hyaline, elliptical-fusoid, aseptate or uniseptate. Conidia very thin walled, hyaline, aseptate, cylindrical, rounded at the ends and possess a mucilaginous coat.

With *A. pinicola*, perennial cankers are rare and then found on the main stem as smooth elongated flattened depressions covered with bark. Apothecia are verumpent, sessile or with a very short central stalk, 2-4mm in diameter. Asci are clavate, interspersed with hair-like paraphyses. Ascopspores are long, narrow, 1-6 celled, hyaline. Conidia are narrowly ellipsoid to bacillary, 1 celled, hyaline. *Atropellis* spp. may be identified using a colorimetric test: a fragment of apothecia turns 5% aqueous KOH a bluish green.

Timber of *Pinus* from countries where the disease occurs should have had the bark removed. Any material with canker lesions should be carefully inspected with particular attention to younger branches and twigs.

2. Delimitation

No information.

3. Monitoring

No information.

4. Commodities

Commodity specific phytosanitary measures exist to prevent the introduction of *Atropellis* spp. into the EPPO region (Anon, 2009).

References: 17 retained out of 23 retrieved, 6 added, 2 used for the summary

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Bean golden mosaic bigeminivirus

Common name(s): BGMV, bean golden mosaic, bean golden yellow mosaic
Taxa: Viruses: Geminiviridae: Begomovirus
EPPO A1 list: No. 204
EU Annex designation: I/A1

Organism

The principal cultivated hosts of BGMV are *Phaseolus vulgaris* and *P. lunatus*. The different isolates have been found to infect a range of wild Fabaceae although BGMV is restricted in its host range to Fabaceae. Symptoms of golden mosaic virus have been seen on legumes in many countries of tropical America.

BGMV is transmitted by the whitefly *Bemisia tabaci*. Adults can acquire the virus in as little as 6 min. BGMV is retained for days or weeks, and through the moult. It does not multiply in the vector and is not transmitted directly to progeny. Non-vector transmission is by mechanical inoculation by grafting, but not by contact between plants. The virus is not transmitted by seed or pollen. The normal source of inoculum is adjacent bean crops, or leguminous weeds. The disease continues to increase in importance in Latin America and the Caribbean, especially in situations where bean crops are planted alongside sources of the vector. The increasing abundance of the *B. tabaci* biotype B on many hosts favours early infestation. Symptoms development depends on temperature: symptoms are more pronounced and appear earlier at 27-30^{o}C than at 21-24^{o}C (Haber *et al*., 1991).

1. Detection

Symptoms of BGMV include vein yellowing, netting and clearing, extending to bright-yellow interveinal chlorosis. Leaves emerging after symptoms have first appeared curl down, fail to expand and become stiff and leathery. Seed set is poor (Haber *et al* 1991). The recommended indicator plant is *Phaseolus vulgaris* cv. Top Crop, which give conspicuous systemic vein chlorosis and golden mosaic. Morphology: geminate particles are 18-20nm in diameter and 30nm in length. Isolates BGMV-PR and BGMV-BZ have been fully sequenced (Howarth *et al*., 1985; Gilbertson *et al*., 1993).

Preparations of BGMV are immunogenic and serological detection methods have been developed (Abouzide *et al*., 2002). The virus reacts in standard gel diffusion tests and is also detectable by ELISA, dot blot hybridisation, polymerase chain reaction (PCR), DNA probes and nucleotide sequence analysis. Multiplex polymerase chain reaction (PCR) primer pairs and nucleic acid hybridization probes have been developed to differentiate bean-infecting begomoviruses (Potter *et al*., 2003). Molecular hybridisation-based detection of the three begomoviruses in bean samples, including BGMV, was accomplished using a general probe obtained by mixing full-length DNA-A clones of the three begomoviruses and specific probes comprising part of the common region of each viral genome (Rodriguez-Pardina *et al*, 2011).

Swanson *et al* (1992) found BGMV to be serologically related to bean calico mosaic, cotton leaf crumple, Serrano golden mosaic and squash leaf curl geminiviruses; a probe for its DNA-A detected nearly all American geminviruses, while its DNA-B gave a weak positive reaction with a probe for bean calico mosaic geminivirus. A strong cross-reaction between sap from squash leaf curl infected squash and bean golden mosaic virus antiserum was detected by ELISA (Dodds *et al*., 1984). BGMV also has some serological relationship with the Old Work mungbean yellow mosaic geminiviruses

(Shimizu *et al*., 1987), but in general the Old World legume geminiviruses are quite distinct from BGMV.

2. Delimitation
No information.

3. Monitoring
Surveys of snap beans during February to April in the USA sampled 100 randomly chosen plants, 25m from the plot boundary (Blair *et al*., 1995; McMillan et 1994). Beans were examined at four growth stages; pre-flowering, flowering, pod fill and maturity. Plants were rated visually as diseased or not diseased based on the typical symptoms of BBMV (Blair *et al*., 1995; McMillan et 1994). Confirmation of virus infection in representative samples was done by dot blot hybridisation (Blair *et al*., 1995).

4. Commodities
No information.

References: 37 retained out of 69 retrieved, 6 added, 11 used for the summary

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Shimizu, S. I., Ikegami, M., Fujii, H. (1987). Serological relationships between mung bean yellow mosaic virus and other whitefly transmitted geminiviruses. Journal of Agricultural Science Tokyo Nogyo Daigaku 32: 139-143.

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Beet curly top virus (non-European isolates)

Common name(s): BCTV, curly top, yellows of tomato, green dwarf
Taxa: Viruses: Geminiviridae: Curtovirus
EPPO A2 list: No. 89
EU Annex designation: II/A1

Organism

This virus affects sugarbeet and a wide range of other crops, including potato and tomato. Various other hosts have been identified from the following families: Chenopodiaceae, Solanaceae, Brassicaceae, Violaceae, Geraniaceae, Cucurbitaceae, Caryophyllaceae, Fabaceae, Asteraceae, Linaceae and Apiaceae. Globally widely distributed, the virus is present in the EPPO region.

1. Detection

ELISA techniques were developed to identify individuals of *Circulifer tenellus* (Baker) that were carrying beet curly top virus (Mumford 1982). The comparison between indirect and two-step ELISA showed that indirect ELISA was two times more sensitive than the two-step one for detecting beet curly top virus (Ali *et al*. 1993). A dot blot hybridization system using digoxigenin-labelled probes and colorimetric visualization was developed for detection of plant viruses; the method can be carried out in diagnostic laboratories using minimal equipment, suggesting its applicability for use in routine plant virus diagnoses (Harper and Creamer 1995). A PCR procedure was used to for the detection of beet curly top virus in insects (Soto and Gilbertson 2002, Chen *et al*. 2004). Techniques combining either tissue printing or blot hybridization, or rolling circle amplification (RCA) and restriction fragment length polymorphism (RFLP) were compared. Although they easily detected beet curly top virus with certainty, both exhibited apparent false positive results which have been scrutinized in closer detail (Homs *et al*. 2008).

2. Delimitation

No information available.

3. Monitoring

Surveys were carried out to evaluate the incidence and distribution of viruses infecting sugar beet in Pakistan (Arif *et al*. 1991) and on pepper in New Mexico (Hubble *et al*. 2003, Creamer *et al*. 2005).
The incidence of virus was analysed on weed hosts (Creamer *et al*. 1996, Davis *et al*. 1998). The vector insect, *Circulifer tenellus*, was collected from the field and tested for virus using PCR (Creamer *et al*. 2003). The detection used to evaluate the presence of virus on sugar beets in Iran were done using tissue-blot immunoassay (TBIA) and confirmed by electron microscopy (Farzadfar *et al*. 2006); ELISA was used to identify the virus (Pourrahim *et al*. 2007, Massumi *et al*. 2009). A molecular technique was shown to be useful in distinguishing beteen viruses, discriminating among strains or for describing related curtoviruses (Baliji *et al*. 2004, Strausbaugh *et al*. 2008, Massumi *et al*. 2009).

4. Commodities

No information available.

References: 23 retained out of 69 retrieved, none added, 17 used for the summary

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Baliji, S., M. C. Black, R. French, D. C. Stenger, and G. Sunter. 2004. Spinach curly top virus: A newly described Curtovirus species from southwest texas with incongruent gene phylogenies. Phytopathology 94:772-779.

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Davis, R. M., H. Wang, B. W. Falk, and J. J. Nunez. 1998. Curly top virus found in perennial shrubs in foothills. California Agriculture 52:38-40.

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Pourrahim, R., S. Farzadfar, A. R. Golnaraghi, and A. Ahoonmanesh. 2007. Incidence and distribution of important viral pathogens in some Iranian potato fields. Plant Disease 91:609-615.

Soto, M. J. and R. L. Gilbertson. 2002. Development and application of a PCR detection method to study the distribution and rate of movement of Beet curly top virus in the beet leafhopper (Circulifer tenellus). Phytopathology 92:S78.

Strausbaugh, C. A., W. M. Wintermantel, A. M. Gillen, and I. A. Eujayl. 2008. Curly Top Survey in the Western United States. Phytopathology 98:1212-1217.

Beet leaf curl virus

Common name(s): BLCV, leaf curl of beet
Taxa: Viruses: Rhabdoviridae: rhabdovirus
EPPO A2 list: No. 90
EU Annex designation: II/A2

Organism

Beet leaf curl virus is vectored by *Piesma quadratum (*Hemiptera: Piesmatidae). In order for the insect to acquire the virus, it must actively feed for at least 30 minutes (Proeseler 1978). The virus is established in Germany, Poland, Slovenia and Turkey and has also been found in the Czech Republic and Slovakia. Yield can be reduced by up to 75% and sugar content and quality can also be affected (Bittner 1998).

1. Detection

There are no serological methods available for detection of this virus. Identification is achieved through observation of the symptoms in the host plant and morphology of the virus particles as determined through viewing under electron microscopy (Eisbein and Proll 1978, Proeseler 1983).

2. Delimitation

No relevant references found.

3. Monitoring

Monitoring of the vector, *P. quadratum*, can be undertaken. Studies have shown that this species occurs in large numbers in sugar beet crops for 2-3 years and then the numbers reduce (Korcz et al. 1997, Korcz 2001).

4. Commodities

No relevant references found.

References: 13 retained out of 19 retrieved, 1 added, 6 used for summary

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Beet necrotic yellow vein virus

Common name(s): BNYVV, rhizomania
Taxa: Viruses: Benyvirus
EPPO A2 list: No. 160
EU Annex designation: I/B

Organism:
All cultivated forms of *Beta vulgaris* are susceptible to the virus (sugar beet, fodder beets, beetroots, mangolds, and spinach beets) and also spinach. Weeds do not carry the virus in the field, although Chenopodiaceae may be infected by mechanical inoculation.
The virus is mainly found in the small roots and is less concentrated in the tap-root. The virus only occasionally enters the aerial parts and has never been observed in seeds. PCR studies have shown the existence of two major strains of BNYVV, A and B and a more recent P strain. BNYVV is transmitted to *Beta* spp. in the soil by *Polymyxa betae*, a soil protozoan. The protozoan is present in most soils where beet has been grown and is not known to cause significant damage in itself. The viral particles have been observed in the cystosori of the protozoan, which can preserve the virus in the soil for many years. The virus produces striking hairy root symptoms and results in severely diminished sugar yield and root weight.
The main means of spread is roots of infected plants, infected beet stecklings and soil containing *P. betae* carrying BNYVV. Suga rbeet waste, washing water and agricultural equipment (especially harvesters) has been shown to be the main carriers at a local level. Stable manure may also play a role in transmission as *P. betae* is capable of passing undamaged through the digestive tracts of animals. Water is important in the spread of the vector. Disease resistant cultivars are available.

1. Detection
Beets grown in heavily infested fields show characteristic symptoms on developed roots: uncoordinated proliferation of partially necrosed small roots pepper-and-salt beard. The root is often constricted (funnel shaped) and cutting the roots shown browning of the vascular ring or the whole tip of the root. In less heavily infested fields the symptoms may be less extensive and may affect only one lateral root without constriction and possibly without the beard. The presence of tumour-like deformations, especially on the rootlets is characteristic. The most useful leaf symptom is visible at the end of the growing season after rainfall, with leaves becoming very pale-green, translucent and upright. The leaf yellowing followed by necrosis along the veins is highly characteristic but infrequent. BYNVV can cause latent infections with no visible symptoms, especially under cool spring conditions.
In beet the most efficient and easy detection method is an ELISA test, done on raw juice extracted from lateral roots or from the tip of the taproot (Putz, 1985). The sensitivity threshold is 2-6ng of virus per g of tissue. Jezewska and Piszczek (2001) found that BNYVV detection by ELISA was much easier using leaves instead of roots as material for diagnostic tests. Results are more reliable than using indicator plants (*Chenopodium quinoa*). Quick test ELISA methods are available (Schaufele *et al*., 1995). In soil or adherent soil beet plants are grown in suspect soil and an ELISA test performed on their roots (Merz and Hani, 1985). Bait plant tests to estimate soil infestation with BNYVV using pre-grown sugar beet seedlings can be used to estimate the level of infestation (Goffart *et al*., 1990) as well as calculate yield losses. The optimum time for bait testing is 6 weeks for the English test and 3-4 weeks for the French test, however if a more rapid method is needed, RT-PCR or Taq-Man RT-PCR can be done after 3 weeks with the English test (Henry *et al*., 1995) However these tests are not reliable enough for detecting very low levels of infestation and are unsuitable for

establishing if fields are free from the virus. The use of the nested PCR assay is recommended for applications where its improved sensitivity over standard RT-PCR is necessary, for example in the early detection of infection from bait-test soils and for quarantine and breeding purposes (Morris *et al*. (2001).

The EPPO diagnostic protocol for BNYVV describes the symptoms and use of ELISA and RT-PCR, immunocapture, Taqman RT-PCR, electron microscopy and soil tests for the identification of the virus (Anon, 2006; Harju *et al*., 2005). ELISA testing is normally sufficient for diagnosis but PCR and TaqMan PCR can be performed as required. For screening tests ELISA is the best and most cost-effective general screen. PCR is more sensitive than monoclonal indirect or amplified ELISA for detection of BNYVV. A gain in sensitivity, time and labour savings made by avoiding the needs for post-PCR gel electrophoresis are some of the main advantages of real-time over conventional PCR, however, machinery and consumables are relatively expensive. Positive identification of BNYVV (in the original plant or indicator plant) should be made using ELISA and/or PCR methods. A confirmation of the presence of the virus may be required using a distinct method from that originally used.

2. Delimitation

No information.

3. Monitoring

Plants showing symptoms of the disease should be sampled. Samples for testing can also be identified from distinct yellow patches in beet crops (e.g. identified from aerial photography or remote sensing). A fork or spade should be used to dig up the roots taking care to lift the beet whole with root tip and laterals. Each sample should consist of the lower third of the taproot of 5 or 6 plants showing symptoms. For laboratory based tests the sugar beet samples should be thoroughly washed in cold water and dried. For soil samples a total of 2.5kg of field soil should be taken by walking in a W shape across each sampling area (Anon, 2006).

4. Commodities

No information.

References (232 retained out of 477 retrieved, 2 added, 8 used for the summary

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Bemisia tabaci (Gennadius) (European / non-European populations)

Common name(s): Cassava whitefly, cotton whitefly, sweet-potato whitefly, tobacco whitefly
Taxa: Insecta: Hemiptera: Aleyrodidae
EPPO A2 list: No. 178
EU Annex designation: I/B, I/A1

Organism

Bemisia tabaci, the tobacco or sweet-potato whitefly, is considered one of the most invasive pest species globally that can infest a wide range of host plants (De Barro *et al*. 2006, Li *et al*. 2011). This phloem-feeding insect is able to vector a range of plant pathogenic organisms, rendering it of very high economic importance. At northern latitudes the pest can only persist in glasshouses whereas in warmer areas the insect is also found on outdoor crops and is capable of overwintering. The host range for *B. tabaci* is vast with over 360 plant species recorded from China alone (Li *et al*. 2011). This whitefly exists as a number of biotypes that have differing biological attributes and host preferences, of which the most important are the B and Q biotypes (De Barro *et al*. 2000). Recently, phylogenetic studies have made it clear that *B. tabaci* is actually an assemblage of >20 species that are morphologically indistinguishable although here, in the absence of updated nomenclature, we continue to refer to it as a single species.

1. Detection

Bemisia tabaci is a small insect and the larvae, and to a lesser extent the adults, are extremely difficult to differentiate from related species. Microscopic speciation of *B. tabaci* is generally accomplished through examining the puparia (Malumphy *et al*. 2010). As a result, visual confirmation of identity is reliant on detailed examination by a trained taxonomist. However, in recent years a number of immunological and molecular tests have become available for ascertaining identity. Biochemical tests to identify whitefly species, and to differentiate biotypes, were initially based on electrophoretic banding patterns, particularly those associated with non-specific esterase diversity (Prabhaker *et al*. 1987, Wool *et al*. 1994). However, more advanced ELISA-based assays have been developed that can readily differentiate whitefly species infesting plant (Symondson *et al*. 1999) although molecular tests are now more commonly used.

Many of the tests currently used are based on PCR techniques that amplify regions of the mitochondrial oxidase 1 gene (mtCOI). At its most basic, a diagnostic region of the mtCOI gene is amplified and sequenced, leading to confirmation of the species using sequence homology. However, this process is time consuming and TaqMan methods and other real time assays can provide a rapid diagnosis of the pest without the need for sequencing (Ueda 2006). In many cases, the primary aim of the tests is not just to identify *B. tabaci* but to also differentiate biotypes, particularly the B and Q biotypes (Jones *et al*. 2008, Papayiannis *et al*. 2009). Such assays are suitably sensitive to facilitate the identification of the remains of *B. tabaci* within the guts of predator organisms (Zhang *et al*. 2007). Several RAPD-PCR techniques have similarly been employed to determine the identity of *B. tabaci* and to ascertain its biotype (Samih *et al*. 2006, Shankarappa *et al*. 2007).

A simple, cost effective and accurate method for detecting *B. tabaci* on sticky traps has recently been published by Malumphy *et al*. (2010). This process involves inspecting insects under low magnification and differentiating insects using pigmentation patters, a process that gives 98% accuracy. A highly novel method for the detection of *B. tabaci* involves the use of machine vision to identify whitefly adults on sticky traps (Solis-Sanchez *et al*. 2009). Whilst this method can adequately differentiate whitefly from other types of insects and count them, it is not known as to

whether it can differentiate whitefly species. Another simple but elegant method can be used to detect the presence of feeding nymphs on plants through the use of magnesium oxide coated glass slides that can be used to visualize honeydew falling from the plants (Wang *et al*. 2009).
The collection of whitefly, visual inspections to observe larvae or sticky traps to catch adults are the main methods employed to obtain insects. In the case of sticky traps, the optimum colour determined by several studies appears to be yellow (Berlinger 1980, Abdel-Megeed *et al*. 1994, Al-Ajaln 2005). Traps are used in both the glasshouse and the field context (Butler and Wilson 1984, Butler *et al*. 1985b). Trap height is an important aspect and they should be placed at the most efficacious height for a given crop (Byrne *et al*. 1986, Atakan and Canhilal 2004). The orientation of the trap is also important, with traps frequently placed horizontally on the ground or at canopy height with the sticky side uppermost (Lynch and Simmons 1993). Several variants of the basic yellow stick trap have been evaluated, including flat and cylindrical designs, the latter appearing to be the more efficacious (Byrne *et al*. 1986, Attique *et al*. 1999). It has also been observed that placing yellow traps on a black background increases catches. (Kim and Lim 2011). In other cases, traps equipped with lime-green light-emitting diodes have been shown to increase trap counts (Chen *et al*. 2004) whilst some non-sticky traps have been developed (Chu *et al*. 1996). The selectivity of traps with respect to catching whiteflies as opposed to their natural enemies has been investigated and yellow sticky traps appear to have the highest non-whitefly catches compared to non-sticky and colourless variants (Hoelmer *et al*. 1998)
Some non-sticky traps have also been equipped with lime-green LEDs, such as the cup trap described by Chu *et al*. (2003). Other capture methods include the aerial netting of flying insects (Bellows *et al*. 1988) vacuum sampling, and beating (Brewster *et al*. 1997). Infrared photography has been used to detect areas of very high infestation in cotton crops in the USA (Meyerdirk *et al*. 1986). The commonly used “black pan” method, whereby insects are beaten from infested plants onto a black cake tin in which a layer of oil has been applied is described by Naranjo *et al*. (1995).

2. Delimitation

Of the references accrued to date, none specifically refer to attempts to delimit the extent of occurrence of this pest within a geographic area. Further examination of the references is ongoing toi ascertain whether more information can be found in this area of surveillance.

3. Monitoring

Surveys monitoring for *B. tabaci* in the field and glasshouse contexts are extremely numerous in the literature. Typically, most monitoring exercises use yellow sticky traps of one type or another (Gerling and Horowitz 1984, Gocmen *et al*. 1987, Diraviam and Uthamasamy 1992, Lynch and Simmons 1993, Legaspi and Carruthers 1995). In some cases, yellow water traps are also used (Mohanty and Basu 1991, Ahmad and Aslam 2002). In some cases, the insect is monitored solely on a visual basis though counting insects present on specific leaves within a crop (Muhammad *et al*. 2001). In Arizona, whitefly populations have been subject to area-wide monitoring through weekly “leaf turn” visual inspections across 18,000 acres of cotton (Jech and Husman 1998a, b). Plastic cup traps have been used to monitor adult *B. tabaci* in Turkey although sampling regimes were not well articulated (Karut *et al*. 2005).
The monitoring of immature whitefly in the USA has been undertaken through sampling leaf discs which were seen to be good predictors of whole leaf counts (Legaspi *et al*. 1997). It is important to know where *B. tabaci* adults occur on plants to enable counting using the leaf turn method as it has been demonstrated that adult *B. tabaci* will often congregate within certain zones (Lin *et al*. 2006). In Croatia, where *B. tabaci* first occurred in 2000, glasshouses have been monitored for 30

different crops through the hanging of sticky traps and the visual inspection of the undersides of leaves, which showed poinsettia to be the most commonly infested of the crops assessed (Lynch and Simmons 1993).

In the USA the spread of *B. tabaci* and the nature of the biotypes present has been very closely monitored with surveys of insects being widely conducted throughout the USA and biotype status of the collected whiteflies determined using several of the techniques described above (McKenzie *et al*. 2008, McKenzie *et al*. 2009). In one survey undertaken, 100 suction samples per field were taken using a D-Vac insect vacuum and the collected insects returned to the laboratory for identification. The leaf turn method has also been successfully employed in United States as part of ongoing monitoring that typically sampled 15 leaves per field to build an extensive picture of infestation within a given region (Jech and Husman 1998b).

A monitoring methodology has been developed in Israel that has been designed with the objective of determining the optimal dates for spraying insecticides (Melamed-Madjar *et al*. 1982). This procedure involved placing yellow sticky traps horizontally above cotton plants at 400 m intervals along the periphery of fields. A simple monitoring method appropriate for resource poor countries is described by Renou *et al*. (1988) that involves using a magnifying glass to inspect cotton for the indistinct larval stages of the whitefly. Sampling methods in cantaloupe, based on a model, have been developed that produced reliable predictions of infestation levels (Tonhasca *et al*. 1994). Further sampling details for immature whitefly and eggs are described by Laurentin *et al*. (2002) for sesame plants. Sampling methodologies for several other crops has been described and discussed (Butler *et al*. 1985a, Gocmen *et al*. 1987, Hirano *et al*. 1995, Singh 1995, Barbosa *et al*. 2000, Gencsoylu 2007, Karut and Kazak 2007)

An extensive whitefly survey has been recently conducted in Sardinia (Nannini *et al*. 2009). In this case adults were monitored in glasshouses and outdoors using yellow sticky traps, whilst visual inspections of leaves for immature stages were also conducted. An extensive comparison of methods for detecting the presence and monitoring for *Bemisia tabaci* is provided by Naranjo *et al*. (1995). This work came to the conclusion that the leaf turn method is the most efficient method for estimating the abundance of *B. tabaci* in cotton. Furthermore, it showed that traps placed at ground level were more effective in capturing whiteflies than those placed at canopy height. Details of the sampling regimes necessary to provide an accurate estimate of abundance are also given in this paper. An extensive survey of *B. tabaci* across Florida describes a novel collection method whereby insects were captured from the plants using a modified hand-held cordless vacuum cleaner (McKenzie *et al*. 2004). The survey was conducted across 15 different crops with multiple sampling events undertaken in each field. The spatial distribution of *B. tabaci* within bean fields has been examined in Brazil, indicating that the insect tends to be regularly distributed throughout the crop (Pereira *et al*. 2004), an important aspect to consider when developing monitoring strategies.

4. Commodities

Bemisia tabaci is frequently transported with traded plant material and is routinely surveyed for at points of entry into countries. Interceptions of *B. tabaci* on traded plants are frequently reported, often following the inspection of imported ornamental plants (Karnkowski 1999). The trade in poinsettia has been implicated in the spread of *B. tabaci* through the USA and New Zealand (Drayton *et al*. 2009) that necessitates the routine inspection of imports of this plant, typically using the leaf-turn method. The need to identify the non-mobile immature stages of *B. tabaci* necessitates that leaf inspections are undertaken for these types of inspections. Unfortunately, information on the methods used is scant. Details of UK interceptions made at ports of entry are

detailed by Baker and Cheek (1993) although little details pertaining to methodology are provided.

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Black raspberry latent ilarvirus (BRLV)

Common name(s): BRLV
Taxa: Viruses: Bromoviridae: Ilavirus
EPPO A2 list: No. 147
EU Annex designation: II/A1

Organism

BRLV is closely related to tobacco streak ilarvirus. BRLV infects only *Rubus* spp. particularly *R. occidentalis* but also *R. idaeus*. BRLV is transmitted in pollen to plant pollinated. A non-flower means of transmission is postulated for some spread in *Rubus* (Converse, 1980). BRLV is in itself an insignificant pest of *Rubus* but the decision to consider it as a quarantine pest was partly based on the possibility of synergistic effects of mixed infections but mainly on the wish to produce and maintain virus-free *Rubus.* BRLV are mainly dispersed by human movement of infected seeds or plants, or by pollen. Plants of *Rubus* from countries where BRLV occurs should come from a reliable certification scheme in which particular attention has been paid to preventing pollen-transmitted re-infection.

1. Detection

Infected *Rubus* plants are usually symptomless, as are plants grown from infected seed. There may be a decrease in vigour and cane number. Detection can be done by mechanical inoculation of *Rubus* sap to herbaceous test plants (*Chenopodium quinoa* and/or *Cucumeris sativus*) or by ELISA (Anon., 1991). The former is simple but requires up to 1-3 weeks for symptoms to appear. Diagnosis is made from sap from infected herbaceous plants against a range of suitable antiserum. The latter is probably the most reliable and sensitive but is strain-specific BRLV is often unevenly distributed in plants so that samples from several positions on the plant should be tested.

2. Delimitation

No information.

3. Monitoring

No information.

4. Commodities

No information.

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Bursaphelenchus xylophilus (Steiner and Buhrer) Nickle

Common name(s): Pine wood nematode, pine wilt disease
Taxa: Nematoda: Aphelenchoididae
EPPO A1 list: No. 158
EU Annex designation: II/A1

Organism

Bursaphelenchus xylophilus (pine wood nematode, PWN) is the causal agent of pine wilt disease. It is vectored by *Monochamus* spp. Pine species show different susceptibilities to PWN. *Bursaphelenchus xylophilus* can also be found in dead trees of *Abies*, *Chamaecyparis*, *Cedrus*, *Larix*, *Picea* and *Pseudotsuga*, but these genera are not known to be affected by pine wilt disease

Bursaphelenchus xylophilus is native to North America, but was introduced to Japan, probably through imported timber and has spread to China, Taiwan and Korea, where it has caused extensive damage. Economic damage has resulted as a result of both loss of trees and quarantine regulations applied to exports. It was first detected in Europe in Portugal in 1999 (Mota *et al*., 1999). Originally thought to be confined to one area, the whole of Portugal is now considered to be an 'affected area' (Mota *et al*., 2009). A single outbreak in Spain was notified in 2008 (Anon, 2009). Since 2000, EU member states have been required to conduct surveys for PWN (Anon, 2000).

A list of pests that includes *B. xylophilus*, which are priorities for the preparation of diagnostic protocols, has been approved by the Commission on Phytosanitary Measures (CPM) (Clover *et al*., 2010).

1. Detection

Detection of PWN has relied on taking samples from trees or other wood sources, extracting and collecting the nematodes, usually using a Baermann funnel. Identification is based on morphological features. The morphology of *Bursaphelenchus* spp can be very similar and techniques using sex pheromones and DNA analysis have been used to differentiate species Molecular techniques have also been used to detect the presence of PWN in trees and other wood sources.

The survey in Portugal involved collecting wood samples from different parts of the tree and extracting the nematodes in water using different techniques. Nematodes were collected using a Baermann funnel. Identification was made based on morphological features and DNA analysis using an ITS-RFLP procedure.

A restriction fragment length polymorphism analysis of the amplified internal transcribed spacer region of ribosomal DNA (ITS-RFLP) technique was developed for classification of *Bursaphelenchus* species by Hoyer *et al*. (1998). This was able to differentiate five different *Bursaphelenchus* species including *B. xylophilus*.

A real-time polymerase chain reaction (RT-PCR) assay was developed that was highly specific and sensitive, detecting as little as 0.01 ng of *B. xylophilus* DNA. The assay was also successfully used to detect B. xylophilus in field samples (Cao *et al*., 2005). A PCR assay using heat shock protein 70 gene sequences was developed for detection of *B. xylophilus* and used to detect their presence in wood samples from lodgepole pine (*Pinus contorta* var. *latifolia*) trees in British Colombia, Canada (Leal *et al*., 2005). The method detected nematodes after collection by the Baermann funnel method and removed the need to culture or recover nematodes before analysis (Leal *et al*., 2005, 2007). Other PCR based methods using different primers have also been developed (Braasch *et al*., 1995; Abad, 2004; Francois *et al*., 2007; Takeuchi and Futai, 2009

A nested PCR method has been developed for detection of PWN in pine wood (Takeuchi *et al*., 2005). This used a modified CTAB method to extract the DNA and this was analysed by PCR followed by a nested PCR. A two hour extraction was sufficient to obtain DNA. This method was stated to be an improvement over methods requiring removal of nematodes from wood samples using the Baermann funnel technique, which relies on mobility of the nematodes. The nested PCR method could detect a single PWN juvenile in 80 mg of wood tissue (Takeuchi *et al*., 2005). A nested PCR method was also used for detection of PWN in asymptomatic trees in Japan (Takeuchi and Futai, 2007).
A PCR-based method without a nematode extraction stage has been developed for detection of PWN in *Pinus massoniana*. The procedure can be completed within 5 hours (Hu *et al*., 2011).
A loop-mediated isothermal amplification assay has been developed, which as it does not require expensive equipment or specialized techniques, has potential to be used in the field (Kikuchi *et al*., 2009).
An ELISA based method has also been developed that is able to detect 0.1µg of nematode protein on the surface of pine wood (Bai *et al*., 2005).Other methods developed for detection of *B. xylophilus* have been based on chemotaxis. An attractant and trap for the detection of PWN has been developed (Zhao *et al*., 2007). This provides a non-destructive method for detecting the nematodes and lower numbers of nematodes can be detected than when using collection with a Baermann funnel (Zhao *et al*., 2009). The trap was used to look at within-tree distribution of PWN and recommendations for sampling positions based on symptoms of a tree were provided (Zhao *et al*., 2009).
Numerous surveys for detection of *B. xylophilus* within EU countries have been undertaken (see for example McNamara and Stoen, 1988; Abelleira *et al*., 2002; Akbulat *et al*., 2006; Behalova, 2006; Magnusson *et al*., 2007). These surveys have taken wood samples and nematodes have been collected and identified using morphological features or molecular techniques. The surveys are based on the EC Pinewood Nematode Survey Protocol 2000 (Anon, 2000).
A detailed description of sampling for and detection of *B. xylophilus* is given by Schröder *et al*. (2009). This recommends that calculation of a suitable number of samples for detection of *B. xylophilus* should use data from previous monitoring and findings of *B. mucronatus* as this species has the same insect vector and is taxonomically closely related to *B. xylophilus*. It is also stated that the survey strategy should include consideration of the distribution of *Monochamus* species, the range of the insect's host tree species, areas of commercial forest, the state of health and recent logging history of the forests, presence of wood processing facilities, points of wood import (e.g. harbours, airports) handling, transport and storage of imported wood and wood products and entrance and storage points of wood packaging.

2. Delimitation

Surveys to determine the extent of infestation in pine trees in Portugal were undertaken and an initial area of infection established. This has since been revised and the whole of Portugal is designated as an area in which *B. xylophilus* occurs (Mota, 2009).

3. Monitoring

Surveys have been undertaken in Canada to establish the distribution of PWN. It was found to occur in all provinces of Canada except Prince Edward Island (Bowers *et al*., 1992). Surveys have also been conducted in China (Cheng, 1988; Yang and Wang, 1988).
Magnusson *et al*. (2007) describe the results of surveys conducted for PWN in Norway between 2000-2006. The method for the surveys is provided as an appendix to the report. Based on

previous experience on the occurrence of the closely related nematode species *B. mucronatus* in cutting wastes, it was estimated that PWN would be detected in 1 out of 1000 samples showing evidence of *Monochamus* activity. Based on the probability of a positive find and the degree of confidence it was calculated that 3000 samples should be inspected. In total 3165 samples were analysed and *B. xylophilus* was not detected although nematodes were found in 85.4% of the samples. The lower than expected occurrence of *B. mucronatus* (3 out of 1000 samples of wood) led to the conclusion that potential niches for *B. xylophilus* were lower than expected and that 9984 samples should be examined as a minimum to state that this species is absent in Norway.

4. Commodities
No relevant references

References: 226 retained out of 483 retrieved, 4 added, 29 used for summary

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Cadang cadang viroid

Common name(s): CCCVd, Coconut cadang cadang cocadviroid
Taxa: Viroids: Pospiviroidea: Cocadviroid
EPPO A1 list: No. 192
EU Annex designation: II/A1

Organism
Coconut cadang cadang is a lethal disease of coconut palms that occurs in the Philippines, where it has caused extensive losses, and the Solomon Islands. The disease is caused by the coconut cadang-cadang viroid, which occurs as a small (246-247 nucleotide) or large (296-297 nucleotide) form, depending on the stage of infection ((Imperial *et al*. 1981). Symptoms of the disease include the presence of spots on the leaflets and, as the disease becomes more severe, there is a reduction in the size of the leaves and the fruits are few, small, misshapen and scarified. The incidence of the disease increases with increasing age of the palms.

1. Detection
Tests for detection of cadang-cadang viroid based on polyacrylamide gel electrophoresis and molecular hybridisation have been developed (Schumacher *et al*. 1983, Hanold and Randles 1991). These tests are definitive for the viroid as they test for size, structure and nucleotide sequence (Hanold and Randles 1991).
A dot-blot hybridization assay has been used for detection of cadang-cadang viroid and other coconut pathogens (Barker *et al*. 1985, Hodgson *et al*. 1998), but a disadvantage of the earliest forms of the assay was the need for a radio-labelled probe. Photo-biotin labelled DNA probes have been developed to replace the use of radio-labelled probes (McInnes *et al*. 1989). These had comparable sensitivity and specificity to ^{32}P-labelled DNA probes under the same conditions.
The presence of a cadang-cadang like viroid in the Solomon Islands was reported for the first time in 1989 (Hanold and Randles 1989). The viroid was detected from acid extracts of leaves using a hybridization assay with a p-labeled complementary RNA probe representative of the full sequence of the cadang-cadang viroid. Both monomeric and dimeric forms of the viroid were detected (Hanold and Randles 1989).
In-situ hybridization with a biotin-labelled RNA probe has been used to determine that the coconut cadang-cadang viroid is located in the nucleolus and nucleoplasm of cells of infected leaves of oil palm (Bonfiglioli *et al*. 1994).
More recently a ribonuclease protection assay (RPA) has been developed (Vadamalai *et al*. 2009). The RPA is a relatively robust, specific, sensitive, liquid-hybridisation-based RNA diagnostic method that uses enzymes resistant to inhibitors. It complements direct sequencing as it has the potential to detect single nucleotide mismatches between the probe and the target RNA (Vadamalai *et al*. 2009). The technique was used for detecting cadang-cadang viroid sequences in coconut and oil palm and has shown a high incidence of these sequences in a typical commercial oil palm plantation in Malaysia. The technique was also used to confirm that a viroid closely similar to coconut cadang-cadang viroid 246 occurs in Sri Lanka.

2. Delimitation
No relevant references found.

3. Monitoring
A survey, based on visual assessment of symptoms, with an assessment of the stage of the disease

was carried out in the Philippines to determine the incidence of coconut cadang-cadang viroid (Zelazny and Pacumbaba 1982). It was concluded that the incidence of the disease had declined (Zelazny and Pacumbaba 1982).

4. Commodities

No relevant references found.

References: 30 retained out of 64 retrieved, 1 added, 10 used for summary

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Carneocephala fulgida Nottingham

Common name(s): red-headed sharp-shooter
Taxa: Insecta, Hemiptera, Cicadellidae
EPPO list: as vectors in A1 list Nos 137 & 166 *Xylella fastidiosa*
EU Annex designation: I/A1

Organism
Carneocephala fulgida has three/four generations per year, overwintering as an adult and oviposition from late February to early March. The second generation generally moves into vineyards. It is recorded as a vector of Pierce's disease (*Xylella fastidiosa*) in grapevines and alfalfa dwarf diseases. It has been found on numerous species of grasses and sedges, by far its most common host plant for feeding and laying eggs is Bermuda grass (*Cynodon dactylon*). It occurs from Mexico and western Arizona north to northern California.

1. Detection
No information available.

2. Delimitation
No information available.

3. Monitoring
A survey of cicadellid vectors was carried out on commercial orchards (Purcell 1980). The tool used was a conventional sweep net, which was useful in phenological studies and for estimates of adult abundance in short, dry vegetation on warm days, but inadequate for estimates of nymphal densities (Purcell and Frazier 1985).

4. Commodities
No information available.

References: 5 retained out of 9 retrieved, none added, 2 used for the summary

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Carposina niponensis (Walsigham)

Common name(s): Peach fruit moth
Taxa: Insecta: Lepidoptera: Carposinidae
EPPO A1 list: No. 163
EU Annex designation: II/ A1

Organism

The peach fruit moth, *Carposina niponensis*, has two sub species – *niponensis* confined to the Far East and a pest of Rosaceae and *ottawana* confined to Canada and feeding only of *Cornus* and *Ribes*. The latter does not attack Rosaceae and is not considered a pest. The hosts of *C. niponensis* are apples, peaches and pears with apricots, plums, quinces also noted. *Carposina niponensis* overwinters as hibernation larvae in cocoons in the soil, though some larvae may overwinter in fruit in storage. The larvae pupate in the spring in fresh cocoons on the surface of the soil and the moths emerge about 12 days later. The flight period starts in late May to early June and end in mid June in Korea, with a 2nd generation of adults flying from mid-August to early September. The emergence with the first generation of moths in Japan is well synchronized with the growth of the main apple cultivars.

1. Detection

Damage to peach may be confused with that due to *Cydia molesta* in Japan and Korea. Damage to apples resembles that caused by larvae of the fruit fly *Rhagoletis pomonella* and not by larvae of *Cydia pononella*. Several larvae may feed in each fruit. Infested apples exude a sticky gum, pears turn yellow and apricots ripen unevenly.

Male moths are detected in apple orchards using lures comprising of a mating disruption sex pheromone. The extract of female abdominal tip was analyzed with gas chromatography and found to contain two compounds,(Z)-7-eicosen-11-one and (Z)-7-nonadecen-11-one (Han *et al*., 2000). In field-trapping, however, a lure containing (Z)-7-eicosen-11-one only showed the highest male attractancy (Han *et al*., 2000). Yin *et al*. (2011) found that trap design affected trapping efficiency, with the Wing trap more efficient against *C. sasakii* in fields. The effective trapping radius was 20-25m, trap catches were significantly higher at that distance than at 15 m or 30 m. Trapping efficiency was also connected with vegetation cover with catches higher in areas of open forest canopy without low shrub cover (Yin *et al*, 2011). Field experiments have indicated that the black green light was the most effective in trapping moths of *C. niponensis* compared to pheromone lures (Hou *et al*., 1994).

Haishi *et al*., (2009), Ihara *et al*. (2008) and Koizumi *et al*., (2010) used magnetic resonance imaging (MRI) apparatus, to non-invasively observe infestations of the peach fruit moth larvae in harvested apple fruits. Toyoshima, *et al*. (2008) evaluated the ability of a near infrared (NIR) spectroscopy device to detect apples ("Fuji" cultivar) injured by the peach fruit moth. The larvae of the internal apples feeders, *C. sasakii* and *Grapholita molesta* (a non- quarantine pest), are difficult to differentiate morphologically. Several polymorphic regions of mitochondrial DNA of both species were sequenced and used for developing specific striction sites and polymerase chain reaction (PCR) primers (Song *et al*., 2007; Hiroshiri and Sekine, 2011). Based on these sequences, three diagnostic PCR-restriction fragment length polymorphism (RFLP) sites were detected and validated for their practical uses. Also, species-specific PCR primers were devised to develop diagnostic PCR method for identifying the internal feeders (Song *et al*., 2007; Hiroshiri and Sekine, 2011).

2. Delimitation

No information.

3. Monitoring

In China an experiment using sex pheromone traps was conducted with 11-year-old trees of apple cultivar Red Fuji grown in a 0.7 ha orchard with spacing of 3.3 m x 3.3 m. Each of the sex pheromone traps contained 500 micro g of pheromone. Five traps were hung in the orchard in late May and replaced in mid August. The number of trapped adults was checked regularly. Results showed there were 2 peaks of the adult numbers, in early June and early September (Feng *et al*., 2005).

In China sex pheromone traps were installed to monitor *C. niponensis* in a jujube orchard from late June to the end of August, with the attractants changed once a month (Lui *et al*., 2001).

Kim *et al*. (2003) evaluated the single pheromone component (Z)-7-eicosen-11-one impregnated into rubber septum and put into 1C Trece traps suspended 1.5m in fruit tree orchards against *C. sasakii*. Males responded to the pheromone in a dose-dependent manner in the range of 0.1 to 10.0mg. There was no difference in males caught in traps with pheromone replaced every 20 days and the original lures up to 25 days (with 1mg pheromone).

Oku *et al*. (1986) determined that 10 pheromone traps per Ha was the critical density of traps in apple orchards. This capturing range is narrower than other fruit moths and may indicate the narrower flight range of *C. niponensis*.

The Taiwanese government has strict quarantine requirements on the importation of host fruits of *C. sasakii* from Korea (Anon, 2011). Pheromone traps must be used with Z7-20-11Kt as lure and hung at about 1.5m above ground during active seasons of adult peach fruit moth. At least one trap shall be set per hectare with farms smaller than this designated as 1 hectare. Traps must be checked every two weeks or more frequently to survey the occurrence of peach fruit moth. If 3 or more peach fruit moths are found at a single trap, effective pest control measures shall be taken immediately. Pheromone lure must be renewed constantly. Complete records of monitoring and control must be kept for inspection. Field surveillance shall be done 10-15 days prior to harvest. At least 10 plants must be examined for orchards smaller than 1 hectare. For those larger than 1 hectare, at least 5 additional plants shall be examined per 0.5 hectare (orchards less than 0.5 hectare is recognized as 0.5 hectare). At least 20 fruits must be checked for each plant. If any peach fruit moth is found, fruits from that orchard are prohibited to export to Taiwan during the whole production season. Complete records of field surveillance must be kept for inspection.

4. Commodities

No information.

References: 51 retained out of 78 retrieved, 1 added, 14 used in the summary

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Yin, H., Liu, X. Ma, R., Li, P. (2011). Factors influencing on trapping efficiency of sex pheromone for *Carposina sasakii* in fields. Chinese Journal of Biological Control 27: 63-67.

Cephalcia lariciphila (Wachtl)

Taxa: Insecta: Hymenoptera: Pamphilidae
Common name(s): web-spinning larch sawfly
EPPO A2 list: No. 72 (deleted in 1984)
EU Annex designation: II/B

Organism
Adults fly in May-June, males often being more common. Eggs are deposited singly on short-shoot needles. Larvae spin a web-tube at the bases of the short shoots and remain in the web while consuming the needles. Most feeding takes place in July. The old larvae drop to the ground where they remain free as eonymphs without a cocoon 5-20 cm below the surface. In September they develop into pronymphs which overwinter. A development cycle of 1-2 years is normal but, in some places, e.g. the Netherlands, up to 44% of the population was reported to have at least a 3-year generation cycle. During outbreaks, population densities of 57-372 nymphs/m2 of soil were found.

1. Detection
C. lariciphila cuts the needle at the bases and pulls it into the web where it is eaten. This is in contrast to all other sawfly larvae feeding on *Larix*, which leave part of the needle uneaten. *C. lariciphila* feeds only on short-shoot needles when they are available, otherwise long-shoot needles are eaten. Trees at the edge of the stands seem less attacked than those in the middle of the stands. Sawflies were most active at 16 degrees C with 20% air humidity and numbers caught decreased steeply up to 0 degrees C and 80% air humidity. The daily flight activity began at 10 a.m. and ended after 5 p.m. Both Malaise traps and the yellow sticky boards should be used in forest practices from the middle of April (Holusa and Kuras 2010).
Virgin females and dichloromethane extract of crushed virgin females were highly attractive to males. Mated females became unattractive to males within 10 min after mating. When mated females or males were placed in traps together with virgin females there was no evidence of an anti-attractive pheromone or pheromone mask. Traps baited with virgin females sited 0-0.5 m above the ground captured over 7 times the number of males as traps at 1, 2 or 4 m, supporting visual observations that the preferred flight level for males is very near the ground. Horizontal board traps with an acetate surface coated with 'Stikem Special' were superior to Pherocon 1C, vertical board and gipsy moth traps in that order. The observations and results suggest that the best applied uses of *C. lariciphila* pheromone would be in survey and detection, and in male disruption techniques.

2. Delimitation
No information available.

3. Monitoring
One difficulty affecting monitoring is the presence of snow patches and ice on the ground during the period of *C. lariciphila* swarming. It is not easy to sample nymphs from the frozen ground earlier; therefore, it is impossible to monitor nymphs at the end of winter or at the beginning of spring. The monitoring of adults helps to identify the first signs of *C. lariciphila* occurrence and it is also easy to collect swarming adults using both sampling methods tested in this study.
The Malaise traps are more effective for collecting sawflies, but on the other hand, the yellow sticky boards are less expensive and more easy to use in practical forestry for detecting the end of

swarming and estimating the maximum of eggs laid in the tree crowns. These traps are useless for monitoring and analyzing population densities because males regularly overfly them repeatedly (Holusa and Drapela 2004). Nevertheless, this behaviour can also be an advantage; e.g. using five traps on a large area (several hectares) is enough to detect flying adults. It seems that both methods can be useful for studying other *Cephalcia* species; however, the yellow sticky boards can sometimes be rendered ineffective by dust deposits in the spruce forests (Puza et al. 2007). Both methods of monitoring, the Malaise traps and the yellow sticky boards, should be used in forests from the middle of April. The traps can be substituted by a visual observation from the second half of April, when the temperature is higher than 10C. Observation should take place at 2–3 p.m.

4. Commodities
No information available.

References: 117 retrieved, 10 retained, none added, 3 used

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Ceratocystis spp.

Taxa: Fungi: Ascomycota: Hypocreomycetidae:

Preliminary remarks

The genus *Ceratocystis* Ellis & Halst includes a large number of species responsible for significant economic losses to both agricultural and forest crops worldwide. In the past there has been a systematic confusion, it is now accepted that *Ceratocystis* and *Ophiostoma* are two distinct groups in separate orders of the Ascomycota, even if the two genera have clearly evolved similar morphologies. There is a large number of species; most are serious pest of broadleaves in forest and urban environment (*Ceratocystis fagacearum, Ceratocystis fimbriata f.spp. platani, Ceratocystis virescens*). The spreading of *Ceratocystis spp.* is promoted by insect vectors.

Ceratocystis fagacearum (Bretz)

Common name(s): Oak wilt

EPPO A1 list (and its putative vectors *Arrhenodes minutus, Pseudopityophthorus minutissimus* and *P. pruinosus*)

EU Annex designation: I/A1

Organism

C. fagacearum attacks *Quercus spp.* and is carried by insect vectors, among which *Pseudopityophthorus spp.* are mainly found on *Quercus* although other hardwood hosts have been recorded, but also the sap beetles *Colopterus truncatus* and *Carpophilus spp. a*re considered important vectors of the oak wilt pathogen (Cease and Juzwik 2001, Ambourn et al. 2005).

1. Detection

The identification was based on the isolation and morphological analysis. Recently a nested and real time PCR assay was developed for the detection of *C. fagacearum* (Wu et al. 2011); more general studies point to develop a rapid and reliable PCR-based RFLP identification and to consider phylogenetic relationships among the better-known species of Ceratocystis and close genera (Kim et al. 1998, Witthuhn et al. 1999). Surveys were carried out at national or regional, these have permitted the first report of some *Ceratocystis* species, as it happened in five new counties in Kansas and Nebraska in 1975-76 (Landis 1977), while in another survey *Ceratocystis fagacearum* was not found (Hammerli and Stadler 1989).

2. Delimitation

No information available.

3. Monitoring

Sampling has carried out to evaluate the distribution and incidence of *C. fagacearum* in different US states.

4. Commodities

Wood is the main commodity for *Ceratocystis* spp. Specific phytosanitary measures were provided for the imports of oakwood (bark removal, fumigation) (Burdekin 1979, Rutze and Liese 1983).

Recently there was new attention paid to woodchip, where *Ceratocystis* spp. may establish during storage (Kliejunas et al. 2001, Lanfranco et al. 2003).

Ceratocystis fimbriata Ellis and Halsted

Common name(s): Ceratocystis blight, mango blight, black canker of aspen (there are many)
EPPO A2 list: No. 136
EU Annex designation: II/A2

Organism
C. fimbriata f.spp. platani attacks only *Platanus* spp. The organism was introduced from the USA to several Southern European ports at the end of the Second World War and spread rapidly in Italy and more slowly in France. The fungus may be transmitted by root contact. Anastomosis between roots of *Platanus* trees is possible and the pathogen can infect the uninfested tree. It is transmitted by contaminated pruning tools and terracing machinery which causes damage to the roots.

1. Detection
The identification was based on the isolation and morphological analysis. In the recent years new techniques are acquiring more importance; loop-mediated isothermal amplification (LAMP) was developed for the detection of *C. fimbriata*, but not for the forma specialis of *Platanus* (Fukuta et al. 2009). More general studies were carried out to develop a rapid and reliable PCR-based RFLP identification method and to consider phylogenetic relationships among the better-known species of *Ceratocystis* and close genera (Witthuhn et al 1999, Kim et al 1998). To detect *C. fimbriata f.sp. platani* in wood samples or soil, a trap technique has been described (Grosclaude et al. 1988a, b): healthy branches of *Platanus acerifolia* are stripped of their bark and placed in close contact with the wood or soil sample and are then incubated in a moist chamber or in water at room temperature. Perithecia develop on the branches within a few days.

2. Delimitation
No information available.

3. Monitoring
Survey were carried out at national or regional to evaluate the spreading of pathogens in Italy and France (Bondavalli et al. 1998, Chapin and Chauvel 2007).

4. Commodities
Wood is the main commodity for *Ceratocystis* spp. Recently there was new attention paid to woodchip, where *Ceratocystis* spp. may establish during storage (Kliejunas et al. 2001, Lanfranco et al. 2003).

Ceratocystis virescens (*= Ceratocystis coerulescens*)

Common name(s): Sapstreak disease of maple
EU Annex designation: II/A1

Organism
Ceratocystis virescens is a pathogen on *Acer saccharum*. There is a single record from *Liriodendron tulipifera*. It has also been found as a saprophyte on logs of a number of other woody species

1. Detection
The information available about this species are few; moreover there was a systematic confusion (Zajonc and Wulf 1997), so the development of new technique could be useful to better define the situation (Harrington et al. 1996).

2. Delimitation
No information available.

3. Monitoring
No information available

4. Commodities
Wood is the main commodity for *Ceratocystis* spp. Recently, there was renewed attention paid to woodchip, where *Ceratocystis* spp. may establish during storage (Kliejunas et al. 2001, Lanfranco et al. 2003).

References: 19 retained out of 772 retrieved, none added, 18 used for the summary

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Chapin, E. and G. Chauvel. 2007. Phytosanitary overview for 2006 of tree plantations, shrubs and clumps in green spaces. PHM Revue Horticole 490:40-44.

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America. Page viii + 134 General Technical Report - Forest Products Laboratory, USDA Forest Service. USDA Forest Service, Forest Products Laboratory, Madison.

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Lanfranco, D., S. Ide, C. Ruiz, I. Vives, and H. Peredo. 2003. Phytosanitary characterization of Eucalyptus spp. and native species chips. Bosque 24:47-54.

Rutze, M. and W. Liese. 1983. Fumigation of oak logs with methyl bromide for control of Ceratocystis fagacearum. [German]. Holz-Zentralblatt 109:1533-1535.

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Wu, C. P., G. Y. Chen, B. Li, H. Su, Y. L. An, S. Z. Zhen, and J. R. Ye. 2011. Rapid and accurate detection of Ceratocystis fagacearum from stained wood and soil by nested and real-time PCR. Forest Pathology 41:15-21.

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Cercospora angolensis T. Carvalho & O. Mendes

(=*Phaeoramularia angolensis* (T. Carvalho & O. Mendes) P.M. Kirk
Taxa: Fungi: Ascomycota: Dothidiomycetidae
Common name(s): Citrus leaf spot, citrus fruit spot
EU Annex designation: II/A1

Organism

The fungus is restricted to species of citrus including grapefruits, limes, mandarins, oranges, rangpur limes, rough lemons and sour oranges. The disease has spread from Angola and Mozambique to countries further north and is absent in the EU. The disease is supposed to be the most serious at altitudes above 600m. Under natural conditions infection of the leaves and fruit is most likely by conidia although the mechanism involved is unknown and the possibility of transfer by insects cannot be discounted. The fungus is only known from its anamorph. Internationally, transfer on the leaves of planting material or on fruits seems the most likely pathway.

1. Detection

Leaf symptoms initially appear as greenish-yellow patches. A maturity the leaf spots are amphigenous, mainly hypophyllus, 4-10 mm in diameter, pale-brown to brown, blackish-brown when sporulation is dense, surrounded by a dark-brown margin and a yellow halo, the centre often becoming detached resulting in a shot-hole spot (Kuate, 1998). During wet weather the lesions sporulate and become black. On young fruits, brown necrotic lesions form. These are usually circular, slightly sunken with a surrounding ring of raised epicarp, giving fruit a blistered appearance. During wet weather the lesions sporulate and become black. In young fruits, a generalised necrosis sometimes forms, resulting in premature abscission of the fruit. Fruits and leaves are much more susceptible than stems, on which symptoms are rare. Infected fruits are juiceless, unsaleable and may drop prematurely (Kuate, 1998). Severe infection of trees can result in defoliation through the fall of young leaves.
Morphology is described in the EPPO datasheet and by Carvalho and Mendes (1953) and Kirk (1986). Morphological characteristics and rDNA sequence data were used to compare a new disease of citrus with *Phaeoramularia angolensis* (Pretorius *et al*., 2003).

2. Delimitation

The spread of *C. angolensis* in Guinea was established by taking suspected leaf and fruit samples, from 25 trees in several localities and analysing in the laboratory (Diallo, 2001).

3. Monitoring

No information.

4. Commodities

The highly restrictive measures already taken by most citrus-growing countries for the import of plants of citrus outside the EU region would protect against the introduction of *C. angloensis*.

References: 25 retained out of 56 retrieved, 1 added, 5 used for the summary

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Diallo, M. T. S. (2001). Progression of the citrus leaf and fruit spot disease (*Phaeoramularia angolensis*) in Guinea. Fruits (Paris) 56: 37-43.

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Kuate, J. (1998). Citrus leaf and fruit spot disease caused by *Phaeoramularia angolensis*. Cahiers Agricultures 7: 121-129.

Pretorius, M. C., Crous, P.W., Groenewald, J. Z., Braun, U. (2003). Phylogeny of some cercosporoid fungi from Citrus. Sydowia 55: 286-305.

Cercospora pini-densiflorae (Hori & Nambu) Deighton

(= *Mycosphaerella gibsonii Evans*)
Taxa: Fungi: Ascomycota: Dothidimycetidae
EPPO A1 list: No. 7
EU Annex designation: II/A1

Organism

Previously, the fungus was only known in its imperfect state but the teleomorph was found and identified as *Mycosphaerella gibsonii*, anamorph: *Pseudocercospora pini-densiflorae* (Hori & Nambu) Deighton.

Cercospora pini-densiflorae can be very damaging on 1- to 2-year-old seedlings of *Pinus caribaea*, *P. densiflora* and *P. thunbergii*, and on seedlings and in young plantations of *P. halepensis*, *P. pinaster* and *P. radiata*. It can also attack *P. canariensis*, *P. luchuensis*, *P. massoniana*, *P. merkusii*, *P. resinosa*, *P. strobus* and *P. sylvestris*. Resistance has been reported in *P. clausa*, *P. elliotii*, *P. kesiya*, *P. patula* and *P. rigida*. Some of these susceptible *Pinus* spp. (such as *P. halepensis*, *P. pinaster*, *P. radiata* and *P. sylvestris*) are widely cultivated in nurseries and are present in European forests, and many other pines have been introduced as ornamental trees. *C. pini-densiflorae* overwinters as mycelial masses or immature stromata in the tissues of diseased needles. The primary infection source consists of airborne conidia produced in the spring from these needles and spread in the wind or by rain-splash. Transplanted seedlings with diseased needles also serve as an infection source. Conidia germinate between 10 and 35°C (25°C being optimal). The incubation period varies with environment but is thought to be around 6 weeks.

1. Detection

First records of the fungus are reported for the Philippines (Kobayashi 1987, Kobayashi and Zhao 1987, Kobayashi et al. 2003) and India (Reddy and Pandey 1973).

2. Delimitation

No information available.

3. Monitoring

No information available.

4. Commodities

It is unlikely that the fungus could spread from Africa and Asia to Europe as wind-borne spores. However, it could enter on infected seedlings and on cut branches of *Pinus* and, in view of the long incubation period, latent infections present a hazard.

References: 15 retained out of 379 retrieved, none added, 4 used

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Kobayashi, T. and J. Z. Zhao. 1987. Two Fungi Associated with Needle Blight of Cunninghamia-Lanceolata. Nippon Kingakukai Kaiho 28:289-294.

Reddy, M. A. R. and P. C. Pandey. 1973. Cercospora needle blight of Radiata Pine in India. Indian Forester 99:308-309.

Cherry leaf roll virus (in *Rubus*)

Common name(s): CLRV
Taxa: Viruses: Comoviridae: Nepovirus
EPPO A2 list: No. 148
EU Annex designation: II/A1

Organism

Cherry leaf roll virus (CLRV) infects a range of woody hosts, including several *Rubus* (blackberry etc) species. The virus is widespread in the EPPO region and also occurs in New Zealand. The disease in other hosts has recently begun to occur in North America. The disease causes leaf mottling, stunting and death in *Rubus*. In Cherry, the trees show poor vigour, stunted leaves and poor fruit quality. Unlike several other nepoviruses, transmission is via physical movement of infected material or pollen (Bush 2002). Most information available refers to the disease in non-*Rubus* hosts.

1. Detection

Serological methods have traditionally been used for the detection of this virus. However, recently an accurate and sensitive PCR method has been developed (Kumari 2009).

2. Delimitation

No information

3. Monitoring

Monitoring in New Zealand has been conducted based on the observation of the physical symptoms in raspberry and serological tests (Jones and Wood 1978, 1979). Work in the Czech Republic has surveyed >100 populations of *Rubus* (Pelikan 1989, Anonymous, 1991, Spak *et al*. 1997) but little further information is available.

4. Commodities

No information

References: 11 retained out of 14 retrieved, 2 added, 8 used for summary

Anonymous. 1991. Outbreaks and new records. Czechoslovakia. Cherry leaf roll nepovirus newly recorded in Rubus in Czechoslovakia. FAO Plant Protection Bulletin 39:183-184.

Bush, M. R. 2002. Cherry growers must contain the Cherry Leaf Roll Virus- A new cherry disease to Pacific Northwest. Basin Business Journal:2.

Jones, A. T. and G. A. Wood. 1978. The occurrence of cherry leaf roll virus in red raspberry in New Zealand. Plant Disease Reporter 62:835-838.

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Kumari, S. 2009. Detection of cherry leaf roll virus and strawberry latent ring spot virus by one step PCR. Plant Protection Science 4:4.

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Cherry rasp leaf virus

Common name(s): American cherry rasp leaf, flat apple
Taxa: Viruses: Comoviridae: Cheravirus
EPPO A1 list: No. 127
EU Annex designation: I/A1

Organism
CRLV is a viral disease of cherries, peach and apple as well as a number of other species that are typically asymptomatic. It occurs in North America, and Oceania but is absent from the EPPO region (Anonymous 2001). The virus is transmitted by a nematode vector (Hansen *et al*. 1974). Symptoms vary with host and include outgrowths, leaf rolling and fruit malformation

1. Detection
Detection of this virus can be achieved through grafting onto indicator species, such as *Chenopodium amaranticolor* (Jones *et al*. 1985, Li *et al*. 1996). RT-PCR techniques have been developed that detect the virus and confirm it as being the same pathogen as the causal agent of flat apple disease (James *et al*. 2000, 2001, James 2004, Susaimuthu *et al*. 2007).

2. Delimitation
No information available

3. Monitoring
Little information was available with regards monitoring efforts for CRLV are available, beyond brief notes of incidences in North America (Luepschen *et al*. 1974, Parish 1977). No more recent examples of monitoring were identified by the literature search

4. Commodities
Commodity transport of CRLV has been recorded in the UK (Jones *et al*. 1982) and general quarantine procedures for *Malus* and *Prunus* described (Adams 1991).

References: 34 retained out of 40 retrieved, none added, 12 used for summary

Adams, A. N. 1991. Quarantine Procedure Other Viruses of Malus and Prunus Inspection and Test Procedures. Bulletin OEPP 21:251-256.

Anonymous. 2001. Cherry rasp leaf virus. Page Map 303 Distribution Maps of Plant Diseases. CAB International, Wallingford.

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Jones, A. T., M. A. Mayo, and S. J. Henderson. 1985. Biological and Biochemical Properties of an Isolate of Cherry Raspberry Leaf Virus from Red Raspberry. Annals of Applied Biology 106:101-110.

Jones, A. T., A. F. Murant, S. Badenoch, and M. A. Mayo. 1982. Rubus viruses. Report, Scottish Crop Research Institute, 1981. 1982. 111-113., Invergowrie, Dundee.

Li, T. S. C., K. C. Eastwell, and A. J. Hansen. 1996. Transmission of cherry viruses by approach grafting from herbaceous to woody hosts. Canadian Journal of Plant Pathology 18:429-432.

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Parish, C. L. 1977. A relationship between flat apple disease and cherry rasp leaf disease. Phytopathology 67:982-984.

Susaimuthu, J., J. Thompson, and K. L. Perry. 2007. A potato strain of Cherry rasp leaf virus and its detection with a broadly applicable set of degenerate picorna-like virus primers. Phytopathology 97:S113.

Choristoneura spp. (non-European)

Taxa: Insects: Lepidoptera: Totricidae

The genus *Choristoneura* Lederer, 1859 (Lepidoptera, Tortricidae) comprises about 40 species, with 20 described from the palaearctic region and 17 from the nearctic region and one from the afrotropical region, whereas holoarctic or intercontinentally introduced species have not been recorded yet. Most are serious pests of conifers (*C. fumiferana*, *C. occidentalis*), but there are some species harmful to broadleaves (*C. conflictana*, *C. rosaceana*).

Choristoneura fumiferana Clemens

Common name(s): Spruce budworm
EPPO A1 list: No. 206
EU Annex designation: I/A1

Organism
Choristoneura fumiferana is the most important defoliator of the northern *Picea* and *Abies* forests of the Eastern United States and Canada. It can also be found on *Pseudotsuga*, *Pinus* and occasionally on *Tsuga* and *Larix.* Several significant host plants of *C. fumiferana* are widely grown in European forests and plantations

1. Detection
Many field studies tested the pheromone of *C. fumiferana,* furthermore the trap type (non-sticky and sticky) and the release rate were evaluated to maximise the trapping efficiency (Sanders and Meighen 1987, Grimble 1988, Grant 1991, Sanders 1996). Coating the inside of sex pheromone-baited funnel traps with insect trapping adhesive can be a solution to avoid the escape of individuals captured and the repellent effect of killing agent (Sanders 1986). An important aspect is that species tend to be very difficult to identify using morphological characters, so the mitochondrial DNA (mtDNA) could be used for species diagnoses within the *Choristoneura* complex (Sperling and Hickey 1995, Lumley and Sperling 2011). A survey of *C. fumiferana* was carried out in Czechoslovakia during 1983. No individual was trapped in this survey program (Brewer et al. 1985).

2. Delimitation
The geographical distribution of *Choristoneura* species (Lepidoptera: Tortricidae) feeding on *Abies*, *Picea*, and *Pseudotsuga* were studied in western Canada and Alaska through use of pheromone-baited traps. The range of all species was more extended than previously known, sometimes adding hundreds of kilometres to the known range (Shepherd et al. 1995).

3. Monitoring
A network of pheromone traps program has been placed throughout the range of the moth (Sanders 1988), the resultant maps can be used to predict incipient outbreaks and predict defoliation (Lyons et al. 2002).

4. Commodities

The movement of infested nursery trees contributes to the spread of *C. fumiferana* (Tagestad 1975*)*.

Choristoneura occidentalis Freeman

Common name(s): Western spruce budworm
EPPO A1 list: No. 207
EU Annex designation: I/A1

Organism
Choristoneura occidentalis occurs principally on *Pseudotsuga menziesii* and also on other forest trees such as *Abies spp* and *Picea spp.*

1. Detection
A study in laboratory and in the field was carried out to test the efficiency of pheromone with different trap types (Angerilli and McLean 1984, Sweeney et al. 1990). An important aspect is that species tend to be very difficult to identify using morphological characters, so the mitochondrial DNA (mtDNA) could be used for species diagnoses within the *Choristoneura* complex (Lumley & Sperling 2011; Sperling & Hickey 1995).

2. Delimitation
The geographical distribution of *Choristoneura* species (Lepidoptera: Tortricidae) feeding on *Abies*, *Picea*, and *Pseudotsuga* were studied in western Canada and Alaska through use of pheromone-baited traps. The range of all species was more extended than previously known, sometimes adding hundreds of kilometres to the known range (Shepherd et al 1995).

3. Monitoring
Monitoring programs are curried out at regional level (Pogue and Lavigne 1984) also with the aim to predict populations (Harris et al. 1981).

4. Commodities
No information.

Choristoneura rosaceana (Harris)

Common name(s): Oblique banded leafroller
EPPO A1 list: No. 208
EU Annex designation: I/A1

Organism
Choristoneura rosaceana is a polyphagous pest, but preferred hosts are predominantly Rosaceae; it is a rather important pest in orchards, especially in apples but is not considered a problem in forests.

1. Detection
The efficiency of pheromone and the trap is tested in numerous field studies, furthermore the trap type (non-sticky and sticky), colour and the release rate were evaluated to maximise the trapping efficiency (Knodel and Agnello 1990, Myers et al. 2009); difference in the response to sex

pheromone blend was highlighted at regional level (Thomson et al. 1991). An important aspect is that species tend to be very difficult to identify using morphological characters, so the mitochondrial DNA (mtDNA) could be used for species diagnoses within the *Choristoneura* complex (Sperling & Hickey 1995). A survey of *Choristoneura rosaceana* was carried out from 1982 to 1983 in India and from 2002 to 2004 in Lithuania using traps baited with the specific synthetic sex pheromone blend, and no individuals of *Choristoneura* were trapped in both surveys (Bhardwaj 1987, Ostrauskas et al. 2010).

2. Delimitation
No information.

2. Monitoring
Monitoring programs are carried out in orchard to predict the population level and to the development of effective management strategies against this important pest of apple (Fadamiro 2004).

4. Commodities
No information.

Choristoneura conflictana (Walker)

Common name(s): Large aspen tortrix
EPPO A1 list: No. 205
EU Annex designation: I/A1

Organism
Choristoneura conflictana occurs mainly on *Populus tremuloides*, but can also feed on other associated broad-leaved trees.

1. Detection
The pheromone was tested in field trials, furthermore a combined pheromone-based monitoring system was developed for *C. conflictana* and *Malacosoma disstria*, another important Lepidoptera defoliator of trembling aspen *Populus tremuloides* in western Canada (Evenden 2005, Jones et al. 2009). The male of *C. conflictana* were captured also in traps baited with *C. fumiferana* pheromone, even if the author suggest that male *C. conflictana* were not attracted by the pheromone, but blundered into the traps (Sanders 1993).

2. Delimitation
No information.

3. Monitoring
No information.

4. Commodities
No information.

Other *Choristoneura* species

Detection and monitoring

The pheromone was identified and monitoring program were developed also for other species of non-European *Choristoneura,* as for example *Choristoneura orae* and *Choristoneura pinus* (Gray et al. 1984, Pendrel 1985). An important aspect is that species tend to be very difficult to identify using morphological characters, so the mitochondrial DNA (mtDNA) could be used for species diagnoses within the *Choristoneura* complex (Lumley & Sperling 2011; Sperling & Hickey 1995).

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Chrysanthemum stem necrosis virus

Common name(s): CSNV
Taxa: Viruses: Bunyaviridae: Tospovirus
EPPO A1 list: No 133
EU Annex designation: II/A1

Organism
CSNV occurs on florists' chrysanthemum and tomato (Anonymous, 2005). In artificial inoculation studies CSNV induced symptoms on a large number of test plants. CSNV was first recognised on chrysanthemum in Brazil (and may occur in other South American countries) and outbreaks have previously occurred in the Netherlands, Slovenia and U.K. CSNV is transmitted and spread in nature by insects of the thrip family in a persistent manner. *Franklineilla occidentalis* and *F. schultzei* but not *Thrips tabaci* are vectors of CSNV. In Europe the major vector of CSNV is probably *F. occidentalis* which is a major pest in glasshouses and some field crops in southern Europe.

1. Detection
On chrysanthemum it is difficult to distinguish between symptoms caused by CSNV and tomato spotted wilt virus (TSWV). *Dendranthema stramonium* has been identified as a suitable indicator plant (Verhoeven *et al*., 1996). In chrysanthemum symptoms of CSNV can result in complete necrosis of the stem resulting in wilting of sections of plants. In Brazil, symptoms can include necrotic lesions surrounded by yellow areas on leaves followed by necrosis on stems, peduncles and floral receptacles (Duarte *et al*., 1995). In U.K. symptoms included distinct dart stem lesions with some leaf necrosis (Mumford *et al*., 2003). On inoculated tomatoes systemic symptoms have been described as chlorotic and necrotic lesions, chlorosis, rugosity and severe growth reduction (Verhoeven *et al*., 1996). Diagnosis of disease is by electron microscopy.
Detection methods for CSNV include the use of ELISA testing, western immunoblot analyses and polymerase chain reaction (PCR) (Anonymous, 2005; Bezerra *et al*., 1999; Bezerra-Agasie *et al*., 2006; Verhoeven *et al*., 1996; Mumford *et al*., 2003). CSNV was detected in thrips from affected glasshouses using TaqMan (Anonymous, 2005). Transcriptase reverse-polymerase chain reaction (RT-PCR) and dot blot hybridization with digoxigenin-labeled probes have been used to detect several tospoviruses including CSNV (Eiras, *et al*., 2001). Kuwabara *et al*. (2010) improved a one-step multiplex reverse transcription-polymerase chain reaction (multi-PCR) method. The multi-PCR system is composed of a universal degenerate primer and five virus-species-specific primers that amplify bands unique to the viruses including CSNV.

2. Delimitation
No information.

3. Monitoring
No information.

4. Commodities
No information.

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Chrysanthemum stunt viroid

Common name(s): CSVd, Chrysanthemum stunt mottle viroid
Taxa: Viroids: Pospiviroidae: pospiviroid
EPPO A2 List: No. 92
EU Annex designation: II/A2

Organism
The main hosts of Chrysanthemum stunt viroid are florists' chrysanthemums (*Dendranthema morifolium*) and related ornamentals including *Chrysanthemum prealtum*, *D. indicum* and *Tanacetum parthenium*. CSVd is closely related to potato spindle tuber viroid and cucumber pale fruit viroid. CSVd can be eradicated by returning chrysanthemum plants to vegetative growth followed by clonal prorogation through meristem culture (Angarita 1999).

1. Detection
CSVd may be detected by graft inoculation of indicator species or by chip-budding. Early techniques for the detection of CSVd used polyacrylamide gel electrophoresis (PAGE) (Mosch *et al*. 1978, Horst and Kawamoto 1980). A spot hybridization technique detected the viroid in 0.1 mg of infected leaves, which was an improvement from the electrophoresis method (Macquaire *et al*. 1984). An alternative "return"-gel electrophoresis method was able to detect a viroid concentation as low as 800 pg/g tissue (Schumacher *et al*. 1986).
Recombinant plasmids have been developed as probes for CSVd (Candresse *et al*. 1988, Chen *et al*. 1988, McInnes *et al*. 1989, Meldrais Ya *et al*. 1989) and improved sensitivity compared with PAGE techniques. Reverse transcription-polymerase chain reaction (RT-PCR) has also been used for detection of CSVd(Kusunoki *et al*. 1993, Hooftman *et al*. 1996, Arts *et al*. 1997). The sensitivity of gene diagnostic methods for detecting chrysanthemum stunt viroid (CSVd) in 2M LiCl-soluble nucleic acids extracted from chrysanthemum plants was examined. Different molecular methods have been compared. In one study dot blot hybridization using DIG-labelled cRNA and cDNA probes and RT-PCR was compared. The cRNA probe was at least 25 times more sensitive than the cDNA probe and had a superior signal-to-noise ratio. RT-PCR was 5 times more sensitive than hybridization using the cRNA probe. The combination of RT-PCR and hybridization on microplate well expanded the detectable range and was at least 25 times more sensitive than agarose gel analysis of the RT-PCR products (Hataya *et al*. 1999). In another study the use of dot-blot hybridization using DIG-labelled cRNA probes, RT-PCR and TaqMan were compared (Mumford *et al*. 2000). Sensitivity comparisons showed that the TaqMan assay was more sensitive than either RT-PCR (100 times) or hybridization (1000 times) (Mumford *et al*. 2000). A reverse transcription loop-mediated isothermal amplification (RT-LAMP) method was as sensitive as RT-PCR for the detection of CSVd (Fukuta *et al*. 2005). More recent studies have examined tests for simultaneous detection of several plant viroids (Ragozzino *et al*. 2004, Hosokawa *et al*. 2006, Hosokawa *et al*. 2007, Monger *et al*. 2010).

2. Delimitation
No relevant references found.

3. Monitoring
No relevant references found.

4. Commodities

No relevant references found.

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Chrysomyxa arctostaphyli Dietel

(= *Melampsoropsis arctostaphyli* Arthur)
Common name(s): Common yellow witches' broom rust, spruce broom rust
Taxa: Fungi: Basidiomycota: Coleosporiaceae
EPPO list A1: No. 8.
EU Annex designation: I/A1

Organism
The spruce broom rust, *Chrysomyxa arctostaphyli*, is a dioicous pathogen developing on *Picea* spp. and the Eurasian *Arctostaphylos uva-ursi*. The main *Picea* spp. concerned are *P. abies, P. engelmannii* and *P. sitchensis*. It has also been inoculated successfully to *P. glauca* and *P. mariana* (Savile 1950, Ziller 1974). Fungal mycelium invades the spruce bark and sapwood of branches and trunks.

1. Detection
The first symptom of the spruce broom rust is needle etiolation in summer. The release of dormant buds results in perennial witches' brooms with yellow-green needles, which will show a yellow-orange appearance. Needles subsequently die and fall in the autumn. Witches' brooms of conifers not caused by rust remain green throughout the year. Trees lose vigour and spike tops, dead branches and mortality are common. Symptoms are similar to *Melampsorella caryophyllacearum* on *Abies*. They may be distinguished by host and by the looser, larger brooms on *Picea* (Mathiasen and Hawksworth 1983). A survey of the occurrence of broom rust of black spruce (*Picea mariana*) caused by *Chrysomyxa arctostaphyli* was made in Newfoundland (Singh 1978). The rust occurred sporadically throughout the island, varying in incidence and intensity. The average number of brooms per tree was 6.6. Height and diameter growth were less in infected than in uninfected trees, but no tree mortality could be attributed to the disease. The incidence and intensity of *Chrysomyxa arctostaphyli* on spruce varied (max. 62% in some areas), but no tree death appeared to be associated with infection. The rust was more frequent on trees >15 cm of diameter at breast height (Singh 1978). A detailed description about *Chrysomyxa arctostaphyli* transmission, new distribution records and new hosts in NA is given (Merrill et al. 1993, Cannon 2007).

2. Delimitation
No information available.

3. Monitoring
The results of a wide roadside survey carried out in Wyoming to evaluate the conifer disease showed the occurrence of patch mortality and crown mortality of *Picea engelmannii* due to *Chrysomyxa arctostaphyli (*Lundquist 1993). In Newfoundland a survey showed that *C. arctostaphyli* occurred sporadically throughout the island, varying in incidence and intensity. The growth of trees was less in infected as opposed to uninfected trees, but no tree mortality could be attributed to the disease (Singh 1978).

4. Commodities
EPPO recommends that all countries should prohibit importation of plants for planting (except seeds and tissue cultures), and cut branches of *Picea* from North America and Canada (Anonymous 1999). Long-distance dispersal is most likely on infected plants, although intercontinental spread is possible by wind-blown aeciospores; the latter can survive storage for several months. A review of the main alien pests and diseases of importance to the UK, their likely means of entry and

measures taken to prevent this is reported (Phillips 1979).

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Ciborinia camelliae Kohn

Common name(s): Flower blight, petal blight
Taxa: Fungi: Ascomycota: Sclerotiniaceae
EPPO A1 list: No. 190
EU Annex designation: II/A1

Organism
Ciborinia camelliae is a fungal disease of *Camellia* species, namely *Camellia japonica, C. japonica subsp. rusticana, C. reticulata and C. sasanqua* (Iriyama 1980). It is a serious disease of camellias in Japan and has spread to the USA, Canada, Australia and New Zealand. In the late 1990s it was found in Europe (Anonymous 2003) and its presence in Italy was confirmed in 2001 (Garibaldi *et al*. 2001). Infection causes camellia flower blight, the first symptom of which is darkening of the veins on the petals. This is followed by the formation of brown spots or blotches, which spread until the whole flower turns brown and drops.

1. Detection
Detection is by morphological identification both *in vivo* and *in vitro* (Anonymous 2003). A detailed account of the key characteristics, together with figures, is provided in a EPPO PM 7/15(1) (Anonymous 2003). A characteristic sign of the disease is the white to whitish-grey ring of felt-like sterile mycelium around the base of the petals. On older infections the slimy black patches in the centre of one or two petals are also characteristic. These consist of massed spermodochia or stromata bearing conidiophores (phialides) and chains of brown microconidia. Their presence, when confirmed microscopically, is very diagnostic. The flattened sclerotia developing later at the bases of the petals are initially covered by a layer of dense, whitish-grey, felt-like mycelium. Finally, they become hard and black, reaching the size and shape of dried grapes, often adhering together in a ring after decomposition of the remaining flower parts. An experienced mycologist can make a definitive identification based on these observations, but confirmation can be made by examining the colony morphology *in vitro*.
The fungus can be isolated by excising and surface-sterilizing small pieces of petal tissue from a leading edge, or the petal bases bearing the white mycelium ring, and plating on potato dextrose agar (PDA). Colonies of *C. camelliae* have a white, felt-like, surface mycelium with a radial growth rate of up to 42 mm per week on PDA at 18°C. These produce spermodochia, which are diagnostic for *C. camelliae*, after about a week. The spermodochia are small, wet, black, microconidial stromata, 1 mm in diameter, produced in concentrically zoned sectors. They become confluent after 4 weeks and are immersed in a water-soluble, black mucilage. They consist of branching clusters of dark-brown, barrel-shaped cells bearing phialides in whorls on an inflated central cell. The phialides are hyaline to brown-walled, about 6µm long, 2–2.5µm broad, each with a prominent, deeply cupulate collarette where the microconidia are borne. The microconidia are brown-walled, globose to obovate, 2.5–4µm in diameter, uninucleate, containing a large guttule. Some spores hang together in short chains but, when separated, a small basal appendage can be seen, often bearing two fringes of wall material where the appendage was previously attached to the subtending microconidium.

2. Delimitation
No relevant references found

3. Monitoring

A monitoring scheme has been set up in the Piedmont region of Italy to monitor spread of the disease since its introduction (Gullino *et al*. 2001). Surveys have been undertaken in New Zealand to monitor the spread of the disease since its introduction in 1993 (Taylor and Long 1998, Taylor *et al*. 1999).

4. Commodities

The disease affects *Camellia* spp. only.

References: 17 retained out of 77 retrieved, none added, 6 used for summary

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Circulifer haematoceps (Mulsant & Rey)

(=*Neoaliturus haematoceps* (Mulsant & Rey))
Common name(s):
Taxa: Insecta: Hemiptera: Cicadellidae
EU Annex designation: II/A2

Organism
Leafhoppers in the genus *Circulifer* may be characterized as being small, males 2.5-3.6 mm and females 2.7-3.8 mm. The head is slightly wider than the pronotum with the anterior margin rounded. The overall colour is brown or stramineous with variable darker markings. *C. haematoceps* is one of the vectors of *Spiroplasma citri* (citrus stubborn disease). Like many other cicadellids, this leafhopper feeds on a wide range of hosts (field crops, fruit trees, ornamentals, wild plants and weeds). *C. haematoceps* has been found particularly on the ornamental *Matthiola incana* and on the wild plants *M. sinuata* and *Salsola kali*. The insect is not particularly associated with citrus, on which feeding is incidental.

1. Detection
C. haematoceps is strongly attracted to the colour yellow. Yellow, opaque plastic cards coated with a sticky substance are an effective survey tool for monitoring adult leafhopper. Besides yellow sticky-traps suction-traps (e.g. Johnson-Taylor) have been tested to monitor the population dynamics of leafhoppers. However, *C. haematoceps* populations are better represented in yellow sticky-trap catches than they are in suction traps (Kersting et al. 1997).

2. Delimitation
No information available.

3. Monitoring
The commonest flight strata at a host site was at ground level, where significantly higher numbers of adults were trapped than at heights of 0.3, 0.6, 0.9, 1.5, 2.1 and 2.7 m above the ground. Ground level is therefore the optimum location for a yellow sticky trap when monitoring *C. haematoceps*. The adult activity is greatest between 17.30 h and 19.30 h and most concentrated within a 30 min period just before sunset. Very few individuals are usually captured during hours of high light intensity and during the night. During the period of greatest activity only males are trapped (Kersting and Baspinar 1995). *Circulifer* sp. often move to non-crop habitats during the season, but could be effectively collected on weeds throughout the whole summer by sweep-netting (Klein et al. 2001). Populations are usually monitored using weekly intervals (Munyaneza et al. 2008).

4. Commodities
No information available.

References: 9 retained out of 145 retrieved, none added, 4 used for the summary
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Klein, M., P. G. Weintraub, M. Davidovich, L. Kuznetsova, T. Zahavi, A. Ashanova, S. Orenstein, and E. Tanne. 2001. Monitoring phytoplasma-bearing leafhoppers/planthoppers in vineyards in the Golan Heights, Israel. Journal of Applied Entomology 125:19-23.

Munyaneza, J. E., A. S. Jensen, P. B. Hamm, and J. E. Upton. 2008. Seasonal occurrence and abundance of beet leafhopper in the potato growing region of Washington and Oregon Columbia Basin and Yakima Valley. American Journal of Potato Research 85:77-84.

Circulifer tenellus (Baker)

(= *Neoaliturus tenellus* (Baker), *Eutettix tenellus* Baker)
Common name(s): beet leafhopper
Taxa: Insecta: Hemiptera: Cicadellidae
EU Annex designation: II/A2

Organism
Leafhoppers in the genus *Circulifer* are characterized as being small, males 2.5-3.6 mm and females 2.7-3.8 mm. The head is slightly wider than the pronotum with the anterior margin rounded. The overall colour is brown or stramineous with variable darker markings. *C. tenellus* is one of the vectors of *Spiroplasma citri* (stubborn disease). Like many other cicadellids, the Mediterranean vectors of stubborn disease feed on a wide range of hosts (field crops, fruit trees, ornamentals, wild plants and weeds). *C. tenellus* has attracted particular attention on sugar beet (*Beta vulgaris* var. *saccharifera*) in North America because it acts as the vector for beet curly top hybrigeminivirus; it is not especially associated with sugar beet in the Europe.

1. Detection
As other leafhoppers, *C. tenellus* is significantly attracted to the colour yellow. Yellow, opaque plastic cards coated with a sticky substance are an effective survey tool for monitoring adult beet leafhopper. Traps with different hues of yellow (wavelengths between 510 and 588 nm) have shown no significant differences between trap catches (Meyerdirk and Oldfield 1985). Suction traps can also be used but are generally less effective and more costly. Direct sampling using sweep-netting can be associated to the sticky traps to sample leafhoppers in non crop-habitats (e.g. hedges or field margins).

2. Delimitation
No information available.

3. Monitoring
The commonest flight strata of *C. tenellus* at a host site is at ground level, where significantly higher numbers of adults are usually trapped than at heights of 0.3, 0.6, 0.9, 1.5, 2.1 and 2.7 m above the ground. Ground level is therefore the optimum location for a yellow sticky trap when monitoring *C. tenellus* (Meyerdirk et al. 1985). During monitoring it should be borne in mind that *Circulifer* spp. often move to non-crop habitats during the growing season. During this period the species could be effectively collected on weeds throughout the whole summer by sweep-netting (Klein et al. 2001). Generally, populations are monitored using weekly intervals (Munyaneza et al. 2008).

4. Commodities
No information available.

References: 9 retained out of 145 retrieved, none added, 3 used for summary

Klein, M., P. G. Weintraub, M. Davidovich, L. Kuznetsova, T. Zahavi, A. Ashanova, S. Orenstein, and E. Tanne. 2001. Monitoring phytoplasma-bearing leafhoppers/planthoppers in vineyards in the Golan Heights, Israel. Journal of Applied Entomology 125:19-23.

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Citrus greening bacterium (*Liberibacter africanus* Jagoueix, Bové & Garnier)

Common name(s): Vein phloem degeneration, greening, leaf mottling
Taxa: Bacteria: Proteobacteria: liberibacter
EPPO A1 list: No. 151
EU Annex designation: II/A1

Organism
Citrus greening bacterium, as the name implies, is a pathogen largely restricted to species of *Citrus*. The bacterium occurs in two forms: the heat-sensitive form (*Liberobacter africanum*) and the heat-tolerant form (*Liberobacter asiaticum*). Trees affected are generally stunted, have sparse yellow foliage, and show twig dieback. Sometimes, at the early stages of infection, symptoms are seen only on one part of the canopy.

1. Detection
A multiplex polymerase chain reaction (PCR) method has been developed for the simultaneous detection of Citrus yellow mosaic virus (CYMV) and fastidious Citrus greening bacterium, Candidatus *Liberibacter asiaticus* (CLa) in sweet orange trees. The technique would also prove highly useful in disease surveys, nursery certification and quarantine applications (Baranwal et al. 2005). Molecular techniques have been demonstrated to be useful for the practical detection of the bacterium (Das, 2009) and improved methods continue to be developed (Gopal et al. 2004).

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
No information available.

References: 5 retained out of 7 retrieved, none added, 3 used for the summary

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Citrus mosaic badnavirus

Common name(s): CiMV
Taxa: Viruses: Caulimoviridae: Badnavirus
EPPO A1 list: No. 285
EU Annex designation: II/A1 – as Citrus mosaic

Organism

This virus (CiMV) affects various species of *Citrus*, primarily orange and is absent from the EPPO/EU region, occurring mainly in Asia, particularly the Indian sub-continent (Anonymous 2004). Symptoms include chlorotic, mosaic patterns on leaves (Rao and Narasimham 1974) that eventually form a uniform leathery texture in mature leaves (Ahlawat *et al*. 1985). It is mechanically vectored (Tanaka and Imada 1974), although there are some suggestions of insect vector involvement (potentially aphids).

1. Detection

A range of assays have been evaluated for the rapid and accurate detection of CiMV. Methods include the use of ELISA (Usugi and Tsuchizaki 1982, Shohara 1989) and DAC-ELISA (Prasanna *et al*. 2002). More recently, several molecular approaches have been developed including a number PCR techniques (Roy *et al*. 2005, Baranwal *et al*. 2007, Ghosh *et al*. 2007, Ghosh *et al*. 2008). A Japanese report of the use of indicator plants (Goutoucheng; sour orange hybrid) is likely to have used a serologically related virus, and not CiMV (Anonymous, 1997).

2. Delimitation

No information available

3. Monitoring

Older examples of monitoring are based on the visual assessment of symptoms (Reddy *et al*. 1974). Collections of budwood for indexing via grafting to indicator plants has also been employed as a means of determining the prevalence of CiMV (Bhagabati *et al*. 1989). Other monitoring efforts have used the collection of leaves that have subsequently been analysed using DAC-ELISA (Prasanna *et al*. 2002).

4. Commodities

No information available

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Ghosh, O. K., A. Balaji, B. Kanchan, and V. K. Baranwal. 2007. PCR based detection of citrus yellow mosaic disease from

Vidarbha region of Maharastra. Indian Phytopathology 60:520-526.
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Citrus tristeza virus (European isolates)

Common name(s): CTV, Tristeza, seedling yellows, quick decline
Taxa: Viruses: Closteroviridae Closterovirus
EPPO A2 list: No. 93
EU Annex designation: II/A2 for European strains

Organism

The Closterovirus *Citrus tristeza virus* (CTV) (Closteroviridae), with monopartite genomes, infects all species, cultivars and hybrids of citrus (Che et al. 2003). It is the main viral disease of citrus affecting production worldwide and, depending on the strain, may induce total groove destruction (Bassanezi et al. 2006, Catara and Tessitori 2006, Melo et al. 2008). CTV is transmitted by several aphid species such as *Aphis gossypii*, *A. spiraecola*, *Toxoptera aurantii* and *T. citricida* (Ullman and Falk 2003, Herron et al. 2006, Nolasco et al. 2008).

CTV populations are among the more complex of plant RNA viruses with unusual mixtures of strains and defective RNAs (dRNAs) (Mawassi et al. 2000). CTV has flexuous particles of 10-12x2000 nm with single stranded, positive sense RNA of ~19 256 nucleotides which encodes 12 open reading frames that potentially code at least 17 proteins. Virus coat protein (CP) is ~25 kDa (Ahlawat and Pant 2003). The genomic RNA of CTV isolate T318A from Spain (19252 nucleotides) was completely sequenced. It showed strong sequence similarities with the severe isolates SY568 from California and NUagA from Japan, and distant relationships with mild non-stem pitting isolates T385 from Spain and T30 from Florida (Ruiz-Ruiz et al. 2006). Albiach-Marti et al. (2000) have developed a procedure to purify rapidly and easily a sufficient quantity of native p25 CP to allow comparison of five isolates of CTV by serological analysis of peptide maps, using monoclonal and polyclonal antibodies.

This virus has been discovered in Greece (Dimou et al. 2002), in Palestine (Jarrar et al. 2000), in Albania (Stamo et al. 1999), in Lebanon (D'Onghia et al. 1998), in United Arab Emirates (Al-Shuraiki 1997), in Italy, in Spain (Ballester-Olmos et al. 1993).

1. Detection

Direct Tissue Blot Immunoassay-ELISA (DTBIA-ELISA) and DAS-ELISA techniques have been compared to establish an effective and efficient method for CTV detection (Ruiz-Garcia et al. 2009). The DTBIA-ELISA technique was more sensitive and more capable of positive prognosis than DAS-ELISA in the diagnosis of CTV in trees with recent and unknown infection. This method was 54.9% more economical than DAS-ELISA and the diagnoses require only 17% of the time invested for DAS-ELISA. However, due to the requirements of the current Mexican official norm (NOM-031-FITO-2000), this method is recommended as a fast technique to discriminate positive trees in field sampling to be later verified with the official method of diagnosis (DAS-ELISA).

Ruiz-Ruiz et al. (2009) identified several CTV variants by quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) using a general primer set and three TaqMan locked nucleic acids (LNA) probes targeting sequences characteristic of severe, mild (non-SY, non-SP), and T36-like isolates. Similarly, Yokomi et al. (2011) have developed an economical and practical strategy to screen for potential severe CTV strains using a two-step system. Step 1 was to screen all samples with serology using a broad-spectrum and the strain-discriminating MCA13 monoclonal antibody. Step 2 was to test MCA13-reactive isolates with reverse transcription quantitative polymerase chain reaction assays using three CTV strain-specific TaqManReg. probes. This allowed categorizing MCA13-reactive isolates into at least three distinct CTV genotype groups which included potentially severe strains associated with VT, T3 or T36 genotypes vs. a non-

standard T36 (T36-NS) genotype associated with a mild phenotype. This assay could be used to find trees infected with VT or T3 genotypes which could be targeted for eradication or CTV management programs and/or further genetic characterization or biocharacterization in citrus plant indicators. In parallel, Adkar-Purushothama et al. (2011) have developed a specific and sensitive reverse transcriptase-nested polymerase chain reaction assay (RT-nPCR) for the detection of CTV from naturally infected citrus samples. This RT-nPCR technique could be used for diagnostic applications and surveillance (Liu et al. 2010, Adkar-Purushothama et al. 2011).
According to Tessitori et al. (2011), two Corsican isolates of CTV have been previously characterized, the K strain from Marumi kumquat, known to be symptomless on Mexican lime, and the Cal-1 from calamondin inducing stem pitting, but no sequences of *p20* gene are published or available in GenBank. In Corsica, two additional CTV strains (LA5 and CO3) were recently found by molecular characterization.

2. Delimitation

Knowledge of the strains present in a certain region is fundamental to the design of an adequate strategy and for the selective eradication of severe strains in regions where the virus has become endemic (Barbarossa and Saponari 2010). The strategy for disease control is based on the eradication of virus-infected trees, detected by test plants or immunological methods (Fishman et al. 1983). Mathematical models for CTV infection and spread were also developed. Therefore the enforcement and the implementation of CTV control strategies are highly recommended, at the same time considerable cooperation is needed between growers and nurserymen in order to avoid the risk of disease epidemics in countries (Barbarossa and Saponari 2010).

3. Monitoring

A monitoring activity was carried out in Eastern Sicily (Italy) for the detection of CTV on citrus trees cultivated in nursery conditions. The diffusion of aphids was also surveyed in order to exclude the presence of *T. citricidus*, recently detected in the North-Western area of the Iberian Peninsula. In this survey period a total of 1,544,359 nursery trees were checked utilizing DAS-ELISA test, and 117,000 nursery trees were observed for the identification of aphids species present on new shoots (Riolo et al. 2011). All infected plots were destructed. In 2009, CTV was detected in the protected foundation block for budwood production. The quarantine aphid *T. citricidus* was not observed in the survey period.
The objective of a study carried out in Brazil was to monitor the maintenance of CTV protective isolates stability in selected clones of 'Pera' sweet orange (*Citrus sinensis*), preimmunized or naturally infected by the virus, after successive clonal propagations (Costa et al. 2010). In the field, coat protein gene (CPG) analysis of 33 isolates collected from 16 clones of 'Pera' sweet orange was performed using single strand conformational polymorphism (SSCP). Initially, the isolates were characterized by symptoms of stem pitting observed in clones. Then viral genome was extracted and used as template for the amplification of CPG by reverse transcription polymerase chain reaction (RTPCR). RTPCR products electrophoretic profiles were analyzed using the Jaccard coefficient and the UPGMA method.
In Syria, several citrus varieties were visually inspected and sampled for laboratory analysis (Abou Kubaa et al. 2009). Infected plants were identified by Direct Tissue Blot Immunoassay (DTBIA). In Cuba, the spatiotemporal dissemination of CTV was evaluated by DASI-ELISA in six citrus producing regions, and aphid populations were evaluated in two selected areas (Batista et al. 2008).

4. Commodities

CTV dispersal occurs by propagation of virus-infected plants (rootstock, fruit, seed and budwood), responsible for most virus introductions into new areas, and by several aphid species, important for local spread (Broadbent 1995, Catara and Davino 2006, Recupero and Continella 2006, Barbarossa and Saponari 2010).

The Florida Department of Agriculture & Consumer Services has three major concerns about *citrus tristeza* disease: (1) an increasing reservoir of severe isolates of CTV in budwood sources in Florida, USA that cause quick decline in mature trees and severe stunting of various citrus propagations on sour orange (*Citrus aurantium*) rootstocks; (2) the introduction of exotic isolates of CTV into Florida that cause stem pitting of scions of citrus cultivars on any rootstock; and (3) the introduction of the exotic brown citrus aphid, *Toxoptera citricidus*, which is the most efficient vector of CTV (Schoulties et al. 1987).

References: 375 retained out of 685 references retrieved, none added, 33 used for summary

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Citrus tristeza virus (European isolates)

Taxa: Viruses: Closteoviridae: Closteovirus
EPPO A2 list: No. 93
EU Annex designation: II/A2 for European strains

Organism

The Closterovirus *Citrus tristeza virus* (CTV) (Closteroviridae), with monopartite genomes, infects all species, cultivars and hybrids of citrus (Che et al. 2003). It is the main viral disease of citrus affecting production worldwide and, depending on the strain, may induce total groove destruction (Bassanezi et al. 2006, Catara and Tessitori 2006, Melo et al. 2008). CTV is transmitted by several aphid species such as *Aphis gossypii*, *A. spiraecola*, *Toxoptera aurantii* and *T. citricida* (Ullman and Falk 2003, Herron et al. 2006, Nolasco et al. 2008).

CTV populations are among the more complex of plant RNA viruses with unusual mixtures of strains and defective RNAs (dRNAs) (Mawassi et al. 2000). CTV has flexuous particles of 10-12x2000 nm with single stranded, positive sense RNA of ~19 256 nucleotides which encodes 12 open reading frames that potentially code at least 17 proteins. Virus coat protein (CP) is ~25 kDa (Ahlawat and Pant 2003). The genomic RNA of CTV isolate T318A from Spain (19252 nucleotides) was completely sequenced. It showed strong sequence similarities with the severe isolates SY568 from California and NUagA from Japan, and distant relationships with mild non-stem pitting isolates T385 from Spain and T30 from Florida (Ruiz-Ruiz et al. 2006). Albiach-Marti et al. (2000) have developed a procedure to purify rapidly and easily a sufficient quantity of native p25 CP to allow comparison of five isolates of CTV by serological analysis of peptide maps, using monoclonal and polyclonal antibodies.

This virus has been discovered in Greece (Dimou et al. 2002), in Palestine (Jarrar et al. 2000), in Albania (Stamo et al. 1999), in Lebanon (D'Onghia et al. 1998), in United Arab Emirates (Al-Shuraiki 1997), in Italy, in Spain (Ballester-Olmos et al. 1993).

1. Detection

Direct Tissue Blot Immunoassay-ELISA (DTBIA-ELISA) and DAS-ELISA techniques have been compared to establish an effective and efficient method for CTV detection (Ruiz-Garcia et al. 2009). The DTBIA-ELISA technique was more sensitive and more capable of positive prognosis than DAS-ELISA in the diagnosis of CTV in trees with recent and unknown infection. This method was 54.9% more economical than DAS-ELISA and the diagnoses require only 17% of the time invested for DAS-ELISA. However, due to the requirements of the current Mexican official norm (NOM-031-FITO-2000), this method is recommended as a fast technique to discriminate positive trees in field sampling to be later verified with the official method of diagnosis (DAS-ELISA).

Ruiz-Ruiz et al. (2009) identified several CTV variants by quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) using a general primer set and three TaqMan locked nucleic acids (LNA) probes targeting sequences characteristic of severe, mild (non-SY, non-SP), and T36-like isolates. Similarly, Yokomi et al. (2011) have developed an economical and practical strategy to screen for potential severe CTV strains using a two-step system. Step 1 was to screen all samples with serology using a broad-spectrum and the strain-discriminating MCA13 monoclonal antibody. Step 2 was to test MCA13-reactive isolates with reverse transcription quantitative polymerase chain reaction assays using three CTV strain-specific TaqManReg. probes. This allowed categorizing MCA13-reactive isolates into at least three distinct CTV genotype groups

which included potentially severe strains associated with VT, T3 or T36 genotypes vs. a non-standard T36 (T36-NS) genotype associated with a mild phenotype. This assay could be used to find trees infected with VT or T3 genotypes which could be targeted for eradication or CTV management programs and/or further genetic characterization or biocharacterization in citrus plant indicators. In parallel, Adkar-Purushothama et al. (2011) have developed a specific and sensitive reverse transcriptase-nested polymerase chain reaction assay (RT-nPCR) for the detection of CTV from naturally infected citrus samples. This RT-nPCR technique could be used for diagnostic applications and surveillance (Liu et al. 2010, Adkar-Purushothama et al. 2011).
According to Tessitori et al. (2011), two Corsican isolates of CTV have been previously characterized, the K strain from Marumi kumquat, known to be symptomless on Mexican lime, and the Cal-1 from calamondin inducing stem pitting, but no sequences of *p20* gene are published or available in GenBank. In Corsica, two additional CTV strains (LA5 and CO3) were recently found by molecular characterization.

2. Delimitation
Knowledge of the strains present in a certain region is fundamental to the design of an adequate control strategy and for the selective eradication of severe strains in regions where the virus has become endemic (Barbarossa and Saponari 2010). The strategy for disease control is based on the eradication of virus-infected trees, detected by test plants or immunological methods (Fishman et al. 1983) Mathematical models for CTV infection and spread were also developed. Therefore the enforcement and the implementation of CTV control strategies are highly recommended, at the same time considerable cooperation is needed between growers and nurserymen in order to avoid the risk of disease epidemics in countries (Barbarossa and Saponari 2010).

3. Monitoring
A monitoring activity was carried out in Eastern Sicily (Italy) for the detection of CTV on citrus trees cultivated in nursery conditions. The diffusion of aphids was also surveyed in order to exclude the presence of *T. citricidus*, recently detected in the North-Western area of the Iberian Peninsula. In this survey period a total of 1,544,359 nursery trees was checked utilizing DAS-ELISA test, and 117,000 nursery trees were observed for the identification of aphids species present on new shoots (Riolo et al. 2011). All infected plots were destructed. In 2009, CTV was detected in the protected foundation block for budwood production. The quarantine aphid *T. citricidus* was not observed in the survey period.
The objective of a study carried out in Brazil was to monitor the maintenance of CTV protective isolates stability in selected clones of 'Pera' sweet orange (*Citrus sinensis*), preimmunized or naturally infected by the virus, after successive clonal propagations (Costa et al. 2010). In the field, coat protein gene (CPG) analysis of 33 isolates collected from 16 clones of 'Pera' sweet orange was performed using single strand conformational polymorphism (SSCP). Initially, the isolates were characterized by symptoms of stem pitting observed in clones. Then viral genome was extracted and used as template for the amplification of CPG by reverse transcription polymerase chain reaction (RTPCR). RTPCR products electrophoretic profiles were analyzed using the Jaccard coefficient and the UPGMA method.
In Syria, several citrus varieties were visually inspected and sampled for laboratory analysis (Abou Kubaa et al. 2009). Infected plants were identified by Direct Tissue Blot Immunoassay (DTBIA). In Cuba, the spatiotemporal dissemination of CTV was evaluated by DASI-ELISA in six citrus producing regions, and aphid populations were evaluated in two selected areas (Batista et al. 2008).

4. Commodities

CTV dispersal occurs by propagation of virus-infected plants (rootstock, fruit, seed and budwood), responsible for most virus introductions into new areas, and by several aphid species, important for local spread (Broadbent 1995, Catara and Davino 2006, Recupero and Continella 2006, Barbarossa and Saponari 2010).

The Florida Department of Agriculture & Consumer Services has three major concerns about *citrus tristeza* disease: (1) an increasing reservoir of severe isolates of CTV in budwood sources in Florida, USA that cause quick decline in mature trees and severe stunting of various citrus propagations on sour orange (*Citrus aurantium*) rootstocks; (2) the introduction of exotic isolates of CTV into Florida that cause stem pitting of scions of citrus cultivars on any rootstock; and (3) the introduction of the exotic brown citrus aphid, *Toxoptera citricidus*, which is the most efficient vector of CTV (Schoulties et al. 1987).

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Citrus vein enation virus

Common name(s): CVEV, vein enation, woody gall
Taxa: Viruses (unclassified)
EU Annex designation: II/A2

Organism
Citrus vein enation virus can be transmitted by some aphid species, but a long acquisition period (5-7 days) for transmission by *Aphis gossypii* or *Myzus persicae* is required (Hermoso De Mendoza *et al*. 1994*)*. The primary aphid vector in citrus is *Toxoptera citricidus*. The latent period of the virus in this vector is 2-3 days (Maharaj and Da Graca 1989).

1. Detection
Detection of citrus vein enation virus is based on biological indexing combined with laboratory-based diagnostic techniques. The bioindexing host used is *Citrus aurantifolia* (Mexican lime). Plants are inoculated using bark patches. The efficacy of four Mexican Limes for the detection of citrus vein enation virus is 95.6% or higher, even in the presence of mixed infections (Vidalakis *et al*. 2004). However, the efficacy for citrus vein enation virus biological indexing using four Mexican limes was reduced by coinfection and superinfection treatments with the T30 group Citrus tristeza virus isolates (Vidalakis *et al*. 2004). Dweet Tangor (*C. reticulata* × *C. sinensis*) and Pineapple Sweet Orange (*C.* sinensis) used together can be considered as valuable indicators for citrus vein enation virus as the former is the most sensitive in single infections, and where it is not as sensitive, in multiple infections, Pineapple Sweet Orange is effective (Vidalakis *et al*. 2004).
Enations are seen in 5-8 weeks on the underside of leaves of limes (*Citrus aurantiifolia*), rough lemons (*Citrus jambhiri*) or sour oranges (*Citrus aurantium*). Swellings or galls appear more slowly on stems of *C. jambhiri* or *C. volkameriana*. The virus is not apparently mechanically transmissible, and serological methods for its detection been not been developed.
Transmission electron microscopy of infected young *Citrus jambhiri* leaf veins, both with and without enations, revealed the presence of isometric virus-like particles of approximately 27 nm in diameter in the phloem tissue. Electron-dense, virus-like particles of a similar size were also observed in the hindgut lumen and accessory salivary glands of *T. citricidus*, but these particles were not found in healthy citrus or aphids fed on healthy seedlings (Maharaj and Da Graca 1988).

2. Delimitation
No relevant references found

3. Monitoring
No relevant references found

4. Commodities
No relevant references found

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Clavibacter michiganensis subsp. *michiganensis, sepedonicus, insidiosus*

Taxa: Bacteria: Microbateriaceae: Clavibacter

Although the subspecies are addressed individually below, there are a few methods specifically used for their discrimination. Proteomic analysis using MALDI-TOF MS and gyrB sequencing are powerful diagnostic tools for the accurate identification of *Clavibacter* plant pathogens (Zaluga *et al*. 2011). Southern hybridization with DNA-probes has been developed to differentiate different subspecies of *Clavibacter michiganensis* (Dreier *et al*. 1995); furthermore a DNA probe was developed to differentiation between *C. michiganensis* subsp. *sepedonicus* and subsp. *insidiosus* (Rademaker *et al*. 1992). A specific PCR assay was developed for 5 subspecies of *C. michiganensis* (Pastrik and Rainey 1996); real-time TaqMan-PCR assays were developed for detection, differentiation and absolute quantification of the pathogenic subspecies of *Clavibacter michiganensis* in one single PCR run (Bach *et al*. 2003).

C*lavibacter michiganensis subsp. michiganensis* (Smith) Davis et al.

Common name(s): Bacterial canker, bird's eye
EPPO A2 list: No. 50
EU Annex designation: II/A2

Organism
The main host of economic importance is tomato, but the pathogen has also been reported on other *Lycopersicon* spp. and on the wild plants *Solanum douglasii*, *S. nigrum* and *S. triflorum*. *C. michiganensis* subsp. *Michiganensis* was first described in North America and presumably originated there, now the pathogen has a worldwide distribution.

1. Detection
Artificial inoculation of host plants was used to assess detection threshold (Vaerenbergh and Chauveau 1985). Indirect immunofluorescence staining (IF) gave highly reliable results (Vaerenbergh and Chauveau 1987), and this technique was evaluated with different extraction methods and three media for selectivity in dilution plating were compared (Franken *et al*. 1993). An immunofluorescence colony staining technique (Nemeth and Vuurde 2006) and an indirect immunofluorescence colony staining method (Veena and Vuurde 2002) were also developed; the indirect method is especially suitable for laboratories, seed companies, and quarantine stations which have no facilities for conjugation of primary antiserum (Veena and Vuurde 2002). The extraction method seems to be an important phase in order to obtain good results: grinding the seeds was significantly better than methods that used only soaking (Hadas *et al*. 2005). A non-destructive tomato seed assay, where the bacteria were extracted from tomato seed fibre, was developed (Biggerstaff *et al*. 2000). The optimization of the media for isolation of the pathogen is an important aspect, as different media have been developed (Fatmi and Schaad 1988, Ftayeh *et al*. 2011). The immunomagnetic separation (IMS)-plating can increase isolation rates and improve standard protocols currently used for routine analysis (de Leon *et al*. 2008).
Profiles of fatty acid methyl esters (FAMEs) were proposed as a technique for the characterization of bacteria (Gitaitis and Beaver 1990), as well as serological techniques: e.g. indirect ELISA (Bragard *et al*. 1987, Laroche and Verhoyen 1987) and DAS-ELISA (Kritzman 1989).
Consistent diagnostic methods were augmented by the advent of molecular techniques. A homologous DNA probe was proposed to distinguish the pathogen from an avirulent strain and all the other bacteria tested (Thompson *et al*. 1989). A PCR protocol was developed (Ghedini and Fiore 1995, Sousa Santos *et al*. 1997, Fu *et al*. 2005) whilst a multiplex PCR methodology allowed good results with less labour and rapid results for the simultaneous detection of *C.*

155iliates155155dae 155iliates155155dae and others bacterial pathogens of tomato, but the sensitivity of detection was reduced (Ravnikar *et al*. 2001). The pathogen was detected successfully with a multiplex PCR method with co-amplification of its host DNA. According to the authors this method will avoid false-negative results (Zhao *et al*. 2007). BIO-PCR method was proved a highly specific, rapid and reliable detection technique (Burokiene 2006).
Further methods were developed to improve the efficiency of detection. A one-step method was developed for the detection in symptomless tomato seeds directly using a TaqMan probe, the technique being fast and showing high specificity (Zhao *et al*. 2007), or with a PCR after the DNA isolation using Dneasy Plant Mini Kit from Qiagen (Kamasa and Pospieszny 2002). A real-time PCR was used after the bacterial solution was treated with the DNA binding dye ethidium monoazide (EMA)(Luo *et al*. 2008).
Studies were carried out to compare the different techniques and possible problems. PTA-ELISA and IFAS showed problem of cross-reactions, but this did not happen with PCR, moreover new primers are proposed with higher sensitivity (Kokoskova *et al*. 2010). A comparison of serological and molecular techniques showed that PCR with different primer sets guaranteed high efficiency for virulent types whilst ELISA using a specific monoclonal antibody is the best tool to differentiate the hypovirulent and avirulent types (Kaneshiro and Alvarez 2001). Extraction procedures and the sensitivity of PCR methods for detection in tomato seeds have been compared (Milijasevic *et al*. 2007, Milijasevic *et al*. 2009a).
The electronic nose can be a useful tool, rapid, sensitive, specific, non-destructive and easy-to-use technique for the detection and identification of plant pathogenic bacteria (Momol *et al*. 2004). The combination of different techniques (analysing morphological, cultural, physiological and biochemical characteristics) were used to have a certain confirmation of pathogen presence in a geographical area (Zhang *et al*. 2011).

2. Delimitation
No information available.

3. Monitoring
Specific surveys were carried out to evaluate the occurrence and incidence on tomato in crops (Black *et al*. 1999, Benchabane *et al*. 2000). After the collection of seeds, leaves, stems and fruits, the samples were tested for the presence of pathogen using ELISA and PCR diagnostics (Borboa Flores *et al*. 2009), microbiological testing and PCR (Kleitman *et al*. 2008), macro-restriction pulsed-field gel electrophoresis and rep-PCR (Milijasevic *et al*. 2009b), biochemical and pathogenicity testing on tomato plants (Sarala-Itty and Shetty 2005), electron microscopic studies, hypersensitivity reactions in indicator plants, pathogenicity tests on hosts (Sarala-Itty and Shetty 2005).

4. Commodities
Sampling methods were studied to determine sample size in zero tolerance situations (Clayton and Slack 1988). It was demonstrated that seeds may be a significant source of infection of tomato plants (Tsiantos 1987). The efficacy of a hot water treatment in reducing bacterial disease incidence and severity was demonstrated, but it is not sufficient to suppress the pathogen (Ivey and Miller 2005). Similar results were obtained in another study using hot water and chlorine (Petrovic *et al*. 1999), whereas hydrochloric acid (HCl) treatment effectively to eliminates the pathogen from contaminated tomato seed (Shi *et al*. 2002). Molecular techniques prove to be useful in determining the possible source of introduction (de Leon *et al*. 2009).

Clavibacter michiganensis subsp. Sepedonicus (Spieckermann & Kotthoff) Davis *et al.*

Common name(s): Potato ring rot
EPPO A2 list: No. 51
EU Annex designation: not assigned

Organism
Natural infections causing disease have been found in potatoes only. The bacterial ring rot was found for the first time in Germany in 1906, whilst later reports of detections came from 31 countries distributed over five different continents.

1. Detection
Ultraviolet light and immunofluorescence staining were evaluated for the detection of the infection (Miller 1984), but identification was limited by the presence of associated non-pathogenic bacteria (Crowley and Boer 1982, Gasperini 1984, Malec *et al*. 1987). Combining *in situ* hybridization with immunofluorescence proved to improve microscopic identification (Li *et al*. 1997) and new tools were proposed, such as low-resolution Raman spectroscopy (Schmilovitch *et al*. 2005). Bioassay on aubergines was used for detection, even though sensitivity of the method is limited by the virulence concentration of the bacteria and by the presence of other bacterial and fungal microorganisms (Behn *et al*. 1992).
The optimization of media for the isolation is an important aspect, as different media were developed (De La Cruz *et al*. 1992, Jansing and Rudolph 1998); another important phase is the extraction procedure (by incubation on a rotary shaker) (Dinesen and De Boer 1995).
Several serological tests (immunofluorescence probes, ELISA) were developed (Chang *et al*. 1984, Corbiere *et al*. 1987, Zielke and Kalinina 1988, Boer 1991, Kokoskova and Janse 2009) and the production of new monoclonal antibodies improved ELISA testing markedly (Boer *et al*. 1988). Others serological tests evaluated include passive haemagglutination (RPH) (Koehm and Eggers-Schumacher 1995) and DAS-ELISA with the evaluation of efficiency of monoclonal antibody and polyclonal antibody (Iveta and Blanka 2002). Studies were carried out to evaluate the reproducibility, the influence of sample size, and the incidence of infection and positive/negative thresholds (Boer *et al*. 1988, De Boer *et al*. 1994, De Boer *et al*. 1996, De Boer and Hall 1996, Boer and Hall 2000). Also the 157iliat-fluorescence antibody staining (IFAS) method was evaluated for possible improvement of the technique (Calzolari *et al*. 1982, Brzozowski 2007).
A PCR technique was developed and evaluated for specificity (Firrao and Locci 1994, Karjalainen *et al*. 1995, Li and De Boer 1995, Karjalainen *et al*. 1996) and proved to be very specific because no amplification of associated bacteria was observed (Kokoskova and Mraz 2008). Further evolution of this tool included BIO-PCR and an automated real-time fluorescence detection system (Schaad *et al*. 1999), rep-PCR technique (Fousek and Mraz 2003), real-time PCR (Gudmestad *et al*. 2009), competitive PCR (Hu *et al*. 1468), dig-labelled PCR (Lee *et al*. 2001), nested PCR (Lee *et al*. 2625), direct-PCR and immunocapture-PCR (Min *et al*. 2010), multiplex PCR (Pastrik 2000), and real-time TaqMan PCR-based assay using an internal reaction control (Smith *et al*. 2008). Immunofluorescence test (IF) has permitted the first record of the pathogen in Turkey (Altundag *et al*. 2009). Other methods that have been used successfully for identification in infected potato sample as molecular hybridization with non-radioactive DNA probes (Llamas *et al*. 1993), AmpliDet RNA (Beckhoven *et al*. 2002), and DNA array (Fessehaie *et al*. 2002).
The combination between molecular and serological technique were developed by a PCR combined with enzyme-linked oligonucleosorbent assay (ELOSA) (Baer *et al*. 2001) and a multiplex PCR-ELISA (Mills and Russell 2003). Different techniques, ELISA and immunofluorescence or PCR, can be used for isolation and characterization of the pathogen, to establish unequivocally its identity, with a positive detection further verified by bioassay on eggplant (Boer *et al*. 2005). A comparison between different techniques was carried out, and a prototype lateral flow device (a serological test kit) showed specificity comparable to IFAS and

PCR tests in distinguishing between the pathogen and other closely-related bacteria (El-Badry *et al*. 2009). The comparison among techniques was carried out, pointing at those with higher sensitivity and those highlighting some problems in identification (Westra *et al*. 1994, Slack *et al*. 1996, Ronda *et al*. 1999, Sigillo and Zoina 2002, Pastuszewska *et al*. 2005, Weller *et al*. 2006, Kokoskova and Mraz 2008). The conservation of samples seems to have an influence on results after fluctuations between freezing and above-freezing temperatures (from -20 to +25 °C), as the capability of detection in potato tuber samples decreased (Kokoskova *et al*. 2005).
The evaluation of the accuracy in a laboratory's results can be important to investigate the problems and initiate corrective procedures to avoid mistakes in identification (Reynolds and Owen 2010). The molecular probes for detection and identification of the pathogen showed possible discrepancies and sequencing errors in public sequences, or even typing errors in the oligonucleotides (Arahal *et al*. 2004). It is important to standardize test methods and procedures for test efficiency (Janse 2005).

2. Delimitation
No information available.

3. Monitoring
The disease incidence was evaluated in field and in storage places through IF, ELISA and PCR and pathogenicity tests were conducted on aubergine (Vasinauskiene and Baranauskaite 2003).

4. Commodities
The pathogen was detected on imported tubers and seed potato (Mazzucchi *et al*. 1981, Potocnik and Cvelbar 1999). Freezing and wide fluctuations temperature did not eliminate the pathogen, but the treatment caused a reduction in infectivity in some cases (Nelson and Kozub 1990). Strict application of certification regulations are important to avoid new introductions of pathogens (Boer *et al*. 1989, DeHaan 1994), such as postharvest laboratory testing of seed lots for bacterial ring rot (Boer *et al*. 1989, Kudela 2007). The disinfection of machinery, tools and stores and the application of biocidal products are useful in preventing spread (Kraska 2006). The presence in potato pulp remaining after industrial processing of potato tubers contaminated with bacterial ring rot was evaluated, the results showing that industrial processing seems to be a safe method of disposal of potato tubers contaminated with bacterial ring rot although it does require permanent phytosanitary inspection (Mackowiak-Sochacka *et al*. 2009).

Clavibacter michiganensis subsp. Insidiosus (McCulloch) Davis et al

Common name(s): Bacterial wilt, blight, root foot
EPPO A2 list: No. 49
EU Annex designation: II/A2

Organism
The main host is lucerne, but *Medicago 158iliate*, *Melilotus alba* and other *Medicago* spp. can also be infected. The pathogen is of North American origin and has spread to other continents (Aittamaa *et al*. 2008)

1. Detection
Combinations of immunofluorescence (IF) staining and dilution plating provide a reliable technique for the identification of the pathogen (Nemeth *et al*. 1991). Polyclonal antisera were prepared for use in serological technique (ELISA, DAS ELISA, PTA ELISA), but the highly variable strains of the pathogen were not reliably identified by the polyclonal antibodies (Kokoskova *et al*. 2000).

A PCR technique was developed (Bryxiova and Kudela 2002, Kamasa and Krawczyk 2007 as was a real time PCR that has shown high sensitivity and specificity (Marefat *et al*. 2007). A real-time Taqman BIO-PCR assay and subsequent confirmation of amplicon identity by melting peak analysis, made possible by addition of EvaGreen (TM) to the reaction mix, was deemed useful for routine analysis in a diagnostic laboratory setting (Ward *et al*. 2008).

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
No information available.

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Coleosporium asterum (Dietel) Syd. & P. Syd.

Common name(s): Pine needle rust, Western pine-aster rust
Taxa: Fungi: Basidiomycota: Coleosporiaceae

Organism
Coleosporium asterum is a pest of pine species. Asters and goldenrods act as secondary hosts (Lowe 1972), although different races may be associated with different secondary hosts (Anderson and Anderson 1978).

1. Detection
In Canada, infection begins in autumn, but symptoms are not visible until spring. Reddish dots appear on yellow bands of the foliage. Lesions develop in early summer into flat sided white columns (0.5-1.0 mm high) that rupture and release spores.

2. Delimitation
No relevant references found.

3. Monitoring
No relevant references found.

4. Commodities
No relevant references found.

References: 7 references retained out of 32 references retrieved, none added, 2 used for summary

Coleosporium eupatorii
References: 1 references retrieved, none added, 1 retained, 0 used for summary

Coleosporium paederiae
References: 4 references retrieved, none added, 1 retained, 0 used for summary

Coleosporium phellondendri
References: 4 references retrieved, none added, 0 retained, 0 used for summary

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Conotrachelus nenuphar (Herbst)

Common name(s): Plum curculio, plum weevil
Taxa: Insecta: Coleoptera: Cuculionidae
EPPO A1 list: No. 35
EU Annex designation: I/A1

Organism

The plum curculio, *Conotrachelus nenuphar*, is a weevil species native to North America that attacks pome and stone fruit (Racette *et al*. 1991, Vincent *et al*. 1999) and can be particularly damaging in apple. It is known to be highly adaptable and can quickly acquire new host species. It is absent within the EU/EPPO region. Adults feed on blossom, leaf and fruit material whilst larvae bore into fruit that causes premature drop. Despite significant work on certain aspects of its life cycle, it is considered to be poorly understood from the standpoint of its biology and ecology (Vincent *et al*. 1999)

1. Detection

Adults of the weevil can be readily detected visually as can damage caused by the larvae. Insects can be easily dislodged from the canopy by tapping branches (Prokopy *et al*. 1999b) and limb jarring (Johnson *et al*. 2002). The scarring caused by oviposition is clearly visible and is pictured by Blanc *et al*. (1984). Polythene tunnels hung under trees have been used for population assessments (Blanc *et al*. 1981) although such methods are now superseded. Host odour is known to be a component of attraction of the pests towards suitable oviposition sites (Butkewich and Prokopy 1993, 1997) and has been used in combination with traps (e.g. pyramid traps) for monitoring of the pest (Prokopy *et al*. 1997, 1999a). The design of the pyramid traps and how they are deployed are extensively described by Prokopy and Wright (1998). Other trap designs have also been evaluated but performance was poor when compared with the pyramidal design (Prokopy *et al*. 2000a). The ground-placed pyramid trap, and an alternate Plexiglas square sticky trap, are pictured in Pinero *et al*. (2001).

Attraction to green sticky traps that mimic the colour of tree canopy indicate that visual cues are also important factors in whether a trap will detect this pest (Butkewich and Prokopy 1997), a fact that has influenced trap design (Leskey 2006) and architecture (Lafleur *et al*. 2007). A male-produced aggregation pheromone has also been isolated and characterized (Eller and Bartelt 1996). Males are also strongly attracted to volatiles released by females feeding on fruit, whilst females are attracted to the odour of males although it is likely that fruit odour is much the more important attractant (Leskey and Prokopy 2001). The knowledge that odour plays a strong part in attraction has led to the development of several traps based on odour lures (Prokopy *et al*. 1996). Some of components of fruit (e.g. plum) odour responsible for this attraction have been identified (ethyl isovalerate and limonene) (Leskey *et al*. 2001). Further studies have shown that pyramid traps baited with benzaldehyde plus aggregation pheromone are highly effective (Pinero *et al*. 2001, Johnson *et al*. 2002). Additional work has further elucidated the chemical attractants for the plum curculio and ascertained that foliar and woody-tissue volatiles are also attractive to the weevil (Leskey *et al*. 2005).

Some research has been invested in the development of branch mimicking traps for catching *C. nenuphar*. These include various cylindrical designs of different colours (Leskey and Prokopy 2002). Other “tree trap” designs have been evaluated, again baited with benzaldehyde although non proved to be particularly effective (Prokopy *et al*. 2003). However, despite the various advances that have been made to detect the plum curculio via traps, Lafleur *et al*. (Lafleur *et al*. 2007) claim

that “no reliable monitoring methods other than visual inspections….are available”. This suggests that there is still some work to go to adequately detect the presence of this insect, especially at lower densities and cooler conditions.

2. Delimitation
No information available

3. Monitoring
A number of monitoring-type surveys are described for *C. nenuphar*. Earlier reports deal with visual assessment of fruit damage and/or presence of the insect (Hagley and Hikichi 1973, Goonewardene *et al*. 1977) or oviposition scarring (Alm and Hall 1986) A combination of capture techniques have been employed in general surveys of orchards in China that effectively ruled-out the presence of this pest in that country (Chen and Tseng 1993). In an assessment of the migration behaviour of the pest, damage and visual recording of presence were used to ascertain the presence of *C. nenuphar* with the conclusion that tree jarring constituted the best technique for monitoring the population (Yonce *et al*. 1995). Comparisons of various traps with respect to their efficacy for monitoring the plum curculio indicated the odour-baited Plexiglas traps placed at the base of trees could be effective devices for ascertaining infestation levels (Prokopy *et al*. 2000b), but only in certain circumstances.
Practical recommendations for the use of odour-baited traps as monitoring tools have been developed. These include the type of trap, spacing, effective range and odour-release values needed (Prokopy *et al*. 2004b, a).
The monitoring of the plum curculio can be aided by the use of computer models. For example, the model of Chouinard and Bourgeois (2002) used air temperature to predict the occurrence of the pests as an aid to control. A similar approach was also reported by Lan et a. (2004), whilst elements of the paper by Ulrichs and Hopper (2008) also deal with temperature-based prediction of the occurrence of this pest. A degree-day approach was also taken by Akotsen-Mensah *et al*. (2011). Modelling approaches have also been used to predict the pest’s response to trap attractants, which indicated that efficacy will rise markedly as temperatures approach 20°C (Leskey and Zhang 2007). Other factors influencing trap catch, such as timing (night and day) and wind speed have also been explored (Lamothe *et al*. 2008)

4. Commodities
No information

References: 81 retained out of 116 retrieved, none added, 36 used for summary

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Cowpea mild mottle virus

Common name(s): CPMMV, angular mosaic, pale chlorosis
Taxa: Viruses: Flexiviridae: Carlavirus
EU Annex designation: I/A1

Organism
This virus (CPMMV) infects a moderately wide range of crops, including *Vigna*, *Vicia* and *Phaseolus* species as well as several solaneceous plants and a number of weedy hosts (Iizuka *et al*. 1984, Iizuka and Reddy 1986, Iwaki *et al*. 1986, Gumedzoe 1993b, Mansour *et al*. 1998). The disease causes chlorotic blotches on the host leaves, mottling, leaf-rolling, stunting and deformity as symptoms (Iizuka *et al*. 1984) (Mali and Nirmal 1987) that, as indicated by the virus's name, are often relatively mild. The disease is known to be vectored by the tobacco whitefly, *Bemisia tabaci,* and seed transmission is also reported (Mali *et al*. 1989, Sreenivasulu *et al*. 2008), although not for all crops (Anonymous 1987). The virus is absent from the EU and occurs primarily in Africa, the Middle East and Asia (Jeyanandarajah and Brunt 1993).

1. Detection
Earlier reports of CMMV describe confirmation of identity through particle morphology and/or transmissibility via *Bemisia tabaci* (Iwaki *et al*. 1982, Iizuka and Reddy 1986). Transmissibility and indicator hosts, such as *Chenopodium*, coupled with morphological (SEM examination of particles) and serological identification has also been used in tandem (Iizuka *et al*. 1984, El-Afifi *et al*. 1996). An ELISA method was used to detect for the first time the presence of CMMV in Tanzania following the observation of symptomatic plants (Mink and Keswani 1987) and to detect the disease in medicinal plants in West Africa (Gumedzoe 1993a) and groundnut in Sudan (El-Hassan *et al*. 1997). Similarly, following a detection survey in Argentina prompted by the occurrence of symptoms in common bean (*Phaseolus*), indicated the first occurrence of CMMV within that region (Rodriguez Pardina *et al*. 2004), whilst a similar surveying scenario identified the disease in soybean in Iran (Tavassoli *et al*. 2008). In both these specific instances, ELISA was successfully used as the diagnostic tool. However, ELISA proved unable to identify CMMV from soybean and groundnut seeds taken from infected plants (Horn *et al*. 1991). ELISA has been widely used in several crops for detection of the virus, either using single or mixed antisera (Joshi and Albrechtsen 1992, Gumedzoe 1993a, Sivaprasad and Sreenivasulu 1996).
Sequencing, following RT-PCR has been used to identify a strain of CMMV in Brazil (Almeida *et al*. 2003) and also for differentiation of strains found in Sudan (Tavassoli *et al*. 2008). However, it is not clear as to what extent RT-PCR can be used as a diagnostic tool for this disease.

2. Delimitation
No information

3. Monitoring
Surveys for CMMV have been undertaken in Togo to demonstrate its widespread occurrence in cowpea and other hosts (Gumedzoe 1993a). Similarly, the virus was found to be widespread in the Yemen following a wide-ranging survey of crops for viral-diseases (Walkey *et al*. 1990) and also in northern Argentina (Laguna *et al*. 2006). Surveys of the Yemen, monitoring for viral diseases in general has indicated the occurrence of CMMV in a wide range of crops (Walkey *et al*. 1990). Little information regarding sampling strategies was identified from the literature.

4. Commodities

As there is the potential for seed-borne transmission of CMMV, a small number of studies have focussed on examining the presence of the disease in seeds. Due to the fact that ELISA methodologies are not always successful in detecting the virus within the seed (Horn *et al*. 1991), seedlings are often produced and the ensuing leaf tissue analysed. For example, a survey of 60 seedlots destined for introduction into Africa were surveyed in the mid-1990s and analysed by ELISA following growing-on of aliquots of the seed to demonstrate the seeds were free of the disease (Gillaspie *et al*. 1995). Similarly, seedling analysis was used to assay for the disease in cowpea seedlots from 21 countries with ca 27% of lots shown to be infected (Hampton *et al*. 1992).

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Cronartium quercuum (Berkeley) Miyabe ex Shirai

Common name(s): Easter pine gall rust
Taxa: Fungi: Basidiomycota: Cronartiaceae
EPPO A1 list: No. 252
EU Annex designation: I/A1

Organism

Cronartium quercuum is a fungal rust disease that primarily attacks *Pinus* species, despite its somewhat misleading Latin binomial that tends to suggest oak as a major host. It is found throughout North and Central America, the Caribbean and Asia but is absent from the EPPO/EU region. The disease causes galls to form on affected plants, with younger trees most susceptible to deleterious effects with older hosts more resilient.

1. Detection

Aside from observation of the physical signs of infection (presence of galls), a number of diagnostic techniques have been developed for *C. quercuum*. Germ tube morphology has been used as diagnostic for *C. quercuum* (Dietrich *et al*. 1985). Other early methods involved the staining of sections taken from the galls of infected trees (Matthews 1973) or the use of fluorescence microscopy for examination of the hyphae (Saho 1973). Differentiation within the genus has been demonstrated using isozymes / protein pattern analysis of *Cronartium* spores (Powers *et al*. 1989, Cheng *et al*. 1998b). However, these methods have been, to a degree, superseded by ELISA (Spaine *et al*. 1985, Spaine 1987) and molecular techniques (RAPD/PCR) that can both identify and/or differentiate different *Cronartium* species (Cheng *et al*. 1998a, Ramsfield and Vogler 2004, Ramsfield and Vogler 2010)

2. Delimitation

No information

3. Monitoring

The occurrence and distribution of *C. quercuum* has been ascertained in North America on several occasions. Sampling of galls followed by aeciospores germ tube morphology was used by Dietrich *et al* (1985) in Minnesota. Two summaries of a series of surveys conducted in the United States are also available (Hunt and Lenhart 1986, Lenhart *et al*. 1988).

4. Commodities

No information

References: 28 retained out of 72 retrieved, none added, 12 used for summary

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Cronartium spp. (Non-European)

Taxa: Fungi: Basidiomycota: Cronartiaceae

Cronartium fusiforme (Hedgcock & Hunt) ex Cummins
Common name(s) Southern fusiform rust
EPPO A1 list: No. 9
EU Annex designation: I/A1

Cronartium (*Endocronartium*) *harknessii* (J.P. Moore) Hiratsuka
Common name(s) Western gall rust, pine-pine gall rust
EPPO A1 list: No. 11
EU Annex designation: I/A1

Cronartium kamtschaticum Jørstad
Common name(s): Japanese white pine rust
EPPO A1 list: No. 18
EU Annex designation: I/A1

Cronartium comandrae Peck
Common name(s): Comandra blister rust
EPPO A1 list: No. 249
EU Annex designation: I/A1

Cronartium comptoniae J.C. Arthur
Common name(s): Sweetfern blister rust
EPPO A1 list: No. 250
EU Annex designation: I/ A1

Cronartium himalayense Bagchee
Common name(s): Chir pine blister rust
EPPO A1 list: No. 251
EU Annex designation: I/ A1

Other species considered include: *C. ribicola*, *C. flaccidum*

Organism

This summary deals with the non-*C. quercuum* species of *Cronartium.* All species attack *Pinus*, although some only infect a restricted number of species. Similarities of methods for detection and monitoring are shared with those of *C. quercuum*, and those of importance to the other species are briefly discussed here.

1. Detection

Detection for surveys is typically based on damage symptoms and the presence of galls/cankers/blisters that are characteristic of the diseases. The gall section staining method of Matthews has been show to be effective in identifying several *Cronartium* species (Matthews 1973), whilst separation of species within the genus can be achieved using isozymes analysis (Powers *et al*. 1989). Diagnosing the identity of the exact *Cronartium* species from within a genus

of closely related species has given rise to several discriminating tests. *Cronartium ribicola* (pine stem rust) can be differentiated from species of the genus using RAPD. Similarly, *Cronartium flaccidum* can be diagnosed using RFLP analysis (Moricca and Ragazzi 1998). Notably, molecular and morphological investigations have revealed hybridization between *C. ribicola* and *C. comandrae* (Joly *et al*. 2006). Likewise, PCR methods have been developed for the separation of *Cronartium harknessii* (Ramsfield and Vogler 2010). A review of such techniques has recently been produced for what is a rapidly developing field (Spring and Thines 2010).

2. Delimitation
No information

3. Monitoring
There are numerous examples of surveys undertaken for the various rusts (and other diseases associated with economically important forest tree species). A good example of a sampling methodology is provided by Geils and Jacobi (1984) when surveying for *C. comandrae* based on a visual assessment of canker presence. A statistical method for sampling for rusts in *Pinus* has been described by Lavallee (1972) whilst sampling methods for *C. fusiforme* have also been described (Phelps 1974, Swindel 1983). Hunt (Hunt 1983) describes a survey for *C. ribicola* whereby stands of host trees were examined for the presence of diagnostic cankers. Similarly, pines in the Alps were surveyed visually for *C. flaccidum* on the basis of pathogenic symptoms (Ragazzi 1984). Similarly, other *Cronartium* species have been surveyed for through searching-out the typical signs of disease within *Pinus* stands and/or other hosts (Geils and Jacobi 1984, Dietrich *et al*. 1985, Lundquist *et al*. 1992, Bergdahl and Teillon 2000, Campbell and Antos 2000). A roadside survey, whereby trees were assessed alongside ca. 800 miles of road allowed data to be accrued on several rust diseases (Lundquist 1993). Further detailed descriptions of sampling methodology are given by Jacobi *et al* (1993) and, in particular, the more recent assessment of *Cronartium ribicola* in Wyoming/Colorado based on visual assessments of *Pinus* (Kearns and Jacobi 2007). Bergdahl and Teillon (2000) also provide a similar level of detail for surveying *C. ribicola* in Vermont. A brief review of *Cronartium ribicola*, including brief details of historical surveys was recently made by Hunt (Hunt 2009). Limited details of a survey for *C. himalayense* are provided by Shukla (1996). No information was recovered by the literature search for *Cronartium kamtschaticum,* whilst no references of value were retrieved for *C. comptoniae.*

4. Commodities
No information

References: 143 retained out of 286 retrieved, none added, 20 used for summary

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Cryphonectria parasitica (Murril) Barr

Common name(s): Chestnut blight/canker
Taxa: Fungi: Ascomycota: Cryphonectriaceae
EPPO A2 list: No. 69
EU Annex designation: II/A2

Organism
Castanea spp. are the main hosts of *Cryphonectria parasitica*, which may also attack *Quercus* spp., *Castanopsis, Acer, Rhus typhina* and *Carya 178ilia*. *C. parasitica* was introduced into North America from the Far East and, in 1938, the pathogen was first discovered in Italy. Following introduction, the fungus spread very rapidly and at the end of the 1960s most parts of southern Europe were affected. Cankers may enlarge so rapidly that the stem becomes girdled without callus formation. Regions above the point of invasion die; the leaves wilt and turn brown but remain hanging on the tree.

1. Detection
The isolation and following analysis of the morphological characters is the method most often used for the identification of fungus (Kazempour *et al*. 2006). The isolates are subdivided between virulent and hypovirulent (Juhasova *et al*. 2006). A PCR technique can be used as an express method for detection of chestnut blight (Popov *et al*. 2010). This molecular technique was developed to identify different isolates of *Cryphonectria parasitica*, showing the suitability of this method to detect possible genetic differences of the dsRNA conserved sequences harboured in hypovirulent field isolates of *C. parasitica* strains (Grimaldi and Catara 1994). Double-stranded RNA (dsRNA) molecules of viruses are found in nature at a very high frequency. Their detection in plants and fungi has been carried out with difficulty due to the complicated dsRNA extraction techniques, which include phenol-chloroform extractions. In a study, an extraction method for isolation of dsRNA is described that is free of phenol and chloroform (Balijja *et al*. 2008). A modified procedure which further improves the dsRNA isolation efficiency from *C. parasitica* is less time-consuming and it requires smaller amount of fungal materials as well as reagents reducing the financial costs and isolation time significantly (Radocz *et al*. 2010).

2. Delimitation
No information available.

3. Monitoring
Survey were carried out the presence and progressive spread of the disease (Saintonge 1998, Carvalheira *et al*. 2000, Scalise *et al*. 2000, Frigimelica *et al*. 2001, Carpanelli 2002, Turchetti *et al*. 2002, Tindall *et al*. 2004, Perlerou and Diamandis 2006, Matosevic *et al*. 2007). The diversity of different isolates was evaluated using a medium that discriminated with high resolution (Cortesci *et al*. 1996). The monitoring and successively evaluation of strains was useful to determine the level of virulence (Elliston 1987, Conedera 1991, Novak-Agbaba *et al*. 2005). The techniques used were double stranded ribonucleic acid (dsRNA) content (Liang and Chen 1990, Peever *et al*. 1997, Lee *et al*. 2005, Lee *et al*. 2006b, Sotirovski *et al*. 2006, Radocz and Tarcali 2007, Zamora *et al*. 2008) that was flanked to morphology in agar culture and virulence in apple fruit (*Malus domestica*) and chestnut sprouts (*Castanea 178iliate*) vegetative compatibility groups (VCG) (Pennisi *et al*. 1992, Turchetti *et al*. 1992, Dunn and Boland 1993, Causin *et al*. 1995, Radocz 1999, Celiker and Onogur 2001, Gouveia *et al*. 2001, Machado *et al*. 2001, Radocz 2001, Aponyi *et al*.

2002, Celiker and Onogur 2002, Aguin *et al*. 2005a, Aguin *et al*. 2005b, Braganca *et al*. 2005, Haltofova 2006, Braganca *et al*. 2007, Liu *et al*. 2007, Vidoczi *et al*. 2007, Aguin *et al*. 2008, Gonzalez Varela and Gonzalez Fernandez 2008, Montenegro *et al*. 2008, Akilli *et al*. 2009, Robin *et al*. 2009, Ackgoz *et al*. 2010, Jankovsky *et al*. 2010, Moghadam *et al*. 2010). A multiplex PCR assay with specific primers was another molecular technique used to determine different isolates (Ackgoz *et al*. 2009). The conversion to hypovirulence was also evaluated (Garbelotto *et al*. 1992, Radocz 1999). Surveys were carried out to know if the silvicultural parameters and management affected the incidence of fungus (Griffin 1989, Guidi *et al*. 1997, Portela and Pinto 2005, Lee *et al*. 2006a, Juhasova *et al*. 2008) or environmental factors (Braganca *et al*. 2009) or the presence of the pathogen in other host (Torsello *et al*. 1994, Radocz and Holb 2002, Haltofova *et al*. 2005, Radocz and Tarcali 2005, Tarcali and Radocz 2006, Ilona *et al*. 2009, Radocz 2009, Radocz and Tarcali 2009).

4. Commodities

The import of plant material was a risk for the introduction of numerous fungi among which *Cryphonectria parasitica*, import and export restrictions were necessary (Jankovsky 1998). *Cryphonectria parasitica* was detected in chestnut plants at the AQIS Post Entry Quarantine facility located at Knoxfield, Victoria (Cunnington and Pascoe 2003). As infected chestnut stems are a source of both virulent and hypovirulent *C. parasitica* strains (Prospero *et al*. 1998), a stem-flow protocol that can devitalise the parasite in barked logs while preserving the commodity characteristics of the wood, through a fast, simple and low-cost treatment, was developed (Nicoletti *et al*. 2005). It can be performed at the European borders whenever *C. parasitica* is or might be present.

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Curtobacterium flaccumfaciens pv. *Flaccumfaciens*

Common name(s): Bacterial wilt, tan spot
Taxa: Bacteria: Actinobacteria: Microbacteriaceae
EPPO A2 list: No. 48
EU Annex designation: II/B

Organism
Curtobacterium flaccumfaciens pv. *Flaccumfaciens* is the causal agent of the bacterial wilt disease of *Phaseolus* spp. and is a systemic bacterium. Economically the most important host is *Phaseolus vulgaris*, but the pathogen can also attack soybeans, peas and cowpeas. *Curtobacterium flaccumfaciens* pv. *Flaccumfaciens* is a seed-borne bacterium and can be transmitted both within and on the seed. It has been known to survive in soil for a least two winters between bean crops rotated with wheat. There are no reports of vectors but the nematode *Meloidogyne incognita* may assist entry by providing wounds. The bacterium can infect in the absence of rain; it has not been observed to enter via stomata. Once within the plant the bacterium colonizes the vascular tissue. Young *Phaseolus* plants 5-8cm tall, may be attacked and are usually killed. If plants survive an early attack or are infected at a later growth stage they may live throughout the season and bear mature seed.

1. Detection
The disease is characterised by the wilting of leaves, or parts of them during the heat of the day followed by recovery as the temperature drops in the evening. As a result of bacterial plugging of the vessels, the water supply is cut off and the leaves turn brown and 183iliate. These typical wilting symptoms may be absent and replaced by golden-yellow necrotic leaf lesions. On young pods, water-soaked spots occasionally appear, the area turning yellowish-green or darker than the rest of the pod. On ripe pods, the lesions are more conspicuous being olive green in colour. Seemingly vigorous plants may bear one or more shrivelled shoots or infected pods which are hidden by healthy foliage.
Bacteria may be detected beneath the seedcoat by means of a combined cultural and slide agglutination test. Direct isolation and PCR can be used as screening tests. Immunofluorescence staining can also be used to detect the bacterium in contaminated seed lots (Calzolari *et al*. 1987). Both monoclonal and polyclonal antibodies raised and screened against *Curtobacterium flaccumfaciens* pv. *Flaccumfaciens* have been shown to be defective in specificity and sensitivity (Calzolari *et al*. 1987; McDonald and Wong, 2000).
Anonymous (2011) describes the diagnostic procedure for extraction from plant material and seeds, presumptive diagnosis with rapid tests and simultaneous isolation of bacterial colonies, identification of *Curtobacterium flaccumfaciens* pv. *Flaccumfaciens* isolates and determination of pathogenicity. Tests for screening and identification include direct isolation of agar media, PCR tests, morphological characterisation, serological methods, fatty acid profiling, molecular methods and pathogenicity tests. PCR protocols have been developed by Tegli *et al*. (2002) and Guimaraes *et al*. (2001).

2. Delimitation
No information.

3. Monitoring
No information.

4. Commodities

Phytosanitary procedures are in place for the field inspection and seed-testing methods of *Phaseolus vulgaris* for *Curtobacterium flaccumfaciens* pv. *Flaccumfaciens* (EPPO/OEPP, 1994). Seeds of *Phaseolus vulgaris* should have been grown in an area where the bacterium does not occur or else that the seed crop should have been free from the bacterium during the growing season.

References: 48 retained out of 151 retrieved, 2 added, 5 used for the summary

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Cydia inopinata (Heinrich)

(=*Grapholita inopinata* (Heinrich)
Common name(s): Manchurian apple fruit moth
Taxa: Insecta Lepidoptera: Totricidae
EPPO A1 list: No. 193
EU Annex designation: II/A1

Organism
Cydia inopinata is a pest of apples, primarily in the far east of Russia and China (Anonymous 2000). Although apples are the most commonly attacked host, the insect can also attacks pears and other members of the Pomoideae (Anonymous 1997). Crop losses result from the feeding chambers created by the larvae as they feed on fruit. The moth is extremely cold hardy and mass emergences of adults occur in June (Lopatina 1978) and July across its native range

1. Detection
Cydia inopinata does not respond to light traps although the sex pheromone of female moths has now been characterized. The pheromone has proven to be a mixture of (Z)-8-dodecenyl acetate☹E)-8-dodecenyl acetate: dodecenyl acetate:dodecanal-1-ol and traps using these components (minus dodecanal-1-ol) have been successfully tries as lures for males (Tanaka et al. 2007)

2. Delimitation
No information

3. Monitoring
No information

4. Commodities
No information

References: 6 retained out of 11 retrieved, 4 added, 4 used for the summary

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2. Delimitation
No information available

3. Monitoring
No information available

4. Commodities
No information available

References: 6 retained out of 11 retrieved, 4 added, used for the summary

Anonymous. 1997. Quanrantine pests for Europe (second edition).

Anonymous. 2000. Grapholita inopinata. Page Map 602 Distribution Maps of Plant Pests. CAB International, Wallingford.

Lopatina, V. V. 1978. The Manchurian apple fruit moth in the Chita region. Zashchita Rastenii 4:47-48.

Tanaka, M., K. Abe, T. Ando, and V. Le Van. 2007. Identification of the sex pheromone secreted by females of Grapholita inopinata Heinrich (Lepidoptera: Tortricidae). Research Bulletin of the Plant Protection Service, Japan:17-22.

Dacus ciliatus Loew

Common name(s): Ethiopian fruit fly, lesser pumpkin fly, cucurbit fly
Taxa: Insecta: Diptera: Tephritidae
EPPO A1 list: No. 238
EU Annex designation: I/A1

Organism
Pumpkin or cucurbit fruit fly, *Dacus ciliatus* , is one of the major pests of Cucurbitaceae (Fetoh 2006, Vayssieres et al. 2008).Copulation occurs with beginning of night, and in laboratory studies, female deposited 322.6 eggs, hatched and complete to give adults a sex ratio 1:1 and the total generation lasted 73.6 days (Fetoh 2006). It is widely distributed in Africa and Asia but not established in the EU.

1. Detection
Host attractants for *D. ciliatus* were identified. The most attractive blend was a mixture of four or five identified acetates (benzyl acetate, hexanyl acetate, (Z)-3-hexenyl acetate, (Z)-3-octenyl acetate, octanyl acetate, (Z)-3-decenyl acetate, and (E)-beta-farnesene). The addition of an equal proportion of (E)-beta-farnesene to this mixture had a deterrent effect (Alagarmalai et al. 2009).

2. Delimitation
No information available

3. Monitoring
No information available

4. Commodities
No information available

References: 95 references retrieved, none added, 33 retained, 3 used for summary

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Dacus cucurbitae Coquillett

(=*Bactrocera curcubitae* (Coquillet))
Common name(s): Melon fly, melon fruit fly
Taxa: Insecta: Diptera: Tephritidae
EPPO list: No. 232
EU Annex designation: I/A1

Organism
The melon fruit fly *Dacus cucurbitae* Coquillett (s an important pest of cucurbits (Lall and Singh 1969, Lee 1972). This pest is distributed widely in temperate, tropical, and sub-tropical regions of the world (Dhillon et al. 2005). Females oviposit in the fruit tissues, and the larvae feed inside the fruit. Pupation occurs in the soil at 0.5 to 15 cm below the soil surface (Dhillon et al. 2005). Present in Asia, Africa, and Oceania but absent from the EU.

1. Detection
The larvae feed inside the fruit rendering chemical control is very difficult at this stage (Liu et al. 1997).*Manihot esculenta* Crantz (Euphorbiales: Euphorbiaceae) can be used as a trap crop to improve suppression of *D. cucurbitae* by providing sites for bait spray applications (McQuate 2011). While methyl eugenol (ME) (4-allyl-1, 2-dimethoxybenzene-carboxylate), cue-lure (C-L) (4-(p-acetoxyphenyl)-2-butanone), and raspberry ketone (RK) (4-(p-hydroxyphenyl)-2-butanone) are powerful male-specific lures; they are used for detection, monitoring, and control of fruit fly (Vargas et al. 2010). However, a closely related compound of cuelure (=the acetate of raspberry ketone), raspberry ketone formate, is somewhat more volatile and has been found to be at least 1.7 times more attractive to both sterile and wild melon flies in field tests conducted in Hawaii (Casana-Giner et al. 2003).
In a study testing the efficacy of different protein sources (viz., peptone, protone, soybean, yeast, malt, casein and a commercial formulation of fruit fly diet in attracting female adults of fruit flies), the adults *D. cucurbitae* showed a significantly higher preference to baits containing commercial fruit fly diet and yeast (4.0 and 3.25/trap/week, respectively) (Kumar and Shashidhar 2007). In the same way, Rousse et al. (2004) showed *Torula* spp., bakers' yeast and beer residues are twice as attractive as Buminal, the only hydrolysate presently registered in France. The addition of acid to Buminal at pH 3, and alkali to bakers' yeast at pH 6 or *Torula* spp. at pH 10.5 increased their attractiveness compared to their initial pHs (respectively 6, 5 and 9). Addition of higher rates of acid or alkali decreased attractiveness. Borax reduces attractiveness of all hydrolysates.
In a series of studies conducted in Hawaii under seminatural conditions, sexually mature females of *D. cucurbitae* were particularly attracted to objects (Tangletrap-coated fruit mimics) of spherical shape (8 cm diameter) colored either yellow, white, or orange; these three pigments as well as cucumber (*Cucumis sativus* L.) odour offered the highest attractivity (Miller et al. 2004, Pinero et al. 2006).
Zhu et al. showed that mtDNA cytb sequences could be used as the molecular marker in identification of *D. cucurbitae* and 5 other fruit fly species (2005).

2. Delimitation
No information available.

3. Monitoring

In India, the peak population (2.83 males per trap) was recorded on the first week of July 2001. The activity of *D. cucurbitae* was monitored using pheromone traps. The mean number of adults was higher in traps placed at 1.5 m above the ground (2.70) than in traps placed at 2.0 m above the ground (0.63) (Pankaj et al. 2002). *Dacus cucurbitae* capture rates were higher inside than outside crop production areas (Vargas et al. 1989).

4. Commodities

The export of horticultural produce, especially fresh fruits and vegetables, presents a risk (Gupta and Khetarpal 2005). In the past, in India, specific imports of fresh fruits have already been allowed on the basis of agreed treatments. Appropriate disinfestation protocols for other fresh fruits are currently being developed in India, particularly with respect to fruit flies, to allow prohibitions of export to Japan and other countries to be lifted (Gupta and Khetarpal 2005).

A survey carried out in one airport, in Japan, showed that sixty one percent (334 times) of all the detections of fruit flies were performed during 5 days storage periods for the fruit. This suggests that careful inspection is not effective enough to detect early stages of fruit flies in fresh fruits (Takeishi 1992).

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Dacus dorsalis Hendel

(=*Bactrocera dorsalis* (Hendel))
Common name(s): Oriental fruit fly
Taxa: Insecta: Diptera: Tephritidae
EPPO A1 list: No. 233
EU Annex designation: I/A1

Organism

The Oriental fruit fly, *Dacus dorsalis* (syn. *Bactrocera dorsalis* (Hendel)) (Diptera: Tephritidae) may overwinter as pupae in temperate zone (Hou and Zhang 2007). Males are strongly attracted to, and compulsively feed upon, methyl eugenol (ME), a compound occurring naturally in various plant families (Shelly 2000) and an effective attractant for many *Dacus* species (Shelly and Dewire 1994, Wee and Tan 2007). The flight activity of *D. dorsalis* was hourly observed using methyl eugenol-baited traps during 24 hours. The results showed that adult males only fly in the daytime, but not in the night, which suggested that light might be a prerequisite for their flying (Liu and Ye 2006).
A key of determination of the subfamily Dacinae has been developed by Lin et al. (2006). *Dacus dorsalis* dry specimens were used for DNA extraction and PCR amplification (Shi and Ye 2005).
This pest is present in China (Yang et al. 1994, Lin et al. 2005, LuXin et al. 2007, Chen et al. 2010), in India (Kapoor 2004), in Taiwan (Wen and Liou 2008), in Hawaii (Wu and Smith 2010) and likely in Africa (Lux et al. 2003).
Recently, Luo and Zeng (2010) found a virus in a parasitized host *D. dorsalis.* In fact, this new rod-shaped nucleocapsids was found inadvertently in *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) and following oviposition, invaded and proliferated in the haemocytes of a parasitized *D. dorsalis*.

1. Detection

A wide range of detection methods are available to ascertain the presence of *D. dorsalis*, but utilization of the methyl eugenol as a sex attractant seems to be the best method. Methyl eugenol (ME) (4-allyl-1, 2-dimethoxybenzene-carboxylate), cue-lure (C-L) (4-(p-acetoxyphenyl)-2-butanone), and raspberry ketone (RK) (4-(p-hydroxyphenyl)-2-butanone) are powerful male-specific lures and are used in current fruit fly programs for detection, monitoring, and control (Vargas et al. 2010). Transparent bottle traps (1000 ml) can also be used to capture *D. dorsalis.* Each bottle trap containing plywood blocks soaked in a mixture of methyl eugenol and malathion (8:1), suspended at a height of 2 m above the ground. According to Ravikumar and Shashidhar (2006) and Shankar et al. (2010), transparent bottle traps with 4 holes with 20 mm size recorded the higher captures. However, the influence of hole size revealed that fruit fly adults were also attracted by the traps with 8 mm dia. holes (9.33 flies/trap/week), probably linked to the quick dispersal of methyl eugenol and the influence of weather factors.
Using near infrared (NIR) image, Saranwong et al. (2011) observed a clear distinction between infested areas with few false positive results in the control fruit. Thus, these results indicate the feasibility of using NIR imaging as the basis for a low-cost, high-speed device for the detection of

the oriental fruit fly in mangoes. In the same way, Yang et al. (2006) previously showed that the digitalized X-ray images can detect injuries caused by *D. dorsalis* at as early as 3 days after implantation of eggs in some fruits. Their results demonstrate that this technique is a useful tool for the non-destructive inspection of the internal injuries of fruit, something which cannot be determined solely with the naked eye.
Recently a novel, visually attractive, rain-fast bait station was developed in Hawaii for potential use against multiple species of pestiferous fruit flies (Pinero et al. 2010). About 42% less GF-120 was used with bait stations compared to foliar sprays, indicating that bait stations can provide a simple, efficient, and economical method of applying insecticidal baits to control fruit flies and a safer alternative to foliar sprays. Leblanc et al. (2010) suggest that torula yeast in water is a more effective attractant than BioLure for male and female *D. dorsalis* while minimizing non-target captures.

2. Delimitation
In Hawai, *D. dorsalis* flies were captured in methyl eugenol traps (Nishida and Vargas 1990, Chu et al. 1994, Froerer et al. 2010) and protein bait traps (Froerer et al. 2010). Their dispersal distance has been measured and many flies were recovered at long distances (between 2-11.39 km) from the release point (Froerer et al. 2010). These outcomes aid in understanding the long tails of spatial distribution of fly movement, and will benefit consideration of dimensions for buffer zones that would be needed for the establishment of infestation-free or low-prevalence zones.

3. Monitoring
Li et al. (2010) had established a prediction model using multifactorial stepwise regression. This model is based on the monitoring data collected from 2003 to 2008 including trapped pests and weather factors (temperature, humidity, precipitation, illumination etc.). It could accurately predict the occurrence quantity and period of oriental fruit fly.
Several monitoring exercises for *D. dorsalis* have been carried out in Asia using methyl eugenol traps (e.g. Divender and Ranjeet 2000, Lu et al. 2006, Rajitha and Shashidhar 2006, Chen and Ye 2007, Dale and Patel 2010, Kao et al. 2010). All these studies have established that weather conditions are key factors in population dynamic of *D. dorsalis.* For example, in Taiwan, the *D. dorsalis* density was monitored every 10 days, during 4 years. Thereby, Kao et al (2010) found that there are two high *D. dorsalis* density peaks per year (April-June and October-December). The results of area-wide *D. dorsalis* control in the sugar apple plantation of Taitung indicated that through the cooperation of farmer's groups, simply by applying 750-850 litres of poisoned methyl eugenol bait a year could effectively reduce the fly density (Kao et al. 2010). A similar study conducted during 2004-2006 in China, showed a high tide period from May to November, with high abundance in August (Chen and Ye 2007).

4. Commodities
Importation of horticultural produce, especially fresh fruits and vegetables represents a risk (Iwaizumi et al. 1995, Gupta and Khetarpal 2005, Zhang and Hou 2005); e.g. avocados exported from Hawaii (Follett and Vargas 2010) but also the fresh fruits carried by passengers (Takeishi 1992).

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Dacus tryoni (Froggatt)

(=*Bactrocera tryon* (Froggatt))
Common name(s): Queensland fruit fly
Taxa: Insecta: Diptera: Tephritidae
EPPO A1 list: No. 235
EU Annex designation: I/A1

Organism

The Queensland fruit fly, *Dacus tryoni* is originally endemic to tropical and subtropical coastal eastern Australia, but now also widely distributed in temperate eastern Australia (Muthuthantri et al. 2010, Clarke et al. 2011). *Dacus tryoni* is a polyphagous species with a very wide host range on both cultivated and wild species (in 25 families) (Muthuthantri et al. 2010). Larvae pupate in the soil (Hulthen and Clarke 2006). Hulthen and Clarke (2006) showed a significant interaction between soil type (loamy sand, loam, sandy clay) and moisture level. But the most significant factor affecting pupae was extremes of soil moisture. Eighty-five percent pupal mortality occurred at 0% soil moisture and 30% mortality at 100% soil moisture.

Adult diet is an important determinant of sexual activity in many tephritid fruit flies (Perez-Staples et al. 2007, Weldon and Taylor 2011). A study of individual flies demonstrated that protein in the adult diet leads to significantly increased sexual activity of both males and females (Perez-Staples et al. 2007). Mating probability increases with age from 4-14 days as the flies began to mature. Overall, access to dietary protein increases longevity for both males and females, although females live longer than males on both diets, i.e. protein (hydrolysed yeast) and sucrose (Perez-Staples et al. 2007).

As shown by Fitt (1984), females exhibit no particular preference for the species of fruit on which they will oviposit; however they greatly preferred to oviposit in loquat fruits, particularly uninfested fruit rather than in those that already contained larvae, even at low densities or newly hatched larvae.

1. Detection

Females of *D. tryoni* can be monitored by McPhail traps baited with protein autolysate or orange concentrate (Dominiak 2006, Dominiak and Nicol 2010). However, Meats et al. (2002) showed that the male lure (cuelure) traps caught very few females. When the food lure traps were based on yeast hydrolysate, they caught mainly females, but they trapped very few flies of either sex after the change to yeast autolysate.

A new method called 'rapid molecular diagnostic technique' has been developed for the detection and identification of quarantine fruit fly species in imported fruits in Singapore (Yap et al. 2002). In New Zealand, Armstrong et al. (1997) evaluated a rapid molecular diagnostic technique for quarantine application, using simple restriction patterns of ribosomal DNA (rDNA) as diagnostic markers. The 18S and 18S plus internal transcribed spacer (ITS) regions were amplified from larval DNA by the polymerase chain reaction (PCR).

2. Delimitation

Cue lure traps could be used for infested area delimitation (Osborne et al. 1997). The captures have shown a regional variation in abundance of *D. tryoni* in the eastern states, which was in accordance with the predictions of a published bioclimatic model.

3. Monitoring

In New Zealand, a system of surveillance has been used using two types of trap, the Lynfield lure trap and the Nakagawa bait trap. The lure traps are loaded with trimedlure, cuelure or methyl eugenol and the bait traps with hydrolysed protein. Over 6900 lure and 1000 bait traps distributed throughout New Zealand are inspected fortnightly (Cowley 1990).

4. Commodities

Adult flight and the transport of infested fruits are the main means of movement and dispersal to previously uninfested areas (Rajan et al. 2004, Dominiak and Barchia 2005). Many *Dacus* spp. can fly 50-100 km.

References: 165 references retrieved, none added, 84 retained, 15 used for summary

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Dacus tsuneonis Miyake

(=*Bactrocera tsuneonis* (Miyake))
Common name(s): Japanese orange fly
Taxa: Insecta: Diptera: Tephritidae
EPPO A1 list: No. 236
EU Annex designation: I/A1

Organism

The Japanese orange fruit fly, *Dacus tsuneonis*, is one of the most important pests of citrus crops in Asia, where it predominantly occurs (Zhang 1989, Wang et al. 2009). Eggs are laid below the skin of the host fruit and larvae bore the underlying flesh. Pupariation is in the soil beneath the host plant and adults emerge in May (Zhang 1989). In China, *B. Tsuneonis* is reported to have a single generation a year and to overwinter as puparia (Zhang 1989). Yasuda et al. (1994) suggest that *D. tsuneonis* has a pupal diapause to facilitate overwintering.

1. Detection

Lin et al. (2007) developed a rapid molecular diagnostic technique for identification of *D. tsuneonis* based on PCR-RFLP analysis of mitochondrial DNA (mtDNA) and ribosomal DNA (rDNA). With four pairs of primers and five restriction endonucleases (Mnl I, Mse I, Ase I, Dra I and Ssp I), two species (*D. tsuneonis* and *Bactrocera minax*) can be discriminated according to the restriction patterns (Lin et al. 2007).

2. Delimitation

No Information available

3. Monitoring

No information available

4. Commodities

Adult flight and the transport of infested fruits are the main means of movement and dispersal to previously uninfested areas (Shutova 1970).

References: 19 references retrieved, none added, 12 retained, 5 used for summary

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Dacus zonatus (Saunders)

(=*Bactrocera zonatus* (Saunders))
Common name(s): Peach fruit fly, guava fruit fly
Taxa: Insecta
EPPO A1 list: No. 302
EU Annex designation: I/A1

Organism
The peach fruit fly, *Dacus zonatus* is a quarantine pest (Anonymous 2005). Its main hosts are guava, mango and peach and it is present throughout much of Asia and North Africa. It is not, however, present in the EU or EPPO regions.

1. Detection
The monitoring and control of *D. zonatus* is based on two strategies: (1) the Bait Application Technique (BAT) relies on protein baits (beef extract used as a source of hydrolysed protein) and (2) the male annihilation technique (Al-Eryan 2008).
Transparent bottle traps (1000 ml) were used to capture *D. zonatus.* Each bottle trap contained plywood blocks soaked in a mixture of methyl eugenol and malathion (8:1), suspended at a height of 2 m above the ground. According to Shankar et al. (2010), transparent bottle traps with 4 holes of 20 mm in size (9.37 flies/trap/week) and those with one hole (9.33 flies/trap/week) recorded the best captures. However, the influence of hole size revealed that fruit fly adults were also attracted by traps with 8 mm dia. holes (9.33 flies/trap/week), probably as a result of the quicker dispersal of methyl eugenol and the influence of weather factors. Rajitha and Shashidhar (2005) as Ravikumar and Shashidhar (2007) showed that *D. zonatus* was also attracted to red coloured traps (500 ml).

2, Delimitation
No information available.

3. Monitoring
According to Sanjeev et al. (2008), there is a positive correlation between fruit fly populations and abiotic factors (temperature, humidity and rain). Temperatures below 15°C prevent the growth and development of the fruit flies. Fruit flies capture numbers of were recorded at weekly intervals (Dale and Patel 2010) whilst in 2001, Egypt initiated a project to monitor and control this pest in the Sinai, using the male annihilation technique (MAT) (Cayol et al. 2004).

4. Commodities
In the United Kingdom, the Plant Health and Seeds Inspectorate has intercepted 21 species of non-native fruit flies on plant material imported. *Dacus zonatus* was the most frequently intercepted species. Despite the frequent interceptions on imported produce, so far no non-native tephritid species have become established in the UK (Reid and Malumphy 2009).

References: 94 references retrieved, none added, 52 retained, 9 used for summary
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(Saunders) (Diptera: Tephritidae). Egyptian Journal of Biological Pest Control 18:139-142.

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Reid, S. and C. Malumphy. 2009. Fruit Flies (Diptera: Tephritidae) Intercepted on Plant Produce Imported into England and Wales. Entomologist's Monthly Magazine 145:213-226.

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Shankar, M., S. R. K. Rao, T. Umamaheswari, and K. D. Reddy. 2010. Effect of number and size of holes of trap on capturing efficiency of Bactrocera spp. in mango. Annals of Plant Protection Sciences 18:231-232.

Daktulosphaira vitifoliae (Fitch)

(= *Viteus vitifoliae* (Fitch))
Common name(s): Grapevine phylloxera, vine louse
Taxa: Insecta: Hemiptera: Phylloxeridae
EPPO A2 list: No. 106
EU Annex designation: I/B, II/ A1I

Organism

Daktulosphaira vitifoliae is native to the eastern part United States and was introduced into France around 1860, resulting in severe economic damage to the French wine industry. It has continued to spread throughout the 20th century and in Europe only small areas remain free from phylloxera. The principal hosts are *Vitis* spp, with a strong difference in susceptibility, the American grape species *V. rupestris*, *V. berlandieri* and *V. riparia* being resistant to phylloxera. In contrast, the American grape species *V. lambrusca, V. aestivalis,* and *V. vulpina* are susceptible to phylloxera. European wine grape, *V. vinifera*, is the most susceptible. The insect shows difference in the life cycle according to the host plant. The full life cycle of *D. vitifoliae* on American *Vitis* spp. is a complex alternation between an aerial, leaf-feeding form, gallicolae, and the root-feeding form, radicicolae. On the European species *V. vinifera*, the radicicolae form predominates and the gallicolae form is virtually absent.

1. Detection

The catches were higher on sticky board traps, mounted on vineyard trellises, at 1.3 or 1.5 m above the ground; they declined as traps were mounted progressively higher above the vines (Stevenson and Jubb 1976). The current range of available early detection methods includes ground surveys and aerial imagery, and relies on detection of a 'weak spot' in the vineyard resulting from the expression of non-specific, often non-phylloxera related, plant stress (Bruce *et al*. 2009). Changes in leaf pigment content and composition associated with plant stress offers a real possibility of a phylloxera-specific detection system, a reduction in the leaf chlorophyll content, and an increase in photoprotective pigment concentrations (Blanchfield *et al*. 2007). In addition, a reduction in the ratio of linoleic acid to linolenic acid in the triglyceride component (Tucker *et al*. 2007) was observed in leaves of phylloxera-infested grape vines compared to uninfested vines. Finally, the DNA assay proved to be substantially more sensitive than a standard ground survey for detecting grape phylloxera presence on vine roots in the infested vineyard (Herbert *et al*. 2007).

2. Delimitation

No information available.

3. Monitoring

Systematic surveys were carried out in Australia to know the distribution of *D. vitifoliae* to evaluate the main factor favouring the presence of this insect and to compare with previous studies on distribution (Buchanan 1987). Yellow sticky trap mounted in the canopy of the vineyard (Herrmann and Herrmann 2003) or traps collecting phylloxera emerging from soil (Herbert *et al*. 2008) were used to monitor abundance and phenology, in order to decide control actions in the viticulture industry (Powell *et al*. 2009). Surveys to know the occurrence and distribution of *D. vitifoliae* are carried out also in south-east and east Anatolia (Gunyadin 1972) and in Northwestern Cape Province (Klerk 1972).

4. Commodities

The presence of dispersive stages of phylloxera, particularly crawlers, within the vine canopy represents risk of transfer of the insect on grapes and foliage during vintage operations. *D. vitifoliae* survived all mechanical processes including transportation, crushing/destemming, pressing and production of unclarified juice (Deretic *et al*. 2003). *D. vitifoliae* was also found during check inspections of imported plant material by the Plant Health & Seeds Inspectorate of the United Kingdom Ministry of Agriculture (Seymour *et al*. 1985).

References: 30 retained out of 121 retrieved, none added, 13 used for the summary

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Dendroctonus micans (Kugelann)

(=*Bostrichus micans* Kugelann)
Common name(s): Great spruce bark beetle
Taxa: Insecta: Coleoptera: Scolytidae
EU Annex designation: II/B

Organism

Picea spp. are the usual hosts of *Dendroctonus micans*, that can also attack other coniferous species, primarily *Pinus silvestris* in the northern part of its range and sporadically other *Pinus* spp., some *Abies* spp., *Larix decidua* and *Pseudotsuga menziesii*. With the exception of Ireland, *D. micans* is widespread throughout Europe. In the UK it was first discovered in August 1982 (Fielding et al. 1991), and in France this species has spread in many regions areas beyond its natural distribution (Rolland & Lemperiere 2004). In most part of its range, it can be considered as a secondary pest, only causing damage where trees are already stressed. The young beetles mate in their birth chambers with their sisters and brothers and continue to feed under the bark until emergence. Dispersal of young females may occur in several ways. Some individuals do not emerge but simply bore new galleries at the edge of their own birth chamber. Others emerge but remain on the same tree and establish galleries there. Some fly to colonize new trees.

1. Detection

The detection is based on visual surveying as there is no knowledge regarding the pheromone of this species. The symptoms of attack are the resin tubes produced by the boring beetles (Gilbert et al. 2001). Kairomone traps can be used instead to monitor the specific predator *Rhizophagus grandis* and thus assessing the occurrence of *D. micans* in areas under colonization or in European Union 'Protected Zones' (Meurisse et al. 2008).

2. Delimitation

After the first records in Anonymous (1982), in the following period the distribution of *D. micans* was monitored; the surveys showed that *D. micans* is gradually increasing its range (King & Fielding 1989)

3. Monitoring

Generic surveys are carried out in different countries of Europe, focusing the attention on trees weakened or damaged by natural causes such as weather.

4. Commodities

The trade of infested spruce logs with bark are the most important reason for the spread of the beetle to new areas (Evans et al. 1994)

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Dendrolimus sibiricus Chetverikov

Common name(s): Siberian silk moth, Siberian moth, Siberian conifer silk moth, larch caterpillar
Taxa: Insect: Lepidoptera: Lasiocampidae
EPPO A2 list: No. 308
EU Annex designation: II/B

Organism
Dendrolimus sibiricus is a polyphagous defoliating pest on conifers (*Abies, Pinus, Larix, Picea and Tsuga*). Outbreaks occur over large areas with a periodicity of 10–11 years. *Dendrolimus sibiricus* occupies a wide range from eastern part of European Russia to China and Korea. The first flight of adults usually occurs in the middle of July although there is a large variability. The full life cycle usually takes two years; however in southern parts of the range, one generation can develop in a single year, whereas, in northern part of the its range the completion of a generation can sometimes take three years. The larva is the overwintering stage.

1. Detection
Field trapping experiments showed the good efficiency of a mixture of aldehydes and alcohols, that can be used as a sex attractant to monitor endemic Siberian moth populations in Asia, and for surveillance and detection of the moth in countries where the insect might be accidentally introduced (Klun et al. 2000). A further study evaluated the improvement of the pheromone dispensers, results showing that the rubber septa pre-treated (zinc galvanized with copper and silver (Zn/Cu/Ag)) and stored at -80 °C were as effective as freshly treated septa, however, they should be replaced biweekly with fresh septa for optimal trap effectiveness (Khrimian et al. 2002).

2. Monitoring
Temporal (1995-1997) Advanced Very High Resolution Radiometer (AVHRR) images were used for monitoring of an infestation of a closely related species, *Dendrolimus superans sibiricus*, which occurred in 1993-1996 in central Siberia. This study could be useful in understanding the population dynamics and the environmental factors that facilitate the outbreaks –and possible spread- of this species.

3. Delimitation
Dendrolimus sibiricus is expanding its range westwards. The pheromones traps are a good method for monitoring its presence in Russia. Results obtained in 2001 show that the pest is now widespread in the Republic of Mari El (500 km east of Moscow), while isolated individuals have been trapped in the Moscow region. No *D. sibiricus* were trapped in Tver' Region (between Moscow and Saint-Petersburg) (Gninenko & Orlinskii 2002).

4. Commodities
No information available.

References : 10 retained out of 173 retrieved, none added, 5 used for the summary

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Khrimian, A., J. A. Klun, Y. Hijji, Y. N. Baranchikov, V. M. Pet'ko, V. C. Mastro, and M. H. Kramer. 2002. Syntheses of (Z,E)-5,7-dodecadienol and (E,Z)-10,12-hexadecadienol, Lepidoptera pheromone components, via zinc reduction of enyne precursors. Test of pheromone efficacy against the Siberian moth. Journal of Agricultural and Food Chemistry 50:6366-6370.

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Dendrolimus spectabilis (Butler)

Common name(s): Pine moth, Japanese pine moth
Taxa: Insecta Lepidoptera: Lasiocampidae
EU Annex designation: N/A

Organism
Dendrolimus spectabilis is a defoliator of pine that occurs primarily in the Far East, including Russia, China and Korea. Larval host include Japanese red pine (*Pinus densiflora*) and Japanese black pine (*Pinus thunbergii*) but also other species of *Pinus*, *Abies*, *Cedrus* and *Larix*. The moth can be an extremely damaging pest in outbreak years (Kokubo 1965) and can considerably retard tree growth (Satomi et al. 1997). The pest generally has one generation per year although a second generation occurs in some regions (Kokubo 1965).

1. Detection
In high numbers, infestation is readily detected visually (Kokubo 1965). A female sex pheromone has studied (Sakurai et al. 1973) been characterized as a complex blend of major and minor components ((Z,E)-5,7-dodecadien-1-ol (Z5,E7-12∶OH), (Z,E)-5,7-dodecadien-1-yl acetate (Z5,E7-12∶OAc) and (Z,E)-5,7-dodecadienal (Z5,E7-12∶Ald) and mall amounts of Z5,E7-12∶OAc, Z5,E7-12: Ald). Various blends have been field tested and shown to be effective in capturing male moths (Vu et al. 1979, Vu et al. 1980, Kong et al. 2001, Kong et al. 2003).
Although some work has been undertaken to examine the population genetics of *D. spectabilis* (Zhang et al. 2004, Nangong et al. 2009, Wang et al. 2009) identification of the pest remains reliant on morphological examination (Yamamoto 1981) as opposed to molecular/biochemical techniques

2. Delimitation
No information available

3. Monitoring
The pine moth is monitored across its Asiatic range. Details of sampling are given by Furuta (1985) where trees within quadrats were examined for the presence of egg masses and larvae. A similar method was employed by Kurananga (1975) where eggs larvae and pupae were collected from census plots to study the population dynamics of the pest. The populations *D. spectabilis* have been widely monitored in Korea (Anonymous 1976) using simple visual methods to ascertain the presence of larvae. The presence of the insects has also been monitored through frass fall collected on sheets below trees and through pesticide knockdown (Kobayashi and Yamazaki 1976). The frass fall method was indicated as a good means of estimating *D. spectabilis* population densities.
It appears that although the female sex-pheromone is characterized, there is little evidence for it being exploited as a monitoring tool for this pest

4. Commodities
No information available

References: 45 retained out of 168 retrieved, 1 added, 15 used for the summary

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Diabrotica barberi Smith & Lawrence

Common name(s): Northern corn rootworm
Taxa: Insecta: Coleoptera: Chrysomelidae
EPPO: A1 list No. 210
EU Annex designation: II/A2

Organism

Diabrotica barberi is absent in the EU and EPPO region, but widely distributed in North America. The species eggs are laid in the top 15 cm of soil, at the base of maize plants; they are the overwintering stage. They hatch over a prolonged period. The larvae develop in and on the roots, the young larvae feeding on fine rootlets and the older ones invading the root core. Pupation takes place in the soil. The emerging adults move to the maize plant, feeding on the leaves and silks.
The symptoms of larval feeding on the roots become apparent as noticeable root degradation. As a result, the force needed to pull the plant from the soil decreases (this can serve as an index of damage), and the plants have a greater tendency to lodge. The older larvae burrow in the cortical parenchyma of the roots, and then dig channels in the central vascular tissue. Tunnels in maize roots are thus a characteristic symptom, though they may be due to other species. Adult feeding does not cause any particularly characteristic symptom.

1. Detection

Horizontal starch gel electrophoresis was used to distinguish the larvae of the northern corn rootworm, *Diabrotica barberi* Smith and Lawrence, and the western corn rootworm, *D. virgifera virgifera* LeConte. In a survey of 20 enzyme systems, consistent electrophoretic differences were found in four: isocitrate dehydrogenase (IDH), esterase (EST), acid phosphatase (ACPH), and hexokinase (HK). Second and third instars of the two species were accurately identified with IDH and EST, while ACPH and HK were suitable back-up systems. The differences in these isozymes were consistent in two populations of separate geographic origin (South Dakota and Ontario). The use of electrophoresis proved to be a valuable technique for the precise identification of the NCR (northern corn rootworm) and WCR (western corn rootworm) larvae of these species, even when the specimens were damaged during extraction from the soil (Piedrahita et al., 1985).

2. Delimitation

According to Hammack (2001) one promising approach to local suppression/eradication of *D. barberi* is the exploitation of insect behaviour-modifying chemicals. These include cucurbitacin feeding stimulants and host plant kairomones or their analogues. Because most crop damage from corn rootworms is a result of larval feeding, kairomones attracting adults could be most effectively used to monitor the potential for damaging larval populations in subsequent growing seasons or to interfere with reproduction by mass trapping or annihilation techniques. Several blends of cucurbit blossom volatiles are known to produce a synergistic increase in capture of diabroticite beetles. These blends include equal-weight mixtures of veratrol, indole, and phenylacetaldehyde (VIP) for SCR (southern corn rootworm) and 1,2,4-trimethoxybenzene, indole, and (E)- cinnamaldehyde (TIC) for all three diabroticite beetles species. The author present results of a study in which primarily maize terpenoids, GLVs, and blends of these chemicals for attractiveness to WCR and NCR beetles were tested. The main goal was to assess the blending of individual attractants as a means of improving lure efficacy, emphasizing inclusion of maize headspace volatiles in test blends

(Hammack, 2001).
Due to results of a later study, Hammack 2003 confirms that despite phenological limitations attractants can be used to concentrate adult beetles within field areas. It is suggested to move preovipositional females in margins or other field areas where a variety of corn rootworm control strategies could be applied.

3. Monitoring
Studies were conducted using sticky traps and corn rootworm traps in fields of maize (*Zea mays* L.) after silking had occurred to determine effect of trap color, olfactory attractants, and type of toxic bait dispenser on captures of adult corn rootworm beetles. Yellow traps captured the greatest numbers of *D. barberi*. Tests comparing commercial brands of sticky traps showed that Multigard traps captured more male *D. barberi* than Pherocon AM traps. Capture of *D. barberi* in corn rootworm traps (vial-type traps baited with toxin and feeding stimulant) did not vary with color or attractant (MPE) (Hesler and Sutter, 1993).
Hoffmann et al. (1996) conducted trials in pumpkin fields to test the efficiency of different trap types and positions within the canopy to cucumber beetle and corn rootworm species. Results show that traps with TIC lures captured more beetles than unbaited traps for all species and the greatest response to the lure TIC, varying by time of day, occurred around midday.
A study by Losey et al. (2003) compared common and less well established monitoring methods for *Diabrotica* beetles in maize. A common currently used sampling method involves systematically covering most of the field following a "W" pattern. The feasibility of replacing the current sampling pattern with a simpler and less time-consuming transect (straight line) pattern was assessed. When sampling methods were compared, computer simulations demonstrated that treatment decisions based on transect sampling would have an acceptably low error rate averaging 10% over a range of realistic corn rootworm densities (0-2 adults per plant). Field trials using transect, systematic, and random sampling in each field were used to compare the categorization of adult corn rootworm densities into "above" or "below" threshold with a sequential sampling plan. Efficiency measured in time to reach a decision, number of corn plants evaluated, and time divided by plants observed were compared between sampling methods. The three methods did not differ significantly in the number of plants evaluated or in the categorization of corn rootworm populations. Transect sampling resulted in a significantly shorter time divided by plants observed (38 s), than either systematic (78 s), or random sampling methods (166 s) (Losey et al., 2003).
Nowatzki et al. (2002) developed and validated species- and sex-specific prediction models for the seasonal emergence of corn rootworm beetles in Iowa. The models were fit with a 3-parameter Weibull function using emergence data collected in 57 Iowa cornfields over 5 yr. and were validated with emergence data collected in 21 additional fields from a separate year. As a result, these models do not eliminate the monitoring for adult corn rootworms but should improve the scouting efficiency by allowing growers to focus to work to peaks in population.

4. Commodities
No information available.

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Diabrotica undecimpunctata howardi Barber

Common name(s): Spotted cucumber beetle
Taxa: Insecta: Coleoptera: Chrysomelidae
EPPO A1 list: No. 292
EU Annex designation: I/A1

Organism
Diabrotic undecimpunctata howardi is a polyphagous beetle pest . Adults attack many cultivated plants including most characteristically Cucurbitaceae (e.g. *Cucumis sativus*, *Cucumis melo*, *Cucurbita pepo*, *Citrullus vulgaris*) but also groundnut (*Arachis hypogea*), soybean (*Glycine max*), *Phaseolus vulgaris* and other legumes, maize (*Zea mays*), sweet potato (*Ipomoea batatas*). If flowers are present, adults will feed on them rather than leaves causing reductions in fruit yield. If flowers are not available, adults prefer the foliage of cucurbits to other crops. The larvae feed mainly on the roots of maize, but can also feed on various other plants (cucurbits, legumes, sweet potato, weeds).
The species is absent within the EU and EPPO region, but widely distributed in North America.
After overwintering under leaves in woodland, the adults become active in the spring and feed on the flowers and foliage of many different host plants, fly to cucurbits as soon as they become available. Females lay 200 to 1,200 eggs singly in the soil, close to the bases of larval host plants. The larvae hatch after 7-10 days and bore into the roots of their usually poaceous hosts where they feed for 2-4 weeks, passing through three instars. During the latter part of the third instar, the larvae leave the host plants, burrow into the soil and enter the inactive or prepupal stage of the larval period which usually lasts 6-8 days. Pupation takes place in an earthen shell and lasts 6-12 days.
Adults of the new generation often move from one host to another, starting on the larval host maize (silks) or groundnut and moving onto the cucurbit hosts in mid-summer, and finally onto subsidiary hosts. The beetles feed until temperatures force them to become inactive.
Infested maize plants usually show the effect of larval infestation of the roots when they are 20-50 cm tall. Plants grow poorly, becoming stunted and yellow, but may survive and still produce grain. If the stem is attacked, internal drilling causes the bud to wither and die. Infested cucurbits show adult feeding holes in the leaves and scars on runners and young fruits. Scarring in the crown of the plant is also typical of adult damage (EPPO Data sheets on quarantine pests, *Diabrotica undecimpunctata*).

1. Detection
No information available.

2. Delimitation
The attraction of *Diabrotica* spp. to single-component and multicomponent lures was evaluated in maize fields in Illinois by comparing the relative number of beetles caught on sticky traps over a 24-h period. Initial tests in 1985 showed that a mixture of veratrole (V), indole (I), phenylacetaldehyde (P), trans-anethole (A) and eugenol (E) (=VIPAE mixture) caught 26 times as many *D. undecimpunctata howardi* adults as did untreated control traps and was at least 3 times

as active as any of the traps baited with a single component. Additional tests showed that traps baited with 3-component VIP mixture (veratrol, indole and phenylacetaldehyde at 20 mg per component) caught about 8 times as many beetles as the expected mean additive response with the individual components; therefore, the *D. u. howardi* response was synergistic (Lampman and Metcalf, 1987a).

3. Monitoring

Hoffmann et al. (1996) conducted trials in pumpkin fields to test the efficiency of different trap types and positions within the canopy to cucumber beetle and corn rootworm species. Results show that traps with TIC lures captured more beetles than unbaited traps for all species and the greatest response to this lure occurred around midday.

Whitworth et al. (2002) conducted studies to compare various sampling methods, traps, and trap components for capturing three species of adult corn rootworms in Kansas corn and soybean fields during 1997. Results showed that lure constituents affected the species of beetle attracted to the trap. Traps containing trans-cinnamaldehyde were most attractive to southern corn rootworms. Multigard sticky traps caught more beetles than Pherocon AM sticky traps. Also, lure-baited sticky traps caught more beetles than nonbaited sticky traps. Varying the color of the lure trap bottom did not affect the number caught.

In a study by Jackson et al. (2005) seven kairomone formulations (Trece, Inc., Salinas, CA) were evaluated for their effectiveness as attractants for luring three species of cucumber beetles into Pherocon CRW traps (Trece, Inc.) in cucurbit and sweetpotato fields. The most efficient lures for D. undecimpunctata howardi were TRE8276 (TIC mixture: 500 mg of 1,2,4-trimethoxybenzene, 500 mg of indole, and 500 mg of traps-cinnamaldeyde) and TRE8336 (500 mg of 1,2,4-trimethoxybenzene, 500 mg of traps-cinnamaldeyde, 500 mg of 4-methoxyphenethanol).

More field tests implementing three trap attractants, 1,2,4-trimethoxybenzene, indole and trans-cinnamaldehyde (TIC), trans-cinnamaldehyde, alone, and sex pheromone (10-methyl-2-tridecanone), were conducted in peanut in 12 locations in southeastern Virginia by Herbert et al.,1996. As aresult pheromone traps caught more beetles than TIC or cinnamaldehyde on most sample dates and at most locations. Pheromone traps detected 2 distinct beetle peaks, the first between 16 and 23 June, and the second between 21 and 28 July. Thus the use of pheromone traps could allow for a more precise timing of insecticide applications.

Also investigations using yellow sticky traps without lure in on muskmelon, *Cucumis melo*, field studies were conducted by Lam in 2007.

4. Commodities

No information available.

References: 13 retained out of 86 retrieved, none added, 5 used in summary

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Diabrotica undecimpunctata undecimpunctata Mannerhein

Common name(s): Spotted cucumber beetle
Taxa: Insecta: Coleoptera: Chyromelidae
EPPO A1 list: No. 292
EU Annex designation: I/A1

Organism
Diabrotica undecimpunctata undecimpunctata is a subspecies *of D. undecimpunctata howardi*. The Western Spotted Cucumber Beetle is found only in the USA in Arizona, California, Colorado and Oregon .The species is considered a major pest of many field crops including cucumbers and other squashes, corn, soy. Adults are also reported damaging to garden plants including hibiscus, roses.
The species overwinters as an adult in southern states. Eggs are laid at the soil surface or below at the base of food plants. Larvae hatch in 7 - 10 days and feed for three to six weeks. The larvae pupate at the base of host plants and emerge as adults in 1-2 weeks. They feed on the roots of their hosts and pass through three development stages (instars) over about 2–4 weeks. They then pupate in the soil; adults emerge about 1–2 weeks later. In cooler climates the adult beetles overwinter at the base of their preferred host plants (usually Cucurbitaceae), or in leaf litter or other protected places. They become active again in the spring and lay eggs. In cool climates there may be only one generation a year; in warm climates or in protected cultivation there may be 2 or 3 overlapping generations.
The damage to host plants consists of feeding on fine rootlets by the young larvae; older larvae tunnel in roots from where they may enter the stalk to feed in the crown of young plants. Root systems are reduced and affected plants appear stunted and chlorotic. Severely infested plants may die. Adults feed mainly on leaves, flowers and fruits, causing scarring and shot-hole damage; severely affected leaves may fall prematurely. The yield and market value of the crop are reduced. Beetles also transmit crop diseases such as bacterial wilt.

1. Detection
Sticky traps with and without the attractant TIC (1,2,4-trimethoxybenzene, indole, and trans-cinnamaldehyde) were evaluated in cucurbits for capture of striped cucumber beetle, Acalymna vittatum, spotted cucumber beetle, *Diabrotica undecimpunctata*, western com rootworm, *Diabrotica virgifera virgifera*, and northern corn rootworm, *D. barberi* Smith & Lawrence, in New York and western striped cucumber beetle, *Acalymna trviittatum* (Mannerheim), and western spotted cucumber beetle, *Diabrotica undecimpunctata undecimpunctata*, in California. Increases in TIC per trap resulted in increases in capture of western spotted cucumber beetle. Yellow traps captured more striped cucumber beetle and western spotted cucumber beetle than did white traps. For cucurbits with short plant canopies, traps were most effective when placed close to the ground. In pumpkins with taller canopies, traps at canopy and mid-canopy height captured the most beetles. Traps replaced 2 times per day had greater captures than traps replaced 1 time per day or 1 time every 2 d (Hoffmann *et al*., 1996).

2. Monitoring
No information available.

3. Delimitation
No information available.

4. Commodities

No information available.

References : 2 retained out of 23 retrieved, none added, 1 used for summary

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Diabrotica virgifera virgifera LeConte

Common name(s): Western corn rootworm
Taxa: insecta: Coleoptera: Chrysomelidae
EPPO list A2: 199
EU Annex designation: I/A2

Organism

The western corn rootworm (WCR) *Diabrotica virgifera virgifera* is endemic in North America and invaded Europe since 1992 (Baca 1994). Its spread began in Serbia from where the coleopteran species, which is characterized by the ability to flight over distances to 40 km and a high reproductive rate, continuously disseminated over Europe. At present it is established in many European countries, particularly in the more continental areas of Central Europe. The species hibernates as eggs, which are laid in soil up to a depth of 15 cm at the base of maize plants. Larval hatching proceeds over a prolonged period. The larvae develop in and on the roots, the young larvae feeding on fine rootlets and the older ones invading the root. Pupation takes place in the soil. The emerging adults move towards the maize plant and feed on leaves, tassels and pollen resulting in significantly reduced seed development. This species has only one generation per year. Tunnels in maize roots are thus a characteristic symptom.
Severe damage to the host plant is caused by the intensive feeding of the larvae in the roots and stem, resulting in reduced plant growth. In the USA, it has been estimated that the financial loss due to corn rootworms in terms of treatment costs and crop losses amounts to 1000 million USD annually (Krysan & Miller, 1986).

1. Detection

The treatment of species which are estimated to be a phytosanitary risk for the whole community are regulated the EC Decisions. Basing on this, time limited emergency measures for *D. virgifera virgifera* are to be carried out by all Member states. In compliance with Article 2 of the EC Decision 2003/766/EC and Article 4b of EC Decision 2006/564/EC official surveys regarding the presence of *D. virgifera virgifera* are required to be conducted within EU Member states. Investigations include the intensive survey of potential entrance places for the species e.g. airports, sea ports, trans-shipment locations, etc.
Through applying the EU Decision, reports of this species' spread resulting from local detection surveys in the recent years are numerous. A summary is provided in Baufeld (2009).

2. Monitoring

During a survey in the UK in 2003 for *D. virgifera virgifera* 80 sites were surveyed. The number of beetles caught from 2003 to 2006 was 92, 87, 481 and 27 respectively. The UK was shown to have a limited outbreak in regions to the south and west of London. The spread and population growth of the beetle appears to have been restricted by a combination of crop rotation and the sub-optimal climatic conditions in the UK (Eyre *et al*., 2007).
Based on monitoring conducted in 1997, the occurrence *of D. virgifera virgifera* in Bosnia and Herzegovina was first documented. The pest is distributed throughout the north eastern and northern parts of the country. An accurate estimate of the first time that the pest entered the country is not known, but it is assumed that it appeared in 1995 in the area of Bijeljina from where it has spread towards the western part of Bosnia and Herzegovina. Currently, the pest is spreading rapidly, and if effective measures for eradication are not taken within a short time period it will have spread throughout this country (Festic *et al*., 1998).
A study was initiated to survey for the damage caused by western corn rootworm on maize. Therefore, in 2006 trials were carried out at Iregszemcse in a maize field with a history of

monoculture and row-treated with the soil insecticide Force 1.5 G, followed by laboratory testing of the collected grain samples. Two plots were designed for row-treated and untreated maize plants. The surveys covered the observation of the seasonal flight, damage by beetles, lodging and root injury of maize plants, as well as the determination of weight and chemical composition of ears taken from different plots. During the study, in addition to leaf injury (around 6%), stigma damage (around 8%) was also recorded, though the mass flight of beetles occurred after the flowering of maize. Surveys confirmed the importance of soil treatment for the crop grown in monoculture. There was a significant difference between the plants of the row-treated and untreated plots in lodging and root damage according to the modified Iowa-scale. A statistically confirmed, significant difference was observed among the weight of ears originating from different plots ($P<0.000\ 1$). The increase of grain: cob ratio (the average difference of grain: cob ratio was 8.05%) and forced ripening (average water content (%): s=28,20; k=26,25) due to pest injury were verified. However, laboratory tests did not confirm that the chemical composition of grains would be adversely affected by crop injury (Keszthelyi *et al*., 2007).

As a result of monitoring of the occurrence of Western corn rootworm beetle conducted by the State Plant Health and Seed Inspection Service and Institute of Plant Protection in 2006, the pest was discovered in further spots in Poland. The Western corn rootworm beetle was spotted in 8 provinces.. Over 17,100 adult beetles were caught in about 250 locations, with the majority of them found in Podkarpackie voivodeship. The Plant Protection Institute in Poznan initialised a research programme for the pest and also engaged in work towards the authorization of plant protection agents for use against that pest. The pest control activities implemented to restrict spreading of *D. virgifera* beetle are currently conducted according to the principles defined in the Decree of the Minister of Agriculture and Rural Development from 13th April 2004 on detailed procedures to be followed in control and preventing the spread of Western corn rootworm beetle (Official Gazette Dz. U. 2004 No. 82, item 763). In connection with the rapidly enlarging area of the beetle occurrence in Poland and the inability to eliminate that pest completely, it became necessary to develop effective methods of controlling that pest in existing conditions. The Ministry of Agriculture and Rural Development appointed a team for preparing a new concept of measures to be taken in order to restrict the spreading of Western corn rootworm beetle and its control in areas where it occurs already. The work of this team led to the development of new regulations for controlling that pest in Poland (Konefal *et al*., 2007).

3. Delimitation

After the establishment of *D. virgifera virgifera,*a number of pest management options are available. As outlined before, prevention and control measures, including field hygiene, avoiding maize monocultures, development and culture of resistant maize hybrids, biotechnical measures and chemical control by pesticides when not to avoid are appropriate delimitation measures.

Where heavy infestation occurs, a buffer area round the affected site should be established to contain the pest by re-introducing a rotation of winter cereals or ryegrass before another maize crop, which should be sown as late as possible. It is anticipated that the larvae will thus be starved to death before the new maize crop emerges. For both environmental and economic reasons, it is inadvisable to try to control the pest with insecticides (Furlan *et al*. 2003).

Without any chemical pesticides or GMO use, the Swiss government mandated adherence to strict crop rotation. In addition to the economic benefits of this relatively simple approach, the environment al impact of widespread use of insecticides was limited (Hummel & Bertossa, 2009).

With prior experience gained in Illinois, USA, and the subsequent largely unsuccessful efforts to stop the pest in Europe, eradication efforts, although attempted in France and in the Veneto region of northern Italy,

are not a viable sustainable strategy for the future nor a long term solution. Crop rotation is one of the best cultural management options for controlling this pest although there are limitations to its effectiveness (Shaw *et al*. 1984, Kiss *et al*. 2005). Preventive and agronomic controls, application of soil insecticides (granulates) and seed treatments, application of insecticides, biological regulation and breeding improvements will help to contain this pest (Schwabe *et al*. 2010).
More methods are currently under discussion in Germany, e.g. intensive monitoring using PAL traps, modelling of population dynamics and trans-shipment locations e.g. container ports. Such areas should be surrounded up to a 1 km radius with maize cultivation in rotation. Thus maize would be present as a trap plant to provoke oviposition in this respective zone. Through crop rotation this procedure leads to the possibility that infestations can be eradicated early (personal communication with JKI expert at 05.01.2012).

4. Commodities

No information available.

References: 68 retained out of 483 retrieved, 1 added, 12 used in summary

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Diabrotica virgifera zeae Krysan and Smith

Common name(s): Mexican corn rootworm
Taxa: Insecta: Coleoptera: Chrysomelidae
EPPO A1 list: No. 292
EU Annex designation: I/ A1

Organism

The major host plant of *D. virgivera zeae* is maize. In addition, other plants e.g. Poaceae and members of the families Cucurbitaceae, Fabaceae and Asteraceae are also attacked. The species has been reported from North and Central America.

They are laid from late summer to autumn in the soil, usually near the base of host plants. Each female can lay 200–1200 eggs. The eggs overwinter and typically hatch over a prolonged period from spring to summer. Larvae are 10–18 mm long, yellowish-white with a brown head capsule and three pairs of very short legs. They feed on the roots of their hosts and pass through three development stages. They then pupate in the soil; adults usually emerge in summer or autumn. There is only one generation a year, but completion of the life cycle depends on temperature, food quality and other environmental factors. Young larvae feed on fine rootlets; older larvae tunnel in the roots. Root systems are reduced and affected plants appear stunted, chlorotic and are prone to lodging; severely infested plants may die.

1. Detection

In 2000 and 2001 the influence of distance on rootworm captures by CRW and Pherocon AM traps was assessed. The fidelity of trap captures to population estimates from visual counts of beetles on plants (whole plant samples), and the seasonal population patterns was indicated by each sampling method (Spurgeon *et al*., 2004). According to the authors, the results of the study show that monitoring does not require trap placement far into the field.

Aiming at the development of non-pheromonal attractants for *D. virgifera zeae,* ten volatiles or blends of volatiles that are known attractants of other *Diabrotica* spp. were tested in the field. Traps baited with 100 mg of the attractants captured significantly more male and female *D. v. zeae* than unbaited traps, but the increase in capture was greater for females than for males. Traps baited with a 1:1:1 mixture of trimethoxybenzene, indole and cinnamaldehyde ('TIC') captured the greatest number of females (a 50-fold increase compared with capture on unbaited traps) but did not capture significantly more adults than traps baited with a 1:1:1 mixture of veratrole, indole and phenylacetaldehyde ('VIP') or with indole alone (Lance *et al*., 1992).

A study carried out by Clark *et al*. (2001) was conducted to determine whether 12 pest and non-pest *Diabrotica* species could be separated using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP). To permit genotypic characterization microsatellite markers were used. The described CTAB procedure was used to isolate a similar quality and quantity of DNA as can be obtained by using commercial kits but was less time consuming and expensive. The DVV-D2 loci, a characteristic microsatellite of *Diabrotica* genus, was amplified (Barragan-Valencia *et al*., 2009). For more results regarding polymorphic microsatellite loci for *D. virg. zeae* see Waits and Stolz (2008).

2. Monitoring

No information available.

3. Delimitation

No information available.

4. Commodities

No information available.

References: 7 retained out of 45 retrieved, none added, 5 used in summary

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Diaphorina citri Kuwayana

Common name(s): Citrus psyllid
Taxa: Insecta: Hemiptera: Psyllidae
EPPO A1 list: No. 37
EU Annex designation: II/A1

Organism

Diaphorina citri is a pest of *Citrus spp.*, but recorded also on other Rutaceae. *D. citri* occurs primarily in tropical and subtropical Asia. In the last decade it has also been recorded in Saudi Arabia and parts of South and North America, Mexico, and the Caribbean. Eggs are laid on the tips of growing shoots in between unfolding leaves. Females may lay more than 800 eggs during their lives. Nymphs pass through five instars. Total life cycle requires from 15 to 47 days, depending upon the season. The greatest economic impact caused by this pest it is the ability to transmit three phloem-limited bacteria in the genus Candidatus Liberibacter. These bacteria are presumed to be the causal agents of huanglongbing (HLB) disease, also known as citrus greening.

1. Detection

Diagnostic characters are provided to distinguish *Diaphorina citri* from *Trioza erytreae*, another important citrus psyllid pest, with which it may be confused (Mead 1977). Yellow traps are an efficient system to detect the psyllid (Hall 2009), and trials with traps of different yellow intensity did not highlighted significant difference (Hall *et al*. 2010); no difference were found also between yellow and blue traps (Hall *et al*. 2007). The traps installed at 1.00-1.50 in height showed higher catches than those installed on the ground and at 0.50 and 2.00 meters in height (Chiaradia *et al*. 2008).

2. Delimitation

After the first detection of *Diaphorina citri*, the attention was addressed to evaluate the methods to manage the incursion (Grimshaw *et al*. 2006).

3. Monitoring

Specific surveys (Divender and Ranjeet 2000, Augier *et al*. 2006) and generic survey concerning pests of citrus (Whittle 1992) have been carried out both at national and regional level. Other aspects have been evaluated on the alternate hosts of the psyllid (Lim *et al*. 1990) and its natural enemies (Ameri *et al*. 2006). Monitoring to assess the simultaneous presence of *Diaphorina citri* and the incidence of citrus huanglongbing are becoming increasingly common (Bove 1986, Miyakawa and Tsuno 1989, Koizumi *et al*. 1997, Meyer *et al*. 2007). Both yellow sticky traps and stem-tap sampling (adults falling into a pan after three rapid taps to a branch) consistently provided the desired average precision level (Hall and Hentz 2010).

4. Commodities

Diaphorina citri was found commonly in the fresh foliage of Rutaceae species (especially curry and kaffir lime) that are regularly imported into Britain from outside the European Union (EU) for culinary purposes (Malumphy 2011). Cold or gamma irradiation treatments have been proposed against this pest (McGuire 2000). Another kind of commodities on which the presence of *Diaphorina citri* is found is unprocessed fruits.

References: 40 retained out of 183 retrieved, none added, 12 used for the summary

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Diaporthe vaccinii Shear

Common name(s): Phomopsis canker and dieback, twig blight, fruit rot (and others)
Taxa: Fungi: Ascomycota: Diaporthaceae
EPPO A1 list: No. 211
EU Annex designation: II/A1

Organism
Diaporthe vaccinii is recorded on the stems, shoots and leaves of several cultivated *Vaccinium* species (blueberry, cranberry) and on indigenous species of European *Vaccinium*. The fungal disease can cause severe twig blight and fruit rot resulting in high yield losses under favourable conditions. It is common in temperate climate areas of North America (Canada and USA) and has been reported in Europe: UK, Romania, Lithuania, the Netherlands and Germany (Anonymous 2004; 2009; 2010).

1. Detection
If suspicious symptoms with fruiting bodies are observed on diseased parts of *Vaccinium* spp., morphological identification is possible. But, given the variable nature of *Diaporthe vaccinii* morphology and its morphological overlap with other *Diaporthe* species, it should be confirmed by molecular methods. Identification protocol by sequencing the rDNA internal transcribed spacer region has been developed (Farr, Castlebury *et al*. 2002; Kacergius and Jovaisiene 2010) using a pure culture of the fungus isolate.
A positive diagnosis can be done on infected plants by direct tissue blot immunoassay and plate-trapped antigen ELISA (Gabler, Kacergius *et al*. 2004).

2. Delimitation
No information available

3. Monitoring
In Lithuania, after *Diaporthe vaccinii* detection and infested plants destruction, the NPPO started a monitoring program on several cultivated *Vaccinium* species (2004)

4. Commodities
Export of infected vines from North America to other countries has been the main source of vine infection at new sites. Since *Diaporthe vaccinii* occurs as an endophyte in apparently healthy plants, only certified as disease-free plants should be imported (Smith I.M. 1992).

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Diaspidiotus perniciosus (Comstock)

(=*Quadraspidiotus perniciosus* (Constock))
Common name(s): San José scale
Taxa: Insecta: Hemiptera: Diaspididae
EPPO A2 list: No. 117
EU Annex designation: II/B

Organism
Diaspidiotus perniciosus attacks many fruit trees (apple, peach, pear, plum and *Rubus*), moreover it has also been reported on other 150 deciduous trees and shrubs. *D. perniciosus* is indigenous to Eastern Asia and has spread to many parts of the world. In Europe this scale has established in most countries with the exception of UK, Belgium and the northern countries. Most individuals of *D. perniciosus* overwinter in the 1st larval stage, and the remaining few as gravid adult females. The species completes three to four generations per year in more southern areas and only one in the more northerly parts of Europe and Mediterranean region. Adult males, which have limited flight ability, are mostly carried by wind currents and by birds.

1. Detection
Pheromone trapping techniques for male *D. perniciosus* were evaluated in numerous studies. The component of pheromone 7-methyl-3-methylene-7-octenyl propanoate, its isomer and various homologues, were synthesized and tested showing a good trapping efficiency. The best mixture of pheromone components varies slightly in different trials (Anderson et al. 1981, Hoyt et al. 1983, Sazo R and Campos S 1983, Smetnik et al. 1983, Bichina et al. 1984). Others factors are the optimum number of traps per hectare (from 1 trap/2 ha in old dense orchards to 1 trap/5 ha in young, well-ventilated orchards (Smetnik et al. 1986), the trap type (roof- and funnel-shaped traps work better than cylindrical one; the trap with white light caught more males of *D. perniciosus* than that with ultraviolet light (Bichina et al. 1984); white trapping plates measuring 5x10 cm were more efficient than plates of other colours and dimensions (Kozar 1973), the trap position (the number of male was greatest at 2 m above ground level than at 3 m or 4 m; traps outside of orchards caught more than inside (Smetnik and Konstantinova 1983). In the most part of cases the first records of *D. perniciosus* is due to generic surveys (Mourad et al. 2008, Koehler and Nussbaum 2009). A difficult issue is the identification of the species as the pheromone is attractive to several other scale species; a randomly amplified polymorphic DNA (RAPD-PCR) is used to identify six European *Diaspidiotus* species (Frey and Frey 1995).

2. Monitoring
Monitoring of *D. perniciosus* was carried out in different trials in Switzerland (Bloesch and Staubli 1992) and a relation between pheromone trap captures and crawler densities (Badenes-Perez et al. 2002) was found. Phenology was also studied during the monitoring (Hippe and Mani 1995).

3. Delimitation
In Switzerland, data from pheromone-trap were used to delimit the area of *D. perniciosus*, and to evaluate the proportion of regions with tree-fruit production that were still free from *D. perniciosus* (Schaub et al. 1995).

4. Commodities

The trade of fruit trees for planting and fruit are the most important reason for the spread of the scale to new areas (Dickler 1975, Bogs 1977, Lucke 1990). Fumigation (Bogs 1977) and cold storage (Dickler 1975, Chu 1992) are two possible treatments for the prevention of the introduction of *D. perniciosus.* (Gonzalez and Barria P 1983) discussed the problem associated with the occurrence of the morphologically similar scale insect *Diaspidiotus ancylus* on export apple. Consignments of fruit have been wrongly rejected at ports of entry because of the presence of this species, which live on other hosts. Again, correct identification is essential.

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Didymella ligulicola (K.F Baker, Dimock and L.H. Davis) von Arx

Common name(s): Ray (flower) blight of chrysanthemum
Taxa: Fungi: Ascomycota: Dothideomycetidae
EPPO A2 list: No. 66
EU Annex designation: II/A2

Organism

Didymella, in addition to chrysanthemums, may also infect endives, globe artichokes, lettuces, dahlias, sunflowers, *Rudbeckia* and *Zinnia*. Originally found in North America, the disease has been recorded in many European countries (Fox 1998). All plant parts, including roots, may be attacked, but flowers and cuttings are particularly susceptible.

Didymella ligulicola has a relatively low dispersal potential on its own, but can be transmitted by infected cuttings, plants and flowers of chrysanthemums. Earth attached to roots can also be a source of inoculum. The fungus can develop under a wide range of conditions and, once established, is both difficult and costly to eradicate (Smith, 1992). A computer simulator of chrysanthemum ray blight epidemic development has been used to predict disease outbreaks, could be helpful for monitoring (McCoy *et al*. 1972; McCoy 1977).

1. Detection

Detection is mainly based on visible symptoms although flower and leaf pathologies, as well as those of rotting of cuttings may be confused with other fungal infections. Pethybridge *et al*., (2006) after identifying a *Phoma* species as the anamorph of *Didymella ligulicola* (Pethybridge and Wilson 1998), described a PCR test developed to detect *Phoma ligulicola* in pyrethrum seeds (Pethybridge, Hay *et al*. 2006).

2. Delimitation

No information available

3. Monitoring

No information available

4. Commodities

The fungus can be transmitted by cuttings, plants and flowers. In Slovenia, *Didymella ligulicola* was probably introduced via infected cuttings showing no symptoms at the time of import. The occurrence of the fungus was monitored throughout the whole growing season (Munda and Zerjav 2003).

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Ditylenchus destructor Thorne

Common name(s): Potato tuber nematode, potato rot nematode
Taxa: Nematoda: Tylenchida: Anguinidae
EPPO A2 list: No 123 (deleted 1981)
EU Annex designation: II/A2

Organism

A plant-pathogenic nematode that primarily attacks potato but can also be found affecting garlic, groundnuts, carrots and iris. Infected potatoes produce characteristic discoloured and sunken tubers with wrinkled skin (Anonymous 1975). Aerial parts are frequently asymptomatic in potato although Iris and tulips may show poorly developed leaves. It has a wide geographic distribution and is found throughout the European territory. The nematode has a requirement for cool, moist soil, factors that limit its economic impact on potato production in southern Europe.

1. Detection

Detection of the potato rot nematode can be readily made though physical extraction from sampled substrates (plant material, tubers soil) using sieving and flotation methods (Jordaan *et al*. 1992, Ebrahimi *et al*. 2004, Rajan and Arjun 2006, Svilponis *et al*. 2008). Diagnosis of the nematode can be made through morphological traits (Darling *et al*. 1983, Jones and De Waele 1988, Rajan and Arjun 2006) whilst molecular diagnosis of the nematode is possible using PCR-based assays (Eycken *et al*. 1994, Liu *et al*. 2007). Similarly, molecular techniques can be used to differentiate closely related species of *Ditylenchus* (Subbotin *et al*. 2005). The differentiation of *D. destructor* from closely related species (such as *D. destructor* and *D. myceliophagus*) can also be readily made on the basis of morphological traits that are readily observed under light microscopy and described by Escuer (1998). The physical characteristics of infestation can be used for detection purposes on a number of crops (Protopopov 1971, Bolton 1989, Anwar *et al*. 1992). As the pest has a global distribution, most surveying efforts are given over to monitoring numbers within areas of known occurrence (see details below).

2. Delimitation

No specific information found on delimitations as its specific aim although the need to delimit alien nematodes (*D. destructor* and others) is highlighted by a number of authors (Escuer 1998, Bello *et al*. 2005).

3. Monitoring

A number of procedures for the monitoring for this nematode pest have been described. These typically involve sampling infested plant material and/or soil and subsequently extracting nematodes for identification using the methods described above. Examples of sampling methodologies are described by several authors (Bolton 1989, Venter *et al*. 1992, Ebrahimi *et al*. 2004, Tanhamaafi *et al*. 2005) with extensive details of sampling procedures described by Garcia Alvarez *et al*. (2005) and Jordaan *et al*. (1992). In the case of potatoes, the best example in the literature examined, describes how soil was sampled at 1.5 M intervals along transects 4 M apart within the fields under examination (Garcia-Alvarez *et al*. 2005). In many cases, both soil and tissue (e.g. tuber, root) samples are collected concurrently with a very good description of sampling practices provided by Jordaan *et al*. (1992) where multiple collections were taken within 10 x 10 m quadrats within cereal fields were combined to form a single sample. A more limited description of plant sampling in groundnut is provided by Venter *et al*. 1992. Techniques for collecting plants are

described by Kornobis and Wolny (1997). The long-term monitoring of *D. destructor* in Estonia that has been ongoing since the 1950s and has used the methods and sampling techniques (soil, roots, tubers and aerial parts of the plant) described above (Svilponis *et al*. 2008). Notably, this paper indicated that sampling of the aerial parts of the plant rarely yields nematodes and sampling tubers is much more reliable.

4. Commodities

The widespread trade of seed and ware potatoes necessitates the need for extensive surveying of potatoes as a commodity. Surveying seeds and contaminating soil and packaging associated with them is described by Rajan and Arjun (2006). Seed-potato tuber sampling is described by Stefan (1999) whilst procedures for seeds, bulbs, corms and tubers are detailed by Esser (1996). In the latter example, seeds were collected randomly with ca. half a cup of seed sampled per lot. For bulbs, corms and tubers, individual lots were sampled and diseased examples were searched for and sampled. In addition, random healthy material was also sampled and subjected to extraction and identification of the nematodes. Similar procedures are described for potatoes by Karnowski (1999) where it is indicated that randomly selected bulbs should be cut across the heel to observe the brown concentric rings associated with infestation as an immediate indicator of infection. Other samples can be macerated and extracted using the Baermann funnel method.

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Ditylenchus dipsaci (Kuhn) Filipjev

Common name(s): Stem nematode, stem and bulb eelworm, onion bloat
Taxa: Nematoda: Tylenchida: Anguinidae
EPPO A2 list: No 174
EU Annex designation: II/A2

Organism

A pathogenic nematode that attacks over 450 plant species. Biological races occur that can be either highly polyphagous or very host-specific (Dropkin 1988). The nematode is primarily found on faba beans, leek, garlic, lucerne, maize, oats onions, peas, potato, rye, strawberries, sugar beet, tobacco, as well as a range of other crops and ornamentals, particularly, narcissi. *Ditylenchus dipsaci* is found across most temperate regions of the world, including most of the EU territory. Damage can take the form of stunted growth, wilting, discolouration and leaf distortion, depending on the plant infested, and crop loss can be total in heavy infestation (Dropkin 1988, Greco *et al*. 1991).

1. Detection

Although symptoms can be an indicator of the presence of *D. dipsaci*, correct identification can only be achieved with a degree of certainty through returning material to the laboratory (Rajan 2006). The presence and identity of the nematode can be detected using a number of techniques. In the case of seeds, submersion in water for 24 hours at 10°C causes the nematodes to egress from tissue and facilitates collection (Augustin and Sikora 1989); the nematodes are usually identified using readily identifiable taxonomic traits. Similar methods are described for non-seed plant material (Abbad Andaloussi and Bachikh 2001). Plant material can be extracted for nematodes using the standard Baermann funnel apparatus followed by sieving (Tenente *et al*. 2000, Milano de Tomasel and McIntyre 2001). Centrifugation and floating-off (in, for example, sucrose solution) nematodes is also used as a means of collecting *D. dipsaci* from infested material (Park 2005). The planting (in sterile soil) and growing-on of suspected infested seeds has also been shown to be effective (Bohm and Apablaza C 2005), although results are not immediate. The use of appropriate indicator plants has been used to identify different *D. dipsaci* races (Metlitskii and Kholod 1986). More recently, molecular PCR-based diagnostic tests have been developed for *D. dipsaci* that can readily detect the nematode's DNA from within infested plant material (Subbotin *et al*. 2005). Tests have also been developed that can differentiate races using protein biomarkers that can be identified using SDS-PAGE or MALDI-TOF (Tenente and Evans 1997, Perera *et al*. 2009). An extensive review of methods for the extraction and identification of potato nematodes has described the morphological characters of *D. dipsaci* and the range of PCR methods available that serve as confirmation is provided by Carta *et al*. (2005).

Detection of infested plants can be undertaken through sampling symptomatic plants (e.g. strawberry, lucerne, peas) and extracting nematodes (Harvey and Somerfield 1977, McBurney 1981, Greco *et al*. 1991). Soil sampling is frequently conducted and the collected material is extracted using the Baermann funnel process (Tenente and Evans 1997, Gonzaga *et al*. 2002). Detection surveys are described by a number of authors (Gray *et al*. 1984) and largely use identical procedures to those described below for monitoring.

2. Delimitation

No specific references to delimitation surveys were found in the literature although Bello *et al*. (2005) emphasise the need to delimit the spread of nematodes.

3. Monitoring

Due to its economic importance, *D. dipsaci* is very extensively monitored for. Good descriptions of sampling regimes and sample treatment are provided by several authors (Hawn 1973, Griffith *et al*. 1997, Abbad Andaloussi and Bachikh 2001). Rajan (2006), in a review of the topic, indicates that sampling of soil can generally be confined to the top 20 cm, with 10 samples per hectare appropriate. In particular, the sampling methodology of Hawn (1973) is particularly detailed with respect to how single fields should be sampled as is a method for surveying strawberries used in Korea (Park 2005). Field methods involve taking shallow soil (ca. 20 cm) samples (or plant tissue samples) at regular intervals across a field, typically following transects, and pooling the resultant material and subjecting the material to the extraction / diagnosis methods described above. In the case of plant material, such as lucerne, both symptomatic and healthy plants are collected (Vrain and Lalik 1983). A detailed monitoring of the presence of *D. dipsaci* in the Colorado River basin provides precise details of both above- and below-ground sampling for *D. dipsaci* in lucerne, with emphasis on the microhabitats that the nematode is found in (Simmons *et al*. 2008). A novel method using infra-red aerial photography to assess presence and spread of *D. dipsaci* within areas known to be infested (Cocks *et al*. 1976) although the authors reported significant sources of inaccuracy with this method. Rajan (2006) emphasises the fact that large numbers of small samples within a given area, as opposed to limited numbers of larger samples, provide the most accurate method for accurately determining nematode levels within a given area.

4. Commodities

The ability of *D. dipsaci* to infest a range of crops and to be present in seeds (Green and Sime 1979) necessitates that traded/stored commodities are surveyed for this organism. In the case of Fragariae spp samples of plant material showing signs of disease should be sampled and subjected to the diagnostic tests outlined in section 1 (Anonymous 2008). Rajan and Lal (2005) compiled details of surveys of plant material (mostly seeds) for export (1976-2007) using water extracted samples to demonstrate that there is no evidence for the presence of *D. dipsaci* in India. Extensive surveying of *D. dipsaci* in faba beans and peas in Germany has been described, that indicated that bean seeds were frequently heavily infested and above the tolerance levels (5/300 seeds). Exotic and within-country seed / bulb consignments (including soil/packing materials) are often infested with *D. dipsaci* (Martinez and Granda 1993, Mennan and Ecevit 2001, Lal and Lal 2006). Surveys of stored garlic in Korea revealed high levels of infestation (Na *et al*. 1998). A further example is given in the case of cut carnations in Sri Lanka (Silva *et al*. 2005). A detailed description, albeit somewhat old, of seed sampling and processing of lucerne in New Zealand indicates that 100g samples of seed (or 50 g samples of plant debris) are appropriate quantities for extraction (Wood and Close 1974). Detailed descriptions for sampling bulbs, corms and tubers in dry storage are available and indicate that damaged material should be selected for sampling (Esser 1996).

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Draeculacephala minerva Ball

Common name(s): Grass sharpshooter
Taxa: Insecta: Hemiptera: Cicadellidae
EPPO list: as vectors in A1 list Nos 137 & 166 *Xylella fastidiosa*
EU Annex designation: I/A1

Organism
The green sharpshooter, *Draeculacephala minerva*, is considered to be one of two important species of insect vectors for Pierce's disease (*Xylella fastidiosa*) and alfalfa dwarf diseases. Although it has been found on many species of herbaceous plants, it shows a strong preference for feeding and reproducing on grasses. It usually has three generations per year in California. Beginning in February and March, females insert eggs into the leaves of winter annual or perennial grasses.

1. Detection
Draeculacephala minerva adults have been captured on intercepting mesh traps, yellow sticky cards, and UV-light traps (Cabrera-La Rosa et al. 2008).

2. Delimitation
Not information available.

3. Monitoring
Surveys of cicadellids was carried out within commercial orchards (Purcell 1980) in three almond growing regions (Daane et al. 2011), and in alfalfa fields (Sisterson et al. 2010). The tool used was conventional sweep netting which is useful in phenological studies and for estimates of adult sharpshooter abundance in short, dry vegetation on warm days, but inadequate for estimates of nymphal densities (Purcell and Frazier 1985).

4. Commodities
Not information available.

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Dryocosmus kuriphilus Yasmatsu

Common name(s): Oriental chestnut gall wasp
Taxa: Insecta: Hymenopetra: Cynipidae
EPPO A2 list: No. 317.
EU Annex designation: included but not yet assigned to a category, likely I/A1

Organism
Dryocosmus kuriphilus is a pest of chestnut trees (*Castanea* spp.), although it does not attack some wild North American species (*Castanea alnifolia* and *Castanea pumila*) (Anonymous 2005). *D. kuriphilus* is a univoltine species, reproducing parthenogenetically. The female, emerging from galls from the end of May until the end of July, lays the eggs in the new buds. The female lifetime is short and each female can produce over 100 eggs. Eggs hatch in 30-40 days and first instar larvae remain within the bud. At bud burst in spring, larval feeding induces the formation of green or rose-coloured galls on shoots, petioles and leaves. It is native to China and in the past it was accidentally introduced into Japan (1941), Korea (1958), USA (1974), and Nepal (1999)(Aebi *et al*. 2007).

1. Detection
The main way to *D. kuriphilus* detection is through visual inspection. In Slovenia after a notification that a consignment was sold from an Italian infested nursery, phytosanitary action was taken in 2005 in order to trace back plants from this consignment and prevent establishment of the pest (Knapic *et al*. 2010). In the period 2006-2009, intensive surveys of forests and nut production areas were conducted. In 2007 *D. kuriphilus* was detected, probably due to an earlier introduction of infested plants from Italy (EPPO workshop, Cuneo, 2006).

2. Delimitation
Following the first record, an intensive survey is usually carried out to establish the actual distribution of the insect. *D. kuriphilus* range was reported spreading in a large chestnut area in the south of Piedmont (Italy) (Bosio *et al*. 2010)). In Slovenia, after the first record in 2007, a survey was carried out in 2009 and it showed the spreading of the insect in the stands of *C. sativa* in Primorska region (Jurc, 2009). Also in the eastern USA, where the introduction has taken place in 1974, its geographic range has expanded northward since its introduction, and now encompasses nearly 1.5 million square kilometres.

3. Monitoring
There are no monitoring techniques explicitly detailed at the present time although the approached for detection and delimitation will be applicable to routine surveillance of this pest.

4. Commodities
The introduction and following spread are associated with the movement of infested plant material that stays asymptomatic for a long period (EFSA 2010). The cryptic nature of the insect, lying within dormant buds for much of the year, makes the effectiveness of plant inspections questionable (Rieske 2007). A consignment of 1250 saplings of sweet chestnut infested by *D. kuriphilus* is the probable cause of introduction in Slovenia (Seljak 2006, Knapic *et al*. 2010).

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Anonymous. 2005. Dryocosmus kuriphilus. Bulletin OEPP/EPPO Bulletin 35:422-424.

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Jurc, M. 2009. Sweet chestnut - Castanea sativa Mill.: insects on branches and leaves - Dryocosmus kuriphilus. Gozdarski Vestnik 67:213-228.

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Elm phloem necrosis phytoplasma (*Phytoplasma ulmi* Lee, Martinia, Marcone & Zhu)

Common name(s): Elm phloem necrosis, elm yellows
Taxa: Bacteria: Tenericutes: Acholeplasmataceae: Phytoplasma
EPPO A1 list: No. 26 (as Ca. *Phytoplasma ulmi* (Elm phloem necrosis) and its vector *Scaphoideus luteolus*)
EU Annex designation: I/A1

Organism
Elm phloem necrosis phytoplasma causes a disease called elm yellow that is known from North America, where it is vectored by the leafhopper *Scaphoides luteolus*. In Europe, there are native agents of elm yellow and the North American pathogen is thought to be a distinct agent.

1. Detection
Serological test were carried out for some MLO-associated disease (Jiang et al. 1989) and specific for Elm Yellows Disease (Chang and Chen 1991). Molecular technique were currently the common tool for identification of elm yellows mycoplasma, among these were PCR (Goodwin et al. 1994, Pollini et al. 1997) also in association with RFLP (Bertaccini et al. 1995, Griffiths et al. 1999), and nested PCR (Lee et al. 1994). In the case of nested PCR, the analysis of extra fragment could be important (Ge et al. 2005). A real time PCR method was developed to for the diagnostic screening of elm trees and for the identification of possible insect vectors of EY phytoplasma (Herath et al. 2010). The 16S rDNA sequences, amplified by polymerase chain reaction (PCR) using a primer pair designed on the basis of an MLO 16S rRNA gene, permit of delineate different group and sub group (Lee et al. 1993).
Elm yellows was identified on the basis of foliar symptoms, phloem discolouration, and presence of mycoplasmalike organisms in the sieve tube elements of affected trees for the first time in Canada. Moreover the bark patch-graft transmission confirmed the identification (Matteoni and Sinclair 1989), whereas molecular techniques were useful to provide the first record of Elm yellows phytoplasma in Serbia (Jovic et al. 2008) and China (Zhu et al. 2008).

2. Delimitation
No information available.

3. Monitoring
A survey of cicadellid vectors was carried out on commercial orchards (Purcell 1980). The tool used was a conventional sweep net, which was useful in phenological studies and for estimates of adult abundance in short, dry vegetation on warm days, but inadequate for estimates of nymphal densities (Purcell and Frazier 1985).

4. Commodities
Non information available.

References: 5 retained out of 9 retrieved, none added, 13 used for the summary
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Jovic, J., T. Cvrkovic, M. Mitrovic, A. Petrovic, S. Krnjajic, and I. Tosevski. 2008. New strain of ' Candidatus Phytoplasma ulmi' infecting Ulmus minor and U. laevis in Serbia. Plant Pathology 57:1174.

Lee, I. M., D. E. Gundersen, R. W. Hammond, and R. E. Davis. 1994. Use of mycoplasmalike organism (MLO) group-specific oligonucleotide primers for nested-PCR assays to detect mixed-MLO infections in a single host plant. Phytopathology 84:559-566.

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Pollini, C. P., L. Giunchedi, and R. Bissani. 1997. Immunoenzymatic detection of PCR products for the identification of phytoplasmas in plants. Journal of Phytopathology 145:371-374.

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Purcell, A. H. and N. W. Frazier. 1985. Habitats and Dispersal of the Principal Leafhopper Vectors of Pierce's Disease in the San-Joaquin Valley California USA. Hilgardia 53:1-32.

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Elsinoë species

Elsinoë fawcettii Bitancourt and A.E. Jenkins

Anamorph: *Sphaceloma fawcettii* var. *fawcettii* A.E. Jenkins
Common name(s): Citrus scab, common citrus scab, sour orange scab
Taxa: Fungi: Ascomycota: Dothideomycetidae
EU Annex designation: II/A1 as *Elsinoë* spp.

Elsinoë australis Bitancourt and A.E. Jenkins (= *Sphaceloma fawcettii* A.E. Jenkins var. *viscosa* A.E. Jenkins)

Anamorph: *Sphaceloma australis* Bitancourt and A.E. Jenkins
Common name(s): Sweet orange scab
Taxa: Fungi: Ascomycota: Dothideomycetidae
EU Annex designation: II/A1 as *Elsinoë* spp.

Sphaceloma fawcettii A.E. Jenkins var. *scabiosa* (Mc Alpine and Tryon) A.E. Jenkins (= *Ramularia scabiosa* Mc Alpine and Tryon)

Common name(s): Australian citrus scab, Tryon's disease
Taxa: Fungi: Ascomycota: Dothideomycetidae
EU Annex designation: II/A1 as *Elsinoë* spp.

Organisms
The quarantine pests specified in EU Directive as *Elsinö* spp. include the three species listed here, *Elsinö fawcettii* being the most important and widespread. Although the three forms can be distinguished by morphology and host range, they cause very similar diseases. *Elsinoë fawcettii* causes citrus scab on various species and hybrids in the Rutaceae family worldwide but mainly attacks sour oranges *(Citrus aurantium). Elsinoë australis,* agent of sweet orange scab, is most important on oranges *(Citrus sinensis)* and has a limited geographical distribution (South America, Oceania*)* while *Sphaceloma fawcettii* var. *scabiosa*, most important on lemons *(Citrus limon),*is absent from Europe and EPPO region but has been recorded in Asia, Africa, South America and Oceania. Inoculum for new infections consists of conidia from scabs formed on leaves, twigs and fruits. Dissemination of the pathogen is mostly by rain (or irrigation water) (Anonymous 1990).

1. Detection
Semi-selective media containing antibiotics and fungicides have been developed for isolating *Elsinoë fawcettii* from citrus scab pustules (Whiteside 1986). The possibility of using immunochemical methods to detect this pathogen has been studied (Pelaez Abellan et al. 1986). More recently, molecular methods with specific primer sets allowed species and pathotypes detection of *Elsinoë* from lesions on diseased leaves and fruit (Hyun et al. 2007).

2. Delimitation
In 2010, USDA published a detailed survey protocol in order to determine the extent of the infestation of *Elsinoë australis* in the U.S. (Anonymous 2010).

3. Monitoring
No information available

4. Commodities
In international trade the pathogen can be carried on infected nursery stock, ornamental citrus plants and fruits (Anonymous 1990).

References: 5 retained out of 89, 2 added and 5 used for the summary

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Enarmonia packardi (Zeller)

(=*Cydia packardi* (Zeller))
Common name(s): Cherry fruitworm
Taxa: Insecta: Lepidoptera : Tortricidae
EPPO A1 list: No. 209
EU Annexe designation: II/ A1

Enarmonia packardi feeds on many common fruit crops in the Rosaceae and Ericaceae families. This species is multivoltine and indigenous to North America (CABI/EPPO 1997). In the EPPO region, the potential host range would include species of *Prunus*, *Vaccinium, Pyrus* and *Malus*, as well as other cultivated and wild hosts, especially in the family Rosaceae. Eggs are laid singly on terminal leaves or on fruit and sometimes on fruit stems. Larvae bore into fruits or terminal shoots and mature larvae overwinter on the host in a cocoon.

1. Detection
Most procedures rely on morphological characterization of adults or late instars for species identification. Unfortunately the larvae are internal feeders and the identification of the smaller larval stages of internally feeding Lepidoptera is difficult.
DNA Diagnostics methods based on conventional and quantitative PCR with species-specific primers were adapted for identifying internal feeders of pome fruits (Barcenas et al. 2005). The method was validated as a decision-making tool for quarantine identifications for Mexico by representatives of their phytosanitary agency (Sanidad Vegetal). This method can facilitate identification of internal feeding Lepidoptera intercepted in apple and pear for importing countries.

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
No information available.

References: 1 retained out of 11 retrieved, 1 added, 2 used for the summary.
Barcenas, N. M., T. B. Unruh, and L. G. Neven. 2005. DNA diagnostics to identify internal feeders (Lepidoptera : Tortricidae) of pome fruits of quarantine importance. Journal of Economic Entomology 98:299-306.
CABI/EPPO. 1997. EPPO Quarantine pest. Datasheets on quarantine pests. Cydia packardi. .

Enarmonia prunivora (Walsh)

(=*Cydia prunivora* (Walsh))
Common name(s): Lesser appleworm, plum moth
Taxonomic position: Insecta: Lepidoptera: Totricidae
EPPO A1 list: No. 36
EU Annex designation: II/A1

The main natural host of *E. prunivora is Crataegus spp.* (hawthorn) but it also feeds on many stone-fruits of the Rosaceae family (Anonymous 1992). This tortricid is indigenous on wild *Crataegus* spp. in north-eastern states of USA and adjoining provinces of Canada and has spread onto fruit trees *(Prunus, Pyrus, Malus)* in western part of Canada and USA, but is absent from the far South.
The life and seasonal history is similar to that of the European codling moth, *Cydia pomonella*. Eggs are laid on fruit or on the upper surface of leaves. Larvae tunnel into the fruit at the calix end and in apple they can create a blotchy mine overwinters as a full-grown larva in a cocoon in debris on the ground or in crevices in the trunks of host trees. Pupation takes place in spring and after 2-3 weeks adult start to emerge.

1. Detection
Damage can be confused with that caused by *C. pomonella*. Morphological identification of larvae may prove difficult as they appear similar to those of many other species of *Cydia* and *Grapholita* although they can be separated on the basis of the size. They also can be separated from *E. prunivora* by the absence of an anal fork.
Sex pheromones (*Z*)- and (*E*)-8-dodecenyl acetates have been identified for both, *C. prunivora* and its close relative *Cydia* (*Grapholitha*) *molesta* (Busck) (Gentry et al. 1975(Gentry et al. 1976)), but optimum attraction of males of *C. molesta* to cis-8-dodecenyl acetate required the addition of 6-7% of the trans isomer, whereas attraction of males of *E. prunivora* was best when 2.2% of the trans isomer was present (Roelofs and Carde 1974).
DNA Diagnostics methods based on conventional and quantitative PCR with species-specific primers have been adapted for identifying internal feeders of pome fruits (Barcenas et al. 2005).

2. Delimitation
No information available.

3. Monitoring
Monitoring was achieved using pheromone traps. Studies have examined the factors that influence trap catch in the fields. Pherocon ICP trap (which has top and bottom sections closed) captured the most moths (Vincent et al. 1991). Traps placed between trees at a height of 3 ft captured significantly less *E. prunivora* that those placed at 1.5, 4.5, 6 and 7.5 ft. The addition of 2.5-7.5% of the E-isomer to the pheromone enhanced attraction to both *E. prunivora* and *C. molesta*, and when dodecyl alcohol was added at various rates as a synergist, 2 mu l pheromone with 8 mu l synergist was the most effective combination (Gentry et al. 1974).

4. Commodities
No information available.

References: 16 retained out of 52 retrieved, 1 added, 6 used for the summary

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Vincent, C., M. Roy, and P. Boucher. 1991. Concomitant monitoring of several lepidopteran species in Quebec apple orchards with pheromone traps. Revue d'Entomologie du Quebec 36:15-25.

Endocronartium harknessii (J.P. Moore) Y. Hiratsuka

(=*Cronartium harknessii* E. Meinecke, *Peridermium Harknessii* J.P. Moore)
Common name(s): Western gall rust, pine gall rust
Taxonomic position: Fungi: Basidiomycota: Cronartiaceae
EPPO A1 list: No. 11
EU Annex designation: I/ A1 as *Endocronartium* spp. (non-European)

Organism
Endocronartium harknessii is the causal agent of Western gall rust on many pine species in North America. The most important aecial hosts are jack pine (*Pinus banksiana*) across Canada and lodgepole pine (*Pinus contorta*) and western yellow pine (*Pinus ponderosa*) in western Canada and USA. This pathogen is absent from Europe and EPPO region. Symptoms are galls which form on branches and small stems of *Pinus* of all ages (1977). *Endocronartium harknessii* can be carried considerable distances as wind-borne aeciospores and can survive long periods in the airborne state (Chang et al. 1989).

1. Detection
Isozyme and protein pattern analysis of aeciospores can allow interspecific and intraspecific differentiation within the genus *Cronartium* (Powers et al. 1989). More recently, Ramsfield et al. developed a DNA-based detection method which provides a rapid and sensitive detection protocol for western gall rust within infected host tissue (Ramsfield and Vogler 2004, Ramsfield and Vogler 2010).

2. Delimitation
No information available.

3. Monitoring
No information available

4. Commodities
The pathogen can be carried to new areas on plants for planting of the coniferous aecial hosts. Long incubation periods mean that latent infections easily go undetected unless post-entry quarantine is applied (Anonymous, 1977).

References: 6 retained out of 59, none added, 5 used for the summary

Anonymous . 1977. Data sheets on quarantine organisms. Endocronartium harknessii (J. P. Moore) Y. Hiratsuka. Basidiomycetes: Uredinales. Page 5 Data sheets on quarantine organisms. Endocronartium harknessii (J. P. Moore) Y. Hiratsuka. Basidiomycetes: Uredinales. EPPO, Paris.

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Powers, H. R. J., D. Lin, and M. Hubbes. 1989. Interspecific and Intraspecific Differentiation within the Genus Cronartium by Isozyme and Protein Pattern Analysis. Plant Disease 73:691-694.

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Ramsfield, T. D. and D. R. Vogler. 2010. A DNA-based method for detection of Peridermium harknessii, the causal agent of western gall rust. Australasian Plant Pathology 39:247-253.

Eotetranychus lewisi (McGregor)

Common name(s): Lewis spider mite
Taxa: Arachnida: Acarina: Prostigmata: Tetranychidae
EPPO A1 list: No. 205
EU Annex designation: II/A1

Organism
Eotetranychus lewisi (Lewis spider mite) is a polyphagous spider mite considered to be a minor pest on citrus, but it presents a significant risk to glasshouse poinsettias and other ornamentals in the EPPO region.

1. Detection
There is an EPPO diagnostic protocol for *E. lewis*i (Anonymous 2006). Detection is based on morphological characteristics. Identification requires examination of cleared and mounted specimens of adult specimens of both sexes by transmitted light microscopy. Positive identification of this species is only possible from adult male specimens positioned laterally as the distinguishing characters are found on the aedeagus. This is distinctive in gradually tapering to form a broad sigmoid ventral bend. The distinct distal tip or knob is absent and the dorsal margin of the shaft is concave.

2. Delimitation
No information.

3. Monitoring
The distribution of *E. lewisi* in Taiwan is described (Ho *et al*. 2005). Identification was based on morphological characteristics.

4. Commodities
No information.

References: 9 retained out of 26 retrieved, none added, 2 used for summary
Anonymous. 2006. Eotetranychus lewisi. Bulletin OEPP 36:161-163.
Ho, C. C., S. C. Wang, and Y. L. Chien. 2005. Field observation on 2 newly recorded spider mites in Taiwan. Plant Protection Bulletin (Taipei) 47:391-402.

Epichoristodes acerbella

Common name(s): South African carnation leaf roller
Taxa: Insecta: Lepidoptera: Tortricidae
EU Annex designation: not classified

Organism

Epichoristodes acerbella had been on the EPPO A1 list until it became established within the EPPO region. It was then transferred onto the EPPO A2 list. However, during the EPPO Council session of September 1999, it was agreed to remove *E. acerbella* from the EPPO A2 list.

This moth is indigenous from South Africa, and has been recorded in Africa from Kenya, Madagascar and South Africa. In the EPPO region, it was first found in the mid-1960s in glasshouses in Scandinavian countries. Eradicated there, it appeared in Italy (Zangheri and Cavalloro 1971) and other Mediterranean countries. It is now established in France, Italy, Croatia and Spain and has been reported more recently from the Balkans (Glavendekic 2006). *E. acerbella* is a polyphagous pest on a range of ornamental and wild plants but its main hosts are carnations, chrysanthemums, *Fragaria* and *Rosa.* Number of generation depends on temperature. In Italy and France, *E. acerbella* has four generations in the fields and five to six generations in the glasshouse (Nuzzaci 1973). Females lays 200-240 eggs on the upper surface of the leaves and after hatching the larvae feed, under a shelter of silk, first on the leaves; on carnation they later mine the stems (Zangheri and Cavalloro 1971).

1. Detection

Symptoms are usually visible to the naked eye and identification is possible based on plant damage and morphological characters of the moth (Sola 1974). *E. acerbella* resembles morphologically to the European carnation tortrix moth *Cacoecimorpha pronuba* but the aspect of the plants mine is different and adult larvae distinction is possible (D'Aguilar and Deportes 1974, Sola 1974). Larvae can also be morphologically distinguished from other tortricidae ((e.g. *Cydia pomonella*, *Grapholita molesta*, and *Thaumatotibia leucotreta* (Timm et al. 2008). Genitalia of adult help to confirm identification. In addition, DNA diagnostics based on mitochondrial cytochrome oxidase I gene is an alternative method of identification of any life stage (Timm 2008).

Pheromone traps and light traps can be used to detect *E. acerbella* (Carpita et al. 1982, Del Bene and Rumine 1984).

2. Delimitation

No information available.

3. Monitoring

Pheromone trapping is effective for monitoring moth populations in the open air and in greenhouse of *E. acerbella*, but little information are available (Quaglia 1993).

4. Commodities

E. acerbella can be transported with traded plant material in any of its stages. It is regularly reported by official inspections of plants and plant products imported (Bauer 1976, Silva et al. 2005).

References: 21 retained out of 77 retrieved, none added, 11 used for the summary

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Carpita, A., A. Crovetti, F. Quaglia, and R. Rossi. 1982. A rather specific and highly effective sex attractant for males of Epichoristodes acerbella (Walk.). Frustula Entomologica 3:196-203.

D'Aguilar, J. and L. Deportes. 1974. Epichoristodes acerbella Walker in France (Lep. Tortricidae). Bulletin de la Societe Entomologique de France 79:6-9.

Del Bene, G. and P. Rumine. 1984. The Use of Synthetic Sex Pheromones against Epichoristodes-Acerbella in Carnations. Redia 67:355-366.

Glavendekic, M. 2006. Epichoristodes acerbella (Walker) (Lepidoptera: Tortricidae): a new species in the fauna of Serbia. Acta Entomologica Serbica 11:77-81.

Nuzzaci, G. 1973. Epichoristodes acerbella Walk. (Lepidoptera - Tortricidae). Entomologica 9:147-178.

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Epochra canadensis (Loew)

(=*Euphranta canadensis* (Loew))
Common name(s): Currant flies, yellow currant fly, currant and gooseberry maggot
Taxa: Insecta: Diptera: Tephritidae
EPPO A1 list: No. 41
EU Annex designation: I/A1 (as *Euphranta canadensis*)

Organism
Epochra canadensis used to be a serious pest of *Ribes* spp. and gooseberries, but it is no longer regarded as a significant pest.

1. Detection
No relevant references found

2. Delimitation
No relevant references found

3. Monitoring
No relevant references found

4. Commodities
No relevant references found

References: 0 retained out of 4 retrieved, none added, 0 used for summary

Erwinia amylovora (Burrill) Winslow *et al.*

Common name(s): Fireblight
Taxa: Bacteria: Proteobacteria: Enterobacteriaceae
EPPO A2 list: No. 52.
EU Annex designation: II/A2

Organism

Erwinia amylovora is a gram negative, facultative anaerobic, rod shaped bacteria. This bacterium is motile by peritrichous flagella at 37°C; it is not motile at 28°C. It is negative for the Voges-Proskauer test and positive for gelatin hydrolysis and it releases gas when it undergoes glucose fermentation. *Erwinia amylovora* affects and can cause extensive damage to apple and pear trees, can also affect other plants in sub-family Pomoideae of the family Rosaceae. It received its name, fire blight, from the appearance of the infected leaves and branches, which often appears blackened as if scorched by fire. It can destroy blossoms, shoots, limbs, and even whole trees. It is native to North America but spread to northern Europe in the 1950s and 1960s. It has continued to spread throughout the eastern Mediterranean since the 1980s and throughout Europe. The bacteria can enter the plant through its blossoms, stomata, or wounds on various parts of the plant and is often carried by rain or insects.

1. Detection

Different media are developed to isolate the agent from symptomatic samples, assuring greater accuracy in pathogen determinations (Ishimaru and Klos 1984, Bereswill *et al*. 1998). Three media are actually used in the diagnosis protocol (King's B, SNA and CCT)(Lopez *et al*. 2006). The selective enrichment can improve the efficiency of isolation (Lopez *et al*. 1997); (Ordax *et al*. 2009a) suggested a change of King's B medium by adding copper. *E. amylovora* colonies were early and easily differentiated by a yellow colour and high mucoid. Modification of another medium (Miller-Schroth) was used to detect *E. amylovora* from internal tissues of apple fruits (Mizuno *et al*. 2002). In some cases the identification can be difficult due to colony discoloration in co-cultured bacteria (Stockwell *et al*. 2008). More recently, fast and cost-efficient MALDI-TOF mass spectroscopy was developed for identification of cells from single colonies in liquid medium (Sauer *et al*. 2008, Wensing *et al*. 2011). Examination by ELISA of bacterial suspensions from naturally infected plant material permitted the diagnosis in cases of acute infection (Zielke and Ficke 1987). Other studies confirmed the possibility to use this test for *E. amylovora* detection (Laroche and Verhoyen 1984, Laroche *et al*. 1987, Lin *et al*. 1987a, Sadowska-Rybak and Knoesel 1992, Mraz *et al*. 1999). A mixture of antibodies would improve detection of *E. amylovora* by ELISA,iIncreasing the sensitivity of detection, (McLaughlin *et al*. 1989). The DASI ELISA technique shows a specific and sensitive detection, also in this the enrichment with monoclonal antibodies improved the test performance (Gorris *et al*. 1996). The immunofluorescence was a method used to detect *E. amylovora* in plant tissue (Van Vaerenbergh *et al*. 1987), and possible problems and advantages were evaluated in other studies (Roberts 1980, Laere *et al*. 1985, Lin *et al*. 1987b). A comparison of different serological methods with risk of cross reactions influence of sampling storage was done (Zielke *et al*. 1993).

The molecular approaches are acquiring a greater importance. A DNA hybridization method allowed direct detection of small populations of *E. amylovora* from apple tissue or in blossoms (Hale and Clark 1990, Steinbrenner *et al*. 1990), while a reverse-dot-blot DNA was used to quantify the relative amount of DNA present (Sholberg *et al*. 2006). The PCR represents a rapid method for identification and monitoring of *E. amylovora* and was developed with different primers (Bereswill

et al. 1995, Berger *et al*. 1995, Maes and Crepel 1996, Jeng *et al*. 1999). In some cases some possible problem were highlighted: inhibition by large amounts of contaminating DNA and/or interfering components from plant tissue, which can be compensated by using bacterial growth enhancement and immuno-capture techniques (Guilford *et al*. 1996), pesticide contamination overcomes by isolation and purification of bacterial DNA on Genomic DNA Prep Plus columns (Pulawska *et al*. 1997). Advanced techniques were suggested to improve the efficiency, obtaining a fast and cost-efficient identification of *E. amylovora,* also from flowers and before fire blight symptoms occurred: with novel primer (Taylor *et al*. 2001), nested-PCR (McManus and Jones 1995, Llop *et al*. 2000, Jia *et al*. 2009), real-time PCR (Salm and Geider 2004), real-time Scorpion-PCR (De Bellis *et al*. 2007), Taqman (R) minor-groove-binder real-time PCR (Gottsberger 2010), direct real-time PCR (DRT-PCR) (Kim *et al*. 2007), DNA microarray technique (Sholberg *et al*. 2005), bio-PCR and bio-duplex PCR (Vanneste *et al*. 2008a), LAMP (Temple and Johnson 2011). New primers and procedures were identified also including recent isolates that lack plasmid pEA29 and thus cannot be detected by the previously popular PCR methods based on the detection of this plasmid (Obradovic *et al*. 2007, Mohammadi *et al*. 2009, Obradovic and Kevresan 2010). New technique to real-time detection the Surface Plasmon Resonance (SPR) (Vanneste *et al*. 2008b) or new rapid test, Ea AgriStrip, serological test based on bacteria-specific antibodies useful to take management decision (Braun-Kiewnick *et al*. 2009), another technique through the first report of cloning EAsdiA from *E. amylovora* and as a new target applied for molecular detection (Gao *et al*. 2007).
The promising results obtained with an electronic nose able to identification by direct comparison of the odorous profiles could be a technique used in the future for the identification of plant pathogenic and plant associated bacteria (Spinelli *et al*. 2011). There are studies that summarized the methods of detection (Catara *et al*. 2000, Demsar *et al*. 2001, Gavrilovic *et al*. 2008, Francois *et al*. 2009, Svircev *et al*. 2009) and other that showed among different technique and protocols (Laroche 1983, Lopez *et al*. 2006, Kokoskova *et al*. 2007, Powney *et al*. 2011). The first record or indication of no presence of pathogen relied on several techniques (Jock *et al*. 2000, Sabec-Paradiz *et al*. 2001, Sivicek 2004, Rommel *et al*. 2010).

2. Delimitation
After the first record, a wide survey has commonly showed a spread and new area of infection in numerous countries (Shabi and Zutra 1987, Graberg 1993, Grimm *et al*. 1993, Angelini *et al*. 1998, Bastas and Katircioglu 1999, Nemeth 1999, Saad *et al*. 1999, Sobiczewski *et al*. 1999, Calzolari *et al*. 2000, Nicolaev *et al*. 2002, Schoch *et al*. 2003, Behalova 2004, Keck 2004, Knapic *et al*. 2004, Dreo *et al*. 2006, Baranauskaite *et al*. 2008, Rodi *et al*. 2008). In Australia, after the first record in the Royal Botanic Gardens of Melbourne, a challenging survey in commercial orchards and in nurseries, parks and gardens, has highlighted the absence of *E. amylovora* until 2002 (Rodoni *et al*. 2002). The combination of methods (isolation, enrichment-DASI ELISA, immunofluorescence (IF), plating, bioassay, nutritional and enzymatic tests) were used to obtain more secure identification. The knowledge of real distribution of pest is important to take management decision (eradication and pruning measures, regulations concerning the movement of beehives have been adopted, together with prohibition of the planting of certain ornamental host plants) (Keck 2004), moreover, efficient delimitation survey is useful to define protected zone (Vahala 2004).

3. Monitoring
Specific surveys were carried out to know distribution and incidence of *E. amylovora* in orchards, nurseries and alternative host (Jovanovic and Arsenijevic 1998, Bobev *et al*. 2011). The monitoring foresees the strain identification (with numerous techniques: fatty acid methyl ester (FAME),

morphological, biochemical and physiological tests) for evaluation of presence of resistant strains to streptomycin or copper (Mitrev 1995, Benlioglu and Ozakman 1999, Marutescu *et al*. 2008). A predictive model to assess infection risks by analysing phenological and climatological variables could be important to decide the period and the extent of monitoring (Billing 1996), according to MARYBLYT model (Lightner and Steiner 1992, Bazzi *et al*. 1996, Gouk *et al*. 1999, Kovacs and Nemeth 1999). The monitoring had a high importance to establish protected zones, stop-gap zones and providing "protective phytosanitary zones" (Calzolari and Finelli 2002). The improving knowledge of presence and incidence of *E. amylovora* could be supported by the contribution of professionals involved in private gardens and public green spaces in France (Chapin and Chauvel 2008). *E. amylovora* monitoring can be realized through honeybees that are known to be potential vectors of the pest (Ghini *et al*. 2002). The method of sampling in orchards is another important aspect, a model was developed to determine the number of trees to be sampled in the orchard and the number of blossoms be taken from each tree, which would enable the true colonization incidence of blossoms in the orchard to be estimated at desired levels of accuracy and confidence (Kritzman *et al*. 2003).

4. Commodities

E. amylovora is commonly detected on imported *Cotoneaster spp.* (Anonymous 1974, 1982). Sale of diseased ornamental trees and shrubs, but also factors not easily verified as migrant birds, insects and wind, are among the modes of entry and subsequent early spread of fire blight into New England (Chechet 1980). There are evidences supporting the hypothesis that *E. amylovora* was introduced into Spain by infected plant material (Lopez *et al*. 2002). To avoid introduction, import restrictions were fixed on host plant material and imported scions (Grimm 1987). Another example are the strains requirements of the plant quarantine system for fresh apples imported from New Zealand to Japan (designation of specific orchards in zone free from fire blight infected zone, inspections for fire blight and chlorine dipping treatment of harvested apples, using sodium hypochlorite) (Hale *et al*. 1996). However, (Roberts 2002) suggested that that a buffer of any size provides no phytosanitary security, because no *E. amylovora* was detected in any fruit, even when harvested from trees with or directly adjacent to fire blight. The risk of *E. amylovora* dissemination through mature fruit transport, although low, has been demonstrated, and should be considered in pest risk assessments (Ordax *et al*. 2009b), more likely to survive in association with the dried remnants of the flower part (Hale *et al*. 1987), whereas, through a model, (Roberts and Sawyer 2008) claimed that the risk of importing *E. amylovora* on commercial apple fruit and the concomitant risk of establishing new outbreaks of fire blight is so small as to be insignificant. A nested PCR method was proposed to be used for the detection of *E. amylovora* in imported commercial apple fruits (Jia *et al*. 2009). Use of PCR, nested PCR and real-time PCR has showed that the existence of *E. amylovora* DNA in cherry fruits imported from USA (Shang *et al*. 2010). Models, moreover, were constructed to determine the effect of major parameters on the probability of importing infested fruit (Fahy *et al*. 1991).

Some of the treatments showed efficiency for controlling *E. amylovora* cool storage of mature apple or of propagation material of apple (Aldwinckle and Gustafson 1993, Keck *et al*. 1995, Hale and Taylor 1999), gamma radiation on apples and pears (Janisiewicz *et al*. 1986), fumigation of dormant scion wood with acetic acid vapour (Sholberg *et al*. 2001). Another possibility to pathogen spreading is due to honeybees, and the movement of beehives is regulated in some countries (Holliger *et al*. 2003). Molecular differentiation by PFGE analysis or CRISPR (clustered regularly interspaced short palindrome repeats) can be useful to understand the spread modality in different countries, also the long pathogen persistence in these countries for accumulation of

genomic changes (Jock and Geider 2002, McGhee and Sundin 2011).

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Erwinia chrysanthemi pv. *dianthicola* Burkholder et al.

(=*Dickeya dianthicola* Samson et al.)
Common name(s): Bacterial slow wilt, blackleg of potato, slow wilt of carnation
Bacteria: Proteobacteria: Enterobacteriaceae
EPPO A2 list: No. 53
EU Annex designation: II/A2

Organism
Erwinia chrysanthemi pv. dianthicola is a widespread soft rot pathogen degrading succulent fleshy plant organs such as roots, tubers, stem cuttings and thick leaves. It became of concern for quarantine because of it attacks carnation, although the identity of the pathovar *dianthicola* it is not clear and can be confused with other native pathovars. Recently the pathovar *dianthicola* has been associated with potato disease.

1. Detection
The detection methods combined indirect fluorescent antibody staining (IFAS) and direct isolation (DI) on selective media (Alivizatos 1979, Minardi et al. 1988, Nassar et al. 1996).

2. Delimitation
No information available.

3. Monitoring
The bacterium has been the subject of a monitoring action in Sri Lanka (Silva et al. 2005).

4. Commodities
No information available.

References: 4 retained out of 57 retrieved, none added, 4 used for the summary

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Erwinia stewartii subsp. stewartii (Smith) Dye

(=*Pantoea stewartii* (Smith) Mergaert et al.)
Common name(s): Stewart's disease, bacterial wilt.
Bacteria: Proteobacteria: Enterobacteriaceae
EPPO A2 list: No. 54
EU Annex designation: II/A1

Organism

Pantoea stewartii subsp. *stewartii* (formerly *Erwinia stewartii*) causes Stewart's bacterial wilt and leaf blight of sweet corn and maize. The corn flea beetle is the vector of bacteria that overwinter in the insect's gut. *P. stewartii* subsp. *stewartii* grows in the xylem vessels of corn plants, producing wilt symptoms, and in the intercellular spaces of the leaves, causing lesions (Frederick RD 2001). The bacterium also attacks other Poaceae. *P. stewartii* subsp. *stewartii* is indigenous to America and has been introduced in Europe and Asia.

1. Detection

The detection of *Erwinia stewartii* can be undertaken by ELISA (Khan *et al*. 1997). A comparison of four ELISA procedures to detect *E. stewartii* in pure culture and mixed with corn-seed tissue revealed that the most appropriate procedure was a double-sandwich ELISA using polyclonal antibodies for capture and monoclonal antibodies for detection (Lamka *et al*. 1991). The ELISA test can be efficient also in maize seed treated with fungicides and insecticides (Michener *et al*. 2002, Pataky *et al*. 2004). A PCR technique was developed for identification of *Erwinia stewartii* (Blakemore and Reeves 1153, Blakemore *et al*. 1993, Maes and Crepel 1996), this technique was refined successively with a PCR-coupled ligase chain reaction (LCR) assay (Wilson *et al*. 1994), real-time fluorescent polymerase chain reaction (Qi *et al*. 2003), nested-PCR (Wang *et al*. 2005, Wu *et al*. 2005), real-time TaqMan PCR (Fessehaie *et al*. 2006) and duplex TaqMan real-time PCR (Fessehaie *et al*. 2007). The polymerase chain reaction (PCR) with arbitrary primers was used to develop a DNA probe to identify *E. stewartii* (Blakemore *et al*. 1992). The comparison between a PCR technique and nigrosine selective medium and ELISA showed that the identification of pathogen from cultured cells is rapid using PCR and can be done in less than a working day (5 hours). The sensitivity of the nested PCR is sufficient to amplify DNA from approximately ten bacterial cells (Blakemore *et al*. 1999). The possible problems in the identification of *E. stewartii* by the standard detection methods, particularly ELISA, were highlighted; however, and the PCR technique with specific primer is potentially a good method for *E. stewartii* detection (Watcharachaiyakup *et al*. 2007)
The identification of *E. stewartii* through PCR test and pulsed field gel electrophoresis (PFGE) was evaluated as an additional test to confirm the identity the pathogen (Coplin *et al*. 2002).

2. Delimitation

Not information available.

3. Monitoring

A survey to know the incidence of *Erwinia stewartii* in the Central Valley of Mexico, Leaves showing disease symptoms were taken to the laboratory and analysed with ELISA test using commercial kits (Valencia-Torres *et al*. 2004). The weather data could be important tool to assess the seasonal and site-specific risks associated with the occurrence of Stewart's disease (Nutter *et al*. 2002)

4. Commodities

The inspection of seed material coming from abroad is useful for reduce the risk of introduction and spread of pathogen (Levchenko and Ivanova 2004).

References: 30 retained out of 89 retrieved, 1 added, 21 used for the summary

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Euphorbia mosaic virus

Common name(s): EuMV
Taxa: Viruses: Geminiviridae: Begomovirus
EU Annex designation: I/A1

Organism

Euphorbia mosaic virus (syn. *Euphorbia mosaic bigeminivirus*) belongs to the whitefly-transmitted geminiviruses subgroup (Roberts et al. 1984). It is transmitted to healthy plant by *Bemisia tabaci* (Hemiptera: Aleyrodidae) (Debrot and Centeno 1985, Anonymous 2009), and has various hosts such as Mexican fireplant (*Euphorbia heterophylla*) (Anonymous 2009) and tobacco (Fiallo-Olive et al. 2010). The disease is restricted to Central and South America and the Caribbean.

1. Detection

Geminiviruses can be detected collecting symptomatic leaves and using polymerase chain reaction (PCR), and universal begomovirus primers that amplify a fragment of the coat protein gene (core Cp) (Ala-Poikela et al. 2005, Hernandez-Zepeda et al. 2007).

2. Delimitation

No information available.

3. Monitoring

No information available.

4. Commodities

No information available.

References: 27 references retrieved, 1 added, 16 retained, 6 used for summary

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Hernandez-Zepeda, C., A. M. Idris, G. Carnevali, J. K. Brown, and O. A. Moreno-Valenzuela. 2007. Preliminary identification and coat protein gene phylogenetic relationships of begomoviruses associated with native flora and cultivated plants from the Yucatan Peninsula of Mexico. Virus Genes 35:825-833.

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Eutetranychus orientalis (Klein)

Common name(s): Citrus brown mite, oriental mite, oriental red mite, citrus mite
Taxa: Arachnida: Acarina: Prostigmata: Tetranychidae
EU Annex designation: II/A1 (as misspelt as *Eotetranychus orientalis*)

Organism
This is a widely distributed pest of *Citrus* species that has been found in some Mediterranean countries. Olives, almonds pears, peaches and a number of other crops are also attacked. The mite causes damage to leaves that result in them becoming chlorotic.

1. Detection
This species and other mites can be removed from material through use of the Berlese funnel. The mites can then be identified through microscopic examination (Witters *et al*. 2007). A description of the morphological traits is provided in the EPPO data sheet on this species.

2. Delimitation
No relevant references found

3. Monitoring
Monitoring for this mite often takes the form of tissue sampling (fruits, buds, leaves) followed by laboratory processing and identification (Atalla and El-Atrouzy 1971). Identification is based on morphological traits when examined under the microscope. A surveys of indicate the importance of timing with respect to the peak mite populations to be found on a range of fruit trees (Zaher *et al*. 1974, Gangwar and Lal 1988) – the timings vary for each host and geographic area

4. Commodities
No information specific to commodities although the procedures for extracting and identifying this species are applicable.

References: 95 retained out of 138 retrieved, none added, 4 used for summary

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Florida tomato virus

(=Tomato mottle virus)
Common name(s): ToMoV
Taxa: Viruses: Geminiviridae: Begomovirus
EPPO A1 list: No. 225
EU Annex designation: I/A1

Organism

Florida tomato virus (syn. *Tomato mottle bigeminivirus*) (Geminiviridae: Bigeminivirus) is transmitted by whiteflies *Bemisia tabaci* (Hemiptera: Aleyrodidae) (Polston et al. 1996) or by grafting.

1. Detection

Affected tomato plants show stunted growth, chlorotic mottling, and distortion and curling of leaves (Polston et al. 1995, Garrido-Ramirez and Gilbertson 1998). Begomoviruses were visualized in the anterior region of the midgut and filter-chamber of adult whiteflies *B. tabaci* by indirect-fluorescent-microscopy (Hunter et al. 1998). Additionally, polymerase chain reaction (PCR) was used to detect nucleotide sequences found in the coat protein gene of aleyrodid-transmitted geminiviruses in the tissues of infected plants (Brown et al. 1995).

2. Delimitation

No information available.

3. Monitoring

No information available.

4. Commodities

No information available.

References: 12 retained out of 26 references retrieved, 1 added, 5 used for summary

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Garrido-Ramirez, E. R. and R. L. Gilbertson. 1998. First report of tomato mottle geminivirus infecting tomatoes in Yucatan, Mexico. Plant Disease 82:592.

Hunter, W. B., E. Hiebert, S. E. Webb, J. H. Tsai, and J. E. Polston. 1998. Location of geminiviruses in the whitefly Bemisia tabaci (Homoptera: Aleyrodidae). Plant Disease 82:1147-1151.

Polston, J. E., D. Bois, A. P. Keinath, and D. O. Chellemi. 1995. Occurrence of tomato mottle geminivirus in South Carolina, Tennessee, and Virginia. Plant Disease 79:539.

Polston, J. E., D. O. Chellemi, D. J. Schuster, R. J. McGovern, and P. A. Stansly. 1996. Spatial and temporal dynamics of tomato mottle geminivirus and Bemisia tabaci (Genn.) in Florida tomato fields. Plant Disease 80:1022-1028.

Fusarium oxysporum Schlechtendahl f.sp. *albedinis* (Killia and Maire) W.L. Gordon

Common name(s): Bayoudh disease, fusarium wilt, tracheomycosis of date palm
Taxa: Fungi: Ascomycota: Nectriaceae
EPPO A2 list: No. 70
EU Annex designation: II/A1

Organism
Fusarium oxysporum f.sp. *albedinis* is the causal agent of vascular wilt of date palm (*Phoenix dactylifera*), also known as Bayoudh disease. It attacks mature and young palm trees as well as their basal offshoots. Infected trees usually die .The pathogen can also attack plants grown in date plantations (*Lawsonia inermis*, lucerne and *Trifolium* sp.), these plants being symptomless carriers. The most important means of carry-over is spores and mycelium in the soil. Infection mainly occurs through the roots and dispersal of the pathogen may occur by the means of infected offshoots, soil, symptomless hosts, infected date tissues and irrigation water (Anonymous, 2003).The disease is widespread on dates in Morocco and Algeria, also reported from Egypt and Mauritania but absent from EU. Economic impact is very important and because of Bayoudh disease high potential for spread, risk for other date-producing areas in the EPPO region is serious (Anonymous 1982).

1. Detection
The symptoms of Bayoudh disease are the classical symptoms of vascular wilt and identification made on the basis of external and internal symptoms is not, therefore, reliable. The first external symptoms appear on leaves of the middle crown. Identification should be based on isolation of the fungus and further identification of the pure culture through molecular methods (2003). Specific PCR primers have been developed and can reliably replace conventional isolation and pathogenicity testing procedures (Baayen 2000). PCR with specific primers provides a rapid and reliable diagnostic tool for detection of *Fusarium oxysporum* f.sp. *albedinis* to prevent spread of the pathogen to other date-growing areas (Freeman and Maymon 2000).

2.Delimitation
No information available

3. Monitoring
No information available

4.Commodities
EPPO recommends date-producing countries should prohibit the importation of the following from countries where Bayoudh disease is present: all date-palm material, soil and plant for planting, plants for planting of *Lawsonia inermis* (Anonymous 1982).

References: 6 retained out of 40 retrieved, 1 added, 4 used for the summary
Anonymous (2003). Diagnostic protocols for regulated pests. Fusarium oxysporum f. sp. albedinis. Bulletin OEPP 33(2): 265-269.
Anonymous (1982). Data sheet on quarantine organisms N° 70. *Fusarium oxysporum* f. sp. *albedinis*. EPPO Bulletin 12 (1)(1).

Baayen, R. P. (2000). Diagnosis and detection of host-specific forms of Fusarium oxysporum." Bulletin OEPP 30(3-4): 489-491.

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Gibberella circinata Nirenberg and O'Donnell

Common name(s) Pitch canker of pine
Taxa: Fungi: Ascomycota: Hypocreomycetidae: Nectriaceae
EPPO A1 list: No. 306

Organism
Gibberella circinata is the causal agent of pitch canker disease which may affect all the *Pinus* species but also Douglas fir (*Pseudotsuga menziesii).* This disease, causing cankers that girdle branches, is a serious threat to the pine forests because tree mortality may occur after multiple branch infection. Moreover, *Gibberella circinata* may also be soil-borne, cause root rot even on mature trees and infect seeds externally or internally (without any symptom before seed germination). The anamorph, *Fusarium circinatum*, is a wound pathogen and may occur after mechanical wounds as well as after wood boring insect damages (Anonymous, 2005). Tree infection is done by aerial dispersion of conidiospores or through vectoring by feeding insects (Gordon 2001).
Climate-based models are also developed to predict global risk of pitch canker establishment (Ganley 2009). Management and control of this disease are dependent on accurate and timely diagnosis of the pathogen. Contribution of molecular analysis is important.
In 2000 EPPO published a Pest Risk Assessment (PRA) report about this pathogen. This pest is now in the EPPO A1 list (No. 306) as a quarantine pest recommended for regulation and a diagnostic protocol was recently published (2009).

1. Detection
As for many other fungal diseases, visual inspection, symptomatic plant tissue sampling and isolation on semi-selective medium are the first step for pathogen detection and identification. The composition of several culture media adapted to isolate and identify with morphological criteria any *Fusarium* spp. is detailed (Anonymous, 2009). But to ensure *Fusarium circinatum* identification, molecular methods are often required.
A PCR-RFLP test was developed for use on *Fusarium circinatum* pure culture only (Steenkamp, Wingfield *et al*. 1999). A detailed characterization of pitch canker fungus isolates using PCR-RFLP analysis confirmed the pathogen establishment in Spain (Perez-Sierra, Landeras *et al*. 2007) where mating types, showing virulence differences, were determined by multiplex PCR. As pitch canker could be a threat for New Zealand forest industry, they developed a PCR-based diagnostic method to detect the pathogen within infected host tissues as well as in infested soil (Ramsfield 2008).
In order to develop a fast and reliable diagnostic test independently of the presence of disease symptoms, Schweigkofler *et al*. (Schweigkofler 2004) present a novel trapping approach using filter paper in combination with a rapid molecular method to detect the presence and to quantify inoculum in the air. The test can be used directly on trapped spores, without the need for spores to be germinated.
Compared to more traditional approaches, SYBR-green real-time PCR allows identification with increased sensitivity and higher selectivity independently of the presence of symptoms (Schweigkofler 2004). Recently, a new detection protocol based on a biological enrichment step followed by a real-time PCR assay was developed in order to allow a quick and reliable detection of *Fusarium circinatum* in pine seeds (Loos 2009). A recent study confirmed IGS PCR-based diagnostic procedures specificity (de Wet 2010).

2. Delimitation

Gibberella circinata has been reported in Europe but infested areas remain restricted.
Spore trapping method combined with real-time PCR approach (Schweigkofler 2004) was used to evaluate aerial dispersal; this study provided important epidemiological information (Garbelotto 2008).

3. Monitoring
EPPO (2009) describes sampling procedures to be used for detection on plant tissue or seeds. The current legislation, including the provisional emergency measures (Commission Decision 2007/433/EC) is aimed at limiting the introduction of the organism but it may have only a limited effect on its spread (Anonymous, 2010).

4. Commodities
Although the fungus may be introduced in Europe by several pathways (seedlings, wood, soil and growing substrates, insect vectors), the most important risk of introduction is by seed.
An exhaustive study of seed driers, nurseries and established plantations which investigated seeds, nursery seedlings, wood with resinous cankers, flowers and cones of *Pinus* spp. throughout Galicia was carried out in Spain as soon as *Fusarium circinatum* was detected (Gonzalez Penalta 2008).

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Gilpinia hercyniae (Hartig)

Common name(s): European spruce sawfly
Taxa: Insecta: Hymenoptera: Diprionidae
EU Annex designation: II/B

Organism
Gilpinia hercyniae is a European spruce sawfly that has been introduced to UK and to North America. It is widely distributed throughout the EU/EPPO region. It occurs only on *Pinus* species.

1. Detection
The species can be determined through morphological traits of the adult. The characters of the genitalia were described to distinguish *Gilpinia hercyniae* and *G. polytoma* (Goulet 1981).

2. Delimitation
After the first record of *G. hercyniae*, a survey was carried out to know its real distribution and the spreading in new areas (Wong 1972, Billany and Brown 1977).

3. Monitoring
Surveys were carried out to know the occurrence of *G. hercyniae* (Mohyuddin et al. 1984). The method used for the monitoring were Malaise traps, emergency traps, bark-beetle pheromone baited traps or sweeping (Holusa and Roller 2004).

4. Commodities
No information available.

References: 15 retained out of 128 retrieved, none added, 5 used for the summary

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Globodera pallida (Stone) Behrens

Common name(s): White potato cyst nematode, pale potato cyst nematode
Taxa: Nematoda: Tylenchida: Heterodidae
EPPO A1 list: No. 124.
EU Annex designation: I/A2

Organism

Globodera pallida is an important nematode pest of potatoes, but it attacks other solanaceous plants as well (tomato, eggplant). *G. pallida*, native from Andes Mountains, was introduced to Europe probably in the mid-19th century. The present distribution covers the temperate zone, from sea level to higher altitudes, and the tropics. It is now distributed in Asia, Africa, Europe, South, Central and North America (Trifonova 2006). This cyst forming nematode has sedentary endoparasitic habit. Cysts are persistent tanned sacs derived by the female body and contain the eggs. Second-stage juveniles (J2) emerge from the cysts, penetrate host roots and establish a specialized feeding site (syncytium) in the stele. Adult swollen females retain the eggs, rupture root cortex and protrude from root surface. At the end of the reproductive phase, females die and become spherical brown cysts. These cysts are circumfenestrate and lack bullae.

1. Detection

The morphological features are useful to discriminate between *G. pallida* and *G. rostochiensis* by measurements of 2nd-stage juveniles (Anonymous 1978), spear length (Anonymous 1975), and anal-vulval region (Matveeva 2004). (Hockland 2005) highlighted the importance of conservation of collection and care of their materials. Progress in information technology should create interest in cataloguing material and making it widely available on the Internet. A important phase is the extracting procedures, a study examines the effects of varying sample size on sample processing and cyst extraction using two widely used laboratory flotation methods (Fenwick and Schuiling method) (Bellvert *et al*. 2008); moreover, at low potato cyst nematode density, the examination of live potato plant roots for developing females is more efficient (Wood *et al*. 1983). As *G. pallida* and *G. rostochiensis* are morphologically closely related, numerous biochemical techniques have been developed to separate the two species (Schots *et al*. 1992). The differentiation of two *Globodera* species is obtained through characterization of the thermostable polypeptides by two-dimensional electrophoresis (Bakker *et al*. 1988), by using of a set of three monoclonal antibodies (Schots *et al*. 1992), and by immunoblotting of parasite homogenates (Duncan *et al*. 1997). Recently a practical tool useful in 'non-expert systems' for routine detection and quantification of nematode species has been developed, using antibody- or lectin-coated magnetic beads (Dynabeads) to recover target nematodes from mixtures of specimens (Chen *et al*. 2001). The development of Isoelectric focusing (IEF) has showed to be sensitive to identify samples of potato cyst nematodes (Fleming and Marks 1983), and the Superoxide dismutase isoelectric focusing patterns have been also used (Molinari *et al*. 2010). A large number of diagnostic approaches are based on the polymerase chain reaction (Fleming *et al*. 1993, De Giorgi *et al*. 1994, Kulka and Novitski 1994, Marshall *et al*. 2002, Nakhla *et al*. 2008). The molecular analysis was based for example on evaluation of the homogeneity of sequences of D2/D3 segments of the 28S rDNA (Douda *et al*. 2010), on second internal transcribed spacer (ITS2) sequence variation (Clapp *et al*. 2000), and internal transcribed spacer 1 (ITS 1) and the 5.8 S ribosomal RNA gene region (Zouhar *et al*. 2000). Other techniques, such the two-tube, multiplex real-time PCR were developed (Nakhla *et al*. 2010), and the RAPD PCR (Roosien *et al*. 1993, Fullaondo *et al*. 1997). A DNA probe (Marshall and Crawford 1992) was a useful tool to distinguish the two species. The RAPD-PCR can be useful

to evaluate the pathotypes (Fleming *et al*. 1993, Sedlak *et al*. 2004), and similar results were obtained with AFLP (Folkertsma *et al*. 1996), whereas PCR product melting peak analysis could be used in determination of relative proportions of *Globodera* species in mixed populations (Bates *et al*. 2002). The biochemical techniques were used commonly with the morphological identification to evaluate the presence of *G. pallida* in numerous national surveys (Zasada and Gatt 2000, Palkovics 2003, Knoetze *et al*. 2006, Trifonova 2006, Jogaite *et al*. 2007).

2. Delimitation
No information available.

3. Monitoring
National and regional surveys have been carried out to evaluate the distribution and incidence of potato cyst nematode (Olsson 1981, Talavera *et al*. 1998, Andres *et al*. 2006, Mariann *et al*. 2008). In some cases the biochemical techniques (isoelectric focusing of species-specific protein bands, PCR, PCR-RFLP) were an important tool for the species differentiation (Evans *et al*. 1998, da Cunha *et al*. 2004, Hlaoua *et al*. 2008). The molecular approach was useful also to distinguish the different pathotypes (Marshall 1993, Hlaoua *et al*. 2008). The pathotypes as well the long term monitoring to know the evolution in presence of two cyst potato nematode are important factors for management decision (Minnis *et al*. 2002). There are numerous studies that evaluate sampling patterns, permitting to detect correctly infestation foci of the potato cyst nematode (Southey 1974, Evans *et al*. 2000, Been and Schomaker 2006, Schomaker and Been 2010). A possible development could be the analysis of light reflected from plants to detect cyst nematode presence and distribution in areas of commercial potato production (Heath *et al*. 2000).

4. Commodities
Globodera pallida is commonly intercepted in imported material (potatoes, soil from plants) (Anonymous 1976, Gaar 2001, Plumas *et al*. 2002, Sirca and Urek 2003), and this has been a major factor in the spread of nematode (Trifonova 2006).

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Globodera rostochiensis (Wollenweber) Behrens

Common name(s): Yellow potato cyst nematode, golden potato cyst nematode
Taxa: Nematoda: Tylenchida: Heterodidae
EPPO A2 list: No. 125.
EU Annex designation: I/A2

Organism

Globodera rostochiensis is an important nematode pest of potatoes, but it attacks other solanaceous plants as well (tomato, eggplant). *G. rostochiensis*, native from South America, was introduced to Europe probably in the mid-19th century. It is now distributed in Asia, Africa, Europe, South, Central and North America. Infection occurs when the second-stage juvenile hatches from the egg and enters the root near the growing tip by puncturing through the epidermal cell walls with its stylet and then through internal cell walls. Eventually it begins feeding on cells in the pericycle, cortex or endodermis. The nematode induces an enlargement of root cells and breakdown of their walls to form a large, syncytial transfer cell. This syncytium provides nutrients for the nematode.

1. Detection

Morphological features are useful to discriminate among *Globodera* spp. (Anonymous 1973, Mulvey 1973, Momota and Ohshima 1976) and in particular between *Globodera rostochiensis* and *Globodera pallida* by spear length (Anonymous 1975), stylet length (Hansen 1988), anal-vulval region (Matveeva 2004). The importance of conservation and care of the materials has been highlighted (Hockland 2005). Progress in information technology should create interest in cataloguing material and making it widely available on the Internet. An important phase is the extracting procedures, as sample size, sample processing, and cyst extraction are cumbersome. There are two widely used laboratory flotation methods (Fenwick and Schuiling method) (Bellvert *et al*. 2008), evaluating also the different soil types under different cyst densities (Bellvert *et al*. 2008), but other techniques were also evaluated (Vasyutin *et al*. 2003). The sample preparation, extraction from soil, and laboratory facilities for the detection of potato cyst nematodes were summarized (Turner 1998).

As *G. pallida* and *G. rostochiensis* are morphologically closely related, numerous biochemical techniques were developed to separate the two species. The differentiation of the two *Globodera* species is obtained through characterization of the thermostable polypeptides by two-dimensional electrophoresis investigations (Bakker *et al*. 1988). Recently, a practical tool useful in 'non-expert systems' for routine detection and quantification of nematode species was developed, using antibody- or lectin-coated magnetic beads (Dynabeads) to recover target nematodes from mixtures of specimens (Chen *et al*. 2001). The development of Isoelectric focusing (IEF) showed to be sensitive to identify samples of potato cyst nematodes (Fleming and Marks 1983, Fox and Atkinson 1984) and the superoxide dismutase isoelectric focusing patterns was also used (Molinari 2004). Other developed methods are serological differentiation (Schots *et al*. 1990, Kiss *et al*. 1997) and Curie-point pyrolysis gas-liquid chromatography (PGLC) (Fox and Atkinson 1985b).

A large number of diagnostic approaches are based on the polymerase chain reaction (Fleming *et al*. 1993, Kulka and Novitski 1994, Fullaondo *et al*. 1999, Rysanek *et al*. 2000, Quader *et al*. 2008, Reid 2009).

The molecular analysis was based for example on evaluation of the homogeneity of sequences of D2/D3 segments of the 28S rDNA (Douda *et al*. 2010), on second internal transcribed spacer (ITS2) sequence variation (Clapp *et al*. 2000). Other techniques, such the two-tube, multiplex real-time

PCR were developed (Madani *et al*. 2008, Nowaczyk *et al*. 2008, Toyota *et al*. 2008, Nakhla *et al*. 2010) and the RAPD PCR (Roosien *et al*. 1993, Fullaondo *et al*. 1997). A DNA probe was an useful tool to distinguish the two species (Marshall and Crawford 1992), whereas PCR product melting peak analysis could be used in determination of relative proportions of *Globodera* species in mixed populations (Bates *et al*. 2002).
Different techniques (PCR, IEF and ELISA) for identification of potato cyst nematodes from field soil samples were compared in relation to the relative time taken for the analysis (Ibrahim *et al*. 2001). Pathotypes were identified with antigenic differences (Fox and Atkinson 1985a) and isoelectric focusing (IEF) (Fox and Atkinson 1988) but these techniques did not show good efficiency. Instead, RAPD-PCR allowed the discrimination of pathotypes (Fleming *et al*. 1993), and similar results were obtained with AFLP (Folkertsma *et al*. 1996). New technologies are also involved in the identification such as the example of NanoChip (R) technology (Liu *et al*. 2008).

2. Delimitation
After the first discovery of *G. rostochiensis* intensive monitoring of the distribution of potato cyst nematodes started. The surveys permitted to individuate other infestations and spread of the pathogen in a wide area (Holland 1991, Palkovics 2003a, Grubisic *et al*. 2007), mainly using morphometrics, PCR, PhastSystem (Sirca and Urek 2005). The accuracy of survey was important to define areas free from the potato cyst and to make decisions of managements (short rotations, restrictions apply to the movement of potatoes) (Holland 1991, Sirca and Urek 2005). The conventional sampling/detection methods were considered inadequate for the detection of very low cyst populations, and an innovative bioassay was developed to improve detection while minimizing costs and labour (Rott *et al*. 2010).

3. Monitoring
National or regional survey were carried out to evaluate the distribution and incidence of potato cyst nematode (Anonymous 1976, Olsson 1981, Blaszek 1987, Vasyutin and Yakovleva 1998, Urek and Lapajne 2001, Ananieva 2003, Shahina and Erum 2007, Elekes Kaminszky *et al*. 2008, Bacic 2010). In some cases the biochemical techniques (isoelectric focusing of species-specific protein bands, PCR, PCR-RFLP) were used (Jogaite *et al*. 2007, Bacic *et al*. 2008). The molecular approach was useful also to distinguish the different pathotypes (Cunha *et al*. 2004, Hlaoua *et al*. 2008), that is important in order to know the evolution of the two cyst potato nematodes and take management decisions (Minnis *et al*. 2002, Palkovics 2003b). There are numerous studies evaluating sampling patterns, permitting to correctly detect infestation foci of the potato cyst nematode (Been and Schomaker 2000, Evans *et al*. 2000, Riding and Parker 2000, Been and Schomaker 2006, Schomaker and Been 2010). A possible development could be the analysis of light reflected from plants to detection of cyst nematode presence and distribution in areas of commercial ware potato production (Heath *et al*. 2000) or remote sensing using aerial digital photography. This offers a method with the potential to screen large areas of potato crop and to rapidly identify and locate areas of potato cyst nematode (Stephens *et al*. 2000).

4. Commodities
G. rostochiensis is commonly intercepted in imported material (potatoes, soil from plants) (Hirling 1976, Toida and Ekanayake 1992, Plumas *et al*. 2002, Sirca and Urek 2005). The presence of *G. rostochiensis* cysts (43 full cysts/100 g) was recorded on guano imported from Japan (Krall 1974). These cysts were found to be fully viable and infective, indicating the potential for spread of this nematode. Strict regulations are recommended and include rigid control of potatoes imported in

bulk, strengthened control of seed potato production (Andersson 1975).

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Glomerella gossypii Egerton

Common name(s): Anthracnose, pink boll rot, seedling blight of cotton
Taxa: Fungi: Ascomycota: Sordariomycetidae
EPPO A2 list: No. 71
EU Annex designation: II/B

Organism

Glomerella gossypii is the causal agent of a disease of the seedlings and bolls in cotton, the only known host of this organism. This quarantine pathogen, which is probably indigenous to America, now occurs in most cotton-growing areas throughout the world but tends to be localized in the higher rainfall areas. In the EPPO region, it is locally established in Bulgaria and Romania and reported but not established in Italy (Sicily), Spain and Tunisia.
Glomerella gossypii is transmitted through seed and may also overwinter in infected cotton plant debris. Ascospores which are the primary inoculum source are released from perithecia developed in dead tissues. Usually, only the conidial spore stage is seen on the cotton plant and secondary spread by conidia occurs in rain and wind.
In Côte d'Ivoire, a hemipteran insect is thought to be an important vector. *Colletotrichum gossypii* var. *cephalosporioides*, a form of the anamorph, causal agent of ramulose, a serious disease on cotton, differs from *Colletotrichum gossypii* var.*gossypii* in virulence, aggressiveness, and morphology. The disease caused by *Glomerella gossypii* is most serious on seedlings and bolls, but lesions also occur on the stems and leaves of plants. Seedlings from infected seeds wilt and die (Anonymous, 1982).

1. Detection

EPPO (Anonymous, 1992) gives several detection methods: as contaminated seeds may look healthy, a germination seed test is described. Detection of disease symptoms on the seedlings is detailed here alsoand refers to the Halfon-Meiri and Volcani procedure (Halfon-Meiri and Volcani 1977). *Colletotrichum gossypii* and *Colletotrichum gossypii* var. *cephalosporioides* can be recognized using the identification method based on the growth characteristics of each pathogen (Pizzinatto and Tanaka 1996; Tanaka, Pizzinatto *et al*. 1996). Molecular methods were also developed to distinguish both pathogens (Silva-Mann, Vieira *et al*. 2005)

2-Delimitation

No information available

3-Monitoring

In the phytosanitary procedures related to *Glomerella gossypii*, EPPO recommend field inspections during the growing season in order to look for symptoms on seedling stems and bolls. Because low levels of infection may escape notice and seed infection is not detectable visually, complementary tests are needed.

4-Commodities

Over long distances and in international trade, pathogen movement is only in infected cotton seeds. According to the EPPO specific requirements for *Glomerella gossypii*, cotton-growing countries should certify that the seed crop has been inspected during the growing season and found free from *Glomerella gossypii* or that the seeds were acid-delinted or that representative samples of seeds have been tested and found free from the fungus.

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Gonipterus scutellatus Gyllenhal

Common name(s): Eucalyptus weevil, Eucalyptus snout beetle, gum tree weevil
Taxonomy: Insecta: Coleoptera: Curculionidae
EPPO A2 list: No. 38
EU Annex designation: II/B

Organism

Gonipterus scutellatus is a defoliator of Eucalyptus trees. It was considered to originate from southeastern Australia and to have been introduced in most places where eucalypts have been planted (USA, South America, Western Australia, New Zealand, China, South and East Africa, and southern Europe; (Anonymous 2005, CABI 2010). In Europe, it was first recorded in Italy in 1975 and then spread to most other Mediterranean countries (Sauvard 2010). It only attacks *Eucalyptus* spp. but differences in susceptibility were noted between species. Earlier studies figured out *E. globulus* and *E. viminalis* as the most susceptible species in the introduction range (Richardson and Meakins 1986)) but 'peppermint' species of *Eucalyptus* (*E. pulchella, E. tenuiramis and E. amgydalina*) and hybrids were preferred for oviposition in the native Tasmania (Clarke et al. 1998, Dungey and Potts 2003). However, *G. 'scutellatus*' recently turned out to be a species complex of at least ten very similar (largely cryptic) species that have been confused in all literature, and different species appeared to have been introduced into Africa, America and Europe whereas the true *G. scutellatus* appears restricted to Tasmania and not introduced anywhere in the world (Newete et al., 2011). The species in Spain (including the Canary Islands) and Portugal is *G. platensis* Marshall whereas the species introduced in France seems to be new for Science. All the observed biological traits, including host preferences, have thus to be reconsidered in the light of these new taxonomic findings.

The species considered as *G. 'scutellatus'* in former studies produced from four generations per year in Mauritius to two- three generations in Southern Europe (Arzone and Meotto 1978, Santolamazza-Carbone et al. 2006). The adults emerge from the soil to feed on leaves and growing shoots. Throughout their life, females lay batches of 3±8 eggs within a small brown pod, which is placed on the surface of young leaves, the overall fecundity of a female being about 150-300 eggs (Arzone and Meotto 1978). The larvae feed on leaves and twigs then fall to the ground to pupate in the soil. Overwintering occurs in the adult stage. In a number of countries, the eucalypt weevil has been brought under successful biological control by the introduction of a mymarid egg parasitoid from south-eastern Australia, *Anaphes nitens* Girault (Arzone 1985, Hanks et al. 2000), but recent failures are probably to be attributed to problems in weevil taxonomic identity (Loch 2008).

1. Detection

Adult snout beetles are easily recognized on eucalypts by their distinctive elliptical, rugose body, their short snout and their characteristic habit of 'grasping' the twigs when disturbed. Male and female genitalia were used to distinguish between *G. scutellatus* and *G. gibberus* (Rosado-Neto and Marques 1996) but this criterion has to be reconsidered following the recent taxonomic findings. The legless, yellowish-green with black marks larvae can be easily spotted on the leaf surface. *Gonipterus* larval feeding can be detected by the presence of translucent mine trails on both sides of leaf surface whilst adult feeding is characterized by scalloping to the leaf edge. Larvae are mostly found on adult foliage whilst adults prefer the juvenile foliage, feeding preferentially on growing shoots and leaves.

2. Delimitation

No pheromone, or other types of trap, are available.

3. Monitoring

There is no chemical attractant available. Population surveys consist in counting the number of pods per tree since the pods are quite conspicuous against the leaf (Clarke et al. 1998, Santolamazza-Carbone and Fernandez de Ana-Magan 2004). (Loch 2008) used shoot clipping to the quantify numbers of pods, randomly selecting five shoot tips ca. 30 cm long and containing new foliage that were removed from various parts of each tree. Visual estimation of foliar damage is also used Bulinski and Matsuki, 2002) (Santolamazza-Carbone and Fernandez de Ana-Magan 2004, Loch and Matsuki 2010)

4. Commodities

The modes or routes of introduction have never been established. However, examinations of different temporal and spatial patterns suggest that the introductions were non-random processes (Paine et al. 2010, Sauvard 2010). Adults, eggs and larvae can be transported with eucalyptus plants for planting; larvae and pupae can be transported with soil. The adults can fly to disperse locally; adults may also hitch-hike, e.g. on vehicles.

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Grapevine flavescence dorée

Common name(s) Baco 22A disease, flavescens dorée of grapevine
Taxa: Bacteria: Acholeplasmataceae: Phytoplasma
EPPO A2 list: No. 94 as Grapevine flavescence dorée phytoplasma
EU Annex designation: II/A2

Organism
The pathogen attacks *Vitis vinifera* (grapevine), but *V. riparia* can also be infected naturally. The phytoplasma causing the disease is present locally in some European countries (France, Italy and Spain) and there are reports from USA, South Africa, Mexico, Chile, Australia and New Zealand. Another well-known European grapevine disease, whose aetiology was until recently uncertain and which was therefore treated as part of the "flavescence dorée complex", is "bois noir" which is a graft-transmissible, slow spreading disease. The phytoplasma is transmitted by the leafhopper *Scaphoideus titanus*.

1. Detection
Under electron microscope observation the cell alterations commonly associated with mycoplasma were useful to identify the pathogen (Baldacci and Belli 1973). The first record of Grapevine flavescence dorée in Spain was completed using PCR-RFLP and ELISA technique (Batlle et al. 1997). ELISA has allowed the detection of flavescence dorée-MLO in individual leafhopper vectors during latency and the inoculative state (Boudon-Padieu et al. 1989, Kuszala et al. 1993, Kuszala 1996). Another tool for identifying the mycoplasma is an indirect immunofluorescent staining technique (Lherminier et al. 1989). Cloned DNA probes was suggested although an MLO enrichment procedure using tissue from main leaf veins was necessary to ensure efficient DNA extraction (Daire et al. 1992). It is not so easy find the pathogen in tissues of field infected grapevines and cell necrosis was not always related to the presence of MLOs at the time of observation (Meignoz et al. 1992). An immune-affinity procedure that permits the purification of large quantities of Grapevine Flavescence dorée MLO (FD-MLO) is described (Seddas et al. 1993). The identification in the insect vector was achieved using a PCR technique (Tanne et al. 2001). Later, a real-time PCR assay (TaqMan(R)) was proposed as an alternative and rapid method for the detection of phytoplasmas in grapevine (Bianco et al. 2004, Galetto et al. 2005). The real-time PCR showed higher sensitivity as phytoplasmas were detected in several PCR-negative and in all PCR-positive samples. A data-mining analysis of results from both detection approaches also favoured real-time PCR over conventional PCR diagnostics (Hren et al. 2007). The RT-PCR method proved to be a useful method, as it has the advantage of being a rapid, reliable, and sensitive assay for large-scale screening of grapevines (Margaria et al. 2007). A further development of this technique was proposed that produced an assay five orders of magnitude more sensitive than the RT-PCR method described previously (Margaria et al. 2009). Nanobiotransducers, a family of biomolecules linked to nanostructures with the ability to generate a physical signal upon the occurrence of a molecular event of biological interest, were used for the first time for the detection of an infectious agent in field samples (Firrao et al. 2005).

2. Delimitation
Specific surveys have been carried out to evaluate the spread of flavescence dorée and its vector, *Scaphoideus titanus*, in several different regions (Cazelles et al. 1992, Boubals 1993, Sancassani et

al. 1997). These surveys, in addition to confirming the presence/absence of the disease, also proved useful in shedding light on the cultivars more sensitive to infection (Vercesi and Scattini 2000). After the first record of the disease, quarantine and preventive measures, particularly with regard to control the vector, are recommended (Oresek 2009). Molecular analysis (PCR and RFLP) of leaf samples taken from diseased grapevine were used to delimit the extent of flavescence dorée (Scattini et al. 2000).

3. Monitoring

Surveys have been carried out to evaluate the occurrence and incidence in several different regions (Bertaccini et al. 1996, Posenato et al. 1996, Sancassani and Turco 1996, Oresek and Zemljic 2010). The information concerning the presence of the insect vectors was highlighted in several of these monitoring exercises (Posenato et al. 1996, Bisognin et al. 2003, Giovanni et al. 2004, Magud and Tosevski 2004, Sancassani et al. 2006, Der et al. 2007). In some surveys, the presence of the disease was assessed in the insect vector as well as the plant (Mori et al. 1999, Krnjajic et al. 2007). The tool to identify the pathogen has typically been PCR and RFLP (Bianco and Casati 2000, Morone et al. 2004, Bosco and Marzachi 2011); molecular techniques (e.g. PCR-RFLP) were used to distinguish different strains of infective flavescence dorée phytoplasm (Bertaccini et al. 2000, Credi et al. 2001, Kuzmanovic et al. 2008). The presence of the mycoplasma was also monitored in a possible alternative host (*Clematis vitalba*) (Angelini et al. 2004, Filippin et al. 2009). The monitoring evaluated also the incidence with bois noir phytoplasma (Angelini et al. 2006, Romanazzi et al. 2007), proving the value of molecular techniques in monitoring this and related diseases (Borgo et al. 2006, Duduk and Ivanovic 2006, Botti and Bertaccini 2007, Vicchi et al. 2008, Quaglino et al. 2010).

4. Commodities

Methods (including visual inspection, microscopy and the use of woody indicators) are described for the inspection of fruit trees and grapes for infections by mycoplasma-like organisms (Anonymous 1994). Some studied showed that it was possible to transmit the phytoplasma that causes flavescence dorée by grafting, and that nursery activities can be responsible for the introduction of the disease into a vineyard or new area (Pavan et al. 1997, Rahola et al. 1997, Vindimian et al. 1997, Moyse 2005). The treatment of propagation material by hot water (50°C for 45 min (Rivenez and Bonjotin 1997), 45°C for 3 hours (Bianco et al. 2000) and 50°C for 50 minutes (Raimondi et al. 2004)) was a useful and highly effective control measure against the pathogen; other authors suggested other forms of heat therapy as possible solution (Belli et al. 2000). Overwintering eggs of the insect in propagation material could constitute a pathway of introduction and spread into new regions (Santini and Lucchi 1998).

Specific directives have been issued on the collection of material for propagation to safeguard the health of the grapevines (Sancassani and Posenato 1995, Colla 2004), as the phytoplasm is present in all parts of the plant. Rootstock mother plants can also be apparently healthy carriers of the disease for many years without developing obvious symptoms (Decoin 1997). ELISA (with DAS-, TAS- and DAS-biotin variants) has been proven to be very useful for diagnosis in such cases (Irimia et al. 2010).

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Graphocephala atropunctata (Signoret)

Common name(s): Blue-green sharpshooter
Taxa: Insecta: Hemiptera: Cicadellidae
EPPO A1 list:Nos 137 and 199 (as *Xylella fastidiosa*)
EU Annex designation: I/A1

Organism
This cicadellid insect is primarily of concern in the EU/EPPO region due to its ability to vector the causative agent of Pierce's disease, the bacterium *Xylella fastidiosa* (Feil *et al*. 2000, Janse and Obradovic 2010). This pathogen causes disease in grapes and related *Vitis* species, as well as almonds, lucerne and a number of wild hosts.

1. Detection

Due to the fact that some of the alternate *Vitis* spp. host species for *G. atropunctata* occur in riparian habitats, surveying for the insect in these areas has been reported (Raju *et al*. 1983) although collection details are not provided. In this case, presumably, the insect was identified on purely morphological characteristics.

2. Delimitation

No information

3. Monitoring

Feil *et al*. (2000) report upon the use of yellow 30 x 30 sticky traps to monitor the population build up of *G. atropunctata* within the riparian zones that are responsible for harbouring populations of the insect. The taps were attached to stakes at a height of 75 cm and placed ca 45 m apart, monitored daily and changed every two weeks. Similar trapping procedures for the purposes of monitoring, coupled with visual assessments, are described in the literature (Purcell 1975, 1979). Sweep netting of the insect can also be undertaken (Purcell 1976) as can the use of suction collectors (D-vac) and water pan traps (Purcell 1980).

4. Commodities

No information

References: 16 retained out of 21 retrieved, none added, 7 used for the summary

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Cydia inopinata (Heinrich)

(=*Grapholita inopinata* (Heinrich)
Common name(s): Manchurian apple fruit moth
Taxa: Insecta Lepidoptera: Totricidae
EPPO A1 list: No. 193
EU Annex designation: II/A1

Organism
Cydia inopinata is a pest of apples, primarily in the far east of Russia and China (Anonymous 2000). Although apples are the most commonly attacked host, the insect can also attacks pears and other members of the Pomoideae (Anonymous, 1997). Crop losses result from the feeding chambers created by the larvae as they feed on fruit. The moth is extremely cold hardy and mass emergences of adults occur in June (Lopatina 1978) and July across its native range

1. Detection
Cydia inopinata does not respond to light traps although the sex pheromone of female moths has now been characterized. The pheromone has proven to be a mixture of (Z)-8-dodecenyl acetate:(E)-8-dodecenyl acetate: dodecenyl acetate:dodecanal-1-ol and traps using these components (minus dodecanal-1-ol) have been successfully tries as lures for males (Tanaka et al. 2007)

2. Delimitation
No information available

3. Monitoring
No information available

4. Commodities
No information available

References: 6 retained out of 11 retrieved, 4 added, used for the summary

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Gremmeniella abietina (Lagerberg) Morelet

Common name(s) Brunorstia disease, sclerroderris canker
Taxa: Fungi: Ascomycota: Heliotiaceae
EU Annex designation: II/B

Organism
Gremmeniella abietina is indigenous to Europe and has spread to parts of eastern North America and Japan. The host range of *G. abietina* is mostly confined to species of *Abies*, *Picea* and *Pinus*. Three strains of fungus from Europe, North America and Asia have been identified immunologically and by other methods. The European strain is more virulent and has a wider host range, but appears to produce few ascopsores and apothecia.
The fungus enters the apical buds and developing shoots by germination conidia or ascospores. Wounded needles, buds and shoots are particularly susceptible to infection. After entrance the fungus kills the bud and proceeds downwards into the stem and needle fascicles. Shoots start dying in the following spring. The entire crown may be infected which causes significant loss of foliage, further weakening from secondary attack and finally death. The fungus overwinters as mycelium in the conifer host or as immature fruiting bodies. Small trees such as nursery seedlings are susceptible and die soon after infection. Larger trees take several years to succumb, usually dying one branch at a time. Free water induces discharge of the conidia and ascospores. Wet springs and cool summers, high precipitation, high RH and fog favour serious outbreaks of the disease. Conidia liberated from infected tissue are dispersed under wet conditions by water splash mechanism. Long distance dispersal is thought to occur largely through wind-borne ascopspores. The absence of ascospores of the European strain had implications for disease spread. Transport of infected nursery stock may provide means of long distance dispersal.

1. Detection
The first symptoms may not appear until the winter following infection in the spring, when resin exudation can be observed on the buds. In the spring infected buds fail to flush and year old needles turn orange to brown. A characteristic yellow colouration of the xylem tissues can be seen. Areas of depressed necrotic tissue may be seen on infected shoots. There may be many dead shoots in the crown. Trees often survive and adventitious buds develop below the point of dieback. Pine seedlings in nurseries should be inspected for orange to brown discolouration at the base of needles in May. By July needles and branch tips become brown. Needles fall from branch tips. In the spring and early autumn black pycnidia or light brown apothecia are visible at the base of dead needles or on dead branch tips.
Detection and identification of *G. abietina* is generally by morphological features (Anonymous, 2009). The most distinguishing feature is the presence of pycnostromata and the conidia of the anamorph as well as the ascospores of the teleomorph (EPPO, 2009). Molecular tests (e.g. PCR, RAPD, RAMS) been developed but none allow the identification of all varieties, races and biotypes of *G. abietina* (Borja et al., 2006; Hamelin et al., 2000; Hantula et al., 1996; Zeng et al., 2005). Aerial photography and satellite scanner images have also been used to detect infection in forests (Hame 1991; Hopkins et al., 1979)

2. Delimitation
No information found.

3. Monitoring

Extensive surveys have been conducted by the Canadian Forestry Service and have established the distribution of *G. abietina* in Canadian forests. Surveys include a random search (up to 0.5hr) for disease symptoms, estimation of disease severity and collection of diseased samples for laboratory analysis using microscopic examination of pycnidia or apothecia (Laflamme, G. and D. Lachance, 1987). The disease was only confirmed when fruiting bodies of the fungus were found; symptoms alone were not sufficient. Lachance (1993) described 3 types of inspections conducted in tree nurseries (certification, prevention and autumn inspection) to identify insect pests and pathogens.
In Sweden a new system for forest health monitoring is being established (Wulff et al., 2012). The monitoring system is divided into strategic monitoring, operational inventories of forest damage and research-related monitoring.

4. Commodities

No information found.

References: 96 retained out of 183 retrieved, 1 added, 9 used for the summary

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Wulff, S., A. Lindelow, Lundin, L., Hansson, P., Axelsson, A-L., Barklund, P., Wijk, S., Stahl, G. (2012). Adapting forest health assessments to changing perspectives on threats - a case example from Sweden. Environmental Monitoring & Assessment 184(4): 2453-2464.

Guignardia citricarpa Kiely

Common name(s): Black spot, hard spot, shot-hole, freckle spot, virulent spot, speckled blotch
Taxa: Fungi: Ascomycota: Botryosphaeriaceae
EPPO A1 list: No. 194
EU Annex designation: II/A1

Organism
Guignardia citricarpa, the causal agent of Citrus Black Spot, is a damaging pathogen on *Citrus* spp. occurring in many areas where this genus is cultivated but has not been reported from Europe or North America. It is a foliage and fruit disease. Making citrus fruit unsuitable for the fresh market, this pathogen has a significant economic impact. Severe infections may cause premature fruit drop and latently infected (asymptomatic) fruit at harvest may still develop symptoms during transport or storage. *Guignardia citricarpa* forms airborne ascospores on decomposing citrus leaves and water-spread conidia on fruits, leaves and twigs. Sutton *et al*. provided a detailed description for this pathogen while Sposito *et al*. studied disease dispersion (Sutton and Waterston 1966; Sposito, Amorim *et al*. 2007; Sposito, Amorim *et al*. 2008).

1. Detection
EPPO (Anonymous 2003 and 2009) published a diagnostic protocol in which disease symptoms on fruits, leaves and twigs are carefully described; fruit symptoms vary to such an extent that confusion exists. Pazoti described a computer vision system for the management of Citrus Black Spot (Pazoti, Pessoa *et al*. 2006).
Morphological characteristics allow *Guignardia citricarpa* identification but the species may be confused with the non-pathogenic species *Guignardia mangiferae*. Many authors describe various specific molecular methods to identify the quarantine pathogen *Guignardia citricarpa* (Meyer, Slippers *et al*. 2001; Anonymous 2003; Bonants, Carroll *et al*. 2003; Peres, Harakava *et al*. 2004; Everett and Rees-George 2006; Meyer, Sanders *et al*. 2006; Gent-Pelzer, Brouwershaven *et al*. 2007; Peres, Harakava *et al*. 2007; Baldassari, Wickert *et al*. 2008; Anonymous 2009; Stringari, Glienke *et al*. 2009; Hu, Wang *et al*. 2011)

2. Delimitation
A sampler designed to capture fungal spores directly from plant material can be applied to establish the potential inoculum load available of *Guignardia citricarpa* (Truter, Kotze *et al*. 2004).

3. Monitoring
No information available

4. Commodities
Citrus fruit trade from potentially infected area is the main risk of introduction in Europe. Existing regulations regarding *Guignardia citricarpa* were strengthened (Kehlenbeck and Unger 1998) In a Pest Risk Assessment document published by EFSA, a scientific committee evaluated current phytosanitary regulations pertaining to the export of fresh citrus fruit from the Republic of South Africa to the European Union (EFSA 2009).

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Everett, K. R. and J. Rees-George (2006). Species-specific PCR primers for Guignardia citricarpa and Guignardia mangiferae. New Zealand Plant Protection 59: 141-145.

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Hu, J., Wang N. *et al*. (2011). Duplex qPCR assay to detect and quantify pathogenic Guignardia citricarpa and non-pathogenic Guignardia mangiferae in plant samples. Phytopathology 101(6, Suppl. S): S76.

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Guignardia laricina (K. Sawada) Y. Zhong

(=*Botryosphaeria laricina* (Sawada) Shang
Common name(s): Shoot blight of larch, twig dieback of larch
Taxa: Fungi: Ascomycota: Botryosphaeriaceae
EPPO A2 list: No. 12
EU Annex designation: I/A1

Organism
Guignardia laricina is the causal agent of shoot blight of larch. The main host is *Larix* spp. but *Pseudotsuga menziesii* can be infected too. This fungus was reported in Asia (the most serious disease of *Larix* forests and nurseries in Japan) and in Far East of Russia. Because *Larix decidua,* one of the most susceptible species, is widely distributed in Europe at various altitudes and because *Pseudotsuga menziesii* is an important forest tree, the pathogen is a threat for EPPO region. Primary infection occurs from May to October with ascospores release. The anamorph (*Fusicoccum*) spores appear on the underside of needles and on young sprouts between July and November and can be dispersed by insects or rain. Although young diseased trees do not usually die, their subsequent growth is retarded or stopped. As it has occurred at the 'margin' of the EPPO region (Russian Far East), *Guignardia laricina* was transferred from the A1 to the A2 list by EPPO Council in September 1999 (Anonymous, 1999).

1. Detection
Shoot blight can be detected by visual inspection and fungal fruiting bodies may be observed directly or isolated and cultured on a specific medium (Anonymous 1979).

2. Delimitation
No information available

3. Monitoring
No information available

4. Commodities
In international trade, spread is possible on diseased host trees. EPPO recommends that all countries should prohibit importation of plants for planting and cut branches of *Larix* from Asia (Anonymous 1979).

References: 4 retained out of 22 retrieved, 2 added, 2 used for the summary
Anonymous (1999) Modifications to the EPPO A1 and A2 quarantine lists. EPPO Reporting Service(99/150).
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Guignardia piricola (Nose) W. Yamamoto

(*Botryosphaeria berengariana* f. Sp. *pyricola* (Nose) Koganezawa & Sakuma
Common name(s): Apple ring rot, blister canker of pome fruit
Taxa: Fungi: Ascomycota: Botryosphaeriaceae
EU Annex designation: II/A1

Organism
Guignardia pyricola is the causal agent of apple ring rot. The main host is Japanese pear (*Pyrus pyrifolia*) but European pear (*Pyrus communis*) and apple (*Malus pumila*) are also attacked. The pathogen has been recorded only from eastern Asia and not from EPPO countries. The pathogen infects the branches, shoots, leaves and fruits of its hosts. The pycnidiospores play a significant role in disease spread and are locally rain-dispersed. Infection is favoured by warm humid conditions. Young fruits can be infected early in the season through stomata or lenticels but wounds are needed for infection of mature fruits (e.g. punctures by *Grapholita molesta*). The fungus has been listed as one of the economically important pests of apples and pears in Japan (Smith *et al*, 1992).

1. Detection
Wart bark, twigs die back and dark-brown spots on leaves and fruits can be detected by visual inspections. Molecular identification methods are described by Zhang *et al*. for several *Botryosphaeria* species (Zhang *et al*. 2000).

2. Delimitation
No information available

3. Monitoring
No information available

4. Commodities
In international trade, *Guignardia piricola* may be carried as latent infections on young shoots of planting material. Although fruits are infected, infection occurs on the young fruit, and would be detectable on harvested fruits, rather than only appearing later in storage (post-harvest rot). Accordingly, infected fruits are relatively unlikely to be traded. As latent infections could not be detected, it is justified to prohibit the import of plants for planting of *Malus* and *Pyrus* spp. from infested countries (Smith *et al*, 1992).

References: 4 retained out of 149, 1 added, 2 used for the summary
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Gymnosporangium asiaticum Miyabe G. Yamada

Common name(s): Japanese pear rust, leaf rust of juniper
Taxa: Fungi: Basidiomycota: Pucciniaceae
EPPO A1 List: No. 13
EU Annex designation: I/A1 - as *Gymnosporangium* spp. (non-European)

Organism
Gymnosporangium asiaticum causes Japanese pear rust. It requires *Juniperus* and rosaceous hosts of subfamily Pomoideae to complete its life cycle. The most important aecial host is Japanese pear (*Pyrus pyrifolia*), and possibly other Asian pear species. In Asia the telial hosts are *Juniperus chinensis* and the closely related *J. procumbens* (Anonymous 2006). European pear (*P. communis*) and quince (*Cydonia oblonga*) are recorded as hosts (Anonymous 2006), but there is little information as to the extent that they act as hosts
Telia are produced on stems and leaves of *J. chinensis* in the spring. Teliospores, sampled from *Juniperus chinensis*, germinated at temperatures ranging from 5 to 28°C, with an optimum between 16 and 20°C (Dong *et al*. 2006). In moist conditions basidiospores are produced that disperse and can affect *P. pyrifolia* or other rosaceous hosts. Infection from basidiospores gives rise to pycnia borne on the upper surface of *Pyrus* leaves. The aeciospores are released when the peridium ruptures and are capable of being wind-borne over long distances to *J. chinensis*. After germinating on *J*. *chinensis*, an overwintering latent mycelium is produced. The telial state appears on *J. chinensis* in the spring to begin the life cycle again.

1. Detection
Identification of the *Gymnosporangium* species mentioned is based on host-pathogen relations and morphological characters on plant material. *Gymnosporangium* species are obligate and cannot be cultured on artificial media. Usually, identification can only be done on aeciospores produced on the rosaceous species. A diagnostic protocol for *G. asiaticum* and other non-European *Gymnosporangium* spp has been produced (Anonymous 2006) that provides details of hosts and spore morphology. The inspection of imported *Juniperus*, in particular bonsai, which may have latent infection, is particularly important (Joseph 1981).

2. Delimitation
No relevant references found.

3. Monitoring
No relevant references found.

4. Commodities
No relevant references found.

References: 29 retained out of 57 retrieved, none added, 3 used for summary
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Joseph, E. 1981. Risks arising from the import of Bonsai plants with particular reference to Gymnosporangium

asiaticum. EPPO Bulletin 11:177-182.

Gymnosporangium spp. (non-European)

Taxa: Fungi: Basidiomycota: Pucciniaceae
EU Annex designation: I/A1

Organism
The genus *Gymnosporangium* contains a number of species, five of which are pests recommended for regulation in the EPPO region: *G. asiaticum, G. clavipes, G. globosum, G. juniper-virginianae* and *G. yamadae* (Anonymous, 2006). The species require two different hosts in order to complete their lifecycle. The main hosts include apple, pear, quince and other rosaceous species. During the winter the fungus survives as mycelium on juniper. Spores are airborne and can spread over large distances.

1. Detection
The identification of *Gymnosporangium spp.* is based on host-pathogen relations and morphological characters on plant material (Anonymous, 2006). Usually identification can only be done on aeciospores produced on rosaceous species. The morphology of teliospores can be characteristic but teliospores can only be observed on Juniper hosts. When spores are absent on infected plant material, incubation in a humid chamber can induce spore production (Anonymous, 2006). Anonymous (2006) gives an overview of spore morphology. *Gymnosporangium* species are obligate and cannot be cultured on artificial media. No information on molecular diagnosis has been reported.
Gymnosporangium asiaticum Miyabe ex Yamada causes Japanese pear rust. The aecia mature within a month of infection. Aeciospores infect the alternate hosts *Juniperus chinenis* and J. *procumbus*. The fungus may survive for many years on juniper twigs.
Gymnosporangium clavipes (Cooke & Peck) Cooke and Peck causes quince rust which is an important disease of apples. The fungus infects the fruits but not the leaves of apple. In autumn aeciospores infect young stems of *J. communis* and *J. virginiana* producing galls from which telial horns emerge under wet conditions in the following spring. The galls are perennial and may produce telia for 20 years.
Gymnosporangium globosum (Farlow) Farlow causes American hawthorn rust and infects leaves of hawthorn, apple and pear. It rarely infects fruit. Its alternate host is *J. virginiana* where infection causes galls. After germination of aeciospores and subsequent infection, an overwintering latent mycelium is produced on J. *virginiana* which can stay viable for more than a year.
Gymnosporangium juniperi-virginianae (Schwein) causes cedar apple rust on apples. On apple aecia and pycnia are produced on leaves. The galls on the alternate host J. *virginiana* are annual producing only one batch of telia. Spore release in *G. juniperi-virginianae* as determined by spore traps is initiated by rainfall and continues as long as r.h.is near 100% (Pearson et al., 1976)
Gymnosporangium yamadae (Miyabe ex Yamada) causes Japanese apple rust. Its morphology and disease cycle closely resemble those of *G. globosum.* Pycnial and aecial lesions on apple leaves resemble those of cedar apple rust. Fruit infections are rare. The alternate host is *J. chinensis.* The galls on *J. chinensis* produce telia for a period of one year only.

2. Delimitation
No information.

3. Monitoring

In Canada, pear trees within 1km of each Juniper nursery are surveyed for *Gymnosporangium* annually. If infections are found on pear the junipers in the vicinity are inspected for infection the following spring. In apple orchards, 100 fruit from each of four trees per orchard are sampled (33 fruits from top, middle and bottom of each tree + one extra) (Clarke, 1990, 1991)

4. Commodities
No information.

References: 18 retained out of 78 retrieved, 1 added, 4 used for the summary

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Heliothis armigera (Hübner)

(=*Helicoverpa armigera (*Hübner))
Common name(s): Cotton bollworm, corn earworm, Old World bollworm, scarce bordered straw
Taxa: Insecta: Lepidoptera: Noctuidae
EPPO A2 list: No. 110
EU Annex designation: I/A2

Organism

The cotton bollworm, *Heliothis armigera* Hübner (syn. *Helicoverpa armigera* (Hübner), *Chloridea armigera* Hübner) (Lepidoptera: Noctuidae), is a polyphagous insect pest of cotton and many other crops cultivated (Sarwan and Saini 2009). It overwinters as a pupa in the soil. A morphological and ethological comparison between *H. armigera and H. peltigera* is given by Sannino et al. (1996).

1. Detection

One of the most efficient methods for capturing *H. armigera* moths uses sex pheromone traps (e.g. Sinha and Mehrotra 1993, Loganathan and Uthamasamy 1998, Loganathan et al. 1999, Nandagopal et al. 2003, Li et al. 2010). According to a comparative study on the effectiveness of pheromone traps, the funnel and sleeve traps recorded the highest numbers of captured male moths (Rai et al. 2000). Another efficient method for capturing *H. armigera* moths is utilization of light trapping (Ma et al. 2010, Puskas and Nowinszky 2011).
On the other hand, mitochondrial DNA markers can be used for delineating genetic relationships amongst populations and estimating genetic diversity (Tan et al. 2001, Vijaykumar et al. 2008). In Japan, Orui et al. (2000) survey the occurrence of *H. armigera* by application of PCR-RFLP analysis.

2. Delimitation

No information available.

3. Monitoring

The sex pheromone trap containing (Z)-11-Hexadecenal, (Z)-9-Hexadecenal and (Z)-7-Hexadecenal was highly effective for monitoring *H. armigera* populations (Salem et al. 2008). However, the blend containing (Z)-11-hexadecenal and (Z)-9-hexadecenal with a ratio of 97:3 is also efficient (Dubey et al. 2003, Moral Garcia et al. 2004, Shweta et al. 2004, Su et al. 2006). Trap was installed 2 m aboveground in a cotton field (Nalwandikar and Puri 2005).

4. Commodities

Several insect species including *H. armigera* were found in plant material imported into England and Wales (Seymour et al. 1985).

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Li, X., T. Li, Z.-Z. Lv, Q. Yu, S.-M. Sheng, and H.-T. Wang. 2010. Field trapping efficacy of two kinds of sex pheromone attractant of Helicoverpa armigera. Chinese Bulletin of Entomology 47:396-398.

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Ma, J.-h., Z.-z. Lu, X.-l. Jin, Y.-l. Pan, and G.-z. Gao. 2010. Comparison of lights and capture efficiency of popular branch bundles for capturing moths of cotton bollworm (Helicoverpa armigera). Xinjiang Nongye Kexue 47:2023-2026.

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Puskas, J. and L. Nowinszky. 2011. Light Trapping of the Scarce Bordered Straw (Helicoverpa Armigera Hbn.) in Connection with the Ozone Content of Air. Novenyvdelem 47:37-40.

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Heliothis zea (Boddie)

(=*Helicoverpa zea* (Boddie)
Common name(s): American bollworm, corn earworm, tomato fruitworm, New World bollworm
Taxa: Insecta: Lepidoptera: Noctuidae
EPPO A1 list: No. 195
EU Annex designation: I/A1 - as *Heliothis zea*

Organism
Heliothis zea is a highly polyphagous moth that produces larvae that feed upon a wide a range of economically important crops, including cotton, tomato and maize/corn (Jackson et al. 1984). The pest is found in North, Central and South America and is absent from the EU. In maize/corn, larvae feed upon the developing cobs and bore holes can readily be observed. In other crops bore holes can also readily be seen.

1. Detection
Emergence cages can be used to detect the emergence of overwintering adults though this is inefficient and requires large areas of land to be covered (Roach and Ray 1976). Black-light traps are commonly used to catch adults on the wing in/around a number of crops affected by *H. Zea* (Roach and Ray 1976, Sanger and McLeod 1988, Hayes 1991, Herbert et al. 1991) The traps are omni-directional and insect are preserved in ethanol. Black light trapping has also been used to spatially map *H. zea* populations (Holmstrom et al. 2001)
Various pheromone traps are used for catching male *H. zea*. These contain the synthetic four component sex pheromone “zealure” and can be deployed at distances as close as 50 m to several km apart (Roltsch and Mayse 1984, Sanger and McLeod 1988) although it is though best to keep traps >100 m apart to prevent interaction (Latheef et al. 1993). Various traps have been evaluated with emphasis on retaining captured insects (Hoffmann et al. 1986) which are typically placed 1-1.5 m above ground level. Trapping is conducted during the flight period of the moth (April-September)(Slosser et al. 1987). Evaluations of optimal pheromone blends and trap design have been undertaken, indicating that the Texas ”Hartstack” trap using a blend of contains 4.25 mg of 87% (Z)-l1-hexadecenal, 8% (n)-hexadecenaI, 3% (Z)-9-hexadecenal, and 2% (Z)-7-hexadecenal is generally the most effective as a monitoring tool(Lopez et al. 1994). Area-wide pheromone trapping has been used to assess populations across susceptible cropping areas (Hoffmann et al. 1991). Visual inspections of the crop for eggs and larvae have been routinely used (Roach and Ray 1976, Slosser et al. 1978).

2. Delimitation
Heliothis zea populations are delimited using trapping, particularly pheromone traps, as detailed above. No specific details of surveys were identified.

3. Monitoring
Monitoring is conducted using the procedures described in above (detection). Pheromone traps should be at least 100 metres apart in order to function correctly. Placement of traps has been evaluated and upwind placement outside of the crop has been shown to give the largest trap counts (Caprile et al. 2003). A number of sampling methods for detecting *H. zea* via damaged tomato fruit (on plant) has been evaluated and implemented (Wellik et al. 1979, Steenwyk 1983, Roltsch and Mayse 1984). Comparisons with whole plant and random fruit sampling techniques have been validated (Zalom et al. 1983). Sampling methods include the physical removal of

material for later analysis or sweeping for insects (larvae) (Jackson et al. 1984). Methods tend to be variable with crop assessed and for crops like sorghum, beating the panicle into a bucket is appropriate for detection of larvae (Merchant and Teetes 1992). Sampling in cotton plants has explored the merits of different sampling plans and sought to validate a model determined strategy (Allen et al. 1972) and indicates a maximum of 50 plants are sampled per 20 acres.
Practical monitoring efforts in the USA are numerous and often involve the generation of long-term datasets using pheromone traps and/or black-light traps For example, Goodenough et al. (1988) reported on trapping data from 100 locations over a three year period (location were throughout the southern United States). Three different trap types were used and tarps were always at least 100 m apart with lures replaces every two weeks to indicate that the date of first trapping was very closely correlated with latitude (higher = later). Recently, the trapping of *H. zea* over a 20 year period at a single location was reported in Louisiana (Micinski et al. 2008) and indicated that abundance of the moth had not been affected by the widespread introduction of Bt cotton over that period.

4. Commodities
No information available

References: 216 retained out of 1349 retrieved, none added, 21 used for summary

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Hirschmanniella spp. other than *Hirschmanniella gracilis* (de Man) Luc & Goodey

Taxa: Nematoda: Tylenchina: Pratylenchidae
EU Annex designation: I/A1

Organism
Hirschmanniella spp. are a genus of around 35 plant parasitic nematodes. They can be found on various plants, such as cucumber, squash, watermelon, rice and banana (Devrajan 2001, Abd-Elgawad et al. 2007). In Taiwan, *Hirschmanniella* spp. was found associated with the cotton rhizosphere (Tu et al. 1972). In India, *Hirschmanniella mucronata* was associated with banana plantations (Khan and Abu Hasan 2010). *Hirschmanniella oryzae* was recovered from rice (Coyne et al. 1996, Aly and Shaukat 1999, Poussin et al. 2005, Medina et al. 2009).

1. Detection
Genetic markers such as nuclear rDNA, and portions of the mtDNA genome, have been applied to identify nematode species; however, these molecular methods need to be validated (Szalanski et al. 2001). In *H. mucronata*, the number of head annules and lateral fields, presence of mucro are important taxonomic characters (Randhawa and Khera 1989).

2. Delimitation
No information available.

3. Monitoring
To determine the nematode fauna in crop systems, sampling of soil and roots has to be done ca. 35-40 days before harvest when irrigation water is drained off the paddies and nematode activity is at its peak (Lin 1970, Taya and Dabur 2004). Lin (1970) also showed that *H. oryzae* was occurred mainly in the upper 10 cm of soil. Nematodes were more numerous in loamy soil and loam than in loamy sand, clay loam, silt or silty loam.

4. Commodities
Hirschmanniella caudacrena was detected in roots of aquarium plants imported from Thailand and Singapore to Poland. Such detections indicate that aquarium plants imported from non-European countries are an important pathway for entry to the EPPO region (Revilla et al. 1991, Ryss and Karnkowski 2010).

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Hishimonus phycitis (Distant)

Taxa: Insecta: Hemiptera: Cicadellidae
EU Annex designation: II/A1

Organism
The leafhopper *Hishimonus phycitis* Distant is a vector of witches' broom disease of lime phytoplasma (Shabani et al. 2011).

1. Detection
This insect's identity is confirmed using morphological traits to differentiate it from closely related species. A key for distinguishing seventeen leafhoppers (including *H. phycitis*) associated with vegetable crops, such as aubergine, okra, tomato, beans, amaranthus, spinach, cabbage, cauliflower, coccinia, cowpea and gourds, from different agroclimatic zones of Andhra Pradesh, India is available (Reddy and Rao 2001).

2. Delimitation
No information available.

3. Monitoring
Razvi et al. (2007) suggest that regular sprayings of acid lime trees with effective systemic insecticides during November to March each year can greatly reduce the vector population and can prevent or delay the spread of the disease to a great extent.

4. Commodities
No information available.

References: 13 retained out of 43 references retrieved, none added,, 3 used for summary

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Hypoxylon mammatum (Wahlenburg) J. Miller

Common name(s): Hypoxylon canker, canker of aspen/poplar
Taxa: Fungi: Ascomycota: Xylariaceae
EPPO A2 list: No. 72 (deleted in 1984)
EU Annex designation: II/B

Organism

The principal hosts are, in Europe, the native species *Populus tremula* (especially its mountain race) and the commercially exploited *P. tremuloides*, a North American species. Though at one time it was thought that *H. mammatum* was introduced into Europe from North America, it is now considered more probable that it occurs naturally throughout the temperate zone of the Northern Hemisphere (Pinon 1979, Pinon and Manion 1991).

Spreads through wind-borne ascospores, liberated at high relative humidity and low temperature, first produced 3 years after infection. In the USA, a high proportion of cankers have been found to originate in insect wounds (Ostry and Anderson 2009). As such, it is known to be associated with galls caused by the longhorn beetle *Saperda inornata* (Anderson 1987) and with oviposition scars of cicadas (Ostry and Anderson 1979). In international trade, young plants may carry ascospores or mycelium of the fungus, and so may wood, particularly with the bark remaining; it should be noted that the fungus can exist as a latent infection (Anderson and French 1972).

1. Detection

Symptoms first appear on the bark as slightly sunken, yellowish-orange areas with an irregular margin. Later, the outer bark becomes raised in blister-like patches and sloughs off to expose the blackened crumbling cortex; these blackened areas stand out on the green trunks. Old cankers may be up to 2.5 m in length. Detection of latent infections is a lengthy process (Anderson and French 1972). The top ends of stem sections should be dipped in paraffin and the sections incubated in moist sand at 24°C and 90% RH for 3 months, and observed for fungal development.

2. Delimitation

No information available.

3. Monitoring

A random sample generally would be adequate for estimating the disease incidence in clones (Falk et al. 1989).

4. Commodities

No information available.

References: 6 retained out of 165 retrieved, 1 added, 7 used for the summary

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Ips amitinus (Eichhoff)

Common name(s): Small spruce bark beetle, eight-toothed spruce bark beetle
Taxa: Insecta: Coleoptera: Scolytidae
EU Annex designation: II/B

Organism
Ips amitinus lives in association with several other bark beetle species, often concurrently. The most significant species with which it co-occurs are *Ips typographus* and *Pityogenes chacographus*. The species is distributed in central Europe, but occurs primarily in mountain regions above 800 m. *Ips amitinu*s host plants are mainly *Picea abies*, but also *Pinus, Cembra* and *Pinus montana*.
Bark beetles are usually secondary pests who find the best developmental conditions to be damaged and declining trees e.g. wind break-offs after heavy storms or extreme weather.
Swarming starts at temperatures above 16 °C. Male beetles construct nuptial chambers, made visible by the bore dust falling out of the entrance holes. Females bore egg galleries and lay 30-60 white eggs. Unlike the egg galleries, larval galleries do not reach the sapwood. The development of the first generation is finished after seven to twelve weeks, whereas the development of the second “summer” generation mainly last lesser than ten weeks.

1. Detection
From 1993 onwards Great Britain installed a detection survey on *I. amitinus* in compliance with securing and retaining Protected Zone status set out in Commission Directive 92/70/EEC. The survey is carried out according to the following methodology. Forest stands of spruce and pine comprising more than 25 trees, older than 15 years, are monitored annually. All together 41 permanent survey plots have been installed across Great Britain.
In 1993 only visual surveys were carried out. Starting with 1994 trap logs with pheromone baits were utilized as well. The trap logs have to comply with the following criteria: freshly felled spruce with minimum 2.5 mm thick bark and 12 cm diameter. Ten such trap logs per plot are installed and provided with 2 pheromone baits as minimum or two Theysohn slot traps attached. The final inspections are to be carried out during September.
Furthermore, people involved in forest or plant health matters were provided special trainings. For example forest health days were held to determine extension of infested trees and diagnosis of harmful organisms. To arouse public awareness, meetings with dock workers were organized and an information leaflet on import controls was published (Fielding *et al*. 1993).

2. Monitoring
No information available.

3. Delimitation
According to the living requirements of *I. amitinus* it is very important to avoid situations that promote bark beetle outbreaks e.g. leaving fallen trees *in situ* after extreme weather situations. Most of the delimitating measures are aiming to prevent the gradation of the species within the stands (Pulpan & Kula 2010).
In the German Bundesland of Lower Saxony trap trees and pesticide applications were utilized for delimitating *I. amitinus*. After 1974 pesticide applications were only justified as emergency measure e.g. application upon the litter in a diameter of 2m around infested trees, spraying and dusting during the debarking procedure, before flight pesticide application, surveillance and removing of infested yet standing wood. In years when spring swarming activity is higher than

expected, the number of felled trap trees are to be increased accordingly (Thalenhorst 1974).
More delimitating measures are the debarking infested trees, what is regarded as the most appropriate mechanical measure, use of trap trees and trap trees provided with pheromone baits (Richter 1989).

4. Commodities
Regularly import inspections on relevant entry points are included in the survey in Great Britain (Fielding *et al*. 1993).

References: 11 retained out of 106 retrieved, none added, 4 used for summary

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Ips cembrae (Heer)

Common name(s): Large larch beetle
Taxa: Insecta: Coleoptera: Scolytidae
EU Annex designation: II/B

Organism
Ips cembrae is a bark beetle living mainly on *Larix* and native to Europe and Asia.

1. Detection
Trapping of *Ips cembrae* with a synthetic mixture containing ipsdienol and ipsenol was tested (Rebenstorff and Francke 1982), however the addition of methylbutenol increased the response considerably (Stoakley et al. 1978). A synthetic pheromone (Cembrax) has also been synthesized (Schmidt et al. 1997).

2. Delimitation
No information available.

3. Monitoring
The occurrence of *Ips cembrae* was monitored (Luitjes 1977, Niemeyer 1989, Moraal 1992, Mihalciuc et al. 2001, Pavlin 2001, Krehan and Steyrer 2005a, Krehan and Steyrer 2005b). A monitoring system with pheromone traps was used to obtain information on the condition of bark beetle population (Krehan and Steyrer 2005b, 2006); in addition, trap logs can be useful (Pavlin 1997). Black Theysohn traps baited with the synthetic pheromone Cembrx has been demonstrated to be an efficient combination in evaluating the phenology and abundance of the insect (Pavlin 2001).

4. Commodities
Several records of *Ips cembrae* were made on coniferous timber, especially of larch, imported from Southern Germany and Czechoslovakia into Sweden (Lundberg 1988). Treatment with methyl iodide (Naito et al. 2003), sulfuryl fluoride (Soma et al. 1997), mixtured gas of sulfuryl fluoride (doses of 30 and 50 g/m3) and methyl bromide (Soma et al. 1999) or sulfuryl fluoride and phosphine (Soma et al. 1998) would be a high potential fumigant for imported logs infested with various species of forest insect pests.

References: 21 retained out of 93 retrieved, none added, 18 used for the summary

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Ips duplicatus (Sahlberg)

Common name(s): Northern bark beetle
Taxa: Insect a: Coleoptera: Scolytidae
EU Annex designation: II/B

Organism
Ips duplicatus is a bark beetle living mainly on *Picea* and native to Asia, although it has recently entered Europe.

1. Detection
Morphological identification of adults based on a simple key and the analysis of the gallery systems has been provided (Knizek 2001). Traps baited with pheromones were the most common method for detection and monitoring of this insect. The attractants used were ipslure (Selander and Nuorteva 1980), Pheroprax (Krol and Bakke 1985, Grodzki 1998), 2-methyl-3-buten-2-ol, cis-verbenol and ipsdienol and mixtures of these pheromones (Gavyalis and Yakaitis 1981, Zotova 1987), a combination of ipsdienol, E-myrcenol, and amitinol (Chen et al. 2009), and the Ecolure pheromone (Holusa et al. 2010a). The addition of α pinene increased the attraction of trap (Erbilgin et al. 2007). Traps used were funnel-shaped (Zotova 1987), barrier (Yakaitis 1988), window-slot and cross-barrier (Chen et al. 2010). The colour of window-slot traps showed a significant effect on catches, with dark colours (black and red) being more effective than light colours; traps at a 1.5-2.0 m level caught more beetles than those at either ground level (0-0.5 m) or at 3.5-4.0 m (Chen et al. 2010).

2. Delimitation
No information available.

3. Monitoring
The distribution of *I. duplicatus* was assessed in different countries (Koponen 1975, Turcani and Zubrik 1999). In Czech Republic, the distribution and spatio-temporal of captures of *I. duplicatus* were analysed, highlighting a spreading in 1997-2009 (Holusa et al. 2010b). The survey used pheromone traps baited with different attractants such as ID-Ecolure (Grodzki 2002), Duplodor (Grodzki 2003), ipsdienol and E-myrcenol (Holusa et al. 2003). The traps used were Theysohn traps (Holusa et al. 2003). An index of strength of bark beetles populations was calculated, which takes into account the number of traps and the number of trapped insects (Kunca and Zubrik 2008). The presence and incidence of bark and wood boring pests can be estimated based on infested dead wood harvested (Hilszczanski and Kolk 2001).

4. Commodities
Spruce logs imported from Russia and the Baltic countries were the cause of introduction of *I. duplicatus* in Belgium (Piel et al. 2006).

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Ips sexdentatus (Börner)

Common name(s): Six-toothed bark beetle
Taxa: Insect: Coleoptera: Scolytidae
EU Annex designation: II/B

Organism
Ips sexdentatus is a bark beetle living mainly on *Pinus* and native to Europe and Asia.

1. Detection
Trapping is an efficient method to catch *Ips sexdentatus*, by using ipsdienol, a synthetic attractant consisting of 2-methyl-6-methylene-2,7-octadien-4-ol (Vite et al. 1974, Schonherr et al. 1983), whit the addition of ipsenol (2-methyl-6-methylene-7-octen-4-ol) (Vite et al. 1974). The addition to ipsdienol of 2-methyl-3-buten-2-ol, cis-verbenol (Gavyalis and Yakaitis 1981, Serez and Schoenherr 1985), alpha-phellandren-8-ol (Lozzia 1995), 2-methyl-3-buten-2-ol and alpha –pinene (Sierra and Martin 2004), 2-phenylethanol in an alpha-pinene solution and alpha-phellandren-8-ol enantiomer in a methylbutanol solution (Lebedeva et al. 2006), allowed higher captures of insect. Pheroprax, a synthetic pheromone used for attracting *Ips typographus*, showed that it is also attractive to *Ips sexdentatus* (Rener and Maja 2001). The traps used were the Lindgren multi-funnel and the Theysohn window (Sierra and Martin 2004).

2. Delimitation
No information available.

3. Monitoring
The occurrence and presence of *Ips sexdentatus* were monitored in various areas (Niemeyer 1975, Simionescu et al. 1998, Krehan and Steyrer 2005). A large-scale survey was carried out to sample the most frequently observed species developing on spruce and pine (among them *Ips sexdentatus*) in 898 locations distributed throughout wind-damaged areas (Gilbert et al. 2005). The tools used were traps baited with different attractive substances (Serez 1987). Bark beetle infestations are often scattered throughout the forest landscape and therefore difficult to accurately and rapidly assess. A roadside sampling technique was tested at this purpose and the sampling method relied on the count along stand edges of all dying or dead trees sighted within a fixed distance from the road (Samalens et al. 2007). The methodology of forest insect and disease survey of bark and wood boring beetles were summarized (Lakatos 1999, Milos and Petr 1999). Another study highlighted the importance of the integration large-scale pheromone trapping data with GIS analysis in order to develop a method to prevent and control future population explosions of scolytids (Sanchez Pena et al. 2008).

4. Commodities
Ips sexdentatus was among the insect intercepted at the U.S. ports of entry (Haack 2001).

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Ips typographus (Linnaeus)

Common name(s): Eight-toothed spruce bark beetle
Taxa: Insecta: Coleoptera: Scolytidae
EU Annex designation: II/B

Organism

This bark beetle is widely distributed throughout Europe, the near and far-East. Species of pine are affected, primarily *Pinus abies.* The beetles develop in galleries under the bark and trees are killed due to the pathogenic fungi (blue stain fungi) that are vectored by the beetle. Large outbreaks of the insect can have a catastrophic effect on Pine stands, such as the one that killed trees in Norway that resulted in a 5 million m^3 loss of timber (Bakke 1991). In most places in Europe two or more generations occur, depending on temperature and elevation (Keskinalemdar *et al*. 1987, Keskinalemdar 1995)

1. Detection

A wide range of detection methods are available to ascertain the presence of this insect. Early detection can be made through the use of aerial photography (Arnberg and Wastenson 1973). Surveying by helicopter in Germany has been shown to be capable to detecting large infestations of *I. typographus* (Jager 1985).

Trap logs can be used to detect beetles as felled trunks can be more attractive than standing ones (Klimetzek and Adlung 1977). Extensive work has been undertaken with respect to understanding the chemical communications of *I. typographus* (Bakke 1975, Bakke *et al*. 1977). This has led to the development of methods for the detection of the insect based on attractant mixtures, such as methyl butenol, cis-verbenol and ipsdienol (Niemeyer and Watzek 1977, Klimetzek 1978). In many cases attractants are used in conjunction with sticky traps (Klimetzek 1978) or other trap types. (Adlung *et al*. 1979). Such approaches are widely implemented for the detection of this pest (Vite 1980)

The effectiveness of attractant lures has led to commercialisation of some mixtures (e.g Typolur, Pheroprax and others) and the use of these materials in mass-trapping exercises for the control of the beetle has been shown to be effective (Adlung *et al*. 1979, Klimetzek *et al*. 1979, Vite 1989). The effectiveness of different trap designs has also been explored. The physical sampling and visual assessment of damaged wood (fallen trees etc) was conducted in Hungary to ascertain the relative abundances of a range of forest pest, including *Ips* species (Lakatos 2006)

2. Delimitation

No relevant references found

3. Monitoring

The economic importance of this pest means that it is widely surveyed for across Europe and elsewhere. Monitoring is conducted using traps that dispense attractant lures (Dimitri 1981). Various traps designs have been assessed and placement regimes have been explored (Vaupel *et al*. 1981). Monitoring of *I. typographus* is described for a number of countries, including Germany (Niemeyer 1989), Denmark (Hubertz *et al*. 1991), France (Malphettes and Saintonge 1995), Norway (Malphettes and Saintonge 1995), Italy (Faccoli and Stergulc 1999) and Sweden (Lindelow and Schroeder 2001). In all cases, pheromone traps are used for monitoring purposes. Recent

monitoring of the pest in Romania has used a variety of trap designs including pheromone, widow barrier and polythene film traps (Varga *et al*. 2010). The large areas of forest affected by the beetle in Austria was determined through pheromone trap monitoring, which allowed the volume of wood infested by *Ips* species to be ascertained and to monitor long-term trends in the insect (Krehan and Steyrer 2007, Krehan and Steyrer 2008)
A phenological model (PHENIPS) has been developed based on climate and solar radiation that allows predictions to be made with respect to the time of infestation of trees and the number of generations that will occur (Baier *et al*. 2007a, b, 2009).

4. Commodities
This bark beetle is readily transported in traded timber and constitutes a serious risk to importer nations (Skarpaas and Okland 2009) and it is suggested that risk can be minimized through isolating the storage/importation of timber from forested regions. This process, therefore, minimizes the chances that emergent beetles can spread to woodland. Pheromone traps have been used in timber yards as a means of assessing the degree of infestation (Babuder and Pohleven 1995), whilst visual assessments are also necessary to assess for the presence of larvae.

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Leprosis

Common name(s): CiLV, citrus leprosis
Taxa: Viruses: Rhabdoviridae: Rhabdovirus
EPPO A1 list: No. 284
EU Annex designation: II/A1

Organism
*Leprosis (*syn. *Citrus leprosis* 'rhabdovirus') is a serious viral disease of citrus, which is vectored by *Brevipalpus* mites such as *Brevipalpus phoenicis* (Gonzalez Arias et al. 2009). Of the citrus, the most susceptible species appear to be grapefruit (*C. paradisi*) and orange (*C. sinensis*).

1. Detection
Leprosis virus can be detected in its viruliferous vectors, *Brevipalpus* spp., using specific primer pairs (Kubo et al. 2011). The efficiency of virus transmission by *Brevipalpus* mites is low, so the detection of this pathogen in its vectors constitutes an important tool for studies involving virus-vector relationships, transmission, and monitoring the pathogen prior to the appearance of symptoms in the field.
Leprosis virus can also be detected through extracting total RNA from symptomatic leaves and conducting RT-PCR (Reverse Transcriptase Polymerase Chain Reaction) analyses (Locali et al. 2003, Freitas-Astua et al. 2005, Leon et al. 2008, Ochoa-Corona et al. 2009). A limiting factor for this method is the requirement for high quality samples. Antonioli-Luizon et al. (2004) shown that citrus foliar samples symptomatic for *Leprosis* can be stored for up to 2 weeks before testing if kept in dry, cool conditions. The use of plastic bags for keeping samples for long periods should be avoided.
For phytosanitary purposes, it is important to consider the following main points: (i) both *Leprosis* and its vectors need to be considered; (ii) sweet orange fruits can be infected even more readily than propagation material; and (iii) *Leprosis* does not systemically infect susceptible citrus species, or any of its known hosts (Lovisolo 2001).

2. Delimitation
No information available.

3. Monitoring
A monitoring of the flat mite, *Brevipalpus phoenicis,* was carried out to determine the acceptable sample size for estimating the population level, which is determinant for decisions on control measures for *B. phoenicis* as a vector of *Leprosis* and for *Leprosis* itself (Czermainski et al. 2007, Lopes et al. 2007). To ensure the error in the sample remains at an acceptable level (20-30% of error), 105 plants must be inspected. In fruits it was indicated that that for the acceptable situation to be reached (20-30%), 540 must be inspected (Lopes et al. 2007). However, according to Czermainski et al. (2007) the presence of symptoms, rather than just the presence of the mites, must be taken into consideration in sampling programmes used in disease control.
A rapid molecular fingerprinting method has been developed for identifying *B. phoenicis* and *B. californicus* and facilitating studies on population dynamics of *Brevipalpus* in citrus (Mata et al. 2010).

4. Commodities

Rooted plants or cuttings shipments containing mites known to vector *Leprosis*, represent a risk of introduction/spreading of the virus (Childers and Rodrigues 2005).

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Leptinotarsa decemlineata Say

Common name(s): Colorado beetle
Taxa: Insecta: Coleoptera: Chysomelidae
EPPO list A2: No. 113
EU Annex designation: I/B

Organism

Leptinotarsa decemlineata is one of the most serious pests of potato (*Solanum tuberosum*), although it can attack other cultivated solanaceous plants such as tomato, aubergine, pepper and tobacco although *Solanum* spp. differ in their susceptibility to this organisms (Anonymous 1992). It also attacks wild Solanaceae.

L. decemlineata is a multivoltine species, and the number of generations depends on temperature. The adults overwinter in the soil and after emergence in spring they must feed before mating. The females lay eggs in clusters of 10-40 on the underside of the leaf surface and they can lay an average of 500-1000 eggs over a life time of several weeks. Eggs hatch in 4-12 days and first instar larvae start to feed immediately close to their hatching site and then migrate to apical leaves. Pupation occurs in the soil. The Colorado potato beetle was discovered in south western North America and, since its description in 1824, has spread across America and Canada reaching the Atlantic coast in 1876. It became established in Europe after its introduction from the USA to Bordeaux, in 1922 but several prior introductions from 1876 in the United Kingdom, the Netherlands and Germany did not result in establishment. Since 1922 it spread rapidly into almost all Europe, the Middle East and Central Asia.

1. Detection

The main method for detecting *L. decemlineata* in the field is through visual inspection. Both adults and larvae feed on the edges of the leaves leaving characteristic black excrement on the stem and leaves. The Colorado potato beetle is easily recognized in adults by their characteristically yellow-orange elytra with five longitudinal black stripes, the red to orange colour of larvae changing from during development and the yellow to pale-orange egg masses on the undersides of leaves

For estimating population densities on host plants visual sampling of *L. decemlineata* is more efficient than either the whole plant bag sampling method or sweep netting (Senanayake and Holliday 1988). Interception traps are inefficient for monitoring and sampling Colorado potato beetles in flight because flying adults avoid the traps and the highest level of efficiency for window traps was obtained with the yellow trap, which caught 16% (Boiteau 2000). A method for determining the abundance of *L. decemlineata* was based on the detection of buried diapausing beetles by soil sampling at harvest time (Glez 1983).

2. Delimitation

Following the first record, intensive surveys were carried on in Europe to delimit the distribution of the insect (Anonymous 1977, 1978, 1982). Also in Asia where it has expanded its geographic range, surveys where carried out in Xijiang (Guo *et al*. 2010) and Heilongjiang Province (Liu 2010).

3. Monitoring

In Germany, monitoring of *L. decemlineata* by visual surveys has been performed regularly, and covers the stages that are important for short-term prognosis and cause active damage during the growing season (Koppen *et al*. 1976). Surveys conducted in 1976-83 In Bulgaria provided

information on the regional variations in population dynamics and in life-history parameters (Mateeva-Radeva 1985).

4. Commodities

The introduction and following spread of *L. decemlineata* are associated with the movement of infested plant material. Potatoes, aubergines and tomatoes are the main commodity crops affected by *L. decemlineata*.

However, the pest is primarily a leaf and stem feeder and does not infest the fruit of aubergine or tomatoes. Potato tubers are occasionally attacked (Karaev 1974, Bartlett 1976), but the adult beetle feeds on the surface, and does not burrow into the tuber. There may be a risk of adults contaminating during packaging, and given the ability of the beetles to enter a facultative diapause under adverse conditions, it is possible they could survive for some months in packed fruit. Introduction through importation of non-host plant (Seymour 1974) and with the greatest risk in fresh vegetables from countries where *L. decemlineata* is established is also possible (Bartlett 1978, 1983). The adults disperse locally by flight or long distance assisted with wind; adults may also hitch-hike, e.g. in vehicles (Bartlett 1978).

References: 50 retained out of 789 retrieved, 2 added, 15 used for summary

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Lettuce infectious yellows virus

Common name(s): LIYV, infectious yellows of lettuce
Taxa: viruses: Closteroviridae: Crinivirus
EPPO list A1: No. 212
EU Annex designation: I/A1

Organism
Lettuce infectious yellows (LIYV) is a viral disease of a wide range of crops, including beetroot and sugarbeet (*Beta vulgaris*), lettuce (*Lactuca sativa*) and melon (*Cucumis melo*) as well as a number of weed species (Duffus and Flock 1982, Duffus et al. 1986, EPPO/CABI 1997). The virus is exclusively transmitted by the tobacco/sweetpotato whitefly *Bemisia tabaci* and is not mechanically transmissible (Duffus et al. 1996, Wang et al. 2009). The disease can cause severe losses in lettuce, curcubits and other vegetable crops (Brown et al. 1990, Wisler et al. 1998). Symptoms include interveinal yellowing or reddening, stunting and chlorosis (Brown et al. 1990) and an excellent pictorial example of the effects on lettuce is provided by Duffus and Flock (1982). The disease first emerged in the early 1980s, and is largely restricted to parts of the south and western United States (Wisler et al. 1998) and is not present in the EU.

1. Detection
Early detection methods involved the use of *Bemisia tabaci* to transmit the virus to indicator hosts. However, this is time-consuming and the method has been superseded in part by other methods (Brown et al. 1990). Such methods include an indirect ELISA that is capable of confirming infection in symptomatic plants but not in those without obvious signs of pathology (Brown and Poulos 1989). A simple staining method for infected plant tissue using Azure A has been described that was augmented by a short microwave treatment (Hoefert et al. 1992). A dot-blot hybridization method has been developed that, although initially complex, was shown to work adequately with only basic equipment, making it suitable for routine testing (Harper and Creamer 1995). The first detection of LIYV in Texas was made using virion morphology coupled with ELISA and was noted to coincide with activity of the vector (Halliwell and Johnson 1992). A detection survey in Europe, the Middle East and California that examined tissue acquired from glasshouse and field curcubits using dot-blot hybridization for diagnosis failed to detect LIYV in any of the samples collected.

2. Delimitation
No information

3. Monitoring
Monitoring for the virus involves, in the first instance, visual location of symptomatic plants (Brown et al. 1990). Surveying of weeds around susceptible crops is also recommended (Brown et al. 1990). However, as the disease's transmission and spread is inextricably linked to whitefly populations, the monitoring of *B. tabaci* is also important (Duffus and Flock 1982). This realisation has led to attention being paid to assessing the size and infectivity of *B. tabaci* populations through using a hand-held vacuum device to collect insects from the field to be used immediately for inoculation studies (Cohen et al. 1989). This study also used the release of fluorescent pigments to stain whiteflies in marked release studies for population size estimates. It has been noted that the numbers of viruliferous *B. tabaci* is closely correlated with the growing cycles of adjacent host crops in the USA and populations shift from one to another as they are cropped. As such, the risk in lettuce, for example, increases once melon has been harvested (Blua et al. 1994). The

relationship between vector population densities with disease (Bellows and Arakawa 1986) incidence has been developed can indicate the likelihood of lettuce becoming infected over the growing period.

4. Commodities
No information

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Leucaspis japonica Green

(=*Lopholeucapsis japonica* (Cockerell)
Common name(s): Japanese long scale, pear white scale
Taxa: Insecta: Hemiptera: Diaspididae
EU Annex designation: II/A1

Organism
Japanese long scale or pear white scale, *Leucaspis japonica* (syn. *Lopholeucaspis japonica* Cockerell) (Homoptera: Coccoidea: Diaspididae) attacks mainly *Citrus* spp., also other fruit trees and woody ornamentals. It has 2 or 3 generations in a year and overwinters mainly as 2nd or 3rd instar nymphs (Gan and Zheng 2007).

1. Detection
Attacks by *L. japonica* result in dieback and premature leaf fall, due to senescence of all infested branches. In some cases, the scales may be found in cracks in the bark, and are then difficult to detect on superficial examination (Anonymous).

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
In March 1999, *L. japonica* was intercepted in Italy on bonsai plants of *Acer* imported from China (Pellizzari and Vettorazzo 1999).

References: 39 references retrieved, none added, 8 retained, 2 used for summary

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Lime witches' broom phytoplasma

Common name(s) Oman witches' broom disease, witches' broom of lime
Taxa: Bacteria: Tenericutes: Acholeplasmataceae
EU Annex designation: II/A1 (as witches' broom MLO on citrus)

Organism
The only known natural host of lime witches' broom phytoplasma is *Citrus aurantiifolia*, the small-fruited acid lime which is grown in the Arabian Peninsula. The organism's range is restricted to this region.

1. Detection
Monoclonal antibodies and PCR primers for 16S rDNA amplification have been obtained for the Candidatus *Phytoplasma aurantifolia* isolated on lime trees in Oman (Bove and Garnier 2000). Information was provided on the morphology of Witches' broom disease of lime; moreover, the serological detection of WBDL phytoplasma as well as its relationship with other phytoplasmas are also discussed (Bove and Garnier 2003). Since phytoplasmas cannot be cultured *in vitro*, molecular techniques are needed for their diagnosis and characterization. The titer of phytoplasma cells in the phloem of infected plants may vary according to the season and the plant species, and it is often very low in woody hosts. Different DNA extraction procedures have therefore been tried to obtain phytoplasma DNA at a concentration and purity high enough for effective diagnosis. DNA/DNA hybridization methods were reported to be appropriate for the detection of phytoplasmas, but at present PCR is considered the most suitable (Marzachi 2004). Nested PCR followed by RFLP or cloning and sequencing were used for detection and identification of phytoplasmas in insect bodies (Siampour et al. 2006). Serological technique were developed to the identification of phytoplasma, this tool could be used for its monitoring disease in the field (Mirzai et al. 2010).

2. Delimitation
No information available.

3. Monitoring
A survey were carried out in Iran on the presence of phytoplasmas with PCR used for identification purposes (Salehi et al. 2000, Zirak et al. 2010).

4. Commodities
The possibility of transmission and spread of WBDL phytoplasma through the seed of infected plants was considered, but there was no evidence found that confirmed this hypothesis (Faghihi et al. 2011).

References: 19 retained out of 386 retrieved, none added, 8 used for the summary

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Liriomyza bryoniae (Kaltenbach)

Common name(s): Potato / tomato leaf miner
Taxa: Insecta: Diptera: Agromyzidae
EU Annex designation: I/B

Organism

Liriomyza bryoniae is a highly polyphagous species and a primary pest of important crops such as tomato, cucurbit (particularly melon, watermelon and cucumber) and glasshouse-grown lettuce and beans. It has been reported to complete its life cycle on plants from 16 different families. *L. bryoniae* is common in the wild in southern Europe, although the insect is now common under glass in many other parts of the region. The insect is also present in the Far East and the USA. Female flies puncture the cotyledons or the young leaves of the host plants causing wounds which serve as sites for feeding or oviposition. Females can live for a week or more, while males only up to 3 days. Eggs are mostly inserted in the upper surface of leaves. Females lay on average 100 eggs. Under glass in Europe, the leafminer breeds continuously throughout the spring, summer and autumn months. There are three larval instars which, in total, last 7-13 days. The mature larvae cut semi-circular exit slits in the upper surface of the leaves. After a short period the larvae drop to the ground and then burrow just below the surface of the soil before pupating. During winter, the pupae go into diapause or retarded development until the following spring.

1. Detection

Morphological identification is difficult and possible only in some life stages based on morphological characters (D'Aguilar and Martinez 1979, Darvas and Hatalane 1982, Zlobin and Drugova 2002). In addition, electron-micrographic and geometric morphometric methods were introduced for separating different species (Shiao 2004). An enzyme electrophoresis method for identification of main Agromyzidae species in each stage of the life cycle is also available (Oudman *et al*. 1995). Other molecular techniques such as RAPD-PCR protocol can help in the identification (Chiu *et al*. 2000).

Mine configuration is often considered a reliable guide to the identification of agromyzid species of economic importance (as in many such cases the species are host-specific). However, with polyphagous pest species such as *L. bryoniae*, mine configuration is affected by the host, by the physical and physiological condition of each leaf and by the number of larvae mining the same leaf. This wider range of variation means that identification from mine patterns alone should be treated with caution. Generally *L. bryoniae* produces a looser, irregular serpentine mine.

The use of sticky yellow plastic discs is the most efficient method for detection of adults (Nucifora and Vacante 1982). The yellow colour showed higher performance than aluminum foil colour, the synthetic methyl salicylate can be an effective extra-tool for increasing the attractiveness of traps (Buda and Radziute 2008).

2. Delimitation

Not information available.

3. Monitoring

Surveys of agromyzid leafminers were carried out in Vietnam (Hoa *et al*. 2005), in the region of Kebily (Tunisia) (Gahbiche *et al*. 2001), and in northern Sinai (Egypt) (El-Basiony *et al*. 1996). In the monitoring surveys the yellow sticky trap and the examination of attacked plants are the more common methods employed (Wang *et al*. 1998, Ostrauskas *et al*. 2005). No information is,

however, available on the optimal density and spatial distribution of the traps.

4. Commodities

Agromyzidae are frequently detected on imported material such as young plants, cut flowers and vegetables. For instance in Japan, *L. bryoniae* was detected on paprika plants imported from the Netherlands (Kasugai *et al*. 2001).

References: 24 retained out of 68 retrieved, none added, 14 used for the summary

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Liriomyza huidobrensis (Blanchard)

Common name(s): Serpentine leaf miner, pea leaf miner, South American leaf miner
Taxa: Insecta: Diptera: Agromyzidae
EPPO list A2: No. 283.
EU Annex designation: II/A2

Organism

Liriomyza huidobrensis, the pea leaf miner, is a highly polyphagous species able to colonize plants of 14 families and inflicting severe damage to crops. *L. huidobrensis* originated in South America and has spread to other continents where it has established populations. It is a multi-voltine species, in summer the generation time is between 17 and 30 days. Mating begins about one day after adult emergence and adult life time is between 12 and 14 days. The egg stage lasts three days. Performance is highly dependent on the host plant.

1. Detection

Morphological identification is difficult and possible only in some life stages based on morphological characters (Zlobin and Drugova 2002), such as abdominal colour patterns and genitalia. In addition, electron-micrographic and geometric morphometric methods were introduced for separating different species (Shiao 2004); moreover, an enzyme electrophoresis method for identification of main Agromyzidae species in each stage of the life cycle was developed (Oudman *et al*. 1995). The molecular techniques can help in the identification, such as a PCR assays to distinguish *L. huidobrensis* from associated species on lettuce cropping systems (Masetti *et al*. 2006) and from the morphologically cryptic leafminer *Liriomyza langei* (Scheffer and Lewis 2001). A RAPD-PCR protocol has also been used for identification of 6 species of *Liriomyza* (Chiu *et al*. 2000) spp. The DNA barcoding approach could provide rapid tool for species identification (Scheffer *et al*. 2006).
The sweep-net survey is a good method to determine the presence of insects (Arce de Hamity and Neder de Roman 1984). The yellow sticky boards were the most effective in trap capture (Chavez and Raman 1987) than others colours (Martin *et al*. 2005). The addition of aromatic substance (spruce oil, basil oil, juniper oil and clove oil) increased the number of trapped insects (Gorski and Pawlowska 2005). The best height probably depends from the crops: (Wang *et al*. 1998a) indicate the highest number of capture 10 cm above the top of plants, whereas (Martin *et al*. 2005) indicated that highest captures occurred about 20 cm below the top of the celery crop canopy.

2. Delimitation

After the first record a specific survey was carried out in France to describe and detail the distribution (Trouve *et al*. 1991).

3. Monitoring

A survey of agromyzid leafminers were carried out in Jordan (Al-Ghabeish and Allawi 2001), in Vietnam (Hoa *et al*. 2005), and in Indonesia (Rauf *et al*. 2000) also to evaluate parasitoids and host crops. A survey was also conducted in conventional fields in central and southern Vietnam, where *L. huidobrensis* was widely found on *Allium fistulosum* and *A. cepa* (Ueno 2006). The yellow sticky trap is the most common method used for the monitoring of leaf miners on vegetables in the suburbs of Beijing (Wang *et al*. 1998b) and in Belgium (de Steene and Tirry 2005). Extensive surveys were carried with the collection of foliage infested by leafminers from the representative weedy plants and from lettuce crop in Province of Bologna (Lanzoni *et al*. 2003) and from

horticultural crops in greenhouses of Southern Spain (Cabello *et al*. 1994).

4. Commodities

The leafminer flies, family Agromyzidae are frequently detected on the imported plants such as young plants, cut flowers and vegetables (Kasugai *et al*. 2001). *L. huidobrensis* is among the species with largest number of individuals among the intercepted insect species reported as identified with certainty (Roques and Auger-Rozenberg 2006). The species was intercepted on of plants of *Gypsophila elegans* imported from the Netherlands to the Czech Republic (Bittner and Cerny 1994), on cut flowers at Dublin in the Irish Republic (Cheek *et al*. 1993) and on cut flowers at Florida ports of entry (Steck 1996).

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Liriomyza sativae Blanchard

Common name(s): Vegetable leaf miner, serpentine leaf miner, cabbage/tomato leaf miner
Taxa: Insecta: Diptera: Agromyzidae
EPPO list A2: No. 282.
EU Annex designation: I/A2

Organism

Liriomyza sativae, the vegetable leafminer, is an important pest of Cucurbitaceae, Leguminosae, and Solanaceae. It is highly polyphagous, having been reported on nearly 40 hosts from 10 plant families. The natural range includes most part of South America. Then, it was introduced in North America, Asia and Africa.

Males usually emerge before females. Mating takes place from 24 h after emergence and a single copulation is sufficient to fertilize all eggs laid. Female flies puncture the leaves of the host plants causing wounds which serve as sites for feeding or oviposition. Eggs hatch in 2-5 days according to temperature. The duration of larval development is generally 4-7 days at temperatures above 24°C. *L. sativae* usually pupates externally, either on the foliage or in the soil just beneath the surface. In the southern USA, the life-cycle is probably continuous throughout the year. *L. sativae* completes its life-cycle in 24-28 days during the winter in California (December-January). Adults of *L. sativae* live between 15 and 30 days. On average, females live longer than males.

1. Detection

Morphological identification is difficult and possible only in some life stages based on morphological characters, such as abdominal colour patterns and genitalia (Abe and Kawahara 2001, Zlobin and Drugova 2002). In addition, electron-micrographic and geometric morphometric methods were introduced for separating different species (Shiao 2004). An enzyme electrophoresis method for identification of main Agromyzidae species in each stage of the life cycle is also available (Oudman *et al*. 1995). A RAPD-PCR protocol has also been used for identification of 6 species of Liriomyza spp. (Chiu *et al*. 2000). The DNA barcoding approach could provide rapid tool for species identification (Scheffer *et al*. 2006).

Mine configuration is often considered a reliable guide to the identification of agromyzid species of no economic importance (as in many such cases the species are host-specific). However, with polyphagous pest species such as *L. bryoniae*, mine configuration is affected by the host, by the physical and physiological condition of each leaf and by the number of larvae mining the same leaf. This wider range of variation means that identification from mine patterns alone should be treated with caution. Generally, *L. sativae* produces a looser, irregular serpentine mine (Anonymous, 2005).

The yellow sticky boards were the most effective trap to capture adults than others tested colours (Tryon *et al*. 1980, Fu *et al*. 2005). The trap shape does not influence the efficiency (Chandler 1981). The adults of *L. sativae* are trapped in higher number at the middle plant height than at the top of the plant and at ground level (Zehnder and Trumble 1984). A lure made of the seven components (2-hexenall 3-Hexen-1-ol, 2-Hexen-1-ol, 1-Octen-3-ol, 3-Hexenol acetate, a-Ionone and P-Ionone) has been demonstrated to be highly attractive for *L. sativae* adults in field trapping trials (Wei *et al*. 2005).

2. Delimitation

No information available

3. Monitoring

Specific surveys were carried out on *L. sativae* to evaluate phenology through sticky traps in northern Vietnam (Hofsvang *et al*. 2005) or to know host plant and parasitoids through the collection of leaves showing damage in Okinawa Prefecture (Japan) (Ishida *et al*. 2003) and Vietnam (Tran *et al*. 2005). The presence and incidence of the pest was evaluated in numerous generic monitoring of Agromyzidae in different countries and crops (Velez Angel *et al*. 1980, Schuster *et al*. 1991, Wang *et al*. 1998, Yuan *et al*. 1999, Rauf *et al*. 2000, Chen *et al*. 2003, Fei *et al*. 2008). No information is, however, available on the optimal density and spatial distribution of the traps.

4. Commodities

Agromyzidae are frequently detected on the imported material such as young plants, cut flowers and vegetables (Kasugai *et al*. 2001). Pre-shipment disinfestation treatments are a critical component in the battle to prevent the spread of alien pest species a possible solution could be the combination of cold storage and methyl bromide could fumigation. *L. sativae* pupae on lettuce were also indicated as having no survivors at fumigation with MB 46g/m(3) for 3 hours at 10 degrees C or MB 40g/m(3) for 3 hours at 15 degrees C. These newly estimated MB-saving standards were convincingly effective (Misumi *et al*. 2009).

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Liriomyza trifolii (Burgess)

Common name(s): American serpentine leaf miner, chrysanthemum leaf miner
Taxa: Insecta: Diptera: Agromyzidae
EPPO list A2: No. 131.
EU Annex designation: II/A2

Organism

Liriomyza trifolii, the American serpentine leafminer, is best known as a pest of chrysanthemums and celery, although it has a wide host range. For instance (Saradhi and Patnaik 2004) revealed that 85 plant species has been recorded as hosts of this pest in a survey conducted in Orissa state of Bubaneswar. *L. trifolii* has long been found in the eastern United States and Canada, northern South America, and the Caribbean. However, in recent years it has been introduced also into California, and Europe. It is a multi-voltine species. One study carried out in California showed that under greenhouse conditions in March the life-cycle from egg to adult lasted up to 20 days, and the adults lived for up to 30 days, during which the females laid about 250 eggs each at a daily rate of 17 eggs. The females puncture the leaves both for feeding and for oviposition, and males feed at the same punctures (Parrella *et al*. 1981).

1. Detection

Morphological identification is difficult and possible only in some life stages based on morphological characters, such as abdominal colour patterns and genitalia (Bogs and Braasch 1988, Abe and Kawahara 2001). In addition, electron-micrographic and geometric morphometric methods were introduced for separating different species (Shiao 2004). An enzyme electrophoresis method for identification of main Agromyzidae species in each stage of the life cycle is also available (Oudman *et al*. 1995). A RAPD-PCR protocol has also been developed for identification of 6 species of *Liriomyza* spp. (Chiu *et al*. 2000). The DNA barcoding approach could also provide rapid tool for species identification (Scheffer *et al*. 2006).

Mine configuration is often considered a reliable guide to the identification of agromyzid species of economic importance (as in many such cases the species are host-specific). However, with polyphagous pest species such as *L. trifolii*, mine configuration is affected by the host, by the physical and physiological condition of each leaf and by the number of larvae mining the same leaf. This wider range of variation means that identification from mine patterns alone should be treated with caution. *Liriomyza trifolii* produces a tightly coiled, almost blotch-like mine.

The use of yellow sticky boards is the most effective method to capture the adults while other colors are less effective (Park *et al*. 2001). Contrasting and mixed information was found about the attractiveness of different yellow types. In one study no difference among the different yellow shades, lemon yellow and golden yellow has been found (Durairaj *et al*. 2007). However, other authors found higher performance of fluorescent yellow over golden yellow in greenhouse (Al-Ayedh and Al-Doghairi 2006), while a similar study showed no difference among fluorescent, translucent and opaque traps in the greenhouse but a preference for opaque traps in the field (Chandler 1981). The traps placed at 30 cm above ground level showed the highest number of captures (Chandler 1985, Galande and Ghorpade 2007). Moreover, higher catches was found on vertical traps than on horizontal or 45-degree angle traps (Yathom *et al*. 1988).

2. Delimitation

After the first record a specific survey was carried out in India to delimit the population distribution (Lakshminarayana *et al*. 1992). A similar survey was also carried out in Korea (Han *et*

al. 1996). No information is, however, available on the optimal density and spatial distribution of the traps to delimit the populations.

3. Monitoring

The incidence and phenology of *L. trifolii* was evaluated in specific surveys in several regions. Examples can be found in South India (Srinivasan *et al*. 1995), and Mexico (Valenzuela-Escoboza *et al*. 2010). Generic surveys of agromyzid leafminers were also carried out in Jordan (Al-Ghabeish and Allawi 2001), Vietnam (Hoa *et al*. 2005), in Lithuania (Ostrauskas *et al*. 2005), and Florida (Schuster *et al*. 1991). Extensive surveys with the collection of foliage infested by leafminers were carried in northern Italy (Masetti *et al*. 2004) and from horticultural crops in greenhouses in southern Spain (Cabello *et al*. 1994). The yellow sticky trap is the most common method used in numerous monitoring of leaf miners (Ohno *et al*. 1999, Valenzuela-Escoboza *et al*. 2010). A time-efficient method for estimating the number of insects caught on yellow sticky traps was developed, i.e. the total number of insects on a trap could be estimated by counting only a 2.3-cm-wide vertical strip of the trap (Heinz *et al*. 1992).

4. Commodities

Agromyzidae are frequently detected on the imported material such as young plants, cut flowers and vegetables (Seymour *et al*. 1985, Kasugai *et al*. 2001). For instance, *L. trifolii* was introduced into Canada on chrysanthemum cuttings from Florida (McClanahan 1983), and into England from Malta and Kenya (Seymour *et al*. 1985). The agromyzid appears to have been first introduced into France with gerberas imported from the Netherlands (D'Aguilar and Martinez 1979). In order to prevent the entry of *L. trifolii*, some countries have decided to prohibit the importation of the main host plants of Agromizidae (for example rooted chrysanthemum, gerbera, cucumber, tomato and lettuce plants) (Rautapaa 1984). Pre-shipment disinfestation treatments are a critical component in the prevention of the introduction of this species. Promising alternatives to methyl bromide fumigation are controlled atmosphere, heat treatment, irradiation, and combinations of the above mentioned treatments. All these alternatives have been demonstrated to effectively disinfest perishable commodities, especially tropical cut flowers and foliage and potted plants (Hara 2002). Combination of cold storage and methyl bromide fumigation is a common technique (Mortimer and Powell 1984). The pupae of a close species, *L. sativae*, on lettuce showed no survivors at fumigation with MB 46g/m(3) for 3 hours at 10 degrees C or MB 40g/m(3) for 3 hours at 15 degrees C (Misumi *et al*. 2009).

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Listronotus bonariensis (Kuschel)

Common name(s): Argentine stem weevil, wheat stem weevil
Taxa: Insecta: Coleoptera: Curculionidae
EPPO A1 list: No. 168
EU Annex designation: II/A1

Organism

Listronotus bonariensis (Argentine stem weevil) attacks pasture grasses, in particular *Lolium* spp. Most research on the biology and control of this species has been undertaken in New Zealand, where *L. bonariensis* causes significant economic damage. There is potential for this species to transmit viruses (Smales *et al*. 1995). It is absent from the EU/EPPO region.

1. Detection

Adult weevils can be detected by soil samples, sweep netting, sticky traps and suction traps. Sweep netting is dependent on temperature and wind speed. Numbers of adults caught in emergence and aerial sticky traps were poorly correlated with field populations (Prestidge *et al*. 1985a).

Larvae have been detected visually from tillers cut at ground level or by using a heat extraction technique (Goldson 1978). Just under a third of larvae were found to be present in the thatch rather than in the tillers (Goldson *et al*. 2001). Sampling and extraction techniques for the soil-borne stages have been developed (Barker and Addison 1989). The sampling unit was a 75 mm diam. soil core taken to a depth of 25-30 mm. The cores were transferred directly from the corer barrel into plastic bags. Cores were held in a cool store at 4-5°C for up to 10 days before extraction. The mechanical extraction process involved breakdown of the soil cores to remove invertebrates > 5mm, passing the crumbled soil through sieves and flotation of the fauna in a bath of magnesium sulphate solution (specific gravity ≥ 1.1).

In order to obtain large numbers of weevils for mass rearing of parasitoids, an electric blanket on top of a tarpaulin placed in a paddock has been used (Goldson and Proffitt 1991). Weevils were collected after 2-3 hours from under the tarpaulin either manually or using a small vacuum cleaner. There was a tendency for a greater proportion of parasitized weevils to be found using this method in comparison with sweep netting. It was suggested by the authors that this method could be a useful survey tool to monitor for the presence of this species.

2. Delimitation

No relevant references found

3. Monitoring

Surveys have been undertaken in New Zealand to examine the presence of *L. bonariensis* in ryegrass pasture. These have involved visual detection of this species (Cromey *et al*. 1980, Prestidge *et al*. 1985b, Bejakovich *et al*. 1998).

4. Commodities

No relevant references found

References: 13 retained out of 86 retrieved, 2 added, 9 used for summary

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Little cherry pathogen

(= Little cherry virus 1, little cherry virus 2)
Common name(s): Little cherry disease,
Taxa: Viruses: Closteroviridae: Closterovirus
EU Annex designation: II/A1

Organism
This organism was for a long time of uncertain taxonomic position as the causative agent difficult to isolate although it was strongly suspected to be a virus (Eppler 1998), and was frequently referred to as such in the literature (Vitushkina *et al*. 1997) until its identity was confirmed. It is also known that the disease is caused by at least two highly-related organisms (Rott and Jelkmann 2001, Jelkmann *et al*. 2008). The causative organisms occur as several variants as determined by sequence analysis (Theilmann *et al*. 2004). The disease affects several species of *Prunus* and causes poor growth and maturation in fruit and leaf discolouration with the fruit rendered tasteless (Yorston 1990, Komorowska and Cieslinska 2004). The disease is reported from North America, parts of Asia, Oceania and the EU. The mealy bug *Phenococcus aceris* is known to be the vector (Yorston *et al*. 1981, Raine *et al*. 1986) as are other homopteran species (Eppler 1998).

1. Detection
Earlier methods for the detection of little cherry pathogen involved the microscopic examination of acridine-orange stained tissues (Verbeek and O'Reilly 1976) such as the phloem (Legrand and Verhoyen 1985b). In particular, the observation of stained deposits on the cell walls was seen as diagnostic for the pathogen (Verbeek 1977). The analysis of double-stranded RNA was rapidly adopted for the diagnosis of the disease during the late 1970s and 1980s (Hamilton *et al*. 1980, Legrand and Verhoyen 1985a) and has been regularly used since (Eastwell and Bernardy 1996). Indicator plants have also been used as reliable methods for detecting little cherry pathogen (Zahn 1995). Comparisons between indexing and Northern blotting for DS-RNA indicated the latter to be more reliable and much more rapid (Eastwell *et al*. 1996).
RT-PCR methods have been developed that can rapidly diagnose the disease (Vitushkina *et al*. 1997) and its variants (Rybak *et al*. 2004, Theilmann *et al*. 2004) and have been exploited as part of for detection surveys in a number of countries (Rochon *et al*. 2002, Isogai *et al*. 2004, Matic *et al*. 2007) or discreet regions within a country (Bajet *et al*. 2008). Similarly, RT-PCR has been used to identify the first occurrence of one or the other of the two causal agents associated with the disease (Komorowska and Cieslinska 2008). A multiplex RT-PCR assay has also been described with the ability to detect a range of stone fruit viruses (Matic *et al*. 2010). Some ELISA methods are also described (Theilmann *et al*. 2001) and have been used for detection surveys in Poland (Komorowska and Cieslinska 2004). Serological detection of the disease has, however, been noted to be problematic (Matic *et al*. 2009)

2. Delimitation
No information

3. Monitoring
The status of little cherry pathogen has been closely monitored since the late 1970s in Canada. An excellent description of the surveying methodology is provided by Slykhuis *et al*. (1980) and results of subsequent surveys are available (Yorston *et al*. 1981, Yorston 1990). In this case, surveys were conducted in June and July and samples were taken on the basis of observable symptoms. Diagnosis was conducted via indexing (using buds from affected trees). In the Canadian context,

trees with the disease are marked for later destruction and budwood taken for either grafting, or, more recently, ELISA or molecular diagnosis (Yorston 1990). Reports of monitoring conducted outside North America include instances from Germany (Eppler 1998, 2001, Eppler *et al*. 2001) and Poland (Cieslinska and Morgas 2010).

4. Commodities
No information

References: 63 retained out of 108 retrieved, none added, 30 used for summary

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Longidorus diadecturus Eveleigh and Allen

Common name(s): Needle nematode, sting nematode
Taxa: Nematoda: Dorylaimida: Longidoridae
EU Annex designation: I/A1

Organism
Adult females and larvae of the nematode *Longidorus diadecturus* are vectors of peach rosette mosaic nepovirus (PRMV) (Allen et al. 1982, Tacconi and Ambrogioni 1995a). This nematode is found in Canada, where its principal host is peach (Eveleigh and Allen 1982, Tacconi and Ambrogioni 1995a) and the central USA. The phylogenetic relationships between *Longidorus* and *Xiphinema* nematode species from North America has been inferred from the analysis of 18S rDNA sequences (Neilson et al. 2004).

1. Detection
Nematodes can be extracted from soil samples by combining the roiling-sieving and sugar centrifugation methods (Ye and Robbins 2001). *Longidorus diadecturus* differs from all other North American *Longidorus* species in having weakly developed odontophore flanges and a guide-ring at about mid-odontostyle (Robbins and Brown 1991). A stepwise discriminant analysis of nine *Longidorus* species indicated that the guide ring position, head width, tail length, body length, odontostyle length, and anal body width were the most important variables to identify each species (Ye and Robbins 2004).

2. Delimitation
No information available.

3. Monitoring
Soil samples are taken to a depth of 30 cm using a shovel and stored in plastic bags at 4°C until they can be processed (Van Driel et al. 1990). The runoff from the Fenwick Can Extraction Procedure is then poured through a 250 µm mesh screen. The nematodes can then be washed from the screen into 100 ml test tubes.

4. Commodities
In Europe, obligatory regulations for certification of fruit trees have been applied to slow down the spread of damaging pathogens, including plant parasitic nematodes (Tacconi and Ambrogioni 1995b, Ward and Hockland 1996, Silvestro and Tacconi 1998).

References: 14 retained out of 23 references retrieved, none added, 11 used for summary

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Ye, W. and R. T. Robbins. 2001. Morphological and molecular taxonomy of Longidorus occurring in Arkansas. Phytopathology 91:S146.

Ye, W. and R. T. Robbins. 2004. Stepwise and canonical discriminant analysis of Longidorus species (Nematoda : Longidoridae) from Arkansas. Journal of Nematology 36:449-456.

Margarodes spp

Common name(s): Ground pearls, margarodes
Taxa: Insecta Hemiptera: Margarodidae

A number of species are of concern as potential pests of European grapevine. The three major pests of concern (detailed below) and other related species are dealt with together due to the relatively low volume of literature associated with them.

Margarodes prieskaensis
EPPO A1 list: No. 214
EU Annex designation: II/A1

Margarodes vitis
EPPO A1 list: No. 215
EU Annex designation: II/A1

Margarodes vredendalensis
EPPO A1 list: No. 216
EU Annex designation: II/A1

Organism
Margarodes spp. are subterranean insects related to above ground scale insects that gain their common name – ground pearls – from their distinctive appearance in the soil. Most species are restricted to feeding upon the roots of wild grasses but sugar cane and grape are also attacked (Mariconi and Zamith 1973, Salinas 1975). Cereals can also be economically affected by some species of *Magarodes*, most notably in Turkey (Duran 1976, Ozer 1976).
Species attacking the roots of grapevine lay eggs close to the root at a depth of about 50cm and the subsequent larvae attach themselves and become sessile and develop to appear as peal-like cysts. The damage caused by the feeding of the larvae has been described as similar to that of phylloxera (*Viteus vitifolia*), with a general loss of vigour of the vines leading to the death of the plant in some cases (Toit 1975, Anonymous 1997, 2007)
Margarodes are widely distributed and are found throughout the Americas, the Caribbean, Australia and Southwest Asia although they are absent from Europe. The biology, economic importance and distribution have been addressed by Foldi (2005)

1. Detection
Magarodes species are readily detectable by eye in the soil around the roots of affected plants. Identification of the species can be achieved through descriptions present in the literature (Duran 1971, Ben-Dov 2005, Foldi 2005). This apart, little information is available with respect to detecting this assemblage of coccids in the various crops they affect. The paper Hoffman and Smith (1991) in addition to providing a good overview of the biology of *Magarodes*, provides some SEM photographs of various stages of *M. meridionalis* that may be useful for identification purposes.

2. Delimitation
No information available

3. Monitoring

Very little work has specifically addressed methods for the monitoring of *Magarodes* species. The best description of a monitoring methodology is provided by Hoffman and Smith (1991) who provide details of a monitoring exercise in bemudagrass. Here, soil was excavated from infested experimental plots and taken to the laboratory for the examination of adults, egg masses and cysts.

4. Commodities

No information available

References: 45 retained out of 168 retrieved, 1 added, 15 used for the summary

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Toit, G. D. G. d. 1975. Notes on the biology and behaviour of Sphaeraspis prieskaensis Jak. Entomological Society of Southern Africa, Pretoria.

Melampsora farlowii (J.C. Arthur) J.J. Davis

(= *Chrysomyxa farlowii* Saccardo and Traverso; *Necium farlowii* J.C. Arthur)
Taxonomic position: Fungi: Basidiomycota: Melapsoraceae
EPPO A1 list: No. 15
EU Annex designation: I/A1

Organism
Melampsora farlowii is the causal agent of the hemlock rust. The main hosts are *Tsuga canadensis* and *Tsuga caroliniana*. Rust symptoms appear on leaves and twigs in spring. The fungus fructifications (teliospores) overwinter on twigs and cones killed the previous spring. Teliospores in dead twigs and fallen needles germinate in spring, producing basidiospores that are wind dispersed and infect young cones, needles and stems (Kenaley S. C. 2010). It is the most destructive rust attacking *Tsuga* spp. and economic impact may be important in nurseries growing ornamental trees. Present only in North America, the pathogen is absent from EPPO region (Anonymous 1980).

1. Detection
Visual inspection during spring in commercial nurseries growing hemlocks may allow the disease detection.

2. Delimitation
No information available

3. Monitoring
No information available

4. Commodities
In international trade, *Melampsora farlowii* is liable to be carried on infected host planting material (Anonymous 1980).

References: 1 retained out of 9 retrieved, 2 added, 2 used for the summary

Anonymous (1980). Data Sheets on Quarantine Organisms: *Melampsora farlowii* (J. C. Arthur) J. J. Davis. EPPO Bulletin 10(1): 9-12.

Kenaley S. C., H. G. W. (2010). Hemlock twig rust caused by Melampsora farlowii (Arth.) Davis. Cornell University publications, College of Agriculture and Life Sciences.

Melampsora medusae Thumen

Common name(s): Polar rust
Taxa: Fungi: Basidiomycota: Melampsoraceae
EPPO A2 list: No. 74
EU Annex designation: I/A2

Organism

Melampsora medusae is one of the causal agents of poplar rust (*Melampsora larici-populina* and *Melampsora allii-populina* are the main species involved in this disease in Europe). For *Melampsora medusae*, two *formae speciales* have been distinguished: *Melampsora medusae* f. sp. *deltoidae* and *Melampsora medusae* f. sp. *tremuloidae*. The primary telial hosts are *Populus* spp. while the secondary aecial hosts are conifers (*Larix* spp., *Pseudotsuga* spp. and *Pinus* spp.). Originating from North America it has spread to other continents: in Europe, it has been reported in Belgium, France, Portugal and Spain. Abundant uredinia production on poplar leaves can lead to premature defoliation, growth reduction and even tree dieback after several years of severe infection. This pathogen has a high potential for natural spread: urediniospores and aeciospores can be spread by wind over long distances (Anonymous 1982).

1. Detection

Early symptoms on leaves are small yellowish pustules (uredinia containing masses of urediniospores) on the abaxial leaf surface. They are hardly distinguishable from those caused by poplar rust fungi native to Europe and therefore are not sufficient for diagnosis. *Melampsora medusae* morphological characterization is possible with microscopic observation of urediniospores but it may be difficult to detect them among a large number of other *Melampsora* urediniospores: specific molecular methods are recommended (Anonymous, 2009). Feau *et al*. developed a method for *Melampsora medusae* f. sp. *deltoidae (*Feau, Bergeron *et al*. 2007).DNA barcodes provided a highly accurate means of identifying *Melampsora* taxa (Feau, Vialle *et al*. 2009).

2. Delimitation

Spore traps could be used for infected area delimitation. They allowed observations on *Melampsora medusae* occurrence in Central USA (McCracken, Schipper *et al*. 1984).

3. Monitoring

Kinetic PCR, by quantification of pathogen DNA, may allow *in vivo* pathogen monitoring: it was demonstrated for poplar rust caused by *Melampsora species* (Boyle *et al*. 1546).

4. Commodities

In international trade, the pathogen can be spread on infected planting material of the various hosts (Anonymous 1982).

References: 9 retained out of 55 retrieved, 1 added, 6 used for the summary

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Feau, N., A. Vialle, *et al*. (2009). Fungal pathogen (mis-) identifications: a case study with DNA barcodes on Melampsora rusts of aspen and white poplar. Fungal Biology 113(6): 713-724.

McCracken, F. I., A. L. Schipper, *et al*. (1984). Observations on Occurrence of Cottonwood Leaf Rust in Central USA. European Journal of Forest Pathology 14(4-5): 226-233.

Meloidogyne chitwoodi (Golden et al.) and *Meloidogyne fallax* (Karssen)

Taxa: Nematoda: Tylenchida: Meloidogynidae

M. chitwoodi
Common name(s): Colombia root knot nematode
EPPO: A2 list No 295
EU Annex designation: I/A2

M. fallax
Common name(s): False Colombian root know nematode
EPPO: A2 list No 227
EU Annex designation: I/A2

Organism

The two *Meloidogyne* species are closely related and difficult to distinguish by morphological characters. When compared with *M. fallax, M. chitwoodi* has a much wider range of host plants. Among them are crops e.g. potatoes (*Solanum tuberosum*) and tomatoes (*Lycopersicon esculentum*) that serve as good hosts. Other hosts, comprising cash crops like barley (*Hordeum vulgare*), maize (*Zea mays*), oats (*Avena sativa*) sugar beet (*Beta vulgaris*), wheat (*Triticum aestivum*) are less suitable for their development, but the nematodes are capable maintain populations on them. Results of extensive investigations in the Netherlands showed that bulbs of many ornamental plants serve as hosts as well, a fact that has had a significant impact on entry inspection regimes (den Nijs et al. 2004). *M. fallax*'s only natural host plant is potato (*Solanum tuberosum*). The species has been detected in several EU countries and also in Australasia.

Two races of *Meloidogyne* spp. are known, which are distinguishable by slight differences in host range e.g. Lucerne (*Medicago sativa*) is host to race 2, whereas carrots (*Daucus carota*) are host for race 1. The distribution of *M. chitwoodi* in Europe is currently under investigation in order to find undetected distribution areas. Within the EU the species is reported from Belgium, The Netherlands, Germany and Portugal, but is detected in South Africa and Pacific Northwest of the USA and Mexico as well (den Nijs et al. 2004).

Larvae hatch from eggs in the soil or on the root surface. The juveniles of the second-stage are attracted to the roots and penetrate the roots closely behind the root tip. In this place the nematodes start feeding on cells, which are stimulated to turn rapidly into giant cells. Neighbouring cells begin the typical gall formation associated with infection by this nematode.

Males leave the root and are found free in the rhizosphere or near the protruding body of the female. Adult females have characteristically pear-shaped, pearly-white bodies and they are found embedded in host tissue. Eggs are laid by the female in a gelatinous sac near the root surface.

The root-knot nematodes are able to move only a few metres annually on its own, but it is spread readily through the transport of infested plant and plant products, in soil clinging to farm implements and in irrigation water.

Root-knot nematodes affect root growth, yield and quality of their hosts. The above ground symptoms are not readily apparent, but they may consist of various degrees of stunting, lack of vigour, and wilting under moisture stress (den Nijs et al. 2004). The species hibernate as eggs or juveniles and are able to survive long periods of temperatures below the freezing point.

1. Detection

Extraction and diagnosis methods are described in detail in "Protocol for the diagnosis of quarantine organisms *Meloidogyne chitwoodi* and *Meloidogyne fallax*" (Anonymous, 2004.). A very good overview of diagnosis procedures is provided by EPPO (in this paper it is stated, that molecular methods are to be preferred to morphological ones, when the two *Meloidogyne* species are to be distinguished).
Karssen et al. (1995) discriminated *M. fallax* and *M. chitwoodi* females by their esterase (EC 3.1.1.1) and malate dehydrogenase (EC 1.1.1.37) isozyme patterns, using the general method of Esbenshade & Triantaphyllou (1985) for identification of female *Meloidogyne* species by isozyme electrophoresis. Additionally, the isozyme glucose 6-phosphate dehydrogenase (EC 1.1.1.49) was used to differentiate the two species (van der Beek & Karssen, 1997). van der Beek et al. (1997) used mini two dimensional gel electrophoresis to study the total soluble protein patterns of *M. hapla*, *M. chitwoodi* and *M. fallax*, and confirmed these species to be distinct biological groups. Zijlstra et al. (1995) used PCR amplification and restriction fragment length polymorphism (RFLP) of the internal transcribed spacer (ITS) of ribosomal DNA (rDNA), and recorded distinct differences between *M. hapla* and *M. chitwoodi*. This method is also useful for differentiating mixtures of root-knot nematodes, including *M. chitwoodi* and *M. fallax* and was improved without the need for subsequent enzyme digestion (Zijlstra, 1997). Peterson & Vrain (1996) described a rapid PCR identification method for *M. hapla*, *M. chitwoodi* and *M. fallax* based on amplification of the rDNA intergenic spacer (IGS), without the need for restriction enzyme digestion. In addition, species-specific primers were developed for *M. chitwoodi* and *M. fallax*, based on unique sequences within ribosomal IGS (Peterson et al., 1997). Besides microspic detection molecular methods like PCR is implemented.

2. Delimitation
No information available.

3. Monitoring
The development of appropriate survey and test methodologies within the EU will remain a future task. For Germany the drafting of respective guidelines intended to support the plant protection agencies of the Länder is actually continuing (after a personnel communication with JKI at 18.01.2012).

4. Commodities
The nematodes are able to move only very limited distances, mostly staying in the rhizosphere of their hosts. Spread is generally facilitated by the transport of infested plants and plant products. Therefore, entry inspections for visual symptoms are undertaken (den Nijs et al. 2004).

References: 64 retained out of 539 retrieved, 1 added, 10 used in the summary

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Monilinia fructicola (Winter) Honey

(=*Sclerotinia fructicola* (Winter) Honey)
Common name(s): Brown rot, twig canker
Taxonomic position: Fungi: Ascomycota: Sclerotiniaceae
EPPO A1 list: No. 153
EU Annex designation: I/A1

Organism
Monilinia fructicola causes brown rot mainly on stone fruit (*Prunus* spp.). It may also affect other rosaceous fruit trees (*Malus , Pyrus*) and was even reported on *Vitis vinifera* (Sholberg et al. 2003).The disease may destroy a crop by killing blossoms or by rotting mature fruits, either on the tree or after harvest(2009). Leaves and shoots are also attacked. This fungus does not depend on specific vectors for propagule dispersal; conidia may be dispersed by wind, water and many kinds of vector, e.g. insects and birds (Leeuwen et al. 2001).Three *Monilinia* species and one *Monilinia* anamorph (*Monilia* sp.) may cause brown rot, of which two (*Monilinia fructigena* and *Monilinia laxa*) have long been present in Europe. *Monilinia fructicola* occurs in North and South America, Japan and Australia. It was detected in France in 2001 and isolated outbreaks have been reported in several European countries (Anonymous, 2009).

1. Detection
In European countries were surveys are conducted, visual examinations are performed in nurseries and in stone fruit production orchards to select samples with possible symptoms for immediate testing. Final identification is based on species-specific primers and on methods described in the EPPO diagnostic protocol (2009).To distinguish the quarantine pathogen *M. fructicola* from other brown rot agents (*M. fructigena* and *M. laxa*), an electrophoresis method using total mycelial protein SDS-PAGE was developed in Italy (Belisario et al. 1999). Then specific and sensitive molecular methods were developed (Boehm et al. 2000) and used directly on symptomatic fruits or plants (Fulton and Brown 1997, Forster and Adaskaveg 2000, Ioos and Frey 2000, Boehm et al. 2001, Cote et al. 2004).Molecular identification could confirm the result of the morphological method (Petroczy and Palkovics 2005, Pizzuolo et al. 2006). Recently, an automated DNA extraction method combined with a multiplex real-time PCR based on TaqMan chemistry was developed for fast, convenient, reliable and specific detection (Brouwershaven et al. 2010). Methodologies using chemical treatments for the detection of latent infections in stone fruits were also tested (Moreira and May-De Mio 2007)

2. Delimitation
In South Africa, molecular techniques with species-specific primers for *M. fructicola*, *M. laxa* and *M. fructigena,* based on the EPPO diagnostic protocol for *M. fructicola*, were used to identify presumed positive *Monilinia* isolates and to justify scientifically the regulated status of this pathogen (Carstens et al. 2010). As soon as the fungus was detected in Europe, delimitation surveys were carried out to determine the possible spread of the disease (Albert et al. 2004, Duchoslavova et al. 2007, Munoz et al. 2008, Michel 2009, Hilber-Bodmer et al. 2010).

3. Monitoring
No information available

4. Commodities

Imported stone fruits and nursery stock are the main pathways for *M. fructicola* introduction (Bosshard et al. 2006). This quarantine fungus has a wide host range and the import of plants for planting of *Prunus, Malus, Pyrus, Cydonia* and other *Rosaceae* presents the major pathway for introduction into the EU (Leeuwen et al. 2001).

References: 40 retained out of 214, none added, 21 used for the summary

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Monochamus spp.

Common name(s): Longhorn beetles, sawyer beetles
Taxa: Insecta: Coleoptera: Cerambycidae
EU Annex designation: I/A1 - as *Monochamus* spp. (non-European)

Organism
Monochamus spp. (Coleoptera: Cerambycidae) are the vectors of the pine wood nematode or pine wilt disease, *Bursaphelenchus xylophilus* (Steiner & Buhrer) Nickle. *Monochamus* larvae mainly grow and develop in *Pinus* spp. but also in other coniferous genera as *Abies* spp. and *Larix* spp. (Hwang et al. 2008).
In Asian countries, the Japanese pine sawyer beetle *Monochamus alternatus*, is the main vector of pine wilt disease, the most serious forest disease in Japan (Shoda-Kagaya 2007). In Russia, six species of *Monochamus* beetles are found, but there is no evidence that *B. xylophilus* has ever occurred in that country (Kulinich and Orlinskii 1998). While in USA, *B. xylophilus* and two beetle vectors *Monochamus caroliniensis* and *M. titillator* were found (Esser et al. 1983).

1. Detection
Ovipositing female *M. alternatus* prefer stressed *Pinus massoniana* over healthy trees (Fan et al. 2007b). Volatile compounds from stressed and healthy pine stems were collected using absorbent trap collection method. Significant differences in absolute terpene quantities between stressed and healthy pines occurred for 7 of 10 terpenes. Field trials demonstrated that four terpenes identified from host pines were attractive to *M. alternatus* with (+)-alpha-pinene as the most attractive compound. Ethanol appeared to be an important synergistic compound causing significant increase in attraction (Fan et al. 2007b).
According to Hao et al. (2009), FA01 was the strongest attractants in capturing *M. alternatus*. Cross-vane pan traps baited with the same pine volatiles caught much more *M. alternatus* than multiple tunnel traps. In addition, *M. alternatus* was significantly attracted to lures with at a release rate of 300 mg/d compared with the lures at a rate of 150 mg/d (Hao et al. 2009).
An interesting study carried out by Li and Zhang (2008) showed with field tests that females gnawed significantly fewer oviposition scars on *Pinus massoniana* trees treated with hexane extracts of larval frass than on the control trees. Mean catch of females in traps with mixtures of synthetic compounds (3.80 +/- 1.02) was significantly fewer than those in the control traps (16.80 +/- 1.16). These results suggested that hexane extracts of larval frass and mixtures of alpha-pinene, beta-pinene, 3-carene, limonene, longifolene and butylated hydroxytoluene can deter the females of *M. alternatus* and may be applied in management of this pest.
Fan et al. (2007a) suggest that the contact sex pheromone maybe does not exist in the body of *M. alternatus*. Mature females and males aggregate on host stem by perceiving the host volatiles. In the small environment of the host stem, visual cues may play an important role in the following mating behaviour of *M. alternatus*.

2. Delimitation
Based on the theory and methods of neural networks, climatic data, geographical data and the occurrence in north of Fujian province, an outbreak forecast of *Monochamus alternatus* was established. The results showed that the established neural network model had both a satisfying fit and forecasting precision. The mean forecast precision for occurrence numbers was 97.2% in two survey plots, and the general forecast accuracy was 100% (Chen et al. 2006).

3. Monitoring

Chen et al. (2010) showed that meteorological factors (atmospheric pressure, average temperature, the temperature difference, total precipitation, and average humidity) and environmental factors (slope location, canopy density, coverage degree of ground, elevation, stand age, height of trees and slope aspect) composed the main factors that caused quantitative changes of trapped adults and emergence holes of *M. alternatus* by factor analysis of vectorial progression.

A comparative study on the field attraction effects of different attractant, trap, lure and controlled-releasing amount on *M. alternatus* was carried out by Wang et al. (2005). Their results showed that MA2K05 was the strongest attractant (mean capture = 26.3 individuals each trap) and was attractive for other species of Coleoptera and Hemiptera; while MA2K13 captured 21.3 individuals each trap. Among the lures tested, the best two lures were lures C (60 ml plastic cup with 2 of 5 cm round holes on the cover) and B (20 ml specified controlled-releasing plastic bottle), with a capture efficiency of 34.25 and 20.3 individuals each trap, respectively. Because the attractant volume of lure C was 1.5 times larger than that of lures B and the attractant for lure C was replenished every 3-5 days, while that for lures B could be used for more than a month with once in place, lure B can be considered as the best lure. As for the test traps, the Xuanzhou trap was superior to a Japanese trap design, with a trapping efficiency of 36.4 and 9.7 individuals per trap, respectively. The attractiveness of attractants was significantly improved when it was up to 120 ml (Wang et al. 2005).

4. Commodities

The most dangerous imported products are stones and electronic equipment from PR China shipped in containers with solid wood packaging material (Krehan et al. 2004). In 1999, *Bursaphelenchus* spp. were found in packaging wood (pallets, crates, dunnage) imported with other goods from China to Austria, and their possible vector species of wood- and bark-breeding insects were recorded (Tomiczek et al. 2003). However, due to strong phytosanitary import requirements the number of detected living stages of organism (e.g. wood boring insects, sapwood-nematodes) decreased compared to the years before (Krehan et al. 2004).

References: 99 retained out of 333 references retrieved, none added, 13 used for summary

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Mycosphaerella laricis-leptolepidis K. Ito, K. Sato and M. Ota

(Anamorph: *Phoma yano-kubotae* Kitajima, *Phyllosticta laricis* Sawada)
Common name(s): Needle cast of Japanese larch
Taxa: Fungi: Ascomycota: Mycosphaerellaceae
EPPO A1 list: No. 16
EU Annex designation: I/A1 as *Mycosphaerella larici-leptolepis*

Organism
Mycosphaerella laricis-leptolepidis is the causal agent of the disease known as "needle cast of Japanese larch". The principal hosts are *larix decidua, Larix gmelinii* var. *japonica*, *Larix gmelinii* var. *olgensis* and *Larix leptolepis*. Present in Asia, the pathogen is absent from Europe and EPPO region. Fungus fructifications develop on fallen needles in contact with the soil during the autumn and winter. The ascospores are carried in air currents and infect the current season's needles mostly from late May to mid-June. This pathogen is the most important defoliator of *Larix* in Japan. Trees in plantations are most severely affected but seedlings and saplings may also be attacked (Anonymous 1990).

1. Detection
No information available

2. Delimitation
No information available

3. Monitoring
No information available

4. Commodities
EPPO recommends that all countries should prohibit importation of plants for planting and cut branches of *Larix* from Japan (Anonymous 1990).

References: 1 retained out of 20, 1 new added and used for the summary
Anonymous. 1990. Data sheets on Quarantine pests: Mycosphaerella larici-leptolepis. EPPO Bulletin:1-3.

Mycosphaerella populorum G.E. Thompson

(=*Davidiella populorum* (Thompson) Aptroot
Common name(s) Septoria canker of poplar
Taxa: Fungi: Ascomycota: Mycosphaerellaceae
EPPO A1 list: No 17
EU Annex designation: I/A1

Organism
Mycosphaerella populorum can infect all species of *Populus* native to the USA. The pathogen overwinters on fallen infected leaves and within infected branches and stems. Primary infection occurs in the spring. The appearance of foliar lesions varies within and among hosts. Typically, lesions appear as small, circular to angular, white, greyish, tan, brown, or purplish spots with a dark border. The fungus is native from North America, but is recorded also in Argentina.

1. Detection
The morphological features were described through the CMI (Sivanesan 1990). Molecular techniques are important tools for the identification of this fungus (Feau *et al*. 2005, Feau *et al*. 2006, Weiland and Stanosz 2006).

2. Delimitation
No information available.

3. Monitoring
A survey of *M. populorum* incidence was surveyed in different regions (Spielman *et al*. 1986, Strobl and Fraser 1989, Romo Lozano *et al*. 1992).

4. Commodities
Ascospores or conidia were present within the plantings in nursery, indicating that the trade of plant material could favour the introduction of fungus in new areas (Ostry 1987). Some control measures to avoid their introduction have been suggested (Castellani *et al*. 1981).

References: 11 retained out of 84 retrieved, none added, 9 used for the summary

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stems. Forest Pathology 36:198-208.

Myndus crudus Van Duzee

Common name: American palm cixiid
Taxa: Insecta: Hemiptera: Cixiidae
EPPO A1 list: No. 159 Palm lethal yellowing phytoplasma (and its putative vector *Myndus crudus*)
EU Annex designation: I/A2

Organism
The adults mainly feed on coconuts (*Cocos nucifera*) and other palms, while the preimaginal stages feed on the roots of turf grasses growing in the vicinity of the palms. It has been reported to be native to southern Florida, Cuba, Cayman Islands, Jamaica, Trinidad and mainland Tropical America from Mexico and Central America through northern South America.

1. Detection
A rotary flight-trap was developed to sample flying insects associated with coconut among which *Myndus crudus* (Woodiel and Tsai 1978, Tsai and Mead 1982); blue sticky traps were more effective for sampling the cixiid (Cherry and Howard 1984). A key to *Myndus* species for all of the Americas were presented (Kramer 1979), whereas a key were developed for the genera of Cixiidae in Florida (Mead 1979). The morphological features were illustrated and described for the immature stages (egg and 5 nymphal instars) of *M. crudus* (Wilson and Tsai 1982). A molecular technique has proven useful for the identification of this insect (Brown and Dollet 2007).

2. Delimitation
No information available.

3. Monitoring
A survey of species of Auchenorrhyncha, possible vectors of the pathogen of lethal yellowing diseases, was carried out in Florida and Dominican Republic (Howard and Mead 1980, Howard *et al*. 1981). The methods used for the sampling were yellow sticky traps, palm fronds coated with adhesive, vacuum sampling and visual collection (Meyerdirk and Hart 1982).

4. Commodities
No information available.

References: 19 retained out of 66 retrieved, none added, 10 used for the summary

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Nacobbus aberrans (Thorne, 1935) Thorne and Allen, 1944

Common name(s): False root-knot nematode
Taxa : Nematoda: Tylenchida: Pratylenchidae
EPPO A1 list: No. 144
EU Annex designation: I/ A1

Organism

Nacobbus aberrans is a nematode that causes important economic losses in some countries of the American continent from where it is indigenous. It is an important pest of sugar beet in North America and of potatoes in South America. It has a wide host range, attacking mainly food crops, e.g. cabbage, carrot, cucumber, lettuce, mustard, been, potato, pee, sugar beet, tomato, but also infests many weeds that may ensures pathogen survival in the field when the crop is not present (Tordable et al. 2010).

The taxonomy of the genus *Nacobbus* includes the two species *N. dorsalis* and *N. aberrans*, but the existence of distinct genotypes (Vovlas et al. 2007) and observations on reproductive isolation suggests the existence of additional species (Anthoine and Mugniery 2006).

The nematode is an endoparasite that forms galls in the roots of infected hosts. *Nacobbus* spp. induce similar galls to those caused by root-knot nematodes (*Meloidogyne* spp.) and are, therefore, commonly named the 'false root-knot nematode', but often occur along the root as discrete and rounded swellings, like beads on a string (Anonymous 2009). Above ground symptoms of heavily infested plants include stunting and yellowing.

1. Detection

Nacobbus aberrans can be found in the roots and adhering soil of a wide range of imported plant material which may or may not show external symptoms. In order to identify its presence on an imported commodity it is necessary to carry out the extraction of nematodes with effective methods (Anonymous, 2001).

After extraction, the potential methods of diagnostics are based on morphological characteristics of the species. Mature females can be observed within the roots by means of a dissecting microscope using transmitted light. They can be extracted by dissecting apart the tissues but then should be stored in a 0.9% solution of NaCl in order to avoid possible osmotic disruption in plain water. Other stages of the species can be obtained from plant tissues or soil by suitable extraction techniques

Morphology of all life stage males, are described and compared with similar species and genera in EPPO diagnostic protocol (Anonymous 2009).

Molecular methods can be used to confirm *N. aberrans* identification. Two approaches based on ITS-rRNA were recently developed. The one described is used for specific detection of *N. aberrans* by direct DNA extraction from soil or from tuber combined to a species-specific PCR (Atkins et al. 2005). The other follows the extraction of the nematodes from the soils and roots (Anthoine and Mugniery 2005).

2. Monitoring

No information available.

3. Delimitation

No information available.

4. Commodities

No information available.

References: 11 retained out of 49 retrieved, 1 added, 7 used for the summary

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Naturally spreading psorosis / Citrus ringspot virus

Common name(s): CRSV, Psorosis B, citrus necrotic ringspot, naturally spread psorosis
Taxa: Viruses: Ophioviridae: Ophiovirus
EU Annex designation: II /A1 (as naturally spreading psorosis)

Organism

Two distinct diseases have been called psorosis (Anonymous 1997). The first is psorosis A, classical psorosis, identified by the presence of bark scaling only in the trunk and limbs, and by leaf flecking symptoms when grafted onto suitable citrus indicators. The second is psorosis B, causing rampant bark scaling even in fine twigs, chlorotic blotching in old leaves with gummy pustules in the leaf underside, and sometimes ringspots on fruits also called naturally spreading psorosis or citrus necrotic ringspot (CRSV), which is associated with distinctive virus particles. CRSV affects most *Citrus* spp. and their hybrids. Grapefruits (*Citrus paradise*) and oranges (*C. sinensis*) are particularly susceptible.

Wallace and Drake (1968) first described citrus ringspot as a different bark-scaling disease inducing yellow blotches, vein banding and/or distinct rings in mature leaves of several indicator species, but later evidence suggested that ringspot and psorosis are variants of the same disease (Da Graca et al. 1991, Derrick et al. 1991, Garcia et al. 1991, Navas-Castillo and Moreno 1993, Navas-Castillo et al. 1993). (Navas-Castillo and Moreno 1993) concluded that six of eight ringspot isolates collected from symptomatic trees in Spain could not be distinguished from psorosis on the basis of greenhouse symptoms in indicator plants, cross protection, mechanical transmission to *C. quinoa*, and the presence of the 48 kd band.

Nothing is known on the mode of transmission and the persistence of the vector, but it is mechanically transmitted and can therefore be identified by its reactions on herbaceous indicators. Citrus psorosis, including citrus ringspot, has mainly been spread through propagating material, and in most citrus-growing areas has largely been eliminated as a commercial problem by bud wood certification programs.

CRSV was first described from California but has since been reported from many areas worldwide causing the decrease of tree vigor and production in different region including South America, Mediterranean region and Asia. In India, a different citrus-ringspot disease from the ringspot and psorosis diseases known from other parts of the world was described by (Byadgi and Ahlawat 1995). An isolate of Kinnow mandarin causing serious disease affecting citrus and showing bright yellow ringspots on mature leaves, followed by rapid decline, was purified and was proposed as a new species (Rustici et al. 2000).

1. Detection

Citrus ringspot appears as large, irregular blotches, or ringspots on mature leaves, which are frequently gum-impregnated. Some cultivars show the classical psorosis symptom: shoot necrosis and bark scaling. Fruits may also show ringspot symptoms.

When material is grafted onto grapefruit or orange (this may also occur with psorosis A), citrus ringspot gives shock symptoms. Reactions of different citrus species to ringspot are variable. Local lesions are obtained on mechanically inoculated *Chenopodium quinoa*.

Until recently, biological indexing on indicator hosts has been the only routine diagnostic method available. In recent years, however, serological and molecular assays have been successfully used as a complementary tool to conventional biological indexing (Alioto et al. 2008).

In 1991, an antiserum, produced to purified isolate of citrus ringspot virus (CRSV-4) from Florida, was used to detect the 48-kDa capsid protein in western blots and virus particles by serologically specific electron microscopy (SSEM) (Da Graca et al. 1991). Latter an antiserum usable in double antibody sandwich (DAS) ELISA was produced, and two monoclonal antibodies (mabs) to citrus psorosis virus PsV, an IgG and an IgM were used (Alioto et al. 1999). A highly sensitive triple antibody sandwich (TAS) ELISA making use of the mabs was used (Alioto et al. 2000). Both the DAS and IgG mab-TAS formats detected all CPsV isolates so far tested (from Argentina, Italy, Lebanon, Spain and the USA).
Molecular RT-PCR assays and multiplex RTPCR have been used for ringspot isolates (Martin et al. 2004, Roy et al. 2005, Loconsole et al. 2010). (Martin et al. 2004) compared ELISA to RTPCR; results suggested that both methods can be used for detection of a range of psorosis isolates. However, variation of the viruses in the field might cause problems for any one diagnostic test.

2. Delimitation
No information available.

3. Monitoring
Survey were carried out to detect the presence and spread of the psorosis disease in various countries but little information on Naturally spread psorosis monitoring are available. Earlier monitoring efforts frequently involved visual assessments for symptoms (Bruggen and Yilma 1985), whilst later ones have employed the range of serological and molecular techniques described above.

4. Commodities
No information available.

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Naupactus leucoloma Boheman

Common name(s): White fringed weevil
Taxa: Insecta: Coleoptera: Curculionidae
EPPO A1 list: No. 293
EU Annex designation: I/A1

Organism
Naupactus leucoloma larvae are root feeding pests of a wide range of vegetable crops as well as occurring in pasture where they can be very damaging (King et al. 1982). Affected plants include *Trifolium* spp., *Lotus* spp., maize (*Zea mays)*, potato (*Solanum tuberosum*) and sunflower (*Helianthus annuus*) (East 1977, East and Watson 1978, McGregor and Somerfield 1978) amongst others. The insect is not present in the EU but can be found in North and South America, Australia, New Zealand, South Africa and Japan (Anonymous 1964, Helson 1971, Turner and Franzmann 1981, Lanteri and Mrvaldi 1995, Sato et al. 2002)

1. Detection
Older methods for detecting the species and differentiating it from related curculionid species rely on the morphological traits of the egg, larvae or adults (Harlan and McGuire 1977, Masaki 1998, 2001). However, differentiation of the insects can now be made using molecular methods such as RAPD-PCR (Hardwick et al. 1997). A PCR method to identify *L. leucoloma* and other quarantine beetle species has been developed in Taiwan (Lin et al. 2008). The physical detection of the adults has been achieved by live capture, which has confirmed the northwards spread of *N. leucoloma* in the United States (Voss and Poly 2002).

2. Delimitation
No information

3. Monitoring
The monitoring of the spread of *N. leucoloma* in New Zealand has been conducted through the *ad hoc* collection of the adults (McGregor and Somerfield 1978). Larvae can be sampled using soil cores and East (1980) describes taking 28 cores (10 cm diam.) from a 0.2 ha plot, with the insects removed through sieving and flotation. This paper also provides recommendations for sampling strategies that takes into account available resources. A sampling strategy for *N. leucoloma* in potatoes has also been formulated that involves taking a slice of the hilled rows of approximate 0.3 m^2 with 50 samples taken per 0.2 ha (Matthiessen and Learmonth 1993). These workers also examined the damage caused by soil-inhabiting pests and could confidently ascribe damages seen in the tubers to *N. leucoloma.* The areas where the pest is most commonly found (bottom middle of the row) is also indicated.
The population dynamics of *N. leucoloma* have been examined in Alabama in sweet potato (*Ipomoea batatas*) through the collection of adults and soil core sampling for larvae (Zehnder 1997). Adult population monitoring has shown that damage in potatoes is related to the abundance of weevils the preceding year (Learmonth 1999). Similar, soil-infesting pests, including white fringed weevils, in New Zealand were monitored over a six year period in pasture through visual damage assessments and via soil core sampling followed by extraction (Watson et al. 2000). A similar study in New Zealand used random cores (10 mm x 200 mm) to assess insect re-colonisation in improved pasture (Hardwick 2004) whilst a survey in Chile largely confined itself to collection of adult insects (Aguilera et al. 2009).

4. Commodities

Procedures for the visual identification of the feeding damaged caused by *N. leucoloma* have been published by EPPO (Anonymous 2008). Specifically, the assessment should look for the notching of the leaf margins caused by adult feeding. The larvae cause more severe symptoms that include wilting and yellowing. The eggs are also visible, and remain so for several months, and can be found on the plant. Eggs, larvae and pupae may also be found in the soil and potting material should be examined for this pest.

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Numonia pyrivorella Matsumura

Common name(s): Pear fruit moth, pear moth, pear pyralid
Taxa: Insecta: Lepidoptera: Pyralidae
EPPO A2 list: No. 184
EU Annex designation: II/A1

Organism
The pear fruit moth *Numonia pyrivorella* (Matsumura) (Lepidoptera: Pyralidae) (syn. *Acrobasis pirivorella* (Mats.), *Eurhodope pirivorella* (Mats.)) attacks both wild and cultivated plants of the genus *Pyrus* (Komarova 1984, Xing et al. 1986, Chen and Chou 2001).
The pest overwinters early in the larval stage (first- and second-instar larvae), mainly in flower buds, but also in leaf and fruit buds that die in autumn but do not fall. Pupation takes place at the end of May, and the first adults appear in mid-July; most of them emerge between late July and mid-August (Gibanov and Sanin 1971, Komarova 1984, Xing et al. 1986).
In China, the main parasitoid species attacking *N. pirivorella* were the ichneumonids *Gregopimpla himalayensis* and *Cremastus flavoorbitalis* (syn. *Trathala flavoorbitalis*) and the tachinid *Pseudoperichaeta insidiosa* (syn. *P. nigrolineata*) (Xing et al. 1986).

1. Detection
Light traps with a UV light source should be effective for attracting these moths (Okudai et al. 1972).
Tabata et al. (2009) have concluded that Z9-15:OAc and 15:OAc are sex pheromone components of this species. Two EAD-active compounds were detected in the pheromone gland extract of females. They were identified as (Z)-9-pentadecenyl acetate (Z9-15:OAc) and pentadecyl acetate (15:OAc). The amounts per female gland (mean +/- standard error) of these compounds were 12.9 +/- 2.8 and 0.8 +/- 0.1 ng, respectively. Synthetic Z9-15:OAc (300 mu g) attracted conspecific males in field trapping experiments. When 15:OAc (21 mu g; 7% of Z9-15:OAc quantity) was added, the number of males trapped increased significantly. Catch in traps baited with the mixture of these compounds was greater than that in traps baited with 1-3-day-old virgin females (Tabata et al. 2009).

2. Delimitation
No information available

3. Monitoring
No information available

4. Commodities
Numonia pyrivorella may be spread with fruits and planting material of pear (Chebanov 1977, Shutova 1977). It can be controlled by fumigation with methyl bromide (Chebanov 1977). Fruit infestation rises to 60-70% in some years. Quarantine regulations are in force in the regions affected in the USSR, since the moth is able to acclimatise itself in any region in which pear is grown (Shutova 1977).

References: 10 retained out of 25 references retrieved, none added, 8 used for summary

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Oligonychus perditus Pritchard and Baker

Common name(s): Byakushin-hadani
Taxa: Arachnida: Acari: Teranychidae
EPPO A1 list: No. 217
EU Annex designation: II/A1

Organism
The host plants of *O. perditus* are conifers, mainly of the family Cupressaceae: *Chamaecyparis pisifera*, *Juniperus chinensis*, *J. formosana*, *Thuja orientalis*. *Taxus cuspidata* and *Cryptomeria japonica* have also been recorded as hosts. Currently, no research has been done on biological or ecological features of *O. perditus*, but it is assumed to be comparable with those of *O. ununguis* as the species are closely related. On intercepted bonsai material (*J. chinensis)* in the Netherlands, *O. perditus* has been seen to overwinter in the egg stage, as *O. ununguis* also does. Diapause starts in September or October, and terminates when the majority of winter eggs hatch in April or May. Adult females of *O. ununguis* lay about 45 eggs in their lifetime and the period from egg to egg is 11-23 days. Symptoms of an infestation include discoloured needles; heavily infested plants show browning and distorted growth. The species is distributed in Asia (China, Hong Kong, in parts of Japan, Korea Republic and Taiwan) and the USA. It is not reported from the EU Member states.
As *O. perditus* has limited long-range dispersal and it is primarily transported on planting material via international trade e.g. on bonsai plants. It has been intercepted on juniper.

1. Detection
No information available.

2. Monitoring
No information available.

3. Delimitation
No information available.

4. Commodities
Woody plants are the main commodity for *O. perditus*. Specific phytosanitary measures were provided for the imports of conifers (restricted importation). The article by Yesilayer and Cobanoglu (2010) provides an overview of the quarantine policies and procedures in Turkey e.g. confiscating infested plant material in registered nurseries for at least 2 years, regularly inspections 4-6 times a year for the pest and the report of findings to plant health authorities.

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Opogona sacchari (Bojer)

Common name(s): Banana moth
Taxa: Insecta: Lepidoptera: Tineidae
EPPO A2 list: No. 154
EU Annex designation: I/A2

Organism

Opogona sacchari is a lepidopterous pest of tropical origin. As its common name suggests, it infests bananas although a wide range of other hosts are known, including crops such as sugar cane, potatoes, maize and figs as well as a wide range of ornamental species (e.g. *Sansevieria*, *Dracaenia*, *Hibiscus* species (Ciampolini 1973, Moreton 1974, Perez Padron and Carnero Hernandez 1984). Yucca and *Dracaenia* are particularly important as they tend to be the species found to be infested by import inspections (Anonymous 1988). The caterpillars will also develop in leaf litter present underneath host plants (Heppner et al. 1987). Its biological characteristics have received extensive study (Mourikis and Vassilaina-Alexopoulou 1981, Perez Padron and Carnero Hernandez 1984, Davis and Pena 1990, Bergmann et al. 1995a) and it is a generally well-understood pest. Damage results from the stem/tissue boring activity of the larvae (Davis and Pena 1990) and can cause wilting and, in extreme cases, collapse of the plant as a result of destruction of xylem (Veenenbos 1981). The moth is widely distributed in Asia, Africa, North and South America and also occurs frequently in the EU/EPPO region (Anonymous 1999).

1. Detection

There are numerous reports regarding the first detection of *O. sacchari* in certain countries. In these cases, the insect was probably detected as a result of routine surveillance for other pests. Examples include the detection of the pests in glasshouses in Denmark (Anonymous 1981) and ornamentals in China (Cheng and Yang 1997), Hungary (Tusnadi et al. 1997) and Poland (Labanowski 1999). Barlett (1992) indicates that light traps can be used for detection of this pest although no further details are given. The pest has, on occasion, been detected through observation of the visible damage caused to hosts (Porcelli and Parenzan 1993). Such damage includes visible holes in plant material (approx 2 mm in diameter) and the presence of frass (Tusnadi et al. 1997). The identity of the species is confirmed via morphological characteristics, which are well described (Robinson and Tuck 1997).

Work towards improving detection of the moth has focussed on the development of pheromone traps. Ioneda et al. (1983) demonstrated the females produce an attractive compound. This finding led to various compounds known to be present in lepidopteran pheromones to be tested, with (Z)-11-hexadecenal giving a clear response in males (Rotundo and Tremblay 1982) although this was not confirmed in the field. Subsequent research clarified the composition of the female-produced sex pheromone as 2/3,(*Z*)13-octadecadienal and led to the successful development of a trap for the early detection of the moth in glasshouses (Labanowski 1999).

2. Delimitation

No information

3. Monitoring

Monitoring surveys have been conducted for this pest in banana in Brazil (Sampaio et al. 1983)

through the visual examination of rotted pseudostems (the main upright stem of the plant) for larvae. This survey demonstrated the widespread nature of the pest and indicated that population sizes were affected by a number of environmental variables, with lower levels of rainfall being particularly associated with higher *O. sacchari* numbers. A second survey in Brazil indicated that 53% of the plants examined showed the signs of attack (Bergmann et al. 1995b). Jang et al. (2010) have recently evaluated the efficacy of sticky traps baited with a lure they have identified in papaya stands with promising results. However, the use of this method as a monitoring technique has not been reported upon to date.

4. Commodities

A common route of entry into a country for *O. sacchari* is via infested plant material and produce, particularly bananas and ornamentals (Cintra 1975, Veenenbos 1981, Bartlett 1992). In some cases, the detection of infestation has been achieved through allowing the emergence of adults from materials suspected of being infested (Cintra 1975). It is difficult to detect eggs and smaller larval stages, rendering detection of this pest's presence uncertain at the time of import. Therefore, it has been suggested that post-entry inspections are carried out for a period of three months at the glasshouse destination of the imported hosts (Veenenbos 1981).

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Palm lethal yellowing phytoplasma

(=Cape St Paul wilt phytoplasma)
Common name(s): Awka disease, Cape St Paul wilt, Kaincope disease, Kribi disease
Taxa: Bacteria: Tenericutes: Acholeplasmataceae
EPPO A1 list: No. 159
EU Annex designation: II/A1

Organism
The main host is coconuts (*Cocos nucifera*) but the disease has also been found on dates (*Phoenix dactylifera*) and *P. canariensis*, and 30 species of palm are susceptible to infection. The area of origin of the pathogen is not known, the range include Africa, North and Central America. LY is caused by a phytoplasma, an unculturable cell wall-less bacterium. The phytoplasma has been classified as a member of group 16S rDNA RFLP group 16SrIV, subgroup A (16SrIV-A). It is spread by the planthopper *Myndus crudus*. The phytoplasma is a systemic pathogen that is found only in the phloem tissue (vascular tissue transporting carbohydrates) of palms.

1. Detection
DNA amplification by PCR were used specifically to detect the mycoplasma-like organism (MLO) associated with lethal yellowing disease of palms (Lee *et al*. 1993, Harrison *et al*. 1994b, Martinez-Soriano *et al*. 1994, Cordova *et al*. 2003). DNA probes were developed to for detection (Harrison *et al*. 1992) and a set of techniques (RFLP analysis of PCR-amplified 16S rDNA and DNA hybridization) were used to detect and differentiate African coconut phytoplasmas (Tymon *et al*. 1997).These molecular techniques were useful to analyse the existence of genetic relationships between MLOs associated with coconut lethal decline diseases in different countries of origin, for example indicating that the lethal yellowing MLO from Florida and lethal disease in Tanzania, although very similar, are not genetically identical pathogens (Harrison *et al*. 1994a).
Amplification of a given gene of the phytoplasmas by PCR is the most convenient diagnosis method for the identification of attacks on coconut palms. The problem is that there are at least 28 "groups" of phytoplasmas and only one pair of primers - P1/P7 - commonly used for PCR. As these primers belong to a very conserved gene, false positives are frequent. Consequently, alternative primers specific to one "strain" (or subgroup) have to be used, such as LY-F/LY-R for the Caribbean LY, Rohde primers for LD Tanzania (Dollet *et al*. 2009).
The electron and fluorescence microscopy or/and molecular methods were used to confirm the first report of yellows in date palms in the Arabian Gulf region and in Guatemala (Al-Awadhi *et al*. 2002, Mejia *et al*. 2004). PCR based methods were used also to determine the presence of phytoplasma in insect vector (Edwin and Mohankumar 2007).

2. Delimitation
The spread of lethal yellowing due to mycoplasma is monitored in Texas, with particular attention to susceptible and resistant palm species. It threatens the susceptible palms in the Rio Grande Valley and increases the possibility of spread to other areas (McCoy *et al*. 1980). After the first records, further infections were identified as a result of the annual country helicopter survey of palms. Perimeters of inoculations have been established, plus aerial and ground surveys are being conducted every 3 months (Petersen and Fedelem 1999).

3. Monitoring

Although the symptoms were evident, no mycoplasma-like organisms have been identified in the phloem of diseased palms (Steiner 1978). Coconut palms exhibiting symptoms of lethal yellowing were signalled in different regions (Dabek *et al*. 1976, McCoy *et al*. 1982, Cardena *et al*. 1991, Bonnot *et al*. 2009), a successively more accurate survey highlighted larger outbreaks (Johnson and Harries 1976, Uaciquete and Rao 1998). The use of PCR determined an improving in the identification of mycoplasma (Almeyda-Leon *et al*. 2001, Harrison *et al*. 2002, Myrie *et al*. 2007). The molecular analysis also permitted the identification of genetic differences from isolates originating from different areas (Llauger *et al*. 2002). The spreading of the disease in a stand was useful to define the width of phytosanitary belts and to design sampling schemes (Cecilio Gongora-Canul *et al*. 2004). The status of the disease spread was monitored by both aerial and/or ground surveys (Nkansah-Poku *et al*. 2009).

4. Commodities

The movements of coconut planting material can be useful to understand the risk of introduction and spread of pathogen (Maramorosch and Harries 1998); a report traced the problems and some possible solutions about introduction of phytoplasmas with vegetative parts of plant (Cousin and Boudon-Padieu 2002).

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Parasaissetia nigra (Nietner)

Common name(s): Nigra scale, black scale, hibiscus shields scale, pomegranate scale (and others)
Taxa: Insecta: Hemiptera: Coccidae
EU Annex designation: II/A2

Organism

Parasaissetia nigra is a polyphagous scale insect that feeds on hosts from 95 plant families (European and Mediterranean Plant Protection 2002) including ornamental plants of tropical origin such as *Ficus* and *Hibiscus* and several agricultural crops. This species probably originated in Africa, but has since spread throughout the world, including to within European countries. The insects produce copious quantities of honeydew on which sooty moulds grow, reducing the photosynthetic area of the plant, resulting in defoliation and stunting. This lowers the aesthetic appearance and market value of ornamental plants (European and Mediterranean Plant Protection 2002).

1. Detection

There is a diagnostic protocol produced by EPPO for the identification of this species (Anonymous, 2002). Infested plants may be covered in honeydew and sooty mould, and may exhibit defoliation and stunting. Identification is based on morphological features. As this insect demonstrates a clumped distribution, as much plant material as possible should be examined (Anonymous, 2002).

2. Delimitation

No relevant references found.

3. Monitoring

The abundance of *P. nigra* in pomegranate and mulberry in India showed a positive correlation with temperature and a negative correlation with relative humidity (Shevale and Kaulgud 1998, Shree and Manjunatha 2000). A survey of scale insects in the Liguria region of Italy reported the presence of *P. nigra* as an invasive species (Pellizzari and Sacco 2010).

4. Commodities

No relevant references found.

References: 21 retained out of 96 references retrieved, none added, 4 used for summary

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Pardalaspis cyanescens (Bezzi)

(=*Trirhithromyia cyanescens* (Bezzi))
Common name(s): Tomato fruit fly
Taxa: Insecta: Diptyera: Tephritidae
EU Annex designation: I/A1

Organism
Pardalaspis cyanescens is a fruit fly that is found in Comoros, Madagascar, Mauritius (Anonymous 1974), Réunion, and the Seychelles. It is a pest of tomato plants and other Solanaceae, including *Capsicum* and aubergines (*Solanum melongena*).

1. Detection
Pardalaspis cyanescens can be monitored by traps baited with male lures. This species is attracted to terpinyl acetate but not to cue lure or trimedlure

2. Delimitation
No relevant references found.

3. Monitoring
This species has been monitored in Mauritius on tomato using pheromone traps and larval counts of attacked fruit (Fagoonee 1978).

4. Commodities
No relevant references found.

References: 4 retained out of 9 retrieved, none added, 2 used for summary

Anonymous. 1974. Annual report for the year 1974. Report, Ministry of Agriculture and Natural Resources, Mauritius 1974, publ 1977 2, 139 pp.

Fagoonee, I. 1978. Investigations on the damage caused by insect pests on love apple (Lycopersicon esculentum L.). Revue Agricole et Sucriere de l'Ile Maurice 57:27-32.

Pardalaspis quinaria Bezzi

(=*Ceratitis quinaria* (Bezzi))
Common name(s): Five spotted fruit fly, Rhodesian fruit fly, Zimbabwean fruit fly
Taxa: Insecta: Diptera: Tephritidae
EU Annex designation: I/ A1

Organism
Pardalaspis quinaria is a fruit fly that is found in Angola, Botswana, Cote d'Ivoire, Malawi, Mali, Namibia, South Africa, Sudan, Zimbabwe and Yemen (Anonymous 2005). Hosts are recorded as Citrus spp., guava (*Psidium guajava*), peach (*Prunus persica*) and other soft fruit trees (Anonymous 2005).

1. Detection
Pardalaspis quinaria can be monitored by traps baited with male lures. This species is attracted to terpinyl acetate but not to cue lure or trimedlure (El Tahir and Venkatraman 1970).

2. Delimitation
No relevant references found.

3. Monitoring
Fruit fly species have been monitored in Benin using traps baited with parapheromones (Vayssieres *et al*. 2005, 2009) and in Mali using sex pheromones and food attractants (Vayssieres *et al*. 2004, Vayssieres *et al*. 2007). *Pardalaspis quinaria* was among the species found.

4. Commodities
No relevant references found.

References: 9 retained out of 12 retrieved, none added, 6 used for summary

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Paysandisia archon (Burmeister)

Common name(s): Palm moth
Taxa: Insecta: Lepidoptera:
EPPO A2 list: No. 338
EU Annex designation: II/A2

Organism

The larvae of *P. archon* feed within the trunks and branches of palms (Arecaceae) (Monteys 2002). The moth is a native to South America but is now frequently detected throughout Europe, including the UK, France, Spain, and Italy (Sarto i Monteys et al. 2005) after being initially introduced between 1985 and 1995 via trees imported from Argentina (Reid and Moran 2009). In France adults are observed from June to September and the moth is thought to have an annual or biannual lifecycle, depending on the climate (Sarto i Monteys et al. 2005). The pest is though likely to cause significant damage to ornamental palms, particularly Europe's only native species *Chamaerops humilis*, although it has no economic impact in its native South American range (Reid and Moran 2009, Lopez-Vaamonde et al. 2010)

1. Detection

The symptoms of attack by this pest are very characteristic and include the presence of sawdust on the crown of the palm, perforated leaves, galleries within the wood, abnormal axillary bud formation and twisted trunks (Sarto i Monteys et al. 2005). The larvae are sometimes visible, aiding detection (Drescher and Dufay 2002)Over the last decade the moth has been detected for the first time in a number of European countries (Reynaud et al. 2002, Riolo et al. 2004, Colazza et al. 2005, Anonymous 2008). For example, the occurrence of this species in Almeria was first detected through the observation of the symptoms of attack followed by destructive sampling of trees to confirm the presence of the moth's larvae (Tapia et al. 2010). A similar situation was also reported from Cyprus where debris surrounding the crown was indicative of attack by this species (Vassiliou et al. 2009). For captured insects, the identity of respective stages (egg, larvae, pupa and adult) can readily be made using morphological characters (Sarto i Monteys and Aguilar 2005, Sarto i Monteys et al. 2005, Anonymous 2008).

2. Delimitation

No relevant references found.

3. Monitoring

Little information is available with respect to monitoring techniques for this pest. The moth has, however, been included in general monitoring activities in Sicily and France (Chapin et al. 2002, Longo 2006). In these cases, the surveys comprised visual assessments of the palms for damage and/or the presence of insects. Techniques beyond visual assessment do not seem to have been explored at the time of writing for monitoring this pest and it uncertain whether chemical communications, such as long-range pheromones, are important in its biology or could be exploited for monitoring and/or detection purposes (Sarto i Monteys and Aguilar 2005, Sarto i Monteys et al. 2011).

4. Commodities

The major route for the spread of this pest has been the global trade in Palms. Whilst no specific literature is available on surveying palms at points of entry, it is clear that examining imported

materials for the presence of this moth is essential (Drescher and Dufay 2002).

References: 41 retained out 45 of retrieved, 2 added, 14 used for summary

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Peach Mosaic Virus (American)

Common name(s): PcMV, American peach mosaic
Taxa: Viruses: Flexiviridae: Trichovirus
EPPO A1 list: No. 27
EU Annex designation I /A1

Organism

Peach American mosaic disease was originally considered peach American mosaic as an A1 quarantine pest (OEPP/EPPO, 1978). In the 1980 it was thought that the causal agent of American peach mosaic was the peach latent mosaic viroid (PLMVd) that also causes the peach yellow mosaic in Japan because of the similarity in symptoms, host-range, etc., After a period of confusion on the identity of the pathogen it is again now clear that there is a North American probable closterovirus which causes a mite-transmitted mosaic disease of peach. A flexuous clostero-like virus is associated with peach mosaic disease and has been isolated from an affected peach tree cv. Elberta.

Peach American mosaic virus affects only *Prunus* spp.: peach (*Prunus persica*), nectarine (*P. persica* var *nectarina*), almond (*P. dulcis*), apricot (*P. armeniaca*), *P. besseyi*, *P. serrulata* and several species of plum. Peaches and nectarines are the main economically affected hosts, as the disease on susceptible cultivars deforms the fruit so that it becomes unsalable.

American peach mosaic was first observed in 1931 in Texas (US) and soon after that in Colorado, and southern California (limited to areas south of the Tehachapi Mountains). The disease was then reported throughout the peach-growing states of the south west of the USA. In Mexico, symptoms of peach mosaic were reported in the 1950s in the north (Chihuahua, Baja California and Coahuila) and later, peach mosaic was detected in several states of the central highlands (Aguascalientes, Guanajuato, Hidalgo, Michoacan, San Luis Potosi, Sonora, Zacatecas).

Peach American mosaic virus is easily graft-transmissible to healthy peach using fruit, leaf, root, or bud tissues and a contact period as short as two days. Attempts to transmit the agent through dodder have failed. The vector of the disease is the peach bud mite *Eriophyes insidiosus* which feeds and reproduces on developing leaf within the bud but is not known to be present in Europe but it has been possible to transmit the virus through another vector, the peach-potato aphid *Myzus persicae* (Desvignes et al. 1988).

1. Detection

The first signs of disorder become apparent on the trees during the second year after planting and include: delays of 4-6 days in leaf emergence, flowering and maturity; pink broken lines on the white petals in warm temperatures; irregularly shaped, flattened, colourless fruit, with cracked sutures and enlarged pits; open habit; bud necrosis. Some isolates cause mosaic, blotch, calico and necrosis of the leaves, whereas others induce stem pitting and leaf twisting (Anonymous 1999).

Peach American mosaic can be detected in the glasshouse on indicator plants (peach seedlings, e.g. cvs. Elberta, GF305, Rio Oso Gem) by grafting (Desvignes 1976). A polyclonal antibody (PAb) was produced and can be used in enzyme-linked immunosorbent assay ELISA and Western blot analysis to detect the virus but this PAb cross-reacted with cherry mottle leaf trichovirus viruses which is related serologically and share common *Prunus* hosts.

An RT-PCR procedure using a single oligonucleotide primer pair that allows simultaneous detection and differentiation of the two viruses was developed (James and Upton 1999, 2001). Finally A

reverse transcription-polymerase chain reaction procedure was developed for reliable and specific detection of PcMV. This will be an asset for stone fruit virus certification (James et al. 2006)

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
No information available.

References: 11 retained out of 19 retrieved, 1 added, 7 used for the summary

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Phony disease of peach

(=*Xylella fastidiosa* subsp. *multiplex* Schaad et al.))
Common name(s): Leaf scald of plum, leaf scorch of elm/oak/plane, peach virus 4
Taxa: Bacteria: Proteobacteria: Xanthomonadaceae
EPPO A1 list: No. 166
EU Annex designation: I /A1

Organism
Phony peach disease (PPD) is caused by a xylem limited bacterium *Xylella fastidiosa*. Strains of this bacterium are causal agent of Citrus variegated chlorosis (CVC), Pierce's disease (PD) of grapes and other disease of woody plants such as, plum leaf scald, and leaf scorch of almond, coffee, elm, oak, oleander, and sycamore.
Several pathogenic varieties of the bacterium have been described, that are often host specific and strains from peach have been identified as *X. fastidiosa* subsp. *multiplex* (Janse and Obradovic 2010).
PPD occurs in America from North Carolina to Texas. The bacterium is transmitted by root grafts and natural transmission occurs via insects feeding on xylem sap, mainly by sharpshooter leafhoppers (Janse and Obradovic 2010). The bacterium overwinters in the xylem of the host plants.

1. Detection
PPD first symptoms are stunted young shoots with denser and darker green leaves than healthy trees. Moreover they show early blooming and both leaves and flowers remain on the shoots longer than normal. Twigs on diseased trees have shortened internodes and increased lateral branching. Fruit production is severely impaired and fruits size quality and number are reduced. Symptom development is often slow (up to 18 months or more after infection) and may be present in one scaffold limb or in the entire tree. Trees are generally not killed but are more susceptible to other diseases and arthropods (Janse and Obradovic 2010).
(EPPO 2004) and (Janse 2010) describe the diagnostic protocols for the detection and identification of *X. fastidiosa* only for citrus and grapevine, but most could be suitable for peach.
The bacterium can be detected using peach root and twig section with a microscope equipped at least dark field or phase contrast optics (Wells et al. 1980) and by Immunofluorescence (Weaver et al. 1980, Wells et al. 1980) or enzyme like immunoabsorbent assay ELISA (Wells et al. 1981).
DNA hybridization probes and general (generic) primers have been developed for non-grapevine strains by (Firrao and Bazzi 1994). A recently multi-primer polymerase chain reaction (PCR) system, random amplified polymorphic DNA (RAPD)-PCR, and sequence analysis of the 16S-23S rDNA inter-genic spacer region (ISR) allowed the characterization of peach strains of *X. fastidiosa subs. Multiplex* (Hernandez-Martinez et al. 2006).

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities

No information available.

References. 1 retained out of 1 retrieved, 7 added, 8 used for the summary

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Peach Rosette Mosaic Virus

Common name(s) PRMV. Rosette mosaic of peach
Taxa: Viruses: Comoviridae: Nepovirus
EPPO A1 list: No. 219
EU Annex designation: I/A1

Organism

The causal agent of peach rosette mosaic virus disease and grape decline is the peach rosette mosaic nepovirus (PRMV). The main host is the American grape species *Vitis labrusca*, but some cultivars of *V. vinifera*, and French-American hybrids are also susceptible. PRMV is also an important pathogen of peaches, and to a lesser degree, blueberries. In addition, several weed species have been shown to be hosts for the virus: dandelion (*Taraxacum officinale*), horse nettle (*Solanum carolinense*), and curly dock (*Rumex crispus*).
PRMV is one of the North American nepoviruses of fruit trees (indigenous in Western Ontario, and in Michigan), and has not extended its range to any other continent. Several nematodes species has been recorded as vectors. The dagger nematode *Xiphinema americanum*, has been known to be a vector in Michigan, USA and Ontario, Canada. A species of *Criconemoides* was reported to be a vector in Michigan, this may have been *C*. (= *Macroposthonia*) *xenoplax*, as later referred from the same area (Ramsdell and Myers 1974). *Longidorus diadecturus*, which occurs in many parts of eastern North America, has been reported as a vector of PRMV, but only in a few peach orchards of Essex County, Ontario (Allen et al. 1982).
PRMV was shown to be seed-borne in grapevine cv. Concord and also in *Taraxacum officinale (*Ramsdell and Myers 1978). When infected vineyards are removed, PRMV can survive for a number of years in viruliferous nematodes that feed on infected root material.

1. Detection

The virus causes leaf malformation, shortening of cane internodes and crooked cane growth in grapevines. Infected vines exhibit reduced vigor and are predisposed to winter injury and death. Vines may die after several years. Over time, a circular pattern of missing or dead vines is commonly observed in mature vineyards. This disease has also been called grapevine degeneration, grapevine decline, berry shelling disease, and delayed budding disease. In peaches, shortened internodes, rosetting and mosaic of leaves, result from infection.
Virus isolates from peach with rosette mosaic and from grapevine with decline in Mich. [RPP. 54, 1375] had a similar host range, in vitro properties and morphology and were related serologically. Particles were isometric, av. 28 nm diam. Isolates from both sources, where transmitted manually, induced rosette mosaic on peach and decline on grapevine. Virus isolates recovered from infected grapevines varied in virulence and serological differences could be detected by cross absorption and immunodifusion tests (Dias and Cation 1976)
Purified PRMV preparations showed that the virus contains 2 RNA species and a single protein (Dias and Allen 1980).
The infection can be tested through indexing. Grapewines indexed on *C. quinoa*. These biological tests are comparatively longer than enzyme-linked immunosorbent assay (ELISA), but they are reliable. *Chenopodium quinoa* indexing was even presented as a more effective method of detecting PRMV in immature grapevine leaf tissue than ELISA (Ramsdell et al. 1979).

2. Delimitation

No information available.

3. Monitoring

Monitoring-type surveys are described in the literature for different countries. In the USA, grapevines of the cv. Concord were sap indexed to *C. quinoa* on an individual vine basis and surveyed for 4 years to determine the rate of spread (Ramsdell and Myers 1978). In Ontario the presence and host range of PRMV was identified by Elisa (Stobbs and Schagen 1996). Other surveys were undertaken to determine the broad range of viruses infecting crops and are not specific for *PRMV*. For example in the south of Poland, monitoring of blueberry plant by indexing and Elisa did not revealed the presence of the virus (Nowak 2009), but few samples were found infected with PRMV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) in surveys in western Anatolia (Azery and Cycek 1997)

4. Commodities

Plants for planting of *Vitis labrusca*, *V. vinifera* and French-American grapevine cultivars and all cultivars of *Prunus persica* from North America should come from areas free from PRMV. Such material may be tested for the presence of PRMV by ELISA. All plants for planting from North America should be free from soil (Anonymous 1997). The presence of viruses in samples of imported fruit propagation materials with phytosanitary certificate dependent on Turkish Regulation on Agricultural Quarantine was tested on different samples by ELISA and DAS-ELISA in 2000-2005 but they were free from PMRV (Ozdemir and Kaya 2008).

References: 13 retained out of 46 retrieved, 1 added, 11 used for the summary

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Peach rosette phytoplasma

Common name(s): Peach rosette
Taxa: Bacteria: Tenericutes: Acholeplasmataceae: Phytoplasma
EPPO A1 list: No. 138
EU Annex designation: I/A1

Organism
Peaches (*Prunus persica*) are the principal host of peach rosette mycoplasm, but most *Prunus* spp., *P. dulcis* (almonds), *P. armeniaca* (apricots), *P. domestica* (plum), and *P. avium*, *P. cerasus* (cherries) can be infected. Ornamental and also wild species *P. angustifolia*, *P. hortulana* and *P. munsoniana which* can act as natural reservoirs of the pathogen can be infected (Anonymous 1986). It can also be artificially transmitted to herbaceous hosts. No vector has been described to date but spread from wild hosts suggests that a vector must be involved. It was first found in Georgia in 1881, and now extended to southeastern states as far west as Texas.

1. Detection
Symptoms are similar to those caused by the peach rosette mosaic nepovirus (Anonymous 1997). On peach, new shoots have very short internodes. The leaves of the older shoots fall in early summer, leaving only bunches of young leaves on the tips of naked shoots. The most severely affected trees die during their first year of disease. Other fruit trees (almond, plum) show similar symptoms. Infected plum trees are seriously stunted (Anonymous 1986).
Peach rosette mycoplasm can be tested on peach seedlings (cv. Elberta or GF305) in the field, but 4 years are needed for results to be certain. It can also be tested on the same indicators in the glasshouse, symptoms appearing up to 3 months after inoculation (Anonymous 1986). An EPPO phytosanitary procedure for fruit tree phytoplasmas gives details (Anonymous 1994).
Phytoplasmas can be detected by electron microcopy, both transmission and scanning DAPI, TEM, that provides reliable and accurate methods for detecting phytoplasmas in plants but they are not specific. In 1995, DNA extraction, PCR amplification techniques and RFLP analysis was used for the first time to characterize a peach rosette phytoplasma isolate (Marcone et al. 1995), however more recently DNA isolated from symptomatic and healthy plum trees was used to amplify 16S rDNA and 16S-23S rDNA intergenic spacer (IS) fragments by direct and nested PCR (Zirak et al. 2009).

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
The pathogen is most likely to be spread internationally in infected planting material. Healthy planting material of *Prunus* should evidently be free from peach rosette phytoplasma.

References: 2 retained out of 6 retrieved, 3 added, 5 used for the summary
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Anonymous. 1994. Quarantine procedures No. 57. MLOs in fruit trees and grapevine. Bulletin OEPP/EPPO Bulletin 24:339-342.

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Peach X disease phytoplasma

(=Western X disease phytoplasma)
Common name(s): Peach x-disease, cherry buckskin, peach yellow leafroll, leaf casting yellows
Taxa: Bacteria: Tenericutes: Acholeplasmataceae: Phytoplasmas
EPPO A1 list: No. 140
EU Annex designation: I/A1

Organism
Peaches (*Prunus persica*) are the principal host of peach rosette phytoplasma, but cherries (*Prunus avium* and *P. cerasus)* and *P. salicina* are also highly susceptible to this disease. Other *Prunus* spp. can be infected, for example almonds (*P. dulcis*), apricots (*P. armeniaca*) and plums (*P. domestica*), but without impact on the trees. The wild chokecherry (*P. virginiana*) is an important natural reservoir of peach X-disease phytoplasma in eastern USA. It can also be artificially transmitted to herbaceous hosts, e.g. celery (*Apium graveolens*). It has been shown to occur in herbaceous weeds in orchards (*e.g. Medicago hispida*).
The disease has been reported to occur in Canada (British Colombia, New Brunswick, Ontario) and in north-eastern and mid-western United States. Two forms of peach X-disease used to be distinguished: eastern X-disease and western X-disease, with slightly different symptoms but they are now considered to be due to strains of the same pest (Jiang et al. 1989).
X-disease can be transmitted by several species of leafhopper, especially *Paraphlepsius irroratus* in eastern USA, and *Scaphytopius acutus*, *Colladonus montanus* and to a lesser extent *Colladonus geminatus*, *Fieberiella florii* and *Graphocephala confluens* (Anonymous 1997). These leafhoppers acquire the X-disease pathogen while sucking juices from the leaves of X-disease-infected chokecherries. Wild chokecherry is an important reservoir for the X-disease phytoplasma. A few weeks later the leafhopper can inject the agent into healthy leaves while feeding. These leafhoppers are usually not considered pests of peach and cherry. Other forms of transmission are by budding or grafting, but the phytoplasma itself can be very irregularly distributed in the plant (according to strain). Transmission is also possible to various herbaceous plants by use of dodder (*Cuscuta* spp.).

1. Detection
This disease is most easily identified on peach. Symptoms are predominantly foliar, but the fruits may also be affected. The first symptoms of infections are leaves on isolated branches curling inward and developing irregular, yellow to reddish or purple spots. The spots soon drop out, leaving a shot hole effect and tattered leaves. Leaves on affected branches fall prematurely, starting at the base of the branch.
A good diagnostic symptom is the presence of a mixture of healthy and diseased branches on the same tree occurring primarily during the first and second years of infection. Two to three years after initial infection, most branches will show symptoms. Young trees die 1-3 years after first symptom appearance. Chronically infected older trees may survive several years but yield little or no fruit (Anonymous 1997).
On cherries the reaction to X-disease depends of the rootstock. On *Prunus mahaleb* rootstocks die rapidly because the rootstock is resistant, and a hypersensitive reaction occurs at the graft union. On other rootstocks, decline is slower (Kirkpatrick et al. 1995). Leaves are smaller and red-tinged, sometimes with enlarged stipules; fruits mature late, have short pedicels and bland-flavoured

watery flesh. On trees propagated on mazzard rootstock fruit are small and pink at harvest and have a bitter flavour.
Peach X-disease phytoplasma can be tested on peach seedlings (cv. Elberta or GF305) in the field, but 4 years are needed for results to be certain. It can also be tested on the same indicators in the glasshouse, symptoms appearing up to 3 months after inoculation (Anonymous 1997).
An EPPO phytosanitary procedure for fruit tree phytoplasmas gives diagnostic details (Anonymous 1994). Serological methods (ELISA, immunosorbent electron microscopy) have been successfully tested for the detection of the pathogen in partially purified preparations from celery, an artificial herbaceous host (Sinha and Chiykowski 1984), and in the leafhopper vectors (Sinha and Chiykowski 1986). Monoclonal antibodies specific to peach X-disease phytoplasma have been obtained (Jiang et al. 1989). Peach X-disease phytoplasma has now become one of the type organisms for molecular studies on phytoplasma. Thus, a variety of serological or nucleic acid-based techniques exist that can distinguish the pest from related organisms. Molecular technique including DNA probes and PCR are now available for the detection and identification of X-disease in *Prunus* (Zirak et al. 2009*)*. Simple fluorescence microscopy with DAPI reagent can be used to detect the pathogen in mid-veins and petioles of peach and *P. virginiana* (Douglas 1986).

2. Delimitation
No information available.

3. Monitoring
No information available.

4 Commodities
No information available.

References: 1 retained out of 7 retrieved, 8 added, 8 used for the summary

Anonymous. 1994. Quarantine procedures No. 57. MLOs in fruit trees and grapevine. Bulletin OEPP/EPPO Bulletin 24:339-342

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Zirak, L., M. Bahar, A. Ahoonmanesh, and 2009. Molecular characterization of phytoplasmas related to peanut witches' broom and stolbur groupsinfecting plum in Iran. Journal of Plant Pathology 91:713-716.

Peach yellows phytoplasma

Common name(s): Peach yellow, little peach disease, red suture disease
Taxa: Bacteria Tenericutes: Acholeplasmataceae: Phytoplasma
EPPO A1 list: No. 139
EU Annex designation: I/ A1 (as peach yellows mycoplasm)

Organism
Absent in the EU, although it has been recorded in Italy but the record was invalided (Anonymous 1996) and probably refers to European stone fruit yellow mycoplasm which causes the peach yellow disease in Italy since the early 1980s.
Peach and nectarine (*Prunus persica*) are the main host of peach yellows phytoplasma but apricots (*P. armeniaca*) and almonds (*P. dulcis*) are also affected. This disease is transmitted by the American leafhopper *Macropsis trimaculata* in its native range (North America). It has an incubation period of 1-3 years in orchards and less than 16 days in greenhouse. Leaf buds develop prematurely, the leaves are yellow and the trees forms spindly branched shoots. Although severely affected trees may die this pest is much less important than the peach X-disease mycoplasm.

1. Detection

Detection of peach yellows by visual inspection is difficult. The peach yellows agent was shown to be a phytoplasma by the electron microscope studies. Bio-molecular tests now show affinities with peach X-disease phytoplasma (Anonymous 1994).
The infection can be tested through grafting on peach seedlings in the fields or in greenhouse, symptoms appearing respectively 4 years and 3 months after inoculation (Anonymous 1994). DAPI (diamidino-2-phenylindole) fluorescence test is a more rapid method to detect phytoplasma infections: microtome sections are stained with DAPI and examined by epifluorescence microscopy (Seemüller 1976). However more sensitive molecular method using phytoplasma-specific PCR primers should be used for identification (Smart 1996).

2. Delimitation

No information available

3. Monitoring

No information available

4. Commodities

EPPO recommended (Anonymous 1990) that *Prunus* planting material should come from a field inspected for disease during the growing season. Any vegetative material shipped from infested countries, should be subject to an official certification scheme, with particular emphasis on preventing re-infection of healthy material by airborne vectors.

References: 1 retained out of 5 retrieved, 5 added, 5 used for summary

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Anonymous. 1996. Peach yellows phytoplasma. In: Quarantine pests for Europe 2nd edition. CAB INTERNATIONAL, Wallingford, UK.

Smart, C., Schneider B, Blomquist CL, Guerra LJ, Harrison NA, Ahrens U, Lorenz KH, Seemller E, Kirkpatrick BC. 1996.

Phytoplasma-specific PCR primers based on sequences of the 16S-23S rRNA spacer region. Applied and Environmental Microbiology 62:2988-2993.

Pear decline phytoplasma

(=*Phytoplasma pyri* Seemüller & Schneider)
Common name(s): Parry's disease, decline of pear, leaf curl of pear, moria disease
Taxa: Bacteria: Acholeplasmataceae: Phytoplasma
EPPO list A2: No. 95
EU Annex designation: I/A2

Organism
Phytoplasmas cause severe damage and heavy economic loss in many European perennial fruit crops. The spread is mainly caused by the use of infected planting material and the transmission by psyllid vectors as well as grafting.
Symptoms of *Phytoplasma pyri* infection include the development of premature autumn leaf colour on affected trees, most cultivars develop a premature red colour, but some may develop a premature yellow colour. Leaf cupping or curling appears besides usually premature leaf drop. In the following spring, affected trees suffer from weak growth and sparse pale foliage. The severity of the spring symptoms can vary from absence to death. There may be a line of necrotic tissue in the bark at the graft union between scion and rootstock. The premature autumn leaf colour symptoms associated with pear decline may also have several other causes. Water logging, root damage, ring barking caused by feeding animals, some bacterial cankers, rootstock and variety incompatibility can all give rise to symptoms resembling those caused by phytoplasma infection.

1. Detection
Whereas, monitoring or sampling procedures are not explicitly described in the literature research on detection methods are reported on in several scientific papers.
Common methodologies for the detection of *Phytoplasma pyri* are PCR and ELISA test. According to Del Serrone, P., S. La Starza, *et al*. (1998) Pear trees with proliferation and decline symptoms were sampled in orchards in Hungary and tested in the laboratory using RFLP analysis and PCR-ELISA techniques.
A combination of nucleic acid extraction and nested PCR was used to identify Pear decline phytoplasma broadly over European countries and Lebanon (Myrta *et al*. 2006, Duduk *et al*., 2005, Choueiri *et al*., 2007, Topchiiska and Sakalieva, 2001).
A total of 1500 commercial plots of approximately 1 ha each, comprising 15 different localities were surveyed in September 2000. Results showed that the nested-PCR, using specific primers to detect the DNA from PD phytoplasma, is the most accurate method to identify the total percentage of affected trees (Garcia-Chapa *et al*. 2003).

2. Monitoring
No information available.

3. Delimitation
No information available.

4. Commodities
No information available.

References 19 retained out of 58 retrieved, none added, 6 used in summary
Choueiri, E., P. Salar, *et al*. (2007). First report and characterization of pear decline phytoplasma on pear in Lebanon.

Journal of Plant Pathology 89(Suppl.3): S75.
Del Serrone, P., S. La Starza, *et al*. (1998). Occurrence of apple proliferation and pear decline phytoplasmas in diseased pear trees in Hungary. Journal of Plant Pathology 80(1): 53-58.
Duduk, B., S. Botti, *et al*. (2005). Occurrence of pear decline phytoplasmas in Bosnia and Hercegovina." Journal of Plant Pathology 87(1): 75.
Garcia-Chapa, M., A. Lavina, *et al*. (2003). Occurrence, symptom expression and characterization of phytoplasma associated with pear decline disease in Catalonia (Spain). Journal of Phytopathology 151(11-12): 584-590.
Myrta, A., M. Martini, *et al*. (2006). First report of apple proliferation and pear decline phytoplasmas in Kosovo. Journal of Plant Pathology 88(1): 125.
Topchiiska, M. and D. Sakalieva (2001). Detection of pear decline phytoplasma by polymerase chain reaction in Bulgaria. Bulgarian Journal of Agricultural Science 7(6): 611-614.

Pepino mosaic virus

Common name(s): PeMV
Taxa: viruses: Flexiviridae: Potexvirus
EU Annex designation: Not listed

Organism

As a member of the genus Potexvirus, Pepino mosaic virus (PepMV) infects mainly solanaceous plants, including tomato, potato and tobacco. It was detected for the first time on pepino plants (*Solanum muricatum*) in Peru in 1974. Since then more detections followed from the Netherlands and Great Britain in 1999, where the virus was found on greenhouse tomato (*Lycopersicon esculentum*). Subsequently, PepMV has been detected in several other European countries e.g. Spain 2000 and North America. PepMV is considered as highly infectious, seedborn and readily mechanically transmittable (Ling 2008). So far five genotypes of PepMV have been detected: EU,LP,CH2, US2 and US1 detected in the EU, Peru, Chile and the US (Hasiow 2008). Artificial inoculation studies have shown that PepMV can also infect potato (*Solanum tuberosum*) and eggplant (*Solanum melongena*). Also several natural weed hosts are reported serving as potential infection reservoirs (Cordoba *et al*. 2004). Reported symptoms are leaf distortion, chlorosis, mosaic, blistering of the leaf surface, green striations on the stem and fruit marbling and spotting as well as general discoloration (Cordoba *et al*. 2004). Severely affected plants become stunted and distorted and it can result in the loss of fruit quality to total loss of the crop (Soler *et al*. 2010). Depending on the genotype, the symptom expression can vary from mild to severe in tomato, as found in a study conducted in Belgium (Hanssen *et al*. 2009).

1. Detection

A survey on tomato viruses in open field cultivation was carried out in three provinces in Spain, comprising all the relevant tomato producing farms within this area, during summer in 2007. On 228 selected survey plots, divided into farmer used own seed and farmer used commercial seeds, 1300 individual adult plants, that were approaching the end of the growing cycle, were sampled and tested for PepMV infection. In most cases, five plants were surveyed ar random from each field. Farmer seed plots were planted with local cultivars, conserved by the farmers themselves. On the contrary the commercial seed plots were planted with purchased seeds of modern cultivars, including F1 hybrids and lines. All samples were tested by DAS ELISA using specific antisera (Soler *et al*. 2010).

In a survey of central and southern Peru, 65 isolated wild and 4 cultivated populations of *Lycopersicon*, as well as 6 populations of other *Solanaceae* species, were tested for the presence of PepMV and other viruses. The survey was carried out in March 2000. The plants were visually inspected for virus symptoms and passport data were taken as well. Only young leaves were taken as samples and subsequently analysed using DAS ELISA in the laboratory. Extracts of 20 positive specimen of the DAS-ELISA test were mechanically inoculated on tomatoes in order to confirm the results (Soler *et al*. 2002).

Further detections of PepMV were reported from Ecuador in 2005, Poland in 2002 and 2005 and Hungary 2006, unfortunately no survey methods were described (Soler *et al*. 2005, Pospieszny et a. 2007, Takacs *et al*. 2006).

Several well established procedures for the testing and detection of PepMV are reported in the literature in detail. Predominantly used diagnosis procedures are given here: DAS ELISA (Soler *et al*. 2005) and DAS ELISA with subsequent mechanical inoculation (Soler *et al*. 2002) was evaluated as has Real- time RT-PCR (Ling *et al*. 2007, Hasiow *et al*. 2008), IC-RT-PCR (Pospieszny *et al*. 2007,

Mansilla *et al*. 2003), Multiplex one-step RT-PCR (Alfaro-Fernandez *et al*. 2009) and Quantitative Real- time QPCR (Gutierrez-Aguirre *et al*. 2009)

2. Monitoring

As the systematic literature search has not provided information about the current situation in Germany (or EU) a short summary is given in this paragraph. A personal communication from a JKI Expert (05. 01. 2012) indicated that detection surveys are carried out in tomato propagation and in tomato producing farms and commodity entry to Germany as well. Depending on the different plant material these surveys are taking place on a regular- or irregular basis or *ad hoc*.

The survey in Germany is done according to Entscheidung 2004/200/EG. Sampling procedures testing, analysing and reporting is in accordance with "Leitfaden zur Durchführung des Monitoring zum Vorkommen von Pepino mosaic virus (PepMV) in Deutschland im Rahmen der Entscheidung 2000/325/EG der Kommission (Artikel 4)".

3. Delimitation

No information available.

4. Commodities

No information available.

References: 36 retained out of 197 retrieved, none added, 14 used for summary, 1 personal comm. added)

Alfaro-Fernandez, A., J. Angel Sanchez-Navarro, *et al*. (2009). Simultaneous detection and identification of Pepino mosaic virus (PepMV) isolates by multiplex one-step RT-PCR. European Journal of Plant Pathology 125(1): 143-158.

Cordoba, M. C., L. Martinez-Priego, *et al*. (2004). New natural hosts of Pepino mosaic virus in Spain.Plant Disease 88(8): 906.

Gutierrez-Aguirre, I., N. Mehle, *et al*. (2009). Real-time quantitative PCR based sensitive detection and genotype discrimination of Pepino mosaic virus. Journal of Virological Methods 162(1/2): 46-55.

Hanssen, I. M., A. Paeleman, *et al*. (2009). Pepino mosaic virus isolates and differential symptomatology in tomato. Plant Pathology 58(3): 450-460.

Hasiow, B., N. Borodynko, *et al*. (2008). Development of a real time RT-PCR assay for detecting genetically different Pepino mosaic virus isolates. Journal of Plant Protection Research 48(3): 295-301

Ling, K. S., W. P. Wechter, *et al*. (2007). Development of a one-step immunocapture real-time TaqMan RT-PCR assay for the broad spectrum detection of Pepino mosaic virus." Journal of Virological Methods 144(1/2): 65-72.

Ling, K.-S. (2008). Pepino mosaic virus on Tomato Seed: Virus Location and Mechanical Transmission." Plant Disease 92(12): 1701-1705.

Mansilla, C., F. Sanchez, *et al*. (2003). The diagnosis of the tomato variant of pepino mosaic virus: An IC-RT-PCR approach. European Journal of Plant Pathology 109(2): 139-146.

Pospieszny, H., B. Hasiow, *et al*. (2007). Detection of Polish strains of Pepino mosaic virus. Progress in Plant Protection 47(2): 266-270.

Soler, S., C. Lopez, *et al*. (2005). Natural occurrence of viruses in Lycopersicon spp. in Ecuador." Plant Disease 89(11): 1244.

Soler, S., J. Prohens, *et al*. (2002). Natural occurrence of Pepino mosaic virus in Lycopersicon species in Central and Southern Peru. Journal of Phytopathology 150(2): 49-53.

Soler, S., J. Prohens, *et al*. (2010). Viruses Infecting Tomato in Valencia, Spain: Occurrence, Distribution and Effect of Seed Origin. Journal of Phytopathology 158(11-12): 797-805.

Takacs, A., G. Kazinczi, *et al*. (2006). Natural virus infection of tomato plants in Hungary. Cereal Research Communications 34(1(II)): 689-691

Pepper mild tigré virus

Common name(s): PepMTV,
Taxa: Viruses: Geminiviridae: Begomovirus
EPPO: Added in 2000 but removed from the EPPO Alert list in 2001 because it is covered by the list of *Bemisia*-transmitted viruses in EU regulations
EU Annex designation: I/A1

Organism
Pepper mild tigré begomovirus (PepMTV) is a geminivirus transmitted by the tobacco whitefly *Bemisia tabaci*. It is very often associated with another geminivirus, Chino del tomate virus (CdTV), in causing tigré disease. It was first described in Mexico on *Capsicum* (Brown et al. 1989).
The virus causes important diseases on its main host *Capsicum annuum* (cvs Jalapeno and Serrano). Other host includes tomato (*Lycopersicon esculentum*), tabacco (*Nicotiana tabacco)* and datura (*Datura stramonium*).
There is very little further information available for this species.

1. Detection
PepMTV causes mild interveinal chlorosis, veinal distortion and mild stunting on peppers, and on tomatoes, it causes leaf curling, mild interveinal chlorosis and moderate stunting. Molecular identification could be done using sequence (Idris et al. 1994, Brown et al. 2001).

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
The PepMTV is transmitted by *Bemisia tabaci*. International movement is possible in peppers and tomatoes infected with virus, but it is not transmitted by seeds (Marchoux et al. 2008).

References: 2 retained out of 5 retrieved, 2 added, 4 used for the summary

Brown, J. K., O. P. Campodonico, and M. R. Nelson. 1989. A whitefly-transmitted geminivirus from peppers with tigre disease. Plant Disease 73:610.

Brown, J. K., A. M. Idris, I. Torres-Jerez, G. K. Banks, and S. D. Wyatt. 2001. The core region of the coat protein gene is highly useful for establishing the provisional identification and classification of begomoviruses. Archives of Virology 146:1581-1598.

Idris, A. M., G. K. Banks, and J. K. Brown. 1994. Development of a diagnostic assay for whitefly-transmitted geminiviruses using PCR. Phytopathology 84.

Marchoux, G., P. Gognalons, and K. Gébré Sélassié. 2008. Virus des Solanacées. Du génome viral à la protection des cultures. Quae, Versailles, France.

Peridermium kurilense Dietel

(=*Cronartium kamtschaticum* Jørstad)
Common name(s): Japanese white pine rust
Taxonomic position: Fungi: Basidiomycota: Cronartiaceae
EPPO A2 list: No. 18
EU Annex designation: I/ A1 as *Cronartium* spp. (non-European)

Organism
Peridermium kurilense is the causal agent of "Japanese white pine rust". The aecial hosts are the five-needled pines *Pinus cembra* var. *sibirica* in Russia and *Pinus pumila* in Japan, possibly also *Pinus strobus.* The telial hosts are species of *Castilleja* and *Pedicularis*, possibly also *Ribes* (Anonymous 1983). The pathogen is present in Russia and Japan but absent from Europe. Infection of the *Pinus* hosts is characterized by fusiform bark swellings which may later develop into cankers. The fungus can be carried considerable distances as wind-borne aeciospores and can survive very long periods in the airborne state.

1. Detection
Morphological examination and molecular methods were combined to identify a rust fungus on *Pinus pumila* in Russia: *Peridermium kurilense* and *Endocronartium sahoanum* var. *hokkaidoense* morphologically similar were separated using PCR-RFLP analysis (Imazu et al. 2000).

2. Delimitation
No information available.

3. Monitoring
No information available

4. Commodities
These rusts can enter on plants in infected coniferous hosts destined for planting. As a result of the long incubation period of this disease, latent infections can easily remain undetected (Anonymous 1983).

References: 5 retained out of 8 retrieved, 2 added, 2 used for the summary

Anonymous. 1983. EPPO Data sheet on Quarantine Organisms n° 18: *Peridermium kurilense*. EPPO bulletin.

Imazu, M., Z. M. Azbukina, M. Kakishima, K. Fukushima, K. Nishimura, and M. Miyaji. 2000. Identification of a rust fungus on Pinus pumila collected in the North Kurils, Russia. Mycoscience 41:139-144.

Phellinus weirii (Murrill) Gilbertson

Common name(s): Laminated butt rot, yellow ring rot.
Taxonomic position: Fungi: Basidiomycota: Hymenochaetaceae
EPPO A1 list: No. 19
EU Annex designation: I/A1

Organism
Phellinus weirii is the causal agent of yellow ring rot. It can attack the roots of many conifer species although *Pseudotsuga menziesii* is the principal host in North America. The pathogen is also present in Asia (China, Japan) but absent from European countries and the EPPO region. Healthy tree roots are infected by contact with infected roots. Natural dispersal occurs only over short distances. The fungus can persist within the roots and stumps of *Pseudotsuga menziesii* for 50 or more years (Anonymous, 1977).

1. Detection
In extensive forests, aerial photographic techniques can be used as tools for detecting root disease foci (Johnson and Wear 1975). High resolution multi-spectral imagery combined with automated procedures seems to be viable for detecting affected trees when severe symptoms are present (Leckie, Jay et al. 2004).
Yu X. et al. developed an immunological method to detect *Phellinus weirii* fungal protein (Yu, Zamani et al. 2002). Molecular methods have been used to separate 2 closely related species of *Phellinus* (Lim, Yeung et al. 2005)and more recently, specific primers applied to real-time PCR allowed an accurate, sensitive and rapid detection of the pathogen (Wu, Gao et al. 2010).

2. Delimitation
No information available.

3. Monitoring
Aerial photographs were used to estimate the disease spread in conifer stands (Nelson and Hartman 1975). Above and below-ground disease indicators were also used to evaluate the total extent of *Phellinus weirii* root rot centers (Wallis and Bloomberg 1981).

4. Commodities
Movement is most likely to occur by transport of infected coniferous logs or bark (1977).

References: 12 retained out of 78 retrieved, none added, 8 used for the summary

Anonymous (1977). Data sheets on quarantine organisms. Phellinus [Inonotus] weirii (Murr.) Gilbertson. Basidiomycetes: Aphyllophorales. Data sheets on quarantine organisms. Phellinus [Inonotus] weirii (Murr.) Gilbertson. Basidiomycetes: Aphyllophorales. Paris, EPPO: 5.

Johnson, D. W. and J. F. Wear (1975). Detection of Poria weirii root rot centers in the Pacific Northwest with aerial photography. Plant Disease Reporter 59(1): 77-81.

Leckie, D. G., C. Jay, et al. (2004). Detection and assessment of trees with Phellinus weirii (laminated root rot) using high resolution multi-spectral imagery. International Journal of Remote Sensing 25(4): 793-818.

Lim, Y. W., Y. C. A. Yeung, et al. (2005). Differentiating the two closely related species, Phellinus weirii and P-sulphurascens. Forest Pathology 35(4): 305-314.

Nelson, E. E. and T. Hartman (1975). Estimating spread of Poria weirii in a high-elevation, mixed conifer stand. Journal of Forestry 73(3): 141-142.

Wallis, G. W. and W. J. Bloomberg (1981). Estimating the total extent of Phellinus weirii root rot centers using above- and below-ground disease indicators. Canadian Journal of Forest Research 11(4): 827-830.

Wu, C., Z. Gao, et al. (2010). Molecular detection of Inonotus weirii Gilgertson. Journal of Southwest Forestry University 30(5): 53-57.

Yu, X., A. Zamani, et al. (2002). A polyclonal antibody against a Phellinus weirii antigen (Phe w I) differentially detects P. weirii fungal and Douglas-fir host proteins. Canadian Journal of Plant Pathology 24(1): 91.

Phialophora cinerescens (Wollenweber) van Beyma

Common name(s): Philophora wilt, wilt of carnatiom
Taxonomic position: Fungi: Ascomycota: Acomycetes (unclassified)
EPPO A2 list: No. 77
EU Annex designation: II/A2

Organism
Phialophora cinerescens is a vascular wilt pathogen of carnations, which are the main host. A number of caryophyllaceous garden plants serve as secondary hosts. The pathogen is an indigenous European species present in many European countries but is also recorded in Asia and America. The fungus, which is a soil-borne pathogen, can survive saprophytically for many years in soil but has a limited potential for natural spread (Anonymous 1982). Following infection, leaves and stems become bluish-grey in colour. Subsequently, there is rapid wilting of the whole plant. As the disease may have an important economic impact in carnation-cultivating areas, specific phytosanitary measures were included in a general “Certification scheme for carnation” published by EPPO (Anonymous 1991).

1. Detection
Vascular pathogens on cuttings can be detected on incubated stem section isolates. A rapid and early diagnosis can be achieved through fluorescence microscopy (Bonifacio and Rumine 1984).

2. Delimitation
No information available.

3. Monitoring
No information available

4. Commodities
The main pathway for spread is international trade in carnation cuttings (Anonymous 1982).

References: 8 retained out of 180 retrieved, 1 added, 3 used for the summary.
Anonymous. 1982. EPPO data sheet on Quarantine Organisms n° 77: *Phialophora cinerescens*. EPPO Bulletin 12.
Anonymous. 1991. Certification Scheme Pathogen-Tested Material of Carnation. Bulletin OEPP 21:279-290.
Bonifacio, A. and P. Rumine. 1984. Use of the fluorescence microscope in the diagnosis of vascular diseases of carnation. Informatore Fitopatologico 34:43-44.

Phoma andina Turkensteen

Common name(s): Black potato blight
Taxa: Fungi: Ascomycota: Leptosphaeriaceae
EPPO A1 list: No. 141
EU Annex designation: I/A1

Organism

Phoma andina is the causal agent of black potato blight also called Phoma potato leaf spot. The main host is potatoes but other Solanaceae (tomatoes, various weeds) are also attacked. Originating from South America (Bolivia and Peru), the pathogen is absent from Europe and EPPO region but it is a potentially dangerous pathogen for potato-growing areas. The fungus survives in the soil and pycnidiospores splashed from the soil surface infect potato leaves. Infection is favoured by high humidity and rain and occurs in cool weather with temperatures below 15°C. Tubers are not infected.

1. Detection

Black leaf spots on leaves can be detected with visual inspections and typical pycnidia embedded in the affected tissue can be observed microscopically.

2. Delimitation

No information available

3. Monitoring

No information available

4. Commodities

In international trade, the fungus could be introduced on leaves of living material, on dead plant material, on crop residues or soil accompanying tubers. It could certainly survive in temperate zones of the EPPO region

References

Phoma tracheiphila (Petri) Kantschaveli and Gikashvili

Common name(s): Dieback of citrus, mal secco, wilt of citrus
Taxonomic position: Fungi: Ascomycota: Leptosphaeriaceae
EPPO A2 list: No. 287
EU Annex designation: II/A2

Organism

Phoma tracheiphila is the causal agent of "mal secco", a destructive vascular disease of Citrus. The main host species is lemon (*Citrus limon*) but the fungus has been reported on many other Citrus spp. The symptoms appear in spring as leaf and shoot chlorosis followed by a dieback of twigs and branches. Inoculum of *Phoma tracheiphila* is dispersed by wind and rain and enters through wounds in the plant. The fungus can survive within infected twigs in the soil for more than 4 months. The pathogen is present in Europe and in EPPO region (Mediterranean and Black Sea area)(Anonymous 1990, 2007).

1. Detection

After visual inspection, the fungus can be isolated from symptomatic fruits or twigs and identified on the basis of cultural and morphological characters (2007). Immunological methods (ELISA) were first used for disease diagnosis (Nachmias et al. 1979). Analysis of mycelial proteins by PAGE could be helpful for strains identification (2007) while molecular methods were developed for the detection of *Phoma tracheiphila*. Balmas et al. have designed a pair of specific primers for *in planta* PCR detection (Balmas et al. 2005) improving the specificity (2007) of the method previously described by Rollo et al. (Rollo et al. 1990). A further improvement of the real-time PCR protocol and the DNA extraction methods allowed the quantification of the fungus from infected citrus species as well as from soil (Russo et al. 2011).

2. Delimitation

No information available

3. Monitoring

Phoma tracheiphila inoculum in the air was quantified in two infected orchards in Sicily. Monitoring over a period of 16 months gave important information on the pathogen spread and viability (Tuttobene 1994).

4. Commodities

Long distance spread of "mal secco" occurs through infected propagative material and plants (Anonymous 1990).

References: 16 retained out of 65 retrieved, 1 added, 7 used for the summary

Anonymous. 1990. EPPO Data Sheets on Quarantine Pests: *Deuterophoma tracheiphila*. EPPO Bulletin.

Anonymous. 2007. Phoma tracheiphila. Bulletin OEPP/EPPO Bulletin 37:521-527.

Balmas, V., B. Scherm, S. Ghignone, A. O. M. Salem, S. O. Cacciola, and Q. Migheli. 2005. Characterisation of Phoma tracheiphila by RAPD-PCR, microsatellite-primed PCR and ITS rDNA sequencing and development of specific primers for in planta PCR detection. European Journal of Plant Pathology 111:235-247.

Nachmias, A., M. Bar-Joseph, Z. Solel, and I. Barash. 1979. Diagnosis of mal secco disease in lemon by enzyme-linked immunosorbent assay. Phytopathology 69:559-561.

Rollo, F., R. Salvi, and P. Torchia. 1990. Highly Sensitive and Fast Detection of Phoma-Tracheiphila by Polymerase Chain Reaction. Applied Microbiology & Biotechnology 32:572-576.

Russo, M., F. M. Grasso, P. Bella, G. Licciardello, A. Catara, and V. Catara. 2011. Molecular diagnostic tools for the detection and characterization of Phoma tracheiphila. Acta Horticulturae 892:207-214.

Tuttobene, R. 1994. Monitoring of Phoma tracheiphila inoculum. Difesa delle Piante 17:69-74.

Phony disease of peach

(=*Xylella fastidiosa* subsp. *multiplex* Schaad et al.))
Common name(s): Leaf scald of plum, leaf scorch of elm/oak/plane, peach virus 4
Taxa: Bacteria: Proteobacteria: Xanthomonadaceae
EPPO A1 list: No. 166
EU Annex designation: I /A1

Organism
Phony peach disease (PPD) is caused by a xylem limited bacterium *Xylella fastidiosa*. Strains of this bacterium are causal agent of Citrus variegated chlorosis (CVC), Pierce's disease (PD) of grapes and other disease of woody plants such as, plum leaf scald, and leaf scorch of almond, coffee, elm, oak, oleander, and sycamore.
Several pathogenic varieties of the bacterium have been described, that are often host specific and strains from peach have been identified as *X. fastidiosa* subsp. *multiplex* (Janse and Obradovic 2010).
PPD occurs in America from North Carolina to Texas. The bacterium is transmitted by root grafts and natural transmission occurs via insects feeding on xylem sap, mainly by sharpshooter leafhoppers (Janse and Obradovic 2010). The bacterium overwinters in the xylem of the host plants.

1. Detection
PPD first symptoms are stunted young shoots with denser and darker green leaves than healthy trees. Moreover they show early blooming and both leaves and flowers remain on the shoots longer than normal. Twigs on diseased trees have shortened internodes and increased lateral branching. Fruit production is severely impaired and fruits size quality and number are reduced. Symptom development is often slow (up to 18 months or more after infection) and may be present in one scaffold limb or in the entire tree. Trees are generally not killed but are more susceptible to other diseases and arthropods (Janse and Obradovic 2010).
(Anonymous 2004) and (Janse 2010) describe the diagnostic protocols for the detection and identification of *X. fastidiosa* only for citrus and grapevine, but most could be suitable for peach.
The bacterium can be detected using peach root and twig section with a microscope equipped at least dark field or phase contrast optics (Wells et al. 1980) and by mmunofluorescence (Weaver et al. 1980, Wells et al. 1980) or enzyme like immunoabsorbent assay ELISA (Wells et al. 1981).
DNA hybridization probes and general (generic) primers have been developed for non-grapevine strains by (Firrao and Bazzi 1994). A recently multi-primer polymerase chain reaction (PCR) system, random amplified polymorphic DNA (RAPD)-PCR, and sequence analysis of the 16S-23S rDNA inter-genic spacer region (ISR) allowed the characterization of peach strains of *X. fastidiosa subs. Multiplex* (Hernandez-Martinez et al. 2006).

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities

No information available.

References:1 retained out of 1 retrieved, 7 added, 8 used for the summary

Anonymous 2004. Diagnostic protocols for regulated pests. Bulletin OEPP/EPPO Bulletin 34:187-192.

Firrao, G. and C. Bazzi. 1994. Specific identification of *Xylella fastidiosa* using the polymerase chain reaction. Phytopathologia 33:90-92.

Hernandez-Martinez, R., H. Costa, C. K. Dumenyo, and D. A. Cooksey. 2006. Differentiation of strains of *Xylella fastidiosa* infecting grape, almonds and oleander using a multiprimer PCR assay. Plant Disease 90:1382-1388.

Janse, J. D. 2010. Diagnostic methods for phytopathogenic bacteria of stone fruits and nuts in COST 873. Bulletin OEPP/EPPO Bulletin 40:68-85.

Janse, J. D. and A. Obradovic. 2010. Xylella fastidiosa: its biology, diagnosis, control and risks. Journal of Plant Pathology 92 S1.35-S31.48.

Weaver, D. J., B. C. Raju, J. M. Wells, and S. K. Lowe. 1980. Occurence in Johnsongrass or Rickettsia like bacteria related to phony peach disease organism. Plant Disease 64:485-487.

Wells, J. M., B. C. Raju, J. M. Thompson, and S. K. Lowe. 1981. Etiology of phony peach and plum leaf scald disease. Phytopathology 71:1156-1161.

Wells, J. M., D. J. Weaver, and B. C. Raju. 1980. Distribution of rickettsia-like bacteria in peach, and their occurrence in plum, cherry, and some perennial weeds. Phytopathology 70:817-820.

Phyllosticta solitaria Ellis and Everhart

Common name(s): Apple blotch, fruit/leaf blotch of pome fruit, teig cancer of pome fruit
Taxa: Fungi: Ascomycota: Botryosphaeriaceae
EPPO A1 list: No. 20
EU Annex designation: I/A1

Organism
Phyllosticta solitaria is the causal agent of apple blotch on cultivated apple trees as well as on the wild *Malus coronaria*. Present in North America, it is absent from European Union and EPPO region. Overwintering cankers are probably the source of primary inoculum. Disease development usually occurs in the spring. The rainsplash-dispersed spores infect the current year's growth, with new cankers appearing in august. Lesions also occur on the leaves and fruit. Disease incidence and severity are directly correlated with rainfall (Anonymous 1980).

1. Detection
Symptoms can be observed on leaves, twigs and fruit. Detection of latent infections in asymptomatic apple fruit can be done after paraquat treatment (Biggs 1995).

2. Delimitation
No information available

3. Monitoring
No information available

4. Commodities
International movement is only likely on seedlings or planting material with cankers. The fungus can withstand long period of cold storage (Anonymous 1980).

References: 2 retained out of 61 retrieved, 2 new added, 2 used for the summary
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Phymatotrichopsis omnivora (Duggar) Hennebert

Common name(s): Texas root rot, Phymatotrichum root rot, cotton root rot
Taxonomic position: Fungi: Basidiomycota: Rhizinaceae
EPPO A1 List: No. 21
EU Annex designation: I/ A1 as *Trechispora brinkmanni*

Organism
There is confusion as to the identity of this pest and it is considered preferable to consider this pest as *Phymatotrichopsis omnivora* rather than *T. brinkmanni,* which is thought to be its possible teleomorph. This is a serious pathogen of cotton in North America (Anonymous 1979) but its identity is still under definition (Marek 2009; Marek, Hansen et al. 2009; Arif, Marek et al. 2010; Uppalapati, Young et al. 2010; Arif, Ochoa-Corona et al. 2011). It occurs mainly in North America (US and Mexico) and is absent from Europe.

1. Detection
No information available.

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
No information available.

References: 0 retained out of 0 retrieved, 6 added, 6 used for the summary

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Phytophthora fragariae Hickman var. *fragariae*

(=*Phytophthora fragariae* Hickman)
Common name(s): Red core, red stele, Lanarkshire disease
Taxa: Chromista: Pseudofungi : Peronosporales
EPPO A2 list: No. 79
EU Annex designation: II/A2

Organism
The pathogen is widely distributed globally and occurs across the EPPO region e.g. Austria, Belgium, Czech Republic, Cyprus, Denmark, Egypt, France, Germany, Ireland, Italy, Lebanon, Luxembourg, Netherlands, Slovakia, Spain, Sweden, Switzerland, UK, and Slovenia. The pathogen is also present in North America, Oceania, Asia and parts of South and Central America. The principal host is cultivated strawberries (*Fragaria* x *ananassa*). Logan berry is also known to be naturally infected but a number of genera within the tribe Potentilleae within the Rosaceae have been infected artificially.
The potential host range in the EPPO region is all species of *Fragaria* and perhaps certain species of *Rubus*, such as loganberries and blackberries (*Rubus fruticosus*)(Anonymous, 1997).
There is experimental evidence that the fungus can survive as resistant oospores in excess of four years and some field reports suggest that it remains viable in soil for 13-15 years after a strawberry crop. Oospores germinate to form one or occasionally several sporangia. The optimum temperature for germination is 10-15°C. The sporangia release vigorously motile zoospores which swim to the root tips of the host plant where they encyst, attach themselves and form germ tubes which penetrate into the root. The fungus traverses the cortex inter- and intracellularly to the stele, mainly colonizing the pericycle and the phloem. Growth is mostly concentrated within the stele, with the fungus growing along it, but hyphae grow out from the roots to form new sporangia which release more zoospores and initiate new infections on other roots and plants. Non-papillate secondary sporangia are produced within a few days and the fungus can produce many cycles of infection over the winter months.
The stele of infected roots turns red in response to infection and later the root starts to rot from the tip upwards. As the infection progresses, oospores are formed in close association with the stele, probably in the sieve tubes of the phloem. Eventually infected roots rot, due in part to invasion by secondary organisms, leaving large numbers of new oospores in the soil. Planting diseased plants into clean soil contaminates the site for many years.
A number of races of the fungus have been recognized. There is no internationally recognized system for classifying races. Symptoms usually appear on the upper parts of plants that come under stress in late spring or early summer, especially in low-lying, wet areas. Plants often fail to develop or make only stunted growth. They may die just before fruiting or produce a few small fruit. Younger leaves can have a blue-green coloration and older ones turn yellow or red. Digging up the plants reveals a poorly developed and rotted root system.
Lateral feeder roots are usually badly rotted and are commonly lost by the time plants are dug. The adventitious roots rot from the tips upwards and often have a grey to brown appearance at their distal ends, giving the characteristic 'rat-tail' symptom. Cutting open the upper, white, unrotted parts of such roots reveals steles wine-red to brick-red in colour - hence the name red core. The colour can extend for quite long distances above the rotted parts of the roots, right into the crown in highly susceptible cultivars (Anonymous, 1997).

1. Detection

Despite having a 'nil tolerance' in propagation schemes and being a quarantine pest, *P. fragariae* has spread steadily throughout Europe such that only a few Mediterranean countries are now apparently free from it. Field inspections of nurseries are inadequate for detection of red core and

a bait test, although sensitive, takes too long to be useful commercially. The European Union, through its Standards, Measurements and Testing Programme has funded the further development of prototype molecular and immunological tests for red core to routine tests that meet ISO 9002 standards. The principal thrust has been the development of a nested polymerase chain reaction (PCR), supported by immunological tests for rapid screening of large numbers of routine samples. Nested PCR has suitable speed, sensitivity and specificity for testing and is now being used routinely in some laboratories (Duncan et al., 2000).
Polyclonal antibodies anti-PfP IgG and anti-PfM IgG and monoclonal antibody MAb29 were prepared to detect the quarantine pathogen of strawberry, *Phytophthora fragariae*. Laboratory rabbits and mice were immunized using purified and unpurified protein extracts from the mycelial mass of the pathogen. PTA-ELISA was used to test the antibodies. *P. fragariae* was detected in artificially infected strawberries (cultivars Elsanta, Kama and Vanda) by means of PTA-ELISA, immunoprinting and dot blot. Detection of the pathogen was optimum in undamaged roots or roots with necrotic tips only. At a later stage of infection, when whole roots were necrotic, the crown was more suitable for successful detection. To detect *P. fragariae* at the early stages of infection it is recommended to use at least two of the three mentioned immunotechniques (Pekarova et al., 2001).
In Israel, strawberry is a winter producing crop cultivated as a fresh cropping system. Nuclear and foundation propagation material are indexed for viruses and checked for fungal pathogens twice annually, while spring-propagated field nurseries are monitored routinely for disease until summer transplanting to production fields. Wilted plants are routinely sampled in the field by farmers and extension specialists from the Ministry of Agriculture and pathogens detected in the laboratory in Volcani Center. Initially, infected plant tissues were plated-out on selective medium for *Phytophthora* spp. and *Fusarium* spp., semi-selective medium for *Colletotrichum* spp. and *V. dahliae*, and non-selective PDA medium for *Rhizoctonia* spp. and *M. phaseolina*. Cultures isolated from the different media were inspected microscopically for morphological characters typical for the causal agent of disease. *P. fragariae* is identified according to specific-primer PCR amplification (Zveibil & Freeman 2009).

2. Monitoring
A survey for the possible occurrence of red-core disease *(Phytophthora fragariae* var*. fragariae)* was carried out on 250 strawberry production sites in Finland. The fields were inspected visually. A total of 1080 samples of strawberry roots were taken in spring and autumn 1995 and examined visually, microscopically and by isolation in the laboratory. Root-tip bait-plant tests were performed in the glasshouse to look for the latent presence of the fungus. No red-core disease was detected in any of the inspected fields or in the examined samples (Pohto, 1999).

3. Delimitation
No information available.

4. Commodities
General elements of this inspection procedure apply to both inspection in the exporting or the importing country. In the exporting country inspections should be done at the premises of the producer or exporter as long as the whole consignment is still accessible i.e. before packing or loading. Inspection of plants in cool storage should be avoided as this greatly influences detection of insect pests because of their reduced mobility. For quarantine organisms likely to be present on the consignment in a latent stage, field inspections and sampling should be done at the most

appropriate time according to EPPO Standard PM 3/xx. *Field inspection* (in preparation).
Producers, exporters and transporters in the exporting country should be informed of the particular requirements of the importing country for strawberry plants. Inspections of consignments of *Fragaria* plants in the importing country may be done at the point of entry or at the point of final destination, depending on the possibilities of carrying out efficient inspections and provided that the plants remain under official control. In case of inspection at the point of final destination there should be no risk of escape of quarantine pests during transport. When a sample has been taken from the consignment the imported *Fragaria* plants should remain under official control and should not be planted until the final laboratory result confirms absence of the relevant quarantine pests. Plants for planting produced according to the EPPO Standard PM 4/11 (2) *Certification Scheme on 'pathogen-tested strawberry'* or any equivalent phytosanitary certification system, are generally considered to provide higher phytosanitary guarantees and this should be taken into account. Consequently intensity of inspections and testing may be reduced and inspection may mainly focus on pests not covered by the respective certification scheme.
Thorough visual inspections on older leaves of green plants or on their remains still attached to the runners should be done looking for typical angular spots. However young plants and, in particular, 'frigo' plants or plants in tissue culture frequently do not show any symptoms of infections at this stage. Therefore, samples, consisting of one plant for 1000 plants from different parts of the lot with a minimum of 50 plants for small lots and a maximum of 300 plants, should be taken for examination in the laboratory (Anonymous, 2008).
Import and export controls in Russia are briefly outlined in this report (Vasyutin, 1999).
Propagation in the glasshouse and beds with pathogen-free soil are to be organized by the Growers Organization TRF. For the production of runners to be planted by the strawberry producers, the mother material has to be taken from this station. In Sweden strawberry plants cannot be marketed unless they are produced and approved under the certification scheme (Gransbo, 1984). In order to assess the health status of cold-stored runner plants to be used in the 2002 plantings in southern Italy, a survey was carried out. Extensive visual inspections were made and laboratory methods were used to isolate fungal pathogens on suitable media for microscopic examination. Therefore a total of 3000 runner plants of seven cultivars, originating from several nurseries, were examined during storage. The occurrence of *Phythophtora* infections was observed only with a highly sensitive nested PCR assay and *P. fragariae* was found to be not present (Camele et al. 2006).

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Phytophthora ramorum Werres et al.

Common name(s): Sudden oak death
Taxa: Chromista: Pseudofungi: Peronosporaceae
EU Annex designation: not listed but regulated in decision 2002/757/EC

Organism
Phytophthora ramorum is a fungus-like pathogen, which has been identified as the causal agent for Sudden Oak Death. After appearing on the EPPO Alert List, the pathogen is now causing extensive damage in the USA on a variety of endemic *Quercus sp., e.g. Q.chrysolepis*, *Lithocarpus densiflorus*, *Quercus agrifolia*, *Q. kellogii*, *Q. parvula* var, Shrevei. Also host of *P. ramorum* are: *Toxicodendron diversilobatum*, *Rubus spectabilis*, *Rhamnus purshiana*, *Corylus cornuta*, *Pittosporum undulatum* and *Trientalis latifolia*. Also some coniferous species are reported as being hosts, e.g. *Abies magnifica* and *A. grandis.*
In Europe, *P. ramorum* is mainly found on *Rhododendron* and *Viburnum,* but it was also isolated from *Arbutus*, *Camellia, Hamamelis, Kalmia, Leucothoe, Magnolia*, *Pieris and Syringa, Quercus falcata, Fagus sylvatica*, *Quercus ilex, Q. cerris, Castanea sativa* and *Aesculus hippocastanum*. Furthermore it caused infection in conifer trees such as *Larix kaempferi* and *Picea sitchensis.* Two mating types of *P. ramorum* exist: A1 is found in Europe and A2 in North America and the true origin of the pathogen is unknown.
Depending on host species, symptoms vary between wilted shoots as first symptoms, sap oozing, cankers, foliage brown turning resulting in the death of the plant. In Europe, *P. ramorum* mainly causes leaf and twig blight also known as dieback, but except from the UK has never been found causing extensive damage in forests.
The pathogen produces zoospores, which can be transmitted by wind and rain as well as surface water. The pathogen has been detected in European nurseries in Belgium, Czech Republic, Denmark, Finland, France, Germany, Ireland, Italy, Lithuania, Netherlands, Norway, Poland, Serbia, Slovenia, Spain, Switzerland, Sweden, and United Kingdom. In the USA infections have reported in ornamental nurseries in several states: California, Georgia, Louisiana, Oregon, South Carolina, Tennessee, and Washington. In Canada, it was detected in 2003 on 1 *Rhododendron* plant in a nursery under eradication.

1. Detection
The most common methods for the diagnosis of *P. ramorum* are isolation, followed by identification based on growth characteristics in culture and morphological features, lateral flow diagnostic tests, ELISA, conventional PCR, Real-Time Taqman PCR and rhododendron leaf indexing (Turner *et al*. 2008, Bulajić *et al*. 2010). To determine the type of *P. ramorum* the isolates are tested with PCR-RFLP based on Cytochrome oxidase subunit 1 (Steeghs & Gruyter 2006). The new EU guideline for testing procedures describes all appropriate measures in detail (Anonymous, 2006). First indications in the field towards *Phytophthora* infestation can be achieved by using ELISA-Kits (Fera, Neogen).

2. Delimitation
Requirements of Commission Decision 2002/757/EC for appropriate procedures aiming at eradication of the harmful organism are laid down in ANNEX 3c, extracts see below.

Destruction of the infected plants and all susceptible plants within 2 m radius of the infected plants, including associated growing media and plant debris,
For all susceptible plants within 10 m radius of the infected plants, and any remaining plants from the affected lot:

- the plants have been retained at the place of production,
- official additional inspections have been carried out at least twice in the three months after the eradication measures have been taken when the plants are in active growth,
- while during this three months-period no treatments that may suppress symptoms of the harmful organism have been carried out,
- the plants have been found free from the harmful organism in these official inspections

For all other susceptible plants at the place of production, the plants are subject to official intensive re-inspection following the finding and have to be found free from the harmful organisms in these inspections

Appropriate phytosanitary measures have been taken on the growing surface within a 2 m radius of infected plants (Anonymous 2002).

In the case case that the pathogen was found through a survey, measures to delimitate a potential outbreak are undertaken, e.g. cut back of branches or trees, removal of infested plants and the prevention of access to infested areas, woodland clearance with subsequent burning of the contaminated plants, cleaning of re-circulating irrigation water by filtering. In the Netherlands treatment measures included complete removal of the plants or cutting back the plants to below 50 cm. Cutback but still infected plants are enclosed in a survey the following year (Steeghs & Gruyter 2006).
Study results show that *P. ramorum* can survive in potting media if introduced as sporangia or chlamydospores, and infested media or contaminated containers can be sanitized by aerated steam treatment (Linderman & Davis 2006).
Whereas in most countries eradication measures are undertaken by the plant health service staff and costs are borne by the State, in the Netherlands land owners are responsible for the eradication of infested plant material. Given the fact that this disease has a high risk impact for host plants, land owners put the eradication into practice very willingly thus lowering the inoculum pressure and risk for endemic plants in woods and free landscape (Steeghs & Gruyter 2006).
Eradication treatments in Oregon forests consists of cutting and burning infected and exposed host plants, and where possible, injecting herbicide into tanoaks to prevent sprouting. The effort has slowed, but not stopped, long distance dispersal of the pathogen. To monitor the effectiveness of eradication treatments we are revisiting treated sites and sampling soil and vegetation in fixed plots centred on stumps of known infected trees (Kanaskie 2009).
During the first two years of the eradication effort, all host vegetation within 15 to 30 m of infected plants was destroyed. In recent years this distance has been increased to at least 100 m, reflecting recent research findings on spread of the pathogen. Following burning most sites are planted with non-host or conifer seedlings (Kanaskie 2008). British woodlands are also considered at risk. In 2004 the Forestry Commission surveyed 1479 woodlands where *Rhododendron ponticum* was present, and in 2008 both infected and non-infected *R. ponticum* were cleared from approximately 1200 ha of woodlands in England and Wales where *P. ramorum* had been found (Tracy 2009).

3. Monitoring
The potential for infection of native tree species in Europe and the detection of the fungus in

nurseries of several European countries has lead to the implementation of EU emergency phytosanitary measures. As a result, most European countries have conducted surveys complying with Commission Decision 2002/757/EC. In addition research as part of risk assessment efforts is continuing (Anonymous 2002).

In the most part, nurseries are surveyed by visual inspections and samples are taken from suspicious plants showing symptoms. The collected specimen are analysed in the laboratory. The surveys are conducted at least once per year.

Further inspections took place in Garden centres, public- and private gardens. This is common practice carried out by plant health inspectors all across the EU and is widely reported in the literature (Bulajić *et al*. 2010, Běhalová 2006, Heiniger *et al*. 2004, Slawson *et al*. 2006, Steeghs & Gruyter 2006, Hunter 2008, Tracy 2009, Orlikowski *et al*. 2007, Hoyer-Tomiczek 2006, Zerjav & Munda 2005). For the whole EU, positive records were found in 115 nursery plants, 63 plants in public green spaces, and 10 forests. The UK recorded the highest number of cases (96), followed by France (18) (Schroder & Pfeilstetter 2007).

As it is know, that *P.ramorum* can be transmitted by soil or cultivation substrate in containers as well as in irrigation water. Some plant health services additionally examine those materials and water recirculation systems (Themann *et al*. 2002).

In addition to the areas that are more amenable to monitoring, detection surveys are required and thus carried out in open woodland within EU Member States (Anonymous 2002). Widespread infestations with *P. ramorum* were first reported from the USA where, similar to Europe, large surveys are undertaken in nurseries, public- and private green and extended woodlands. States that reported large infested areas are: Oregon, South Carolina, North Carolina, California, Florida, Texas and Oklahoma (Warfield *et al*. 2008, Wamishe *et al*. 2008, Von Broembsen *et al*. 2004, Benson & Warfield 2004, Appel & Kurdyla 2004, Hwang *et al*. 2006, Osterbauer *et al*. 2003). Taking the large expanses of woodland in the USA into account, early detection efforts in Californian and Oregon at-risk forests include monitoring from airplanes and helicopters using hyperspectral imagery for pre-visual detection. Trees under suspicion were mapped and checked from the ground, and samples collected for confirmation of an infection. In addition, annual ground surveys check previously treated areas for newly infected trees. Oregon nurseries, Christmas tree plantations, and other sites are surveyed regularly, as mandated by federal regulations. Streams in and near the quarantine area in southwest Oregon are monitored for *P. ramorum* using rhododendron and tanoak leaves (Osterbauer *et al*. 2004).

Monitoring of sixty-four streams in and near the Oregon quarantine area using leaf baits for early detection of *P. ramorum* has been an important part of the Oregon Sudden Oak Death (SOD) program since 2002. Leaves of rhododendron (*Rhododendron macrophyllum*) and tanoak (*Lithocarpus densiflorus*) were placed in mesh bags, and bags were floated in streams. Leaf baits were exchanged every 2 weeks throughout the year. Leaves were assayed by isolation on selective medium and by multiplex rDNA internal transcribed spacer polymerase chain reaction (ITS PCR). *P. ramorum* was regularly recovered during all seasons of the year from streams draining infested sites 5 years after eradication treatment. *P ramorum* was isolated from streams in 23 watersheds. When *P. ramonum* was detected, intensive ground surveys located infected tanoaks or other host plants an average of 306 m upstream from the bait station. *P. ramorum* was isolated from stream baits up to 1,091 m from the probable inoculum source (Sutton *et al*. 2009). Similar findings are reported for at-risk areas in California (Kliejunas, 2010).

A national survey of forests at risk is conducted by the USDA FS Forest Health Monitoring program and its co-operators. They developed and tested protocols for identifying and surveying forest ecosystems that may be vulnerable to invasion by *P. raramorum*. A detection survey is targeting

areas outside the currently known distribution of *P. ramorum*, including eastern oak forests. Sampling intensity is based on a risk map that identifies areas at high, moderate, and low risks of invasion. Surveys were conducted in 36 States with emphasis on forests near nurseries that received *P. ramorum* infested plants. The cumulative number of locations surveyed during the 2 years now exceeds 1,100, with more than 5,600 samples submitted for laboratory analysis for *P. ramorum*. Detection surveys were continued in 2005 (Tkacz *et al*. 2006).

In Canada annual surveys targeting importing nurseries, botanical and public gardens, and collections of rhododendron societies for *P. ramorum* are conducted. When the pathogen is found, the nursery site is placed under quarantine and all infected plant material has to be destroyed (Kliejunas 2010). Monitoring of residual inoculum levels in soil/leaf debris has been carried out monthly for a period of up to three years in several sites in the south of England to investigate the extent of contamination within the gardens or woodlands and to quantify the effect of season on variation in inoculum levels (Turner *et al*. 2008).

4. Commodities

Requirements of Commission Decision 2002/757/EC for introducing and moving susceptible host plants, woods, bark and plants for planting into or within the EC are laid down in Articles 3, 4 and 5.It is required within the EC and stated in the Annex points 1, 2 and 3, that plants have to be accompanied by a certificate or plant passport to certify the products to be free from *P. ramorum* (Anonymous 2002).

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Pissodes spp

Common names: Pine weevils
Taxa: Insecta: Coleoptera: Curculionidae
EPPO A1 list: No. 44
EU Annex designation: II/A1 – as *Pissodes* spp. (non-european)

Organism

Pissodes spp. (Coleoptera: Curculionidae) are a genus of polyphagous beetle species. For example, *Pissodes castaneus*, which is a major pest in Chile and Uruguay, attacks all species of the genus *Pinus*, as well as some *Abies* and *Pseudotsuga* species (Abgrall et al. 1999). Weevils cause damage by killing the leader (top branch) of a tree, resulting in defects such as crooks and forks that can reduce tree merchantability (Lysak et al. 2006).
In central Maine, *Pissodes strobi* were monitored in a stand of *Pinus strobus*. Overwintering adults emerged in late April, and peak numbers were observed on the trees by mid-May; no adults were found on the trees by early July (Dixon and Houseweart 1983). While, in west-central Alberta (Canada), adults of *Pissodes terminalis* emerged from the litter and began flight in late May, with a peak in mid-June (Langor and Williams 1998). Eggs were deposited from mid-June to late July. These weevils have four larval instars. The first two instars feed only in the phloem. Third and fourth larval instars eventually enter the pith to continue feeding, overwinter, and complete development the following spring. The new generation of adults emerge between mid-July and early August, feed on new shoots for several weeks, and overwintered in the litter. Adults have an obligatory diapause and do not reproduce until winter is completed (Langor and Williams 1998). Williams and Langor (2002) have developed a key of description of mature larvae of the four species of the *Pissodes strobi* complex.
The difference in volatiles of *Pinus koraiensis* before and after damaged by *Pissodes nitidus* may play important roles in host location and feeding behavior of *P. nitidus* (Peng et al. 2009).

1. Detection

Lindgren multiple-funnel traps, baited with (-)-alpha-pinene and (+-)-pityol, captured significant numbers of *Pissodes affinis* and *P. fasciatus*, in a coastal stand of Douglas-fir and western white pine (Miller and Heppner 1999). Pitfall traps baited with live *Pissodes schwarzi* males plus pine sections captured 46 females *P. schwarzi* from June to September 1989, indicating the presence of a male-produced sex pheromone (Maclauchlan et al. 1993). In the same way, *Pissodes nemorensis* also uses a male-produced aggregation pheromone. A field test carried out in Florida showed that the combination of the presumed pheromone components, grandisol (*cis*-1-methyl-2-(1-methylethenyl)cyclobutaneethanol)), its corresponding aldehyde, grandisal, and slash pine (*Pinus elliottii*) bolts acted synergistically to attract large numbers of males and females of *P. nemorensis* (Phillips et al. 1984). Fontaine and Foltz (1982) also shown that traps containing slash pine (*Pinus elliottii* var. *elliottii*) bolts and males captured significantly more weevils than at any other treatment, i.e. slash pine bolts and females, males with females, or no adults.

2. Delimitation

No information available.

3. Monitoring

According to Rieske (2000), *Pissodes nemorensis* was most receptive to fresh pine billets in pit traps compared to ethanol- and turpentine-baited flight traps, and ethanol- and turpentine-baited pitfall traps. However, in using flight traps more weevils were captured at 0.8 m than at 1.6 m. Catches were greatest in traps placed perpendicular to the slope.

4. Commodities

In USA, alien species of *Pissodes* spp. (and also of scolytids) have tended to be intercepted mainly in crating and dunnage (Anonymous 1979).

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Plasmopara halstedii (Farlow) Berlese and de Toni

Common name(s): Downy mildew of sunflower
Taxonomic position: Chromista: Pseugofungi: Peronosporales
EU Annex designation: II/A2

Organism

The oomycete *Plasmopara halstedii* is the causal agent of sunflower downy mildew. Wild and cultivated species of *Helianthus* are significant hosts but many other species from a wide range of genera in the family of Asteraceae were reported as host species too. Present wherever sunflowers are grown, the disease is known on every continent of the world. This pathogen is basically soil-borne but may also be seed and wind-borne. The main symptom is systemic infection of stem, leaves and flower-seed head. *Plasmopara halstedii* oospores may survive as long as 8-10 years in the soil; the disease is extremely difficult to eradicate once it is established (Anonymous 1990).

1. Detection

Morphological identification is possible only in vivo, on the host plant; bioassays are needed for morphological identification from seed and soil samples (Gulya 2004). Seven races of *Plasmopara halstedii* have been recorded and four of these exist in Europe (Gulya et al. 1991). Elisa test is described in the literature (Bouterige et al. 2000) but there is little experience with using this method. PCR diagnostic tests were developed to detect the pathogen in plant tissues (Roeckel-Drevet et al. 1999) and in sunflower seeds (Says-Lesage et al. 2001). Specific primers have been developed for the direct detection of the oomycete in sunflower seed samples (Ioos et al. 2007) and this PCR-based detection test was validated (Ioos and Iancu 2008). EPPO published a detailed diagnostic protocol about this pathogen (Anonymous 2008).

2. Delimitation

No information available.

3. Monitoring

No information available.

4. Commodities

Introduced seed represent the major quarantine risk and should originate from certified disease-free fields and be treated with a proper fungicide (Anonymous 1990). Seed importation must be regulated (Guenin 1990).

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Plum line pattern virus (American)

Common name(s): Plum line pattern, banded chlorosis of oriental flowering cherry
Taxa: Viruses Bromoviridae: Ilarvirus,
EPPO A1 list: No. 28
EU Annex designation: I/A1

Organism
The American plum line pattern virus (APLPV) is an iliarviruses that infects stone fruits, in particular plums and other *Prunus* spp. such as peaches and flowering cherry. Certain ilarviruses are known to be transmitted by pollen, but so far no vector has been demonstrated for APLPV (Anonymous, 1997). The virus is easily transmissible in tree hosts by bark patch grafting, budding and mechanical means, but not by seeds.
It has been transmitted mechanically to numerous species. Furthermore, the purified virus can be transmitted to various Rosaceae and herbaceous species (Anonymous 1997). It is present in North America but absent from Europe although, *Prunus* spp. are potential hosts for the EPPO region.

1. Detection
Stone-fruit trees infected by APLPV generally show striking symptoms which vary seasonally, so visual inspection has practical importance. Disease symptoms on Japanese plum, peach and *P. serrulata* are detailed on EPPO diagnostic protocols (Anonymous 2006). However, similar symptoms on *Prunus* spp. may also be caused by other ilarviruses, e.g. *Apple* mosaic ilarvirus (ApMV) and Prunus necrotic ringspot ilarvirus (PNRSV). Moreover, some host cultivars do not show symptoms, and laboratory tests are required for unequivocal identification of the virus.
Diagnostic protocol for regulated pests: *APLPV* (Anonymous 2006) include a detailed description of the detection and identification methods. The protocols do not include specific information on inspection and sampling (e.g. statistical aspects) but may include specific advice on how to take appropriate samples. The methods used for detection of the virus include grafting on woody indicators, testing on herbaceous indicators, DAS ELISA, molecular hybridization and RT-PCR.
Detection DAS ELISA is a reliable and rapid method however molecular hybridization is more reliable, especially in testing during the non-optimal detection season (Al Rwahnih *et al*. 2004), and therefore it can be considered as a satisfactory alternative for routine diagnosis. Recently, simultaneous detection by one-step RT-PCR of various stone-fruit viruses, including APLPV, was developed by (Sanchez-Navarro et al. 2005).
A decrease in sensitivity was observed when the primer cocktail contained more than five different pair primers. However, comparative analyses showed that the multiplex RT-PCR containing the eight virus pair primers was even more sensitive than the ELISA or molecular hybridization assays. The use of the multiplex RT-PCR technology in routine diagnosis of stone fruit tree viruses is discussed.

2. Delimitation
In Lebanon, DAS-ELISA was used on leaf collected from either symptomatic or symptomless trees during a sanitary survey and leaf samples were tested to detect the presence of American plum line pattern virus (APLPV). The virus was identified serologically in 48% of the total (Choueiri et al. 2006).

3. Monitoring

No information available.

4 Commodities

No information available.

References: 7 retained out of 35 retrieved, 1 added, 4 used for the summary

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Plum pox virus

Common name(s): PPV, sharka, sharka virus
Taxa: Viruses: Potyviridae: Potyvirus
EPPO list A2: No. 96
EU Annex designation: II/A2

Organism

Plum pox disease, PPV, is considered one of the most devastating diseases of stone fruit in terms of agronomic impact and economic importance. The virus attacks apricot, peach and plum trees leading to a reduction in quality, most often in cherries and almond trees. The virus also infects most wild or ornamental *Prunus* species. PPV was described for the first time around 1917 in Bulgaria. Since then, the virus has progressively spread to a large part of the European continent and Middle East. It has been found also in India and in America. Symptoms may appear on leaves, petals, fruits and stones. They are particularly clear on leaves in springtime: mild light green discoloration, chlorotic spots, bands or rings, vein clearing or yellowing, or even leaf deformation. The virus is non-persistently transmitted by a number of aphid species.

1. Detection

Indicators plants graft-inoculated have been used as a diagnostic host for detection of plum pox virus (Seidl and Droblkova 1980), the main indicators plants are *Prunus persica* GF305 and *P. tomentosa* (Rankovic 1980, Gentit 2006). Plum pox virus isolate was identified by immunosorbent electron microscopy (Noel et al. 1978, Kerlan et al. 1981, Himmler et al. 1988). It is important to maintain correctly identified and well-maintained representative strains (Barba et al. 2010). Enzyme-linked immunosorbent assay (ELISA) were developed for PPV identification (Voller et al. 1976, Adams 1978). Another simple and rapid serological method, slot-immunobinding, showed also good efficiency in identifying the virus (Kotuc and Deborre 1994). Immunoprinting-ELISA (direct and indirect) constitues a very simple and sensitive technique that can be used to routinely analyze samples, in addition the technique is very fast (3 h) and the imprinted membranes can be stored for long periods before reacting them with antibodies (Cambra et al. 1994). DASI-ELISA, using the universal monoclonal antibody 5B-IVIA, is presently the most accurate system available for routine PPV detection (Cambra et al. 2006). Monoclonal and polyclonal antibodies have been characterized to improve the sensitivity of serological test (Lopez-Moya et al. 1994, Pasquini et al. 1995, Orban et al. 2007). Further evolution of the diagnostic assay came with a triple antibody sandwich-microsphere immunoassay (TAS-MIA) (Croft et al. 2008). The reliability of detection depended on the relative concentration in different plant tissue and time (Rankovic and Vuksanovic 1985, Polak 1995). For example, in the summer the leaves of the most cultivars will give a negative reaction (Albrechtova 1990) and autumn and winter periods are not suitable for indexing PPV in the bark due to the low values of absorbance (Karesova 1993), however further attempts at solving this issue have been encouraged (Roggero and Lenzi 1985).

Molecular approaches represented a further important tool to PPV identification. A number of different types of polymerase chain reaction have been proposed and different systems of viral target preparation prior to PCR have been developed. To date, the most commonly used assay is the immunocapture IC PCR method (Wetzel et al. 1992, Candresse et al. 1994, Adams et al. 1998, Varveri and Boutsika 1998)and a simple direct tissue blotting PCR assay called print-capture PCR

(PC-PCR) (Olmos et al. 1996). The efficiency of RNA extraction methods was tackled in others studies (Cambra et al. 1998, Pasquini et al. 1999, Ruan et al. 2004, Plesko et al. 2011).
Reverse transcriptase-polymerase chain reaction RT-PCR offer a good levels of sensitivity and specificity, different systems for viral target preparation have been tested and the problems caused by plant materials with high contents of RT-PCR inhibitors were solved (Olmos et al. 2006). A further evolution is the spot real-time RT-PCR , a method for detection of Plum pox virus using conventional ELISA plant crude extracts immobilised on paper without the need of RNA extraction (Capote et al. 2008) and real-time fluorescent RT-PCR (Sherman et al. 2002). Real-time RT-PCR using TaqMan technology showed high sensitivity in the quantitative detection of Plum pox virus (Olmos et al. 2004), good results were obtained by the real-time fluorescent RT-PCR (Wen et al. 2009). (Olmos et al. 2002) proposed co-operational amplification (Co-PCR) a new and highly sensitive method for the amplification of viral RNA targets.
The new direct real-time polymerase chain reaction (drtPCR) procedure was based on crude supernatants collected from peach (*Prunus persica*), the leaves macerated in a buffer that was specially developed for this purpose and named "direct pathogen extract buffer (Kim et al. 2008).
Melt peak analysis is a useful and robust tool for the rapid detection of PCR products and the identification of organisms (Winder et al. 2011) and a novel nucleic acid detection technology based on a primer with a quenched fluorophore and a probe complementary to an internal portion of the amplicon has been developed. The primer probe, named 'Scorpion', fluoresces only upon hybridization with its target (Sialer et al. 2000).
Others molecular techniques are dot hybridization detection of plum pox virus (Varveri et al. 1988, Wetzel et al. 1990), RT-LAMP is a very sensitive, low cost diagnostic tool that should be of value in more accurate determination of the distribution of PPV (Varga and James 2006b), microarray detection provides the greatest capability for parallel yet specific testing, and can be used to detect individual, or combinations of viruses and, using current approaches (Boonham et al. 2007)
The simultaneous detection of different virus could be important the technique used: obtained with a multiplex RT-PCR (mRT-PCR) (Sanchez-Navarro et al. 2005, Jarosova and Kundu 2010, Yardimci and Culal-Kilic 2011).
A greater importance is the individuation of different isolates for this purpose can be used a DAS-ELISA (Asensio et al. 1995), PCR assay combined with RFLP analysis (Hammond et al. 1998), PCR-ELISA (Olmos et al. 1997) real-time RT-PCR (Varga and James 2006a).
There is some studies that compared different technique, evaluating sensitivity and possibility of use (Bittoova et al. 1997, Poggi Pollini et al. 1997, Adams et al. 1998, Faggioli et al. 1998, Mavrodieva and Levy 2004, Cambra et al. 2008, Fiore et al. 2008).
The different technique singly or combined were widely used in the survey of first detection or to signal the not presence of pathogen (for example ELISA test (Llacer et al. 1985, Caglayan and Gazel 1998, Jarrar et al. 2001), ELISA and ISEM and antiserum (Thakur et al. 1994), DAS-ELISA (Choueiri et al. 2001, Marn et al. 2008), DASI-ELISA (Al-Rwahnih et al. 2001), ELISA and molecular techniques (Boulila and Ravelonandro 2004), ELISA and RT-PCR (Navratil et al. 2005), IC-RT-PCR test (Snover-Clift et al. 2007))

2. Delimitation

The delimitation of area with presence of Plum pox virus is important for establish pest-free production sites (Marn et al. 2004). The survey must be accurate it could be necessary carry out more than one inspection in the farm and in neighbouring orchards in the period March-May, before the appearance of vectors, that can spread further the pathogen (Desvignes and Bois 1995).

The survey, moreover, must include all the possible host plants (Myrta et al. 2006). When the origin of infected trees is unknown, the survey has to be carried out in all orchards of growers that had planted these plants (Verhoeven et al. 1998).

3. Monitoring

The survey includes the collection of plant samples having symptoms or symptom-like appearance to a following analysis of material (Arikan et al. 2004), however the infection can be present also in the symptomless samples (Mikec et al. 2008). The survey was specific on plum pox virus (Bunter et al. 2006, Blystad et al. 2007, Alnsour et al. 2010) or more generic on the presence of virus and virus virus-like disease (Rankovic and Dulic-Markovic 1992, Al Rwahnih et al. 2001, Ismaeil et al. 2003). They must evaluate the main stone-fruit trees in wide area (Choueiri et al. 1993, Kolber et al. 1998, Bouani et al. 2004) or in single plant species particularly important in an area (Amenduni et al. 2001). The used techniques were serological: ELISA (Barba et al. 1985, Herrera et al. 1998, Sipahioglu et al. 1999, Buzkan et al. 2005, Matic et al. 2005) DAS-ELISA (Myrta et al. 1998) or most often a combination of different technique were used con indicator plants, IEM test, serological and molecular (Di Terlizzi et al. 1994, Jawhar et al. 1996, Jackeviciene and Staniulis 2003, Bouani et al. 2004, Mandic et al. 2007, Isac et al. 2008, Alnsour et al. 2010). The use of more test is important because the technique can have a margin of error, and in some cases the analysis do not give univocal answers (Mumford et al. 2001, Caglayan et al. 2008).

Monitoring, with the involvement of many countries, is important to evaluate the pathogen status (Dunez 1986, Myrta et al. 1998, Kolber et al. 2001) like so the characterization of Plum pox virus isolates. The isolates identification carried out with biological indexing and serological technique (ELISA, DAS ELISA DASI ELISA) (Kamenova et al. 2002), moreover, the molecular approach is a good tool for the differentiation of isolates RT-PCR followed by a RFLP analysis was used (Navratil et al. 1998, Bianco et al. 2004, Caglayan et al. 2004, Jarausch et al. 2004, Laimer et al. 2005, Briciu et al. 2007, Norkus et al. 2008).

Ornamental and wilt plants, also, must be monitored, because these can be an important source of pathogen not controlled (Elibuyuk 2004, Stobbs et al. 2005, Elibuyuk 2006, Kamenova 2008), a specific monitoring, carried out in Hungary, have evaluated the presence of virus in rivers and lakes (Horvath et al. 1999).

The survey methods were developed to optimize the cost-effectiveness and probability to individuate pathogen presence, ad example in the hierarchical sampling method approximately 25% of the trees were sampled and tested in composites of 4 trees (Hughes et al. 2002).

4. Commodities

Plum pox virus was detected in an illegal consignment of plum budwood and fruit intercepted by Australian Quarantine and Inspection Service inspectors at Sydney International Airport, the virus identification was obtained with ELISA, RT-PCR and electron microscopy (Davis et al. 2002). The primary infection was often due to import of infected plants (Ginanni et al. 1993, Cambra et al. 2004, Lebas et al. 2006, Blystad et al. 2007, Verhoeven et al. 2008). Some tretaments are evalutuated to production of virus free plantlets as cryopreservation (Brison et al. 1997) or on in vitro thermotherapy and subsequent meristem culture led to virus-free plant material, which could be multiplied rapidly by micropropagation techniques (Manganaris et al. 2003, Laimer et al. 2006). The virus is individuated also in accessions of fruit tree germplasm are imported annually into the USA, destinted for USDA and university researchers, commercial interests, national repositories,

and private citizens (Kinard et al. 2001). The possibilities of transmission of plum pox by seed were investigated, but numerous studies seem reject this hypothesis (Eynard et al. 1991, Pasquini et al. 1998, Glasa et al. 1999, Milusheva et al. 2008), whereas the PPV was recorded in the soil (Keldysh et al. 2005). Concerning the coltural pratices, (Sertkaya 2008) suggests that rootstocks produced by cuttings could become main reservoirs for the most important viruses of stone fruits, if the future use of cuttings derived from uncertified mother plants becomes widespread for obtaining genetically uniform rootstocks.

The importance of systems of a regulated certification scheme and the supply of virus-free planting material is indicated in numerous studies (Mumford 2006) and moreover the all imported Prunus material is submitted to biological, serological and molecular analysis for Plum pox virus (PPV) detection (Munoz et al. 2006). In some cases, the analysis of propagation material having CAC [Conformitas Agraria Communitatis] or materials with phytosanitary certificate showed the presence of pathogen (AmbrozicTurk 2006, Ozdemir and Kaya 2008).

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Popillia japonica Newman

Common name(s): Japanese beetle
Taxa: Insecta: Coleoptera: Scarabeidae
EPPO A1 list: No. 40
EU Annex designation: I/A2

Organism

The Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae) is a polyphagous scarab (Allsopp 1996, Hammons et al. 2009). *Popillia japonica* feeds most extensively within trees from mid-morning until late afternoon so long as ambient temperature is >15°C (Kreuger and Potter 2001). It facilitates feeding by the obligate fruit-feeding native Green June Beetle, *Cotinis nitida*, by biting into intact grape berries that *C. nitida*, which has blunt spatulate mandibles, is otherwise unable to exploit (Hammons et al. 2009). Thus, establishment and spread of *P. japonica* throughout fruit-growing regions of the United States is likely to elevate the pest status of *C. nitida* and other pests of ripening fruits in vineyards and orchards.

In addition, P. japonica is a vector of plant viruses. It is able to transmit Southern bean mosaic virus (SBMV) and Bean pod mottle virus (BPMV) (Wickizer and Gergerich 2007).

The majority of adults are not fully mature until mid-August, and oviposition appears to peak at that time. A small portion of the population (ca. 10%) required 2 years to complete its development (Vittum 1986).

1. Detection

The standard Japanese beetle trap manufactured by Trece (Palo Alto, CA) is effective. In addition, the number of beetles captured is higher when trapped beetles are removed daily from traps (Alm et al. 1996). Traps that use a bag for collection of beetles are not as effective because beetles are able to escape through the holes made for drainage (Alm and Dawson 2003).

Traps baited with both 2-phenylethyl propanoate (PEP), a synthetic compound similar to a chemical produced by grapes, and the synthetic female sex attractant (R,Z)-5-(1-decenyl)dihydro-2(3H)-furanone (Japonilure) have also demonstrated their effective (Pierce 1981). The sex pheromone of *P. japonica* is (4R,5Z)-5-tetradecen-4-olide (2) (Zarbin et al. 2004).

2. Delimitation

One study has demonstrated that mass trapping has the ability to reduce an isolated population (Wawrzynski and Ascerno 1998). In this case, four years of mass trapping produced a 97% reduction in *P. japonica* numbers.

3. Monitoring

Adult populations are monitored with pheromone traps. Integrated control involving chemical and microbial insecticides and semiochemicals has been used to suppress populations of this pest (Martins and Simoes 1988).

Trapping systems with attractants for both male and female Japanese beetles are commercially available (Klein and Lacey 1999). In the 1990s, field-collected adult Japanese beetles were treated with conidia of *Metarhizium anisopliae* Sorokin (Lacey et al. 1995). A week after treatment with the fungus, 100% mortality was observed. The delay in mortality and the flight distances observed for fungus-treated beetles indicate good potential for dispersal of *M. anisopliae* via infected adult

Japanese beetles.

4. Commodities

Popillia japonica is transported via imports of fruit and planting material of pear (Chebanov 1977).

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Potato black ringspot virus

Common name(s): PBRSV, Andean calico of potato
Taxa: Viruses: Comoviridae: Nepovirus
EPPO A1 list: No. 246
EU Annex designation: I/A1

Tobacco ringspot nepovirus
EPPO A1 list: No. 228
EU Annex designation: I/A1

Organism
The major hosts for potato black ringspot nepovirus (PBRSV) are potatoes though much of the literature reviewed here details work with other crops, particularly tobacco (as tobacco ringspot nepovirus; TRSV). Several other hosts are known, including species from economically important families such as the Curcubitaceae, Fabaceae and Solanaceae. Whilst the PBRSV appears to be restricted to South America, the probably synonymous organism causing disease in other hosts is wider distributed and is present within the EPPO region. Due to this potential confusion, the hosts relevant to each observation are stated.

1. Detection
Indicator species, such as *Chenopodium quinoa* and *Nicotiana*, are often used for assessing potentially infected material (Yamashita *et al*. 1996, Verhoeven and Roenhorst 2000). A number of ELISA assays have been developed, including ones for TRSV in *Pelargonium* (Newhart *et al*. 1980, Romaine *et al*. 1981, Newhart *et al*. 1982) and various woody/herbaceous hosts (Shiel and Castello 1985). Potato tubers have been successfully analysed for the presence of PBSRV using DAS-ELISA (Schroeder and Weidemann 1990). Direct immune-blotting was demonstrated to be a more sensitive method for TRSV extracted from tobacco and several ornamental species (Powell 1987).

2. Delimitation
No information

3. Monitoring
A wide number of virus-monitoring surveys are described that include the detection of TRSV on a wide range of hosts. These include surveys of grapevine (Uyemoto 1975), clover (Jones and Diachun 1976), gladiolus (Reynolds and Teakle 1976), dogwood (Reddick *et al*. 1979), soybean (Reddick *et al*. 1979) and various other ornamental, grass and crop species (Chiko and Godkin 1984, Kearns and Mossop 1984, Cooper and Edwards 1985, Chiko and Godkin 1986). Good details of sampling methodology for an extensive survey of soybean in Canada, where diagnosis was based on the use of susceptible hosts, is provided by Tu (1986). Sampling of tobacco fields, based on dividing the fields into areas and subsequently collecting material from rows within these areas is described by Mayunga and Kapooria (2003). A methodology used for collecting symptomatic material from glasshouses is also available and reasonably well described (Sanchez-Cuevas and Nameth 2002)

4. Commodities
Little information was identified beyond the sampling of soybean seed in the US and India as part of phytosanitary efforts (Zhu *et al*. 1995, Parakh *et al*. 2005). Methods for analysing imported

tubers have also been described (see above) (Schroeder and Weidemann 1990)

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Potato leafroll virus

Common name(s): PLRV, potato phloem necrosis virus
Taxa: Viruses: Luteoviridae: Poleovirus
EU Annex designation: Not classified

Organism

Potato leafroll virus (PLRV) is one of the most important potato viruses worldwide, where it has caused extensive losses. It can also infects other species of the Solanaceae family including moonflower, *Datura stramonium*, hairy nightshade, *Solanum villosum* and husk tomato, *Physalis floridana*, and some non-solanaceous plants.
It is transmitted by several aphid species, principally the green peach-potato aphid, *Myzus persicae* which is the most efficient. Aphids acquire the virus by feeding on infected plants and will be able to transmit the virus permanently during their lifespan. Spread of the virus between plants can occur from infected plants to nearby uninfected plants by wingless aphid forms and at longer distance by the winged morphs. Aphids can also spread the virus to tubers in storage. Spread of the virus also occurs through the planting of infected tubers that will subsequently give rise to plants affected by the virus.

1. Detection

In potato, first symptoms of infection include the presence of necrotic areas at the margins of the youngest leaves, turning brown and curling inwards toward the center of the leaf. Later, leaf rolling is more obvious and the entire leaf can become necrotic. Plants have stunted growth and produce smaller tuber. Tubers exhibit necrosis of the vascular tissue that may not be apparent at harvest but develops in storage. It causes small brown spots due to the death of specific cell within the tuber tissue.
A range of assays have been described for the rapid and accurate detection of PLRV. Methods include the use of immune electron microscopy (Kojima et al. 1978, Roberts and Harrison 1979, Stanarius and Proll 1986), enzyme-linked immunosorbent assay ELISA (Casper 1977, Gugerli 1979b, a, Tamada and Harrison 1980b, a, Syller 1988) and double antibody sandwich enzyme-linked immunosorbent Assay DAC-ELISA (Marco 1985, Boulton et al. 1988, Heide and Lange 1988). ELISA methods can also be used also to detect PLRV in viruliferous individuals *of M. persicae* (Clarke et al. 1980). More recently, several molecular approaches have been developed including a number PCR techniques, e.g. multi-primer reverse transcription polymerase chain reaction (RT-PCR) for detecting main potato viruses (Shalaby et al. 2002, Peiman and Xie 2006) and fluorescent amplification-based specific hybridization (FLASH-PCR) enable fast and accurate diagnostics of the major potato pathogens (Ryazantsev and Zavriev 2009).
The reliability of ELISA and molecular method was tested on leaves sampled from the field, greenhouse and *in vitro*-grown plants by comparison with a double-antibody sandwich ELISA (DAS-ELISA) procedure. A high correlation between these two methods was observed (Peiman and Xie 2006). Therefore, in spite of newer molecular methods being available, ELISA testing methods still have a place for routine diagnoses of PLRV as described in EPPO standard PM 4/28 (1), and an ELISA assay in a double antibody method was validated for the detection of PLRV antigens in sap (Zahn et al. 2011).
For on-site detection the PLRV an AgriStrip-magnetic assay was developed (Altenbach 2011). This lateral-flow test is based on antigen-antibody reaction combined with magnetic bead technology,

enabling the preceding enrichment of target antigen (PLRV).

2. Delimitation

Little information on delimitation is available beyond a study on the spread of PLVR that was carried out in relation to aphid populations in Scotland (Howell 1974).

3. Monitoring

Surveys for a series of potato viral pathogens have been carried out in various countries. A survey evaluated the prevalence of twelve viruses in 242 samples collected in 16 trips to Tabuk and Hail, northern Saudi Arabia, using ELISAs method to detect viruses associated with potatoes (Al-Shahwan et al. 1997). A survey in Costa Rica was carried out in the northern zone of the Cartago province during the period from February to August 1997 (Hord and Rivera 1998). Terminal leaves were collected from 30 plants in each of 36 fields located at altitudes between 1400 and 3000 m and were analyzed for the presence of potato viruses X, Y, A, M, S and potato leaf roll virus (PLRV) by DAS-ELISA to investigate the prevalence and geographic distribution of viruses.

A European survey was carried out by sending postal questionnaire to 41 institutes in 27 countries to obtain information on the national potato cultivar testing programs in Europe (Wustman and Carnegie 2000). Foliage, tuber and yield characteristics were assessed in all countries but the number of sites and replicates varied considerably between the countries. Susceptibility to the most common diseases was assessed. Many of the assessments were based on records of natural infection although controlled inoculation tests were done more frequently for the more important diseases.

The surveying of tubers as well as the aphid vectors has also conducted in a number of countries. In Hungary, yearly between 1993 and 2000, aphid flight was monitored with yellow pan traps (Basky 2002). Virus infection in seed potato progeny tubers was tested with DAS-ELISA. A simple linear regression analysis showed that the factors examined, i.e. total aphid number, vector number, cumulative vector intensity and age-corrected vector intensity had significant effects on the proportion of PVY and PLRV infected progeny tubers in seed potato fields.

4. Commodities

There are strict quarantine measures in the EPPO region against the introduction of Potato viruses (Anonymous 2004).

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Potato spindle tuber viroid

Common name(s): PSTV, Potato spindle tuber, tomato bunchy top virus,
Taxa: Viroids: Pospiviroidae: Pospiviroid
EPPO A2 list: No. 97
EU Annex designation: I/A1

Organism

Potato spindle tuber viroid (PSTVd) is primarily a disease of potato although other hosts have been reported, including sweet potato (*Ipomoea batatas*), avocado (*Persea americana*) and pepino (*Solanum muricatum*) (Puchta et al. 1990, Querci et al. 1995, EPPO/CABI 1997). The symptoms of the disease vary with host plan. In potatoes foliage appears darkened, spindly with a proliferation of buds, plants eventually becoming stunted and producing small, misshapen (dumbbell-shaped) tubers whilst in tomatoes leaf death and stunting will eventually occur (EPPO/CABI 1997). The disease periodically occurs within the EU as isolated outbreaks, such as those in France (Tassus et al. 2009) and the Netherlands (Verhoeven and Roenhorst 2006). The disease is present in North and South America, parts of Asia and Africa and also some parts of the EPPO region.

1. Detection

There has been significant research into methods to detect this pathogen, especially as mild strains of the viroid often give rise to asymptomatic infections. Early methods used in the 1970s include the use of polyacrylamide gel electrophoresis of viroid nucleic acids and subsequent staining (Morris and Wright 1975, Morris and Smith 1977, Mosch et al. 1978). The electrophoretic subjected to various improvements and its use continues, albeit usually as highly refined methods ("return"-PAGE, "sequential" –PAGE, temperature gradient electrophoresis (TGGE) etc) in conjunction with other methodologies (Musin et al. 2001, Singh 2007). Bioassaying with indicator plants such as tomato as also commonly used at as a diagnostic methods (Schumann et al. 1978) method was more reliable.

Dot-blot hybridization of nucleic acids, following electrophoresis, has been commonly used although early variants of this methodology required radiolabeled probes, typically P^{32} (Salazar et al. 1983, Macquaire et al. 1984, Barker et al. 1985). Later variant replaced the radiolabel with a digoxeginin label that allows the chemoluminescent detection of hybridized nucleic acid for the detection of PSTVd (Ziegler et al. 1991a, b, Welnicki and Hiruki 1993, Welnicki et al. 1994)

More recently, more advanced molecular techniques have been developed and assessed. Mumford et al. (2000) reviewed several methods and indicated that TaqMan detection was 100 x more sensitive than RT-PCR and 100x more sensitive that the older dot-blot hybridization. A variety of molecular methods have been reported upon over the intervening years, mostly based on RT-PCR and TaqMan methodologies (Weidemann and Buchta 1998, Kryczynski et al. 1999), including multiplex and microarray methods (Boonham et al. 2007) (Shamloul et al. 2002).

The above methods have been usefully employed to detect PSTVd as part of surveys aimed at excluding the disease from certain geographic areas. For example Elliott et al. (2001) detected the viroid in glasshouse tomatoes in New Zealand through mechanical transmission to indicator plants and RT-PCR. This discovery prompted detection surveys within further glasshouse crops and field potatoes, revealing the disease at other glasshouses but not outdoors (potato). RT-PCR was also used to confirm the viroid's identify in outbreaks in ornamentals in Greece (Malandraki et al. 2010) and Slovenia (Marn and Plesko 2010). RT-PCR has also been used for detection in cape gooseberry

(*Physalis peruviana)* in Turkey (Verhoeven et al. 2009)

2. Delimitation
No information

3. Monitoring
PSTV has been widely monitored in countries where it occurs. For example, following the introduction of the virus to Australia swift action was taken to contain the disease. Monitoring was instigated in fields around the outbreak site (Cartwright 1984, Mason and Heath 1984). The incidence of PSTV has been monitored in Canada (New Brunswick) whereby seed potato fields were inspected three times during the growing season (Singh and Crowley 1985). Symptoms were visually assessed and verified through infecting indicator plants to show a general decline in the incidence of the disease over the 15 years period of assessments reported upon. Ultimately, continued surveying in New Brunswick showed the eradication of PSTV from that area, a fact that has been put down to the stringent monitoring procedures that had been adhered to (Coates-Milne 1989). Later surveys in Canada whereby randomly collected samples of 400 leaves collected from selected fields (<500 fields) and subjected to a number of diagnostic tests indicated the absence of PSTVd from the country.
n Australia dot-blot hybridization was used to assay potato leaf disks to determine that non out of a total of 18,900 plants assessed were infected with the disease (Schwinghamer and Scott 1986), whilst return-PAGE and planting out similarly demonstrated the absence of PSTVd in the Brazilian main crop potatoes (Avila et al. 1990). An extensive survey throughout the USA (1999-2001) which tested selected crops using dot-blot hybridization determined the seed crop to be free of the disease (Sun et al. 2004)

4. Commodities
Potatoes (seed and ware) and tomato seed constitue routes of dissemniation for PSTVd. For example, Kryczynski (1988) noted the development of tomatoes that, although symtomless, were positive for the disease. Seed potatoes were examined in China with over 50% of the seedlots tested positive for the viroid (Singh et al. 1991). This study also demostrated that the viroid could be detected in seed potatoes stored for up to 21 years. The Canadian Seed Certification Program carries out visual and diagnostic testing of seed potatoes that have been instrumental in eradicating the deisease (Dehaan 1994).

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Potato stolbur phytoplasma

Common name(s): Stolbur, big bud, fruit woodiness, female sterility
Taxa: Bacteria: Tenericutes: Acholeplasmataceae: Phytoplasmas
EPPO list A2: No. 100
EU Annex designation: II/A2

Organism
This phytoplasma causes yellows-type diseases and attacks a range of solanaceous species, including potato and tomato (EPPO/CABI 1997). It is occurs across the EU and Asia although the number of related disease organisms, and the similarities in symptoms, makes the determination of the exact range of this organism difficult.

1. Detection
ELISA and immunofluorescence have been successfully used as a means of detecting potato stolbur (Garnier et al. 1990). In recent years PCR and RFLP analysis of extractions made from plant tissues have been used to diagnose and differentiate phytoplasmas (Paltrinieri 2007). Plant collected recently as part of survey in Serbia similarly used PCR/RFLP to analyse the 16S RNA, as did a similar effort in Romani and Russia (Ember et al. 2011). Stolbur specific amplification is also possible using the Stol11 primers and both of these methods were capable of detecting the disease in asymptomatic plants (Jovic 2011). PCR has also been used to detect potato stolbur in hemipteran species

2. Delimitation
No information

3. Monitoring
A recent paper details the surveying of phytoplasmas associated with Romanian and southern Russian potatoes (Ember et al. 2011). Symptomatic plants were samples on three occasions through the growing period (vegetative, bloom and near harvest stages). Weeds and other crops tomato, pepper, eggplant, beet and maize) in the immediate area were also sampled. The tissue was analysed for phytoplasmas using nested PCR followed by RFLP analysis.

4. Commodities
No information

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Potato virus A

Taxa: Viruses: Potyviridae: Potyvirus
Common name(s): PVA, Common mosaic of potato, mild mosaic of potato, veinal mosaic of potato

Organism
Potato virus A causes mild mosaic symptoms in *Solanum tuberosum* when infected alone, and leaf crinkling and more serious effects when co-infection occurs with potato viruses X and Y (McDonald 1984, Carnegie and McCreath 2010). The disease in vectored by a number of aphid species, including *Myzus persicae* (peach-potato aphid) (Copeland and Mills 1985). The virus is thought to be distributed worldwide (Garg 2005). The disease itself only causes slight yield loss although co-infection with other viruses may have a more serious impact.

1. Detection
A large quantity of research effort has been expended on developing methods for the detection of PVA in host tissues. As a result, over the last 40 years and array of techniques have been described of which the most important developments are described here.
Indexing on susceptible hosts was widely used to determine the presence of this virus e.g. (Gnutova et al. 1990) and frequently uses potato cultivars known to be highly susceptible to the virus (Krachanova et al. 1978). Other plants that have been used to assay for the virus include *Physalis floridana* (husk tomato) that develops distinct lesions when infected with PVA (Singh et al. 1979). Verhoeven and Roenhorst (2003)describe more recent advances in the use of indicator plants for potato-virus detection and a range of suitable plant species. Initial *in vitro* methods for detection of PVA utilized basic serological methods, such as those described by Gnutova and Krylov (1975a, b).
In the 1980 a range of ELISA assays were developed for diagnosis of PVA (Singh and McDonald 1981, Khalil et al. 1984 and employed in surveying efforts for the disease {Deltour, 1987 #215). Whilst ELISA was considered to be a largely sensitive and reliable method for detection of PVA, as discussed by Deltour et al. (1987, Schiessendoppler 1989), several refinements to the concept have been developed over the years. Such improvements include a dot-ELISA described by Schiessendoppler (1990), “quick” ELISA (Filigarova et al. 1991) and an enzyme amplified method (Singh and Somerville 1992). However, a direct tissue blotting serological assay was found to be faster and more sensitive than the ELISA methods that had been developed up to the early 1990s (Samson et al. 1993) as well as having a cost benefit to the user. Although refinements to ELISA methodologies have continued up to the present, particularly with respect to the use of monoclonal antibodies (Browning et al. 1995, Subr et al. 1996), in recent times there has been a shift of emphasis towards developing molecular assays for this virus.
A number of PCR-type assays have been developed for PVA detection. These include the RT-PCR methodologies described by Singh and Singh (Singh and Singh 1998) and Nie and Singh (Nie and Singh 2001). Importantly, the use of molecular techniques facilitates the detection of multiple potato viruses simultaneously (Singh and Nie 2003). As with the ELISA techniques, the RT-PCR approach has received significant attention, with numerous improvements introduced over the years {Wang, 2005 #154}(Du et al. 2006, Fox et al. 2008). Other methodologies available include TaqMan and macroarray approaches that can both differentiate a range of viruses (Agindotan et al. 2007, Agindotan and Perry 2008) whilst at the same time providing improved cost effectiveness.

2. Delimitation
No information available

3. Monitoring
The monitoring of potato disease receives considerable attention and, due to its ubiquitous nature, PVA has been widely detected in general surveys of potato diseases. Many of the surveys described, such as those described from Finland, Croatia, USA, Taiwan and New Zealand (Kurppa 1983, Kajic 1991, Petrunak et al. 1991, Deng et al. 1992, Fletcher et al. 1996) used ELISA techniques from detection. Tissue sampling is described by Fletcher et al. (1996) whereby 130 leaves were selected from each potato field, taken from the mid-upper part of the plant, through following a "W" pattern across the sampling area. Leaves were subsequently stored at 6°C and submitted to analysis within one week. The results indicated low levels of PVA in many of the fields sampled. A similar detection exercise conducted in Saudi Arabia and the former Yugoslavia, however, used much less extensive sampling to indicate the comparative rarity of PVA when compared with other potato viruses (Al-Shahwan et al. 1997, Gavran 1997).
Sampling is also described for a monitoring survey conducted in Costa Rica (Hord and Rivera 1998). Here, terminal leaves from 30plants from each field were sampled, regardless of size or altitude, and submitted to DAS-ELISA with PVA present in 52% of the sites sampled. Other described surveys that provide details of sampling and diagnostic processes include examples from Jordan, Lebanon and Pakistan, amongst others (Mansour 1999, Abou-Jawdah et al. 2001, Bhutta 2008).
In addition to the monitoring of the disease via tissue collection/diagnostic test, in a number of instances the aphid vectors of PVA have been monitored. Here, the aim has typically been to equate aphid activity with the likelihood of virus outbreak. In an example from Hungary this supposition was borne-out through collecting aphids in yellow water traps with a good relationship between higher aphid levels and virus incidence being apparent over an eight year period (Basky 2003). This close relationship has led to efficient methods for monitoring potato virus vectors to be investigated (Lasue and Pinchon 2009).

4. Commodities
Potato virus A can be readily detected in tubers and, therefore, testing seed and ware potato for presence of the disease is a useful way of inhibiting the spread/occurrence of the disease. For example, the post-harvest indexing of tubers has been conducted using indicator plants (*P. floridana*) infected with tissue extracts (Singh 1982). Here, both intact and detached leaves gave identical results, with characteristic lesions (pictured) appearing within one week. In a similar exercise, conducted on both imported and domestic potatoes, the widespread infection of seed potato tubers with a range of viruses, including PVA, was ascertained in the Former Yugoslavia (Rankovic et al. 1986). A long-term monitoring of seed-potato in Brazil has been undertaken for a range of viruses. Here, a scheme provides a testing service for farmers that allows decisions to be made as to the fate of individual lots whilst at the same time generating large quantities of data regarding virus occurrence (Souza-Dias and Betti 2003). The importance of using tests that can discriminate between the various potato pathogens when testing material for certification schemes has been highlighted (Jones 1987).
The common infection of tubers has led to methods being developed for the generation of virus-free material. One example described employed the use of meristem tip culture coupled with chemical treatments to achieve 100% virus free material in Argentina (Munoz et al. 1990).

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Potato virus M

Common name(s): PVM, potato M carlavirus, potato paracrinckle virus
Taxa: Viruses: Flexiviridae: Carlavirus

Organism
Potato virus M is an aphid-vectored pathogen with a world-wide distribution (Garg 2005). The virus causes mottling, crinkling and rolling symptoms in potato leaves with the severity of symptoms being somewhat dependent on the cultivar of potato and, in some cultivars, plants may remain asymptomatic (Chrzanowska and Kowalska 1978). Tomato can also become infected, although the severity of infection was seen to be highly dependent on variety (Agur 1987).

1. Detection
Significant research effort has been expended on developing sensitive, selective and cheap methods for the detection of PVM. In common with many plant viruses, detection before the advent of biochemical and molecular methods was predominantly undertaken using indicator plants. A number of indicator species have been evaluated of which *Phaseolus vulgaris* (bean) has proven to be very effective in generating lesions within 3-5 days (Dziewonska and Ostrowska 1973, Hiruki 1973, Kowalska and Skrzeczkowska 1976, Dedic 1978). Other plants that give rise to lesions following infection include *Lycopersicon* and *Chenopodium* although symptoms tend to take longer to develop than on bean (Kowalska and Was 1976, Krachanova and Ivanova 1978).
A number of relatively simple and effective serological tests were described for PVM detection during the 1970s and early 1980s (La 1974, Mierzwa and Skorko 1974, Sawicki and Zebrowski 1978, Polak 1980). Various improved serological assays have been developed over the years, such as the latex test described by Wiedemann (1983) and Sawicki (1983).
However, during the 1980s more advanced ELISA techniques were developed, including those described by Bokx et al. (1980) and the improved methods of Richter and Kalinina (1983) and Reichenbacher et al. (1984). The reliability of ELISA as a technique for PVM detection was ascertained as being good by Schenk et al. (1986) whilst a later review critically assessed the technique as a tool for potato virus diagnosis (Schiessendoppler 1989). A number commercial kits based on ELISA subsequently became available that showed good specificity and levels of sensitivity (Mierzwa and Skorko 1996)
Double antibody sandwich (DAS) – ELISA has also proven to be an effective and rapid method for PVM detection that is capable of diagnosing the virus in very crudely prepared tissue samples (Kaniewski and Thomas 1988). This procedure was used to successfully detect the presence of PVM in the ornamental *Solanum jasminoides* in the Netherlands. (Verhoeven et al. 2006)
Dot immunoblotting onto plain paper and nitrocellulose membranes has been demonstrated to be a simple and effective way of detecting PVM and other potato viruses although its sensitivity was seen to be considerably less than DAS-ELISA (Heide and Lange 1988). Improved sensitivity of dot-blotting was achieved by Nikolaeva et al. (Nikolaeva et al. 1990) that produced an assay capable of detecting ca. 0.2 pg of purified virus. Samson et al. (1993), however, produced evidence that a simple tissue blotting serological tests could outperform the ELISA tests available at that time.
Various molecular techniques for PVM detection have emerged since the early 1990s in order to address some of the perceived drawbacks of ELISA-based techniques. These include an array of PCR and RT-PCR based assays (Maroon and Zavriev 2002, Gawande et al. 2008, Nie et al. 2008, Treder et al. 2009, Xu et al. 2010). These assays have proven to be even more sensitive than their

antecedents enabling the detection of viral material in very dilute samples.
In addition to the above mentioned techniques, macroarray technology has also been used effectively and affords the ability to differentiate between an array of plant viruses (Agindotan and Perry 2007, Agindotan and Perry 2008).

2. Delimitation
No information available

3. Monitoring
A number of monitoring surveys for potato disease have been reported upon over the years. These include examples from Sweden, Finland, Russia, Poland and Denmark, amongst others that reported varying degrees of infection with PVM across the respective countries (Lihnell et al. 1975) (Pietrak and Chrzanowska 1975, Anonymous 1976, Gorokhova 1976, Kurppa 1983).
The incidence of PVM in Pennsylvania was ascertained from 282 and 120 samples submitted to DAS-ELISA over two consecutive years to ascertain the extent of a range of potato viruses (Petrunak et al. 1988). Similarly, in New Zealand Fletcher et al. (1996) describe their sampling methodology whereby 130 leaves were selected from each potato field, taken from the mid-upper part of the plant, through following a "W" pattern across the sampling area. Leaves were subsequently stored at 6°C and submitted to analysis within one week. ELISA was used in a Saudi Arabian survey to detect PVM in tissue sampled from the fields of the major potato-growing areas of the country (Al-Shahwan et al. 1997). Here, symptomatic plants were actively sampled as the predominant aim of this survey was to determine the number of viruses present and not the relative infection rates.
In the former Yugoslavia, simple random selections of 100 leaf samples from 12 localities were used to determine the extent of PVM (Gavran 1997). Diagnosis was undertaken through the use of indicator plants and the presence of PVM was not unequivocally proven for any locality samples. Other monitoring exercises detailed in the literature include ones from Costa Rica, Mexico, Pakistan, USA ,France and Poland (1998, Ali et al. 2002, Lozoya-Saldana et al. 2002, Baldauf et al. 2004, Kerlan 2005, Mosahebi et al. 2005). Of these surveys, perhaps one of the most extensive was the monitoring completed in Iran where over 8000 leaves from 132 fields across the country were submitted to analysis to give a value 9.0% of locations affected by the virus.

4. Commodities
Potato virus M can be readily detected in tubers immediately after harvest and after periods of time in storage (Hord and Rivera 1998), therefore, testing seed and ware potato for presence of the disease is a useful way of inhibiting the spread/occurrence of the disease. The disease can readily be detected from leaflets taken from tissue culture leaflets by ELISA as a means of ensuring virus-free propagating material (Singh et al. 1996). Seed potato producing areas in the Lebanon were surveyed as part of a seed-certification programme to indicate that over half the samples taken were positive for one or more virus (Abou-Jawdah et al. 2001). In this instance, tuber sampling was undertaken from both symptomatic and asymptomatic plants taken from 69 locations and diagnosis achieved with DAS-ELISA. The finding were compared with imported seed potatoes and shown to be higher than those from other regulated sources.
In order to circumvent the problems associated with producing virus-free seed potatoes, a rapid propagation methodology has been developed to take virus-free single node cutting and grow them on in aphid-proof greenhouses. Following a second generation the plants are subsequently planted in non-potato areas, well removed from any sources of infection (Loebenstein 2007).

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Potato virus S

Common name(s): PVS, potato S carlavirus
Taxa: Viruses: Flexiviridae: Carlavirus

Organism

Potato virus S is an latent virus of potato that can result in yield losses of up to 20% (Wardrop et al. 1989). The virus occurs as two main strains that are mechanically transmissible (Halterman 2012) although transmission is suspected to occur via aphids also (Wardrop et al. 1989). The virus has a restricted host range but occurs world-wide in cultivated *Solanum tuberosum* (Franc and Banttari 1996). Infected plants are often symptomless (Halterman 2012), a fact that has probably assisted its global spread in infected seed potatoes. Where symptoms do appear, it is manifested by chlorosis and roughness of the leaf surface. The disease primarily attacks cultivated potato but a number of wild solanaceous relatives may also become infected (Valkonen et al. 1992).

1. Detection

There is a significant quantity of literature regarding the identification of PVS in infected material. As with other related viruses, the techniques range from traditional biological indexing through to serological, immunological and molecular techniques, amongst others. Due to the weight of literature in this area, only the salient techniques are discussed.

Prior to the advent of more complex techniques, the use of indicator plants was a common means of establishing the presence of PVM in potato. Commonly used plants for such tests include *Chenopodium album* and *Solanum demissum* (Gooth and Webb 1972, Chrzanowska and Was 1974) that both generate characteristic symptoms when mechanically infected. Other plants used for indexing include *Nicotiana debneyi* that has been used to detect PVS in tuber juice (MacKinnon 1974) and *Chenopdiun quinoa* in the leaves(Kaczmarek and Bokx 1977)

Serological tests were commonly used from the 1970 onwards (Gorokhova 1976, Staszewicz 1977, 1979, Konstantinov 1982). Variants, such as latex agglutination and precipitation assays have also been described as being useful methods for the detection of PVA (Polak 1980, Talley et al. 1980, Polak et al. 1983, Fribourg and Nakashima 1984). Another serological method described that provides a simple diagnostic tool is immune-diffusion on agar gels (Richter and Kalinina 1983, Kaniewski and Thomas 1988, Khalil et al. 1988)

During the late 1970 ELISA tests became available that have been progressively improved over the years in terms of sensitivity, specificity and speed (Richter et al. 1977, Banttari and Frane 1982). Such procedures enabled low concentrations of purified virus to be detected (Khalil and Shalla 1982) and the development of commercial kits (Allen and McMorran 1983, Mierzwa and Skorko 1996). Franc and Banttari (1986) evaluated some of the various techniques available at that time and found ELISA to be as sensitive as serological and indexing methods (Braun and Opgenorth 1987) whilst in another evaluation serologically specific electron microscopy was seen to 10x more sensitive. A wide range of other workers report on the use of ELISA in the routine testing for PVS (Dedic 1988) as well as DAS-ELISA, dot ELISA, and enzyme-amplified ELISA variants (Perez de San Roman et al. 1988, Schiessendoppler 1990, Singh and Somerville 1992). The application of ELISA in the surveying for PVS is dealt with below.

Indication have been made that the best stage for sampling plant tissue for submission to ELISAis before flowering and selecting leaves from the middle of the plant (Khalil and Shalla 1982).

Other techniques that have been evaluated over the years include immune-fluorescence

(Weidemann 1981) and virobacterial agglutination(Chirkov et al. 1982). A time-resolved fluoro-immunoassay (TRFIA) with europium-labelled monoclonal antibodies has also been used to assay for a range of potato viruses (Sinijarv et al. 1988)
Molecular techniques that have been developed to identify PVS include nucleic acid spot hybridization (NASH) (Foster and Mills 1990, Nikolaeva et al. 1990). RT-PCR was demonstrated to improve sensitivity >1000 fold over ELISA (Mierzwa et al. 1975) and, as a result, much of the more recent work into detecting PVS has shifted to developing various variants of this procedure (Ptacek et al. 1999, Nie and Singh 2001, Maroon and Zavriev 2002, Singh and Dilworth 2004, Singh et al. 2004). Several multiplex RT-PCR methods, using primer pairs for a range of potato viruses, have provided the ability to simultaneously detect several viruses (Wang et al. 2005, Du et al. 2006, Peiman and Xie 2006, Witek, 2006). The development of DNA microarray techniques has opened up the potential to assay for a number of viruses simultaneously (Boonham et al. 2003, Bystricka et al. 2003){

2. Delimitation
No information available

3. Monitoring
A number of monitoring efforts for PVS are documented. For example, a survey in Queensland found it to be the most prevalent potato disease in that Australian state {Holmes, 1980 #300}. A survey undertaken across Finland used ELISA to detect potato virus from leaves and tubers (Kurppa 1983). A survey across a region of Chile collected apical leaflets from a varied number of plants per locality (1-4 per field) and diagnosed using serological methods to show a PVS infection rate of 38% (Saldias and Apablaza 1984). Similarly, in Colombia, a survey across a number of potato-growing regions indicated that PVS was the most commonly found viral pathogen.
The spread and prevalence of potato viruses in Serbia was monitored through the analysis plant and tuber samples by ELISA to provide a detailed picture of infection rates with respect to variety grown (Milosevic 1989). In Mexico a regional survey collected symptomatic and symptomless plant material and submitted these to ELISA to demonstrate fairly low levels of PVS incidence and higher levels of PVX and PVY. In the USA, a study in Pennsylvania collected leaf samples from various countries (Petrunak et al. 1991). In one year 280 samples were collected through walking diagonal transects and tissue was collected regardless of symptoms and subsequently analysed by DAS-ELISA to give a 38% infection rate for PVS. In Taiwan, monitoring across several regions showed no differences in viral prevalence, with PVS generally detected at low levels using ELISA (Deng et al. 1992). By contrast, in Sudan, regional differences were found to be pronounced with PVS infection rates ranging from 4.2-83.1% (Omer and El-Hassan 1992).
Tissue sampling is described by Fletcher et al. (Fletcher et al. 1996) whereby 130 leaves were selected from each potato field, taken from the mid-upper part of the plant, through following a “W” pattern across the sampling area. Leaves were subsequently stored at 6°C and submitted to analysis within one week. In Saudi Arabia a four year study examined the prevalence of potato viruses in the important growing regions to the north of the country (Al-Shahwan et al. 1997). Samples were collected just before harvest and analysed by agar diffusion tests and ELISA and demonstrated the year on year variation in the incidence of PVS and other potato viruses.
In a survey of the Former Yugoslavia, 100 leaf samples were taken from across 9 localities and bioassayed with indicator plants and submitted to ELISA (Gavran 1997). The results indicated the very low levels of incidence of PVS. Levels in a survey of Fuijan Province in China, however, indicated that the disease was almost ubiquitous (Wu et al. 2005). The high levels of incidence of

PVS and PVY in Syria, as determined in a survey, led to the development of a duplex RT-PCR to simultaneously detect these diseases.

4. Commodities

There have been significant efforts to detect and manage PVS at the commodity stage (i.e. in the traded tubers and seed etc). For example, clones within the Chilean potato collections were examined using serological methods to demonstrate a 53% infection rate for PVS (Contreras M and Banse H 1982). Seed potatoes were tested in New Zealand to indicate that virus-free tuber of various cultivars all became infected with at least one virus within 16 weeks of planting (Fletcher 1984).

Latex agglutination assays and ELISA were used to detect viruses in tissue culture plantlets to facilitate the generation of virus-free stocks (Gallenberg and Jones 1985). Imported and locally sourced potatoes were analysed by ELISA to show the widespread presence of PVS in the elite cultivars kept in collection in the Former Yugoslavia (Rankovic et al. 1986). In the Czech Republic a survey of imported tubers of 209 genotypes was conducted to reveal between 4 and 12% infection, depending on year (Dedic et al. 1996). In Australia, seed potatoes sourced from various locales were analysed by ELISA to indicate relatively low and declining levels of the disease (Jafarpour et al. 1988). In this survey, all tubers (10-50 per location) were planted after dormancy was broken and, after about five weeks, sprouts and leaves were sampled.

In Brazil, the presence of viruses in seed-potatoes was monitored over a 12 year period, with the numbers of tubers tested rising to around 20,000 in some years (Souza-Dias and Betti 2003). The prevalence of PVS and other viruses was confirmed by commercial ELISA-based kits using sap squeezed from the tubers.

A second import issue with respect to controlling PVS is the generation of PVS-free material for propagation. A number of studies have detailed methods that can be used to eliminate the virus, such as chemotherapies (El-Amin et al. 1994, Kim et al. 1996), the screening of propagation material (plantlets/tubers etc) (Njukeng et al. 2007) or heat treatment (Brown et al. 1988)

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Potato virus T

Common name(s): PVT
Taxa: Viruses: Flexiviridae: Trichovirus
EPPO A1 list: No. 247
EU Annex designation: I/A1

Organism

Potato virus T was first described in 1979 from potatoes collected in South America (Salazar and Harrison 1977). The virus infects a range of tuber-bearing *Solanum* species, mostly symptomlessly and is transmitted via the tuber. The virus can infect a number of other plant species when they are inoculated mechanically, including *Chenopodium* and *Phaseolus* species (Salazar and Harrison 1978) whilst, more recently a number of other natural hosts have been identified in South America (Lizarraga et al. 2000). The disease is largely restricted to Bolivia and Peru although evidence of the disease from other regions within the Americas is available (Vasquez et al. 2006).

1. Detection

Chenopdium species (particularly *C. amaranticolor*) serve as good indicator plants for PVT (Salazar and Harrison 1978). The disease can also be detected by ELISA (Schroeder and Weidemann 1990, Vernon-Shirley et al. 1993) but no information on molecular diagnostic tests were available in the literature

2. Delimitation

No information available.

3. Monitoring

A monitoring effort in Costa Rica describes the collection of plants across 30 different locations and subjecting the tissue to ELISA (Vasquez et al. 2006). PVT was found in 39% of samples, providing the first evidence of the disease in Costa Rica

4. Commodities

No information available.

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Potato virus V

Common name(s): PVV
Taxa: Viruses: Potyviridae: Potyvirus

Organism
PVV is a virus mostly restricted to South America and Western Europe. Compared to some of the other potyviruses it has received relatively little attention, possibly as a result of its often mild symptoms although in some cases leaf and stem lesions can be observed (Vetten et al. 1992). *Solanum tuberosum* is its only natural host. The disease is vectored by aphid species such as *Myzus persicae* (Bell 1988).

1. Detection
As with other potyviruses, the predominant methods used for detection of the virus have been based around ELISA procedures. For example, a range of mono- and polyclona antibodies were tested against a range of Andean viral isolates to find that PVV reacted only weakly with them (Fernandes-Northcote and Gugerli 1987). It was, therefore, suggested that specific antibodies would be necessary to detect PVV reliably (Jones 1987) and such procedures have been applied to differentiate PVV from other viruses (Richter et al. 1994)
A range of *Nicotiana* and *Chenopodium* species have been assessed as test plants that can be mechanically inoculated with PVV and other viruses (Verhoeven and Roenhorst 2003).More recently, PVV has been detected by RT-PCR and multiplex RT-PCR (Fox et al. 2008, Liu et al. 2009)

2. Delimitation
No information available

3. Monitoring
Only a few articles report upon the monitoring of PVV. Of those, the best described in an example from Costa Rica where 30 fields were sampled with tissue 20 samples taken at each (Vasquez et al. 2006). The samples were submitted to ELISA to reveal an incidence of PVV of 37%. In another survey, this time in Eritrea, leaf samples were also taken and submitted to DAS-ELISA although the presence of PVV was not confirmed (Biniam and Tadesse 2008). Additional details of monitoring are also provided by several other papers (Jones and Fuller 1984, Carnegie and McCreath 2010)

4. Commodities
In common with many potato viruses, the commodity stage of the production process has received some attention. For example, in Germany 5 tubers were selected from 139 cultivars and the tissue subjected to ELISA and test plants inoculation (Weidemann 1989). In Argentina, a wide-ranging potato collecting exercise was undertaken to catalogue diseases in cultivated and wild potato species (Clausen et al. 2005). The tubers were tested following the breaking of dormancy to reveal the presence of PVV and other viruses in the material

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Potato virus X

Common name(s): PVX, potato latent virus, potato mild mosaic, potato mottle, tomato streak
Taxa: Viruses: Flexiviridae: Potexvirus

Organism
Potato virus X is a ubiquitous disease that occurs wherever potatoes are grown with many strains symptomless or only resulting in a mild mosaic condition. The disease is mechanically vectored and will infect a range of solanaceous hosts, and other crops such as artichoke (Gallitelli et al. 2004, Rana et al. 2005).

1. Detection
The development of fast, accurate and cost effective methods for the detection of PVX has receives a large quantity of research attention, with much duplication of effort. Here we detail the more significant papers concerning the evolution of testing procedures over recent decades. Earlier tests included the use of radial diffusion (Salazar M 1971, Fuchs and Richter 1975), polyacrylamide electrophoresis (Czupryn and Blaszczyk 1974) (Paul 1974) (El-Dougdoug 1996) and microprecipitin tests (La 1974). Haemaggluttination, latex agglutination, virobacterial agglutination and flocculation assays have also proved effective, albeit at quite high viral concentrations (Gnutova and Krylov 1975, Yokoyama 1975) (Chirkov et al. 1982). Of these earlier tests, comparisons seemed to indicate that the microprecipitin assay was the most sensitive (Shanta and Beczner 1978) (Wiedemann et al. 1983). Commercial kits based on some of the procedures listed here were subsequently developed (Talley et al. 1980). Many of the above techniques continued to be used and refined, even after the advent of ELISA and molecular methods (Fribourg and Nakashima 1984) (Araujo and Carvalho 1986).
Immunofluorescence (Weidemann 1981)and immunoelectronmicroscopic (Schenk 1981) methods are also described in the literature.
Tobacco leaves can be used as testing material with necrotic lesions appearing in inoculated tissue after immersion in hot water (50°C) for 40 seconds two days after inoculation .(Foster and Ross 1975). *Gomphrena globosa* has also been widely used as a test plant (Scholz 1982). Interestingly, *G. globosa* has been shown to be extremely sensitive, the extent that it can detect PVX at levels significantly lowers than some of the pre-ELISA testing methods (Franc and Banttari 1986). *Nicotiana occidentalis* has also been shown to be an effective test plant for PVX (Kryszczuk and Chrzanowska 2000) and other tests plants continue to be detailed (Esfandiari et al. 2006)
During the 1980s several ELISA type assays were developed that increased the sensitivity of detection of PVY. Many variants have been tested as the procedure has been continually refined (Bokx et al. 1980). The assay was rapidly demonstrated to diagnose the disease in all parts of the potato, including dormant tubers (Banttari and Frane 1982). The sensitivity of ELISA was demonstrated by Moran et al. (Moran et al. 1983) who examined 57,000 plants to identify a very low level of infection (0.13%). Various improvements in the basic ELISA methodology have been made to both increase the sensitivity of the assay and also reduce costs associated with it (Goodwin and Banttari 1984, Reichenbacher et al. 1984, Banttari and Goodwin 1985). With a view to field testing, an ultramicro-ELISA was developed that enabled visual reading of the colour change to be made without the need for a plate reader (Reichenbacher et al. 1985), a method that was adapted for countrywide use in Cuba (Rivas et al. 1989). The ultramicro-ELISA system has also been adapted for the simultaneous diagnosis of PVX and one or more other viruses (Gonzalez and

Peralta 1993) (Mierzwa et al. 1993).
Even greater sensitivity was achieved using serologically active electron microscopy, which was suggested as a means of confirming questionable ELISA results (Braun and Opgenorth 1987). Several DAS-ELISA techniques have also been developed and tested (Kaniewski and Thomas 1988), including ones that allow the simultaneous detection of viruses using polyvalent antibodies (Perez de San Roman et al. 1988, Bostan and Peker 2009) and has been used as the diagnostic tool in detection surveys in the USA (Murphy et al. 2000). The ELISA technique has also been modified to utilize sap extracts as opposed to ground material (Lizarraga and Fernandez-Northcote 1989). A combination of DAS- and Amplified ELISA was developed to reduce the time and expense of the use of the separate assays (Darda 1998) and to produce a more sensitive assay.
The DAS-ELISA concept was used to develop reagent strips for field use for PVY detection (Reichenbaecher et al. 1990). Similarly, a test detailed by Danks and Barker (2000) adapted ELISA techniques to produce a field test lateral flow kit that could give diagnosis within three minutes a high level of accuracy
Simultaneous detection of a range of viruses has been achieved through the use of IgG indicator paper (Shi and Banttari 1988).Dot immunobinding further increased levels of sensitivity of assays for PVY detection to a level of around 2 pg of virus. (Parent et al. 1985) whilst direct immunoblotting was shown to be a rapid and inexpensive alternative to ELISA (Ju et al. 1993, Samson et al. 1993)
The application of molecular techniques to the diagnosis of PVY began with use of DNA hybridization methods (Baulcombe et al. 1984, Baulcombe and Fernandez-Northcote 1988) that were capable of detecting PVX down to 1pg (Karjalainen et al. 1987). This method was subsequently improved to increase sensitivity significantly (Nikolaeva et al. 1990). Multiplex nucleic acid hybridization methods have also been described (Janczur et al. 2006). Solution hybridization using oligonucleotide probes provided a highly novel method for sap samples that was suggested could for the basis for a field diagnostic kit (Rouhiainen et al. 1991).
After the turn of the century, the development of diagnostic tests for PVY became dominated by PCR based method that proved superior to ELISA/DAS-ELISA in terms of sensitivity (Du et al. 2006). For example, the simultaneous detection of five viruses of potato was enabled through the development of the assay described by Nie and Singh (Nie and Singh 2001) and the others that followed (Shalaby et al. 2002). A method has been developed for the detection of viruses of the Potexvirus using primers designed around conserved sequence motifs (van der Vlugt and Berendsen 2002). PCR / RT-PCR has been optimized in several ways over the years so that it can detect PVY in crude extracts (Agindotan et al. 2003) and simultaneously detect several viruses simultaneously (Singh and Nie 2003, Verma et al. 2003, Wang et al. 2005) (Peiman and Xie 2006, Maoka et al. 2010). A single tube real-time multiplex high throughput assay using fluorescent TaqMan probes has been developed (Mortimer-Jones et al. 2009)
More recently still, further developments have seen the emergence of microarray (Bystricka et al. 2003) (Sip et al. 2010) and macroarray detection methods (Agindotan and Perry 2007, Agindotan and Perry 2008) capable of screening for several viruses at once,

2. Delimitation
No information available

3. Monitoring
Monitoring for PVY has been routinely undertaken throughout potato growing areas of the world.

For example, a survey in Venezuela for both field and glasshouse potatoes showed the widespread occurrence of PVY when samples were used to inoculate the test plant *G. globosa* (Debrot et al. 1980). In Canada, sampled 582 plants showing mosaic-like symptoms, of which 67% were diagnosed as positive for PVX using ELISA (McDonald 1984). In Chile, a survey visited 20 different localities where diseased plants were selected and leaflets taken. Three-30 samples were taken at each location and returned to the lab for diagnosis via serological tests and inoculation onto *N. tabacum* indicator plants to determine the presence of PVX in 24% of the samples (Saldias and Apablaza 1984). Tomato plants in Crete were frequently surveyed during the 1980s with plants showing disease symptoms sampled for sap inoculation tests to indicate that PVX was the second most prevalent virus in glasshouses sampled (Avgelis 1986).
Potato sampling for leaf tissue is detailed by Petrunak et al (1991). Here, leaf tissue was collected from different counties in Pennsylvania (USA) through walking diagonal transects and collecting tissue randomly regardless of symptoms. Viruses were detected through DAS-ELISA.
In a well described survey in New Zealand, sampling of well established potato plants through walking a "W" through the field to randomly collect 130 leaves from the mid-upper part of the plants (Fletcher et al. 1996). The leaves were pooled 10 leaf samples and assayed using DAS-ELISA to show relatively low incidences of PVY.
In a less extensive series of surveys of weeds, *Capsicum* and tomatoes, PVX was detected by ELISA in a number of crop and weed species (Nava et al. 1996, Nava et al. 1997) in Venezuela whereas in a study in Saudi Arabia involving the collection of 242 samples ELISA demonstrated the presence of the virus within the main growing areas (Al-Shahwan et al. 1997). A second survey in Saudi Arabia collected around 250 diseased plant samples to again confirm PVX presence (Al-Shahwan et al. 1998). Similarly, a relatively broad-ranging survey in the Cameroon demonstrated the presence of PVX in a number of growing areas with diagnosis again achieved using ELISA (Fondong 1997).
A country wide survey of the former Yugoslavia 100 plants were sampled for leaves from each of several localities in two consecutive years. A range of indicator plants, including *G. globosa,* were used to confirm infection with further confirmation using ELISA. Most regions were free of PVX although one had an incidence of 23% infection (Gavran 1997).
A potato survey conducted across seven states in Brazil over two years collected over 1200 samples from symptomatic plants and 360 random samples and submitted them to DAS-ELISA. Whilst virus was present in well over half the samples, no PVX was detected.
In Tunisia, a survey of tomato involved sampling within three regions once or twice a year over three years (Ben Moussa et al. 2000). Symptomatic leaves were collected, stored in Petri dishes containing $CaCl_2$ to give a total of 257 samples for analysis by ELISA. No PVX was detected in any location or year. Similarly, a broad-ranging examination of a virus survey of *Lycopersicon* species in Ecuador did not detect PVX in any samples taken (Soler et al. 2005).
Other surveys that have been detailed include: tomatoes in Chile (Castro and Sandoval B 1998), Turkey (Kiran and Ertunc 1998), and Venezuela (Nava 1999), Algeria (Nechadi et al. 2002); pepper in Serbia (Mijatovic et al. 1999, Petrovic et al. 2010); potato in Pakistan (Ali et al. 2002, Muhammad et al. 2007), India (Garg et al. 2003, Santanu and Amitava 2003) and Germany (Zahn 2004); and curcubits in Turkey (Ozaslan et al. 2006).

4. Commodities

In common with many potato diseases, particular attention has been paid to controlling its dissemination through seed and ware tubers. Therefore, a number of surveys have examined the prevalence of PVY in tubers for planting or movement to elsewhere (Darozhkin and Grabenshchykava 1974, Wright et al. 1977). An example of survey is provides by an exercise in

Maine (USA) where tubers were examined from 10 lots and examined both ELISA and test plants to indicate the absence of PVX but the widespread infection of other viruses. A similar survey of tubers in the former Yugoslavia indicated levels of PVX of up to 33% using a commercially available serological kit (Rankovic et al. 1986). A fairly extensive survey of seed tubers from three regions in India examined over 7000 samples by ELISA to give an infection rate of between 4 and 20%.

The elimination of the virus from propagation material has been explored on a number of occasions (Jordan et al. 1978, Hahm et al. 1981, Cassells and Long 1982, Borissenko et al. 1985). In these instances, propagation material, such as meristematic tissue, was closely monitored for viral symptoms using ELISA and other serological techniques, often alongside anti-viral treatments, to ensure no infected material was subsequently allowed to be used as planting material. Workers in this are emphasize the need for early and accurate detection of PVX in plantlets, something that the development of ELISA testing facilitated (Gallenberg and Jones 1985). The value of testing seed potatoes is exemplified by the reductions in incidence of 5 virus in India potatoes by over half following a vast sampling exercise where nearly 40,000 leaf samples from seed potatoes were analysed (Ahmed and Bhutta 1995). Heat treatment can also be method for virus elimination although mortality of plantlets was seen to be high (Hernandez Perez et al. 1995).

A number of other studies give limited details related to either the detection of PVY in commodity potatoes of its elimination (Cadena Hinojosa et al. 1999, Garg et al. 1999, Mumford et al. 2004, Loebenstein 2007, Njukeng et al. 2007).

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Potato virus Y

Common name(s): PVY, Potato acropetal necrosis, potato leaf-drop streak, potato mottle (and others)
Taxa: Viruses: Potyviridae: Potyvirus

Organism
Potato virus Y infects a range of cultivated and wild solanaceous plants. The disease is almost ubiquitous and causes a range of symptoms such as mottling, yellowing, leaf streaking, deformation and necrosis. Infected plants often generate bushy tops and tend to lose lower leaves. The disease is vectored by a number of aphid species, including the peach-potato aphid *Myzus persicae*

1. Detection
In common with many viruses, prior to the advent of more complex biochemical and molecular testing, the use of indicator plant for detection of this virus was extremely common and still plays a role in detection. Indicator plants that have been widely used include a number of *Nicotiana*, *Chenopodium*, *Physalis* and *Capsicum* species (Krachanova and Ivanova 1978, Pop 1981, Valenzuela-Herrera et al. 2003). Biological indexing has also been commonly undertaken through the use of A6 hybrid plants (*S. demissum* x *S. tuberosum*) that reliably develop necroses when challenged with PVY (Chrzanowska et al. 1977, Hahm et al. 1984, Badarau et al. 2010) although the sensitivity of the plants is less that most of the range of tests detailed below (Telesforo Zavala and Cadena-H 1987).
A range of serological assays have been used down the years in the detection and diagnosis of PVY. These include microprecipitin tests (La 1974, Saldias and Apablaza 1984, Paul and Weidemann 1988), flocculation assays (Gnutova and Krylov 1975, Saldias and Apablaza 1984) and latex agglutination assays (Yokoyama 1975, Polak 1980, Chirkov et al. 1982, Fribourg and Nakashima 1984) and reverse passive haemagglutination (Kohm 1993). A number of serological kits have been developed, some that could give rapid field diagnosis of PVY (Talley et al. 1980, Kurppa and Vuento 1987). .
A very wide range of ELISA-based assays have been used to detect PVY and only a few of the many hundreds of examples can be dealt with here. Immunosorbent assays began to be implemented in the late 1970s with a view to providing a more accurate and sensitive diagnosis of plant viruses. A early example was shown to detect PVY at low viral concentrations, a level of efficiency that outperformed the biological assays then available (Gugerli 1978).(Walter and Sander 1979, Singh and Santos-Rojas 1983). Importantly, ELISA has been shown to detect the virus from all parts of the potato plant (leaves, stems, tubers) and enables the relative concentrations of the virus to be determined (Munzert et al. 1981). Comparisons of the range of available techniques indicated that the early ELISA methods were superior to all other methods available from the standpoint of sensitivity (Raeuber et al. 1984) Subsequent to this early work various modifications of the basic technique have been implemented to increase sensitivity, specificity and efficiency (Goodwin and Banttari 1984, Banttari and Goodwin 1985). The DAS-ELISA variant of the procedure also received considerable attention (Kaniewski and Thomas 1988, Perez de San Roman et al. 1988, Richter and Richter 1990, Nono-Womdim et al. 1991) as well as Dot-ELISA (Banttari and Goodwin 1985). Similarly, enzyme amplified ELISA has also been evaluated but was not seen as an improvement of other methods (Singh and Somerville 1992).

The wide range of evaluations of ELISA and related techniques has led to various kits being developed for use in the field (Mierzwa et al. 1992). ELISA remains widely used up the present time as a diagnostic tool, particularly as constant improvements have been made to the technique. In particular, improved efficiency was facilitated through the generation of monoclonal antibodies against PVY (Rose and Hubbard 1986, Sanz et al. 1990) (Ohshima et al. 1990, Singh et al. 1992) that enabled simple procedures to be developed for rapid diagnosis (Gibson 1988, Heide and Lange 1988).

A cDNA hybridization techniques was developed and compared with existing ELISA techniques that, although slightly more sensitive, was not seen as an improvement due to its time consuming nature (De Bokx and Cuperus 1987). A later nucleic hybridization technique did, however, increase sensitivity 20-50 fold over DAS-ELISA (Hopp et al. 1991). Similarly, a dot-blot hybridization was seen to improve sensitivity 1000-fold over the methods existing at the time (Nikolaeva et al. 1990).

Subsequent to these, a number of PCR-based techniques have been devised that have proved to be extremely sensitive. Hatay et al. (2010), for example, produced an elegant RT-PCR assay for PVY that was over 1000 times more sensitive than ELISA. A similar procedure described by Lee (1996) had the benefit of being able to use diluted sample from leaves without a purification/clean-up step.

The progressive developments in PCR techniques has led to procedures for the simultaneous diagnosis and differentiation of several potato viruses simultaneously. These include duplex assays (Singh et al. 1996, Singh et al. 2003, Crosslin et al. 2005) and a number of multiples procedures (Boonham et al. 2000, Shalaby et al. 2002, Wang et al. 2005, Liu et al. 2009).

The application of the methods described here are discussed below. For example, protected tomatoes in Crete have been routinely inspected for viruses (Avgelis 1986). Samples of plants with obvious symptoms were taken and their sap used to inoculate tests plants with serological tests used for confirmation. The results indicated to be the second most prevalent disease in this situation. Tobacco plants in the USA were surveyed through sampling ca. 800 plants per field in North Carolina. A strict sampling regime was used that varied with the size of the field: all plants in every fourth row sampled for 1-2 acre fields, every 12th row for 12-3 acres and every 16th row for larger fields. Viral prevalence was confirmed by visual assessments and serological tests and indicated the near absence of the disease in most locations.

Significant attention has been paid to monitoring vector aphid species and their contribution to the spread of PVY. For example, in New Brunswick (Canada), aphid species associated with potato were monitored in experimental plots (10 x 10 m) using 4 yellow water traps per plot. Of the 63 species trapped, only seven were vector species of which *Myzus persicae* was the most important

2. Delimitation

No information available

3. Monitoring

Potato virus Y has been widely surveyed for in many parts of the world and here we mostly deal some of the more recent and better documented examples. For example, the incidence of PVY in protected tomatoes was conducted through sampling plants with virus like symptoms and using sap to inoculate test plants followed by serological confirmation (Avgelis 1986). The results indicated that PVY as well as several other viruses were present within the houses sampled although the sampling of only symptomatic plants may have led to underestimates of prevalence. A survey in Israel consisted of the sampling of shoot tips with 2-3 leaves from mature autumn

potatoes as well as tuber collections (Marco 1981). The tubers were kept in the dark until dormancy was broken and sprouts had begun to be produced. Tissues were assayed through inoculation onto A6 test plant leaves.

An interesting aspect of surveying for PVY was highlighted in a survey of tobacco in Japan where it was found that the strain of the virus prevalent in that country by not infecting *Capsicum* and *Chenopodium* indicator plants (Suzuki et al. 1983). A detailed account of a survey of tobacco in North Carolina (USA) where over 8000 plants were sampled using a rigorous sampling regime that sampled all plants in every 4^{th}, 8^{th} or 16^{th} row of field depending on the size of the plot (Gooding and Rufty 1987). Virus diagnosis combined visual assessments followed by serological tests to indicate that PVY was present at very low levels with other viruses much more prevalent.

A survey described form Colombia investigated the occurrence of PVY in six potato growing areas (Corzo et al. 1989)with generally low levels of symptoms found although few details were available as to their methodology. An extensive look at the range and incidence of potato viruses in New Zealand, however, gives more extensive details of methodology (Fletcher 1989). In this instance 10 leaves from different plants were sampled at random from 56 crops. Sap from the leaves was used to inoculate *N. tabacum* cv. White Burley test plants. Tissues from any plants that developed symptoms were subsequently assays using DAS-ELISA. This paper also gives extensive details on the experimental host range of PVY and the symptoms that are generated on each plant species as well as the relative susceptibilities of different potato varieties. In a further survey in New Zealand, selected fields were sampled through walking “W” patterns and collecting mid-upper leaves from pre-flowering plants and storing at 6°C until assaying by a commercial DAS-ELISA kit. The results imndicated the varying presence of PVY across most crops sampled with a peak incidence of 76%.

A serious outbreak of disease in tomato in Alabama (USA) prompted a survey to identify the causal agents (Sikora and Murphy 2005). Over 30 fields were sampled and tissue collected from 300 visibly diseased plants. These leaves were than assayed for disease using a commercial ELISA kit. The results demonstrated that a range of viruses were present in the tomato fields with up to 63% of plants PVY positive at one location. This survey also incorporated an aphid monitoring exercise that collected data on the aphids present on 120 randomly selected plants within a one Ha plot. Each sampled plant had the third fully expanded leaf from the top of the stem examined and the presence of aphids was scored. Results indicated that *Aphis gossypii* was the most prevalent aphid on the tomatoes, a finding that suggested that the insects were migrating from other wild or cultivated plants as tomato is not a good host for this species. The rates of infection of plants quite closely tracked the numbers of aphids, indicating a strong causal link between aphid presence and disease outbreak. Another survey in Italy, this time concerning tobacco also highlighted the importance of aphid control as a means of preventing virus infection though using a collection of phytosanitary methods (Cosmi et al. 1999). A later survey in Italy also highlighted the importance of control measures and also indicated that virus-tolerant varieties of tobacco should be introduced into areas where the diseases were particularly problematic (Martini and Cosmi 2001).

A review of the status of viral diseases in Italian tomato crops indicated that surveys up to that point had suggested that PVY was ubiquitous (Parrella and Crescenzi 2005) with 60-80 of the crop affected. The review also notes the genetic variation of the disease.

A survey in Pennsylvania (USA) collected 282 leaf samples from 5 areas across Pennsylvania in one year and 150 the following (Petrunak et al. 1991). Samples were collected along diagonal transects and leaves collected regardless of the symptoms and viruses detected by DAS-ELISA. Rates of PVY ranged from 15-27% of the samples collected in any given area.

As has been alluded to above, aphid monitoring and management also go hand-in-hand with PVY monitoring and control and, therefore, significant work has addressed vector species, their

behaviour and ability to transmit plant viruses including PVY. A number of papers have addressed the abilities of various aphids to transmit PVY and key species have been identified. For instance, the simple experiment of excluding aphids (primarily *Myzus pericae*) from potato plots was to reduce the incidence of virus from 17-29% to 1-6% (Zimmerman-Gries 1979). In Scotland, aphids have been sampled for many years using aerial suction traps with the abundance of the several vector species showing a strong correlation with the incidence of PVY (Turl and MacDonald 1987). This correlation has been used as the basis for a number of PVY forecasting exercises (Sigvald and Sandstrom 1993, Sigvald 1995, Sigvald 2008).

4. Commodities

There has been significant work to protect potato crops from PVY through controlling spread via tubers, particularly seed potatoes. An example of a survey of the extent of infection is given by Wooster (1981) who grew on sprouted eyes from 100 tuber samples and visually assessed plants for symptoms. However, the sample size and difficulty of growing some cultivars led the author to conclude that the process was not suitable for monitoring purposes. In Canada, agar immunodiffusion was used to reliably detect PVY from the sap of infected tubers (Peterson 1973).

In a more advanced survey of commodity potatoes, Rankovic et al. (1986) examined 10 cultivars grown in Serbia, a s well as imported tubers, for a range of viruses using a commercially available ELISA kit. The results indicated a variable incidence of viruses (generally high) present within the samples, with variety appearing to strongly affect the degree of infection. A three year trial by Barker et al (Barker et al. 1992) investigated the use of a novel nylon membrane ELISA for the early detection of PVY in tubers although the effort was only partially successful with the technique only providing positives results in 83% of samples that were known to have the disease.

The need to generate plants free of PVY and other viruses has prompted research into means of generating clean planting material. For example a simple methodology developed in Argentina using meristem tip culturing and chemotherapy followed by close monitoring with ELISA and biological indexing (Munoz et al. 1990). A second method is described for developing nations that involves the exclusion of vector species for the growing on of sprout-cut seedling (Karim et al. 2010). Covering materials and/or insecticide treatments have also been evaluated and assessed by eye to demonstrate that PVY symptoms were markedly reduced over a two year trial period (Hemphill et al. 1988). A number of chemotherapies with antiviral activity have been reported upon and include gibberellic acid, piperony butoxide and abscisic acid (Hecht 1984).

Hot air and treatments have been used to eradicate viruses from potato stocks although they did not appear particularly successful in eliminating PVY (Kaiser 1980). Here potatoes were exposed to temperatures of 37°C for up to 10 weeks or 50°C water immersion for 25-180 minutes with evidence that several viruses could withstand these treatments.

Reduction of the virus in the field was successfully achieved some reductions through the systematic removal of unwanted and diseased plants from fields in Cyprus, a process known as rouging. (Ioannou 1988)

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Premnotrypes fractirostris Marshall

Common name(s) Andean potato weevil
Taxa: Insect: Coleoptera: Curculionidae
EPPO A1 list: No. 143 as *Premnotrypes* spp. (Andean)
EU Annex designation: I/A1

Organism
Premnotrypes fractirostris is one of several species in this genus of weevils (Coleoptera Curculionidae) attacking potatoes in the Andes. None of the species occur in the EU/EPPO region

1. Detection
No information available.

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
No information available.

References : 0 retained out of 88 retrieved, none added, 0 used for the summary

Premnotrypes spp. (non-European)

Common name(s) Andean potato weevil
Taxa: Insect: Coleoptera: Curculionidae
EPPO A1 list: No. 143 as *Premnotrypes* spp. (Andean)
EU Annex designation: I/A1

Organism
Premnotrypes is a genus of weevils (Coleoptera Curculionidae) attacking potatoes in the Andes, including several poorly known species such as *P. fractoristris*, *P. suturicallus*, *P. vorax* (see relevant summaries).

1. Detection
A key was given for identification of species forming the Andean potato weevil complex and in particular *Premnotrypes* spp. (Alcazar and Cisneros 1999). The detection of adults of the *Premnotrypes vorax* in the soil of potato fields is made difficult because the adults closely resemble lumps of soil. A procedure was developed to overcome this problem and enabled adult populations to be surveyed, by sieving samples of soil from the potato field, and washing the final subsample to separate the adults from the particles of soil adhering to them (Calvache Guerrero 1979). Traps consisted of cardboard sheets or paper bags baited with potato plants were used to monitor *P. vorax* between soil preparation and crop emergence (Nino et al. 2000). Net pitfall traps that contained parts of the potato plant as attractant, and traps that contained the same insect in group of males and females as attractants, showed high capture in other studies (Gallegos and Asaquibay 1997, Kroschel et al. 2009) but seem not useful to evaluate the behaviour of the insect (Galindo and Espanol 2004).

2. Delimitation
No information available.

3. Monitoring
A survey on the distribution and damage caused by insects to potato were carried out in some area of South America (Arestegui 1976, Morante et al. 2007). Knowledge of the spatial distribution of insect could be an important factor in optimizing the pest's management; a trial was carried out to evaluate the spatial dynamics of the Andean weevil *Premnotrypes vorax*, with the use of pitfall traps (Perez-Alvarez et al. 2010).

4. Commodities
The spreading into new areas can be done through potato tubers; during the storage, the exposition of tuber to diffuse light can be a method to eliminate the larvae (Yabar 1986).

References: 18 retained out of 77 retrieved, none added, 10 used for the summary

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Premnotrypes suturicallus Kuschel

Common name(s): Andean potato weevil
Taxa: Insect: Coleoptera: Curculinonidae
EPPO A1 list no. 143 as *Premnotrypes* spp. (Andean)
EU Annex designation: I/A1

Organism
Premnotrypes suturicallus is one of several species in this genus of weevils (Coleoptera Curculionidae) attacking potatoes in the Andes.

1. Detection
A key was given identification of species forming the Andean potato weevil complex and in particular *Premnotrypes* species (Alcazar and Cisneros 1999). The pitfall traps were the method to monitor and control the insect in the field (Kroschel et al. 2009).

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
No information available.

References: 5 retained out of 35 retrieved, none added, 2 used for the summary

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Kroschel, J., J. Alcazar, and P. Poma. 2009. Potential of plastic barriers to control Andean potato weevil Premnotrypes suturicallus Kuschel. Crop Protection 28:466-476.

Premnotrypes vorax (Hustache)

Common name(s) Andean potato weevil
Taxa: Insect: Coleoptera: Curculionidae
EPPO A1 list: No. 143 as *Premnotrypes* spp. (Andean)
EU Annex designation: I/A1

Organism
Premnotrypes vorax is one of several species in this genus of weevils (Coleoptera Curculionidae) attacking potatoes in the Andes.

1. Detection
A key was given for identification of species forming the Andean potato weevil complex and in particular *Premnotrypes* spp. (Alcazar and Cisneros 1999). The detection of adults of the *Premnotrypes vorax* in the soil of potato fields is difficult because the adults closely resemble lumps of soil. A procedure was developed to overcome this problem and enable adult populations to be surveyed, samples of soil from the potato field are passed through a succession of sieves of appropriate sizes, and the final subsample is washed to separate the adults from the particles of soil adhering to them (Calvache Guerrero 1979). Traps consisted of cardboard sheets or paper sacks baited with potato plants were used to monitor *P. vorax*, between soil preparation and crop emergence (Nino et al. 2000). Net pitfall traps that contained parts of the potato plant as attractant, and traps that contained the same insect in group of males and females as attractants, that showed high capture in others studies (Gallegos and Asaquibay 1997), but seem not useful to evaluate the behaviour of the insect (Galindo and Espanol 2004).

2. Delimitation
No information available.

3. Monitoring
The knowledge of spatial distribution of insect could be important in order to optimize the pest management. For example, a trial was carried out to evaluate the spatial dynamics of the Andean weevil *Premnotrypes vorax*, with the use of pitfall traps (Perez-Alvarez et al. 2010).

4. Commodities
No information available.

References: 10 retained out of 47 retrieved, none added, 6 used for the summary

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Calvache Guerrero, H. 1979. 'Sieving' method to detect the presence of adults of the potato whiteworm, Premnotrypes vorax in the soil. Revista Colombiana De Entomologia 1982. 5:31-35.

Galindo, P. and A. Espanol. 2004. Capture dynamic of Premnotrypes vorax (Coleoptera: Curculionidae) and the Guatemalan potato tuber moth Tecia solanivora (Lepidoptera: Gelechiidae) in traps with different types of attractants in a potato crop (Solanum phureja). Revista Colombiana De Entomologia 30:57-64.

Gallegos, G. P. and C. Asaquibay. 1997. Integrated control of Andean potato weevil. CIP Circular 22/23:23.

Nino, L., E. Acevedo, and F. Becerra. 2000. Control of adults of white potato grub with foliage traps. FONAIAP Divulga 67:41-43.

Perez-Alvarez, R., J. Arguelles-Cardenas, and E. Aguilera Garramuno. 2010. Spatial distribution of Premnotrypes vorax (Hustache) (Coleoptera: Curculionidae) in potato crops. Revista Corpoica - Ciencia y Tecnologia Agropecuarias

11:11-20.

Prunus necrotic ringspot virus

Common name(s): Peach ringspot virus, plum line pattern virus, prunus ringspot virus (and others)
Taxa: Viruses Bromoviridae: Ilavirus
EU Annex designation: II/A1

Organism

Prunus necrotic ringspot virus (PNRSV) is a plant pathogenic virus belonging to the genus *Ilarvirus* of the family Bromoviridae. It has been reported on cherry (Oliver et al. 2009) but also on peach and myrobalan trees (Polak 2007, Torre-Almaraz et al. 2008) as well as in almond, plum and apricot germplasm accessions (Spiegel et al. 2004) and on roses (Rakhshandehroo et al. 2006, Szyndel et al. 2006). According to Salem et al. (2003), young leaves are the most reliable source for distinguishing between healthy and infected plants.

Kelley and Cameron (1986) found no particles in pollen from PNRSV-infected or non-infected sweet cherry trees. PNRSV was detected by ELISA in all parts of fruit from infected trees pollinated with pollen from healthy trees (Kelley and Cameron 1986). However, further evidence for PNRSV association with the pollen exine (outer coat) was obtained by binding of latex-conjugated antibody to the surface of pollen grains from PNRSV-infected sweet cherry (Hamilton et al. 1984). In a study on the possible role of honeybees, Mink (1983) concluded that they are considered as potential vectors of PNRSV.

Characteristic symptoms are chlorotic line patterns (zigzag pattern), vein-banding and mottles in leaves, in spring-summer and flower abnormalities as phyllody, in autumn. Distortion and reduction in flower size and early leaf drop have been observed on symptomatic plants in winter (Sertkaya 2010). PNRSV is associated with rose mosaic disease (Sertkaya 2010).

1. Detection

The most extensively used methods for detecting viruses are enzyme-linked immunosorbent assays (ELISA) and reverse transcription-polymerase chain reaction (RT-PCR) (e.g., Spiegel 2008, Ulubas 2008, Zhu et al. 2009, Rana et al. 2011). The RT-PCR technique has the added advantage that plant material can be tested at any time throughout the growing season (Mekuria et al. 2003) and allows the amplification of a 282 bp genomic fragment located on PNRSV RNA-3 (Boulila and Marrakchi 2001). Saade et al. (2000), Sanchez-Navarro et al. (2005) and, Youssef and Shalaby (2009) have developed a single-step multiplex RT-PCR for the simultaneous detection and discrimination of three, eight and five viruses, respectively, including PNRSV. This method saves time and reagent costs compared with monospecific RT-PCR that need several reactions for the same number of tests. More recently, Peiro et al. (2012) proposed the use of a unique polyprobe as an alternative to other routinely used detection methods. Transmission electron microscopy (TEM) was also used to detect the presence of PNRSV. The presence of virus particles was confirmed by the DAS-ELISA serological technique (Jakab-Ilyefalvi et al. 2011). However, a deteriorating effect of high temperatures on concentrations of PNRSV in leaves has been observed by DAS-ELISA (Marn and Mavric 2005).

The infected host leaves, dried at 65°C for 2 days and conserved at 4°C in air proof conditions, serve as good sources for detection of viral and viroid pathogens by PCR methods (Sipahioglu et al. 2006).

Ruan et al. (2004) have developed a simple and effective RNA virus extraction protocol for fruit tree (e.g., cherry, plum and peach) tissues containing high polyphenolic compounds and polysaccharides. The extract allows the detection of PNRSV by RT-PCR. The method is based on

silica capture and releasing under appropriate conditions without using organic solvents such as phenol and chloroform. The extracted RNA and transcripted cDNA can be stored at -20°C for a long period of time.
Importantly, Amari et al. (2009) demonstrated the vertical transmission of PNRSV from gametes to seedlings (i.e., next generation), therefore demonstrating a clear means for the virus to persist year on year.

2. Delimitation
Detection and mapping the distribution of PNRSV was undertaken in Washington State over a three year period with 15,000 sweet cherry trees examined (Mink and Aichele 1984). Approximately 28% of trees were infected with PNRSV and diseased trees were reliably identified by ELISA during the winter months, allowing for their removal before flowering. However, it was noted that some growers were reluctant to remove trees solely on the basis of a positive result from the diagnostic test.

3. Monitoring
Field inspections (observation of symptoms) and sample collections from stone fruit trees have been carried out to assess the presence and distribution of viruses in commercial orchards and mother blocks (e.g., Kamenova and Borisova 2002, Sertkaya et al. 2004, Rouag et al. 2008).
In Canada, Cui et al. (2012) confirmed by RT-PCR the presence of PNRSV in ELISA-positive samples. The near full-length genomic RNA3 segment encoding the 5'-proximal movement protein (MP) and the 3'-proximal coat protein (CP) of samples was cloned and sequenced. Phylogenetic analysis of MP and CP genes suggest these isolates belong to two groups, PV96 and PV32, with the former as the predominant group.

4. Commodities
Viruses, including PNRSV, can be found in imported fruit (Ozdemir and Kaya 2008). In Australia, there has been a high incidence of virus infections in prune trees since growers and nurserymen have planted infected material (Barkley et al. 1995). *In vitro* heat therapy has been successfully applied to shoot tip cultures and the virus eliminated from plant material (Snir and Stein 1985), providing a safe way for generating virus-free material for planting.

References: 464 references retrieved, none added, 294 retained, 34 used for summary

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Pseudomonas caryophylli (Burkholder) Starr & Burkholder

(=*Burholderia caryophylli* (Burkholder) Yabuuchi *et al.*)
Common name(s): Bacterial wilt, bacterial stem crack
Taxa: Bacteria: Proteobacteria: Burkholderiaceae
EPPO A2 list: No. 55
EU Annex designation: II/A2

Organism

Pseudomonas caryophylli Burkholder (syn. *Burkholderia caryophylli*, *Phytomonas caryophylli*) is the causal agent of carnation bacterial wilt, a disease that can result in serious crop losses. Occurs within parts of Europe, North America and South America

1. Detection

A selective medium (APCA medium) was developed for the isolation of *Pseudomonas caryophylli*, from both plants and soil (Kawanishi et al. 2009). The resultant composition of the medium per litre was: 0.79 g (NH4)(2)SO4, 1.0 g KH2PO4, 0.5 g MgSO4.7H(2)O, 0.2 g KCl, 2.0 g D-arabinose, 5 mg crystal violet, 50 mg cycloheximide, 50 mg polymyxin B sulphate, 50 mg ampicillin sodium, 10 mg chloramphenicol, 25 mg blue tetrazolium, and 15 g agar. This medium allows the bacterium to be successfully isolated from contaminated soil and plant tissues. It also inhibits almost completely the growth of other plant pathogenic bacteria and soil saprophytes. This selectivity was high enough to detect *P. caryophylli* in contaminated soil.
Khan and Matsuyama (1998) provide details of a Thin Layer Chromatography (TLC) method that was performed for the rapid identification of phytopathogenic bacteria such as *Pseudomonas* spp., *Ralstonia solanacearum* and *Herbaspirillum rubrisubalbicans*.

2. Delimitation

No information available.

3. Monitoring

No information available.

4. Commodities

No information available.

References: 94 references retrieved, none added, 28 retained, 2 used for summary

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Khan, A. A. and N. Matsuyama. 1998. Rapid identification of phytopathogenic bacteria by TLC. Journal of the Faculty of Agriculture Kyushu University 42:281-287.

Pseudomonas solanacearum (Smith) Smith

(=*Ralstonia solanacearum* (Smith) Yabuuchi et al.)
Common name(s): Brown rot, southern bacterial wilt, moko disease, Granville wilt
Taxa: Bacteria: Proteobacteria: Burkholderiaceae
EPPO A2 list: No. 87
EU Annex designation: I/A2

Organism

The bacterium *Pseudomonas solanacearum* (=*Ralstonia solanacearum*) causes the disease known as brown rot or bacterial wilt disease in potato. The diseases caused in other plants are known by a range of other common names. The nematode is considered one of the most serious potato pathogens globally. Spread is facilitated by infected seed potato tubers (Elphinstone *et al*. 1996) and the movement and trade of ware potatoes. In addition to potato, the disease affects over 50 plant families including several members of the economically important solanaceae (pepper, eggplant, and tomato)(Fajinmi and Fajinmi 2010), groundnut (Khetmalas and Sardeshpande 1989), radish (Lin *et al*. 1994), several ornamentals (Kim *et al*. 2002), plantain and banana (McDonald 1985), ginger (Nath *et al*. 2001) amongst others. The disease exhibits wide genetic diversity and is known to exist in a range of strains (races and biovars) with varying pathogenicities and host specificities (Martini *et al*. 2002, Ji *et al*. 2007). In the EPPO region race 3 predominates, which appears to be largely restricted to *Solanum* species. Race 3 biovar 2 is the most commonly transmitted strain via soil and infected plant material (Tahat and Sijam 2010). The distribution of *P. solanacearum* is worldwide and the disease is widespread in Europe (Grousset 1998)..

1. Detection

The diagnosis and detection procedures for *P. solanacearum* have been covered by EU Directive 2006/63/CE (Anonymous 2006). A detailed report on methods for the sampling and detection *of P. solanacearum* is provided by French *et al*.(1985). Here, visual signs of disease are described such a creamy bacterial ooze exuding from the vascular ring. The storage of tubers at 25°C for 4-6 weeks can encourage development of the disease and facilitate detection of the pathogen in latent infections. A simple method to confirm infection can be made by suspending tissue in water whereby milky ooze will drift downwards from the tissue after a few minutes. Indicator plants can also be used to detect the presence in *P. solanacearum* in soils. The growth of the bacteria on semi-selective growth media has been frequently undertaken for detection purposes (Nesmith and Jenkins 1979) and for sample preparation in advance of submission to other diagnostic procedures (discussed below).

Further confirmation of the disease identity can be obtained through staining accumulated poly-3-hydroxybutyrate (PHB) with Nile Blue and examining material using fluorescent microscopy. Immuno-fluorescent antibody staining (IFAS) methods have been developed and used by Janse (1988) and Wolf *et al* (Van der Wolf *et al*. 1998, Van der Wolf *et al*. 2000) to provide a simple and accurate method for determining latent brown rot infections in potato. Various serological techniques, such as ELISA, are also described by French *et al*. (1985), as are early DNA-based methods. ELISA/antibody based assays have been successfully used to detect *P. solanacearum* is South Africa (Bellstedt 2009) and the Netherlands (Griep *et al*. 1998), Canada (Paret *et al*. 2008), France (Priou *et al*. 1999) and Africa (Mwangi *et al*. 2008), amongst others. Elphinstone *et al*. (1996) reviewed the sensitivity of the various earlier methods for brown rot detection and indicated all methods routinely used at that time (semi-selective media culturing, ELISA, PCR, nested-PCR) gave adequate results, particularly after enrichment of the bacterium using semi-

selective broth, the most sensitive assay being plating out on media. Standardized methods were later described by Elphinstone *et al*. (2000).

Black *et al* (1999) (1996) evaluated several methods for detection in Africa, including ELISA, PCR and the BACTID kit; all successfully detected the disease and the merits of each assay are discussed. Over the last decade ELISA methods have been significantly finessed to the extent that a double-antibody sandwich indirect assay can routinely detect the disease in symptomless potato tubers (Caruso *et al*. 2002). An outbreak of brown rot in Portugal was recently detected via routine monitoring followed by diagnosis using laboratory procedures laid down by Directive 2006/63/EC that included plating out on semi-selective media, immune-fluorescent assays and PCR-based tests (Cruz *et al*. 2008).

The molecular detection of *P. solanacearum* can be achieved through a wide range of other, largely molecular, techniques. Notable methods include a method based on fluorescent in situ hybridization (FISH) method of Wullings *et al*. (1998). Random amplified polymorphic DNA (RAPD) analysis has been used to identify *P. solanacearum* strains from several plant species (James *et al*. 2003). Immuno-capture-PCR (IC-PCR) has been successfully used to assay for *S. solanacearum* in soil and weeds, although the limit of detection was not as good as some of the assays detailed below (Dittapongpitch and Surat 2003). A co-operation PCR method has been developed with the sensitivity to identify one cfu/ml^{-1} water, which can assess the spread of the disease via water courses (Caruso *et al*. 2003). Various PCR and Real-time PCR methods are now available for detection in soil and plant tissues with very high sensitivity and specificity (Iglesia *et al*. 2008, Huang *et al*. 2009, Chen *et al*. 2010). Seal (1995) describes a PCR-based method suitable for use in less-developed nations. A range of other PCR methods are described in the literature for potato and several other crops (Glick *et al*. 2002, Ozakman and Schaad 2003, Kang *et al*. 2007, Kumar and Abraham 2008, Grover *et al*. 2009) including some that use bateriophage lysis of the cells (Kutin *et al*. 2008, Kutin *et al*. 2009) and methods for non-culturable cells (Marco-Noales *et al*. 2008). The limit of detection for *P. solanacearum* infecting tomatoes is given for various PCR methods (and selective media) by Perez *et al*. (Perez *et al*. 2008). A novel sampling procedure for the collection and preservation of nucleic acids from plant material using “Flinders Cards” has been developed and is an effective means of collecting brown rot (and other pathogen) samples for analysis (Grund *et al*. 2010). A rapid field detection kit, using a method known as the *Staphyloccoccus aureus* slide agglutination method, has been successfully used in Portugal (Lyons *et al*. 2001) whist portable RT-PCR procedures are now available for in-field molecular diagnosis (Ozakman and Schaad 2003) and this probably represent the current state of the art. We must add the caveat, however, that the very large numbers of similar and sensitive PCR-based assays make the determination of what process is the best very difficult in the absence of extensive comparative data.

Sampling and processing methods for tubers are given in detail (EPPO 1990) that indicate that samples of around 200 tubers should be taken out of lots of 25 tonnes (or less) and subjected to the tests detailed above. Further work has indicated that the probability of detection from field samples (soil/tissue) was highest when sampling 20 points per 1002 (Pradhanang 1999). The statistical elements of sampling of potatoes for analysis have also been extensively discussed by Priou *et al*. (2001).

Other diagnostic tests that have been evaluated for *P. solanacearum* include electronic nose methods (Momol *et al*. 2004) and thin layer chromatographic (TLC) procedures (Rahman *et al*. 1998) although these methods are unlikely to be as discriminating as serological and PCR-based assays and offer no benefit of the other assays detailed here. The demands for the processing of large quantities of samples has led Van der Bilt *et al*. (2008) to develop a high-throughput diagnostic assay in the Netherlands that utilizes immune-fluorescent staining that enables the

rapid turnaround of high numbers of extracts. This procedure is designed to make the processing of large numbers of samples with a high degree of accuracy possible.
Following the incidence of brown rot in the Netherlands, all seed-potatoes are routinely tested following the EPPO guidelines (EPPO 1990); water samples and tissue samples of *Solanum dulcamara* (bittersweet nightshade) have also been sampled on occasion (Janse and Schans 1998). Visual detection inspections of 400,000 tubers were used to first detect *P. solanacearum* in Sweden in the early 1970s and to monitor the disease in later years (Persson 1998). Surveys also showed that *S. dulcamara* was also infected. Continued monitoring since the outbreak indicated that eradication had been achieved in Sweden. A similar situation occurred in the Netherlands in the 1990s where brown rot was first detected in seed-potatoes (Janse and Schans 1998). Eradication measures were instigated (1995-1996) whereby seed-potato lots were only authorized for marketing following testing for presence of the pathogen (>7000 surveys and >115,000 tubers tested). Detection surveys in France are described by de Guenin (1998) that were undertaken following very small outbreaks in the 1990s. In particular, all seed-potato fields are visually inspected whilst stored seed potatoes were tested. Tests for the disease in water, *S. dulcamara* and *S. nigrum*, planted and volunteer potatoes, maize, peas, rape and tomatoes were also implemented. The methodologies for water and field surveys are briefly described.

2. Delimitation

The need to effectively delimit the extent of areas of infection is highlighted by Wright (1998) as part of the measures necessary to prevent the introduction and spread of *P. solanacearum* in the European Union. The importance of potatoes as a crop in Egypt has led to measures being taken to delimit pest-free areas (PFAs). Surveys use the diagnostic approaches detailed above and exploit the accepted tissue sampling methodologies detailed above. An extensive account of the surveying efforts taken in Egypt are detailed by Kabeil *et al*. (Kabeil *et al*. 2008) and involves the testing of tubers and surveying tomatoes, identifying non-potato sources and examining water contamination. Tomlinson *et al*. (2009) reported that the delimitation surveying in Egypt showed that potato brown rot was limited the canals of the traditional potato growing areas of the Nile Delta. Cultivated reclaimed desert was designated as pest-free.

3. Monitoring

Pseudomonas solanacearum is widely monitored for throughout the world on a number of crops. The introduction of *P. solanacearum* into the Veneto region of Italy in the 1990s led to a widespread monitoring effort (Turco *et al*. 1998). In the autumn of 1995 contaminated and potentially contaminated fields were monitored whilst the area of infection was effectively delimited through broadening monitoring out to the surrounding (non-affected) areas. Sampling consisted of taking 200 tubers from each field within the affected area and from fields outside the know area of infection. Similar methods have been used in Portugal using the scheme outlined by EU Directive 2006/63/CE (Cruz *et al*. 2008) that produced the first report of biovar 1 in that country. Surveying for a range of potato disease in Pakistan is described by Khan (Khan *et al*. 1985). Here 5 kg tuber samples were collected at a rate of six samples per field (areas not given) whilst signs of wilting were visually scored in each field. Notably, the observation that visual assessments can be obscured by the occurrence of other diseases (e.g. late blight) indicates the unreliability of this method of assessment in some instances. Ahmad *et al*. (1995) described further surveying in Pakistan that reports upon the occurrence and delimitation of the brown rot in different areas of the country. Similarly extensive surveys have been conducted in Guatemala that indicates the diversity of hosts that the pathogen infects (Perez *et al*. 2008). The visual surveying and collection

of tissue for diagnosis for *P. solanacearum* in tomato in Africa has also been described (Adebayo and Ekpo 2005). Black *et al*. (1999) similarly conducted surveys in a range of vegetables in Africa (Tanzania) and was detected in tomato and potato tissues using semi-selective media and PCR or ELISA techniques.

As watercourses are frequently responsible for the dissemination of *P. solanacearum*, rivers are frequently monitored. For example, in a Spanish example 65 water samples (500 ml) were collected from 5 sites on the Tormes River over the course of a year. Sampling depths of 30-40 cm at 2 m from the bank were used and presence diagnosed through growing on selective media and ELISA (Caruso *et al*. 2005). Similarly, Elphinstone *et al*. (1998) demonstrated that irrigation water was the source of isolated brown rot disease in ware potatoes in the UK

4. Commodities

The potential for the ware potatoes to introduce *P. solanacearum* into new areas means that routine testing of imports is undertaken. Calzolari *et al*. (Calzolari *et al*. 1998) describe procedures in Italy for the examination of potatoes following the sampling regimes described above that were used to determine the import of infected material from Egypt. Testing of imported material following very similar sampling procedures to those outlined above are also carried out in Israel whereby 200 tubers are sampled per 25 tonnes and the disease diagnosed using selective media (Tsror *et al*. 1999). Commodities surveyed in India reveal that *S. solanacearum* is frequently intercepted in imported groundnut (Chakrabarty *et al*. 2004). The testing of tubers in Germany (ware and seed) is described by Muller and Parusel and follows the sampling approaches described above.

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Pseudomonas syringae pv. *Persicae* (Prunier et al.) Young et al.

Common name(s): Bacterial dieback of peach
Taxa: Bacteria: Proteobacteria: Pseudomonadaceae
EPPOA2 list: No. 145
EU Annex designation: II/A2

Organism
The bacterial dieback *Pseudomonas syringae pv. persicae (syn. Pseudomonas mors-prunorum* f.sp. *persicae*) causes decline of stone-fruit trees as apricot and peach (Gardan et al. 1972, Arsenijevic 1980). It is observed on the surface of leaves, branches, buds and flowers of peach trees (Gardan et al. 1972). Occurs in parts of Europe and Oceania

1. Detection
According to Lopez et al. (2010), PCR protocols for toxins and siderophores produced by *Pseudomonas* are not yet specific enough to be used for their diagnosis or detection in plant material. However, they can be used combined with other tests for identification purposes.

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
No information available.

References: 27 retained out of 120 references retrieved, none added, 3 used for summary

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Pseudopityophthorus minutissimus (Zimmerman)

Common name(s): Oak bark beetle
Taxa: Insecta: Coleoptera: Scolytidae
EPPO A1 list: No. 6
EU Annex designation: I/A1

Organism

Pseudopityophthorus minutissimus (Coleoptera Scolytidae) has been commonly recorded from red oak (*Quercus rubra)* but absent in white oak (*Q. alba*), and it is recorded to feed on several other hardwood hosts (McMullen et al. 1955). Two generations of beetles occur per year throughout most of the oak's range, late stage larvae and adults serving as the overwintering stage. *P. minutissimus* has been recorded in southern part of Quebec and Ontario in Canada and parts of the USA. The beetle is considered an important vector of the oak wilt fungus, *Ceratocystis fagacearum*, but the vector's importance seems vary amongst region (Ambourn et al. 2005).

1. Detection

Adults of *Pseudopityophthorus minutissimus* are captured in generic surveys in plantation of living eastern cottonwood trees through ethanol-baited traps. The phenology of this bark beetle was studied with window traps placed in the canopies of recently killed northern pin oaks (Ambourn et al. 2006). In this study canopies were also sampled (in May) through collecting material from the crowns of wilted trees. Braches were taken from the tree and cut into smaller pieces and bagged and the presence of insects, emergence holes and galleries determined in the laboratory. Sub-samples of the collected material were also taken and incubated to allow the insects to emerge naturally so that the presence of *Ceratocystis fagacearum* could be determined. Similar methods were also employed for examining slash (material generated during logging) that had been removed from trees at an earlier date.

2. Delimitation

No information available.

3. Monitoring

No information available.

4. Commodities

No information available.

References: 6 retained out of 10 retrieved, 1 added, 3 used for the summary

Ambourn, A., J. Juzwik, and J. Eggers. 2005. Relative importance of the small oak bark beetle, in the overland transmission of Ceratocystis fagacearum in Minnesota. Phytopathology 95:S161.

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Pseudopityophthorus pruinosus (Eichhoff)

Common name(s): Oak bark beetle
Taxa: Insecta: Coleoptera: Scolytidae
EPPO A1 list: No. 6
EU Annex designation: I/A1

Organism
Pseudopityophthorus pruinosus is a bark beetle (Coleoptera Scolytidae) considered as a potential vector of the fungus *Ceratocystis fagacearum* in North America.

1. Detection
Window traps, placed in canopies of recently killed northern pin oaks, were used to determine seasonal flight of *Pseudopityophthorus*, among them *P. pruinosus* (Ambourn et al. 2006).

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
No information available.

References: 6 retained out of 10 retrieved, 1 added, 3 used for the summary

Ambourn, A. K., J. Juzwik, and J. E. Eggers. 2006. Flight periodicities, phoresy rates, and levels of pseudopityophthorus minutissimus branch colonization in oak wilt centers. Forest Science 52:243-250.

Pterandrus rosa (Karsch)

(=*Ceratitis rosa* Karsch)
Common name(s): Natal fruit fly, Natal fly
Taxa: Insecta: Diptera: Tephritidae
EPPO A1 list: No. 237
EU Annex designation: I/A1

Organism

The natal fruit fly, *Pterandrus rosa* (Karsch) (syn. *Ceratitis rosa* Karsch) (Diptera: Tephritidae) is a polyphagous species (Grout and Stoltz 2007). It is a pest of coffee and a wide range of fruits in Africa (Anonymous 1985). In Kenya, the most important wild hosts are the evergreen shrubs of the Sapotaceae and a range of flowers and shrubs of the Annonaceae (Copeland et al. 2006). The insect is widely distributed throughout Africa and is absent from Europe.

1. Detection

Flies can be collected using McPhail traps baited with parapheromones (methyl eugenol, trimedlure and cue lure) and synthetic food baits (hydrolyzed yeast and three-component lure) or from infested fruits after incubation in the laboratory (Mwatawala et al. 2009).
Baliraine et al. (2004) screened 24 Medfly (*Ceratitis capitata*) microsatellite markers for cross-species amplification in *P. rosa* and two others species. The Medfly-based microsatellite markers can be useful for population genetic studies in the species tested, which would facilitate the tracing of the geographical origin of colonist pest populations, assessment of their invasive potential and risk assessment.

2. Delimitation

No information available.

3. Monitoring

Control methods include cover sprays and bait application (Barnes 2000). The best catches occur before and during the fruit maturation period and when traps are placed on fruit trees at 4 and up to 6 m above the ground (Normand et al. 2000, Vayssleres and Kalabane 2000).
McPhail traps were used to assess the relative performance of commercial protein hydrolysate baits and various home-made autolysates of brewery yeast wastes in order to develop home-made alternatives to imported hydrolysate bait. The locally-made yeast autolysate is produced by a combination of heat autolysis and papain enzyme promoted proteolysis. Fruit fly catches were greater when protein autolysate was prepared with higher concentrations of papain (2.0 or 4.0 g/litre) (Sewoosunkur et al. 2000).

4. Commodities

The risk of introduction of *P. rosa* is higher with incoming travelers than with fruit consignments which are mostly transported by ship (Li et al. 2010).

References: 46 retained out of 85 references retrieved, none added, 10 used for summary

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Puccinia horiana Hennings

Common name(s): White rust of chrysanthemum
Taxa: Fungi: Basiomycota: Pucciniaceae
EPPO A2 list: No. 80
EU Annex designation: II/A2

Organism

Puccinia horiana is the causal agent of chrysanthemum white rust, an economically important disease of florist's chrysanthemum (*Dendranthema* x *grandiflorum*) which has also been observed on many true chrysanthemum species. Chrysanthemum white rust originates from Japan but has now been reported in most countries where florist's chrysanthemums are grown. It is an autoecious (completing the life cycle on one host) rust fungus that produces teliospores and basidiospores. Teliospores germinate *in situ* and form basidiospores which are dispersed by air and cause new infections under appropriate conditions. High humidity and a film of moisture are necessary for spore germination. Typical symptoms are yellow spots on the upper leaf surface and raised pustules on the lower leaf surface. In severe cases, infections of petioles, stems and flowers can occur. Detailed information about this quarantine organism has been published by EPPO in a Datasheet (Anonymous 1982) and in a specific diagnostic protocol (Anonymous 2004).

1. Detection

Typical white rust symptoms on leaves can be detected through visual inspections. Identification protocols rely on macroscopic symptom development and microscopic examination of teliospores. *Puccinia horiana* is easily distinguished from other species by its smooth, hyaline teliospores that always germinate *in situ* on the living leaf. Molecular methods (conventional and real time PCR) were developed (Alaei, Baeyen *et al*. 2009; Pedley 2009) and allow a sensitive and specific detection of *Puccinia horiana* even during the latent stage of infection.

2. Delimitation

A delimitation survey (within 405m) was organized in Oregon after the first occurrence of Chrysanthemum White Rust in a private garden; identification relied on symptoms and microscopic observations of teliospores. No positive result was found within the 211 properties and 25 commercial nurseries inspected (Griesbach, Milbrath *et al*. 1991).

3. Monitoring

No information available

4. Commodities

This pathogen has spread rapidly through infected imported cuttings and is now a serious disease in nurseries in Europe, frequently causing complete loss of glasshouse chrysanthemum crops. Once established, Chrysanthemum White Rust is extremely difficult and costly to eradicate (Anonymous 1982). Imported chrysanthemum cut flowers and pot plants can also spread the disease. A pre-export inspection system used in the Netherlands gives a high level of security that the produce is free from *Puccinia horiana* (Veenenbos 1984*).*

References: 14 retained out of 47 retrieved, 2 added, 6 used for the summary

Alaei, H., S. Baeyen, *et al*. (2009). Molecular detection of Puccinia horiana in Chrysanthemum x morifolium through conventional and real-time PCR. Journal of Microbiological Methods 76(2): 136-145.

Anonymous (1982). EPPO Data Sheet on Quarantine Organisms N°80: *Puccinia horiana*. EPPO Bulletin 12(1).

Anonymous (2004). Diagnostic protocols for regulated pests: *Puccinia horiana*. EPPO Bulletin 34: 209-211.

Griesbach, J. A., G. M. Milbrath, *et al*. (1991). First occurrence of chrysanthemum white rust caused by Puccinia horiana on florists' chrysanthemum in Oregon. Plant Disease 75(4): 431.

Pedley, K. F. (2009). PCR-Based Assays for the Detection of Puccinia horiana on Chrysanthemums. Plant Disease 93(12): 1252-1258.

Veenenbos, J. A. J. (1984). The "Green Corner"; a pre-export inspection system for chrysanthemum cutflowers and pot plants in the Netherlands. EPPO Bulletin 14(3): 369-371.

Puccinia pittieriana Hennings

Common name(s): Common potato rust
Taxa: Fungi: Basiomycota: Pucciniaceae
EPPO A1 list: No. 155
EU Annex designation: II/A1

Organism

Puccinia pittieriana, causal agent of common potato rust, is indigenous to Central and South America where it occurs in mountain valleys, mostly over 3000m. It has not spread to other continents. The main hosts are potatoes and tomatoes but *Solanum demissum* and other wild solanaceae are also attacked. This rust produces only teliospores which, below 15°C, germinate to produce basidiospores . They are dispersed by wind and can immediately infect new host leaves. At higher temperatures, basidiospores are not formed, so spread is favoured by cool conditions: average temperature of 10°C with 10-12 h of free moisture. The pathogen can persist on overlapping potato crops, on solanaceous weeds or in debris in soil. Typical rust symptoms can be observed on leaves and the pathogen can cause severe defoliation (mainly on potatoes). Few publications are available about *Puccinia pittieriana*; a Datasheet about this quarantine pathogen has been published by Anonymous (1988).

1. Detection

Symptoms on leaves can be detected through visual inspections. Identification is based on observation of typical lesions on leaves (smaller and easy to distinguish from those of the only other potato rust, *Aecidium cantensis*).

2. Delimitation

No information available

3. Monitoring

No information available

4. Commodities

The fungus could be introduced on leaves of living material (e.g. material imported for breeding purposes), or on dead plant material, on crop residues or soil accompanying tubers (Anonymous, 1988). Strict post-entry quarantine procedures in the EPPO region, together with equivalent checks before export are fully justified.

References: 2 retained out of 8 retrieved, none added, 1 used for the summary

Anonymous (1988). Puccinia pittieriana Henn. Basidiomycotina: Uredinales. Bulletin OEPP 18(3): 517-519.

Radopholus citrophilus Hüttel *et al*. and *Radopholus similis* (Cobb) Thorne

Taxa: Nematoda: Tylenchida: Pratylenchidae

Radopholus citrophilus

Common name(s): Citrus spreading decline nematode
EPPO A1 list: No. 161
EU Annex designation: II/A1

Radopholus similis

Common name(s): Burrowing nematode, banana toppling disease nematode
EPPO A2 list: No. 126
EU Annex designation: II/A2

The two sibling species are addressed together because in the retrieved references most of studies were carried out on *R. similis* specifying rarely whether it was formerly *R. similis* banana race (i.e. *R. similis*) or *R. similis* citrus race (i.e. *R. citrophilus)*. Indeed, among the relevant references (170) only four quote *R. citrophilus*.

Organism
Radopholus citrophilus and *Radopholus similis* are two sibling species of nematods (Tylenchida: Pratylenchidae) (Kaplan et al. 1996). *Radopholus similis* is recognized to have a wide host range (e.g., Zem and Lordello 1983, Gnanapragasam et al. 1991, Oever and Mangane 1992, Bala and Hosien 1996, Victoria et al. 2005) and cause rhizome necrosis, root-rot, stunting and chlorosis (Ploetz et al. 1992, Bala and Hosien 1996). However, according to EPPO datasheet, *Radopholus similis* (syn. *R. similis* banana race; *R. similis sensu strict*; *Tylenchus similis*) attacks bananas but not citrus while *R. citrophilus* (syn. *R. similis* citrus race) attacks bananas and citrus (see also Huettel and Dickson 1981).
The population of nematodes can vary with crop combinations, soil types and seasons (periods) studied (Sheela 1995, Grislaes Lopez and Lescot 1999).
The distribution of *R. citrophilus* encompasses parts of North and Central America with some reports from the Caribbean and South America. *Radopholus similis* is more widely distributed and is present within Europe as well as across Africa, Asia, Oceania and the Americas.

1. Detection
Ge et al. (2007) and Arzanlou et al. (2009) developed a (duplex and quantitative, respectively) PCR assay for the banana burrowing nematode, *R. similis*. The lowest amount of DNA required for the detection of *R. similis* was 100 pg (Ge et al. 2007).
Extraction of *R. similis* nematodes from banana roots was most efficient when macerated roots were incubated at 27 to 31°C for two days in hydrogen peroxide. The optimum concentration of hydrogen peroxide was 10 ml of 30% solution in 1 litre of tap water. On a 200 cm² sieve area more nematodes were extracted from 20 g of macerated root tissue than from 40 g (Gowen and Edmunds 1973). In a book, Tacconi and Ambrogioni (1995) described the taxonomy, geographic distribution, principal hosts, economic importance, biological cycle, identification and methods of control of nematodes as *R. similis* and *R. citrophilus.*

2. Delimitation
No information available.

3. Monitoring
According to Araya et al. (1999), samples for monitoring nematode populations in bananas should be taken within a horizontal distance of 0 to 30 cm from the plant base and down to 30 cm deep from the soil surface. In addition, Araya and Cheves (1998) suggest that follower suckers which are less than 200 cm in height coming from recently flowered plants (within 8 days of flower emergence) should be sampled to give a more reliable nematode population estimate. Then, nematode extraction can be done using 25 g of fresh roots macerated in a kitchen blender, followed by recovering the nematodes with a 0.025 mm pore size (No 500 mesh) sieve. Frequency of occurrence and abundance of nematodes is calculated as a percentage (number of samples containing a species/ number of samples collected x 100) (Ramclam and Araya 2006, Chavez and Araya 2010).

4. Commodities
Radopholus similis can be imported with banana-planting material, but also with flowers and nursery stocks that are shipped internationally (Wang et al. 2007). The nematode can also be spread by various other plants that are traded across borders e.g. *Marantha makoyana* (Tacconi 1996), *Anthurium* spp. (Cadet et al. 1993), *Maranta leuconeura*, *Philodendron* and *Scindapsus aureus* (Anonymous 1978).

References: 169 retained out of 341 references retrieved, none added, 22 used for summary

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Huettel, R. N. and D. W. Dickson. 1981. Parthenogenesis in the two races of Radopholus similis from Florida. Journal of Nematology 13:13-15.

Kaplan, D. T., M. C. Vanderspool, C. Garrett, S. Chang, and C. H. Opperman. 1996. Molecular polymorphisms associated with host range in the highly conserved genomes of burrowing nematodes, Radopholus spp. Molecular Plant Microbe Interactions 9:32-38.

Oever, H. A. M. v. d. and S. E. Mangane. 1992. A survey of nematodes on various crops in Mozambique. Afro-Asian Journal of Nematology 2:74-79.

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Sheela, M. S. 1995. Analysis of phytonematodes associated with coconut-based cropping system in Kerala. Indian Coconut Journal (Cochin) 26:5-6.

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Raspberry leaf curl virus (American)

Common name(s): RLCV, American raspberry leaf curl
Taxa: Viruses (unclassified)
EPPO A1 list: No. 31
EU Annex designation: I/A1

Organism

Raspberry leaf curl virus is present in North America on a number of *Rubus*, on which it is vectored by aphids, *Aphis rubicola* (small raspberry aphid, present in North America) and *A. idaei* (experimentally demonstrated, present in Europe). The disease is absent from the EU/EPPO region

1. Detection

Aphid transmission to the sensitive indicator species *Rubus phoenicolasius* induces pronounced leaf curl symptoms within 10 days. However, results from graft-inoculation tests usually take longer (Jones 1991) and longer infection periods lead to more pronounced symptoms.

2. Delimitation

No information available.

3. Monitoring

No information available.

4. Commodities

No information available.

References: 4 retained out of 13 retrieved, none added, 1 used for the summary

Jones, J. 1991. Quarantine Procedure Rubus Viruses Inspection and Test Methods. Bulletin OEPP 21:241-244.

Raspberry ringspot virus

Common name(s): RRSV, ringspot disease of raspberry/strawberry/current, Lloyd George raspberry yellow blotch disease
Taxa: Viruses: Comoviridae: Nepovirus
EPPO A2 list: No. 98
EU Annex designation: II/A2

Organism
The Raspberry ringspot virus is present in Europe but localized and subject to quarantine. It is associated with raspberry (*Rubus idaeus*) and with other crops (cherry, strawberry) and transmitted mechanically through plant damage.

1. Detection
Immunological techniques such as indirect and sandwich ELISA and dot immunobinding assays (DIBA) have been carried out for detection of raspberry ringspot virus. It was concluded that sandwich DIBA is suitable for routine assays (Hutschemackers and Verhoyen 1989). A single DAS-ELISA test was developed for the simultaneous detection of several nepoviruses infecting grapevine (Etienne et al. 1991). The procedures for producing monoclonal antibodies (MAbs) against raspberry ringspot virus were developed with aim to increase the sensitivity (Snacken et al. 1992). The serological technique (DAS ELISA) was a useful tool to individuate for the first time the raspberry ringspot (Spak 1995). Among the molecular technique a highly sensitive isothermal AmpliDet RNA system is developed for specific detection of 15 isolates of different nematode-transmitted nepoviruses (Klerks et al. 2001).

2. Delimitation
No information available.

3. Monitoring
The occurrence and outbreaks of raspberry ringspot nepovirus has been monitored in an number of different crops (Bercks and Querfurth 1973, Shukla and Schmelzer 1974, Ahmed and Bailiss 1975, Kilevits and Miltin'sh 1977, Bremer 1983, Anonymous 1985, Honetslegrova and Spak 1995, Janeckova and Svobodova 1995, Nyerges et al. 2001, Subikova et al. 2002, Isac et al. 2004, Karesova et al. 2004, Rakhshandehroo et al. 2005). A sampling regime based on a regular grid lattice with 7 m between sampling points was indicated to be potentially efficient to for detection of nepoviruses and tobraviruses (Brown et al. 1990). A study was carried out to evaluate the optimal time for using ELISA for detection of sap-borne viruses (Kolbanova and Kukharchik 2001). The methods used for virus identification were: indicators plants (Kurcman 1977), electron microscopy and serology (Ahmed and Bailiss 1975), electron microscopy and the alkaline phosphatase ELISA variant (Brown et al. 1990), reactions of indicator plants and physico-chemical and serological properties (Gordejchuk et al. 1977), DAS-ELISA (Honetslegrova and Spak 1995), indicators plants and DAS-ELISA (Isac et al. 2004), ELISA (Janeckova and Svobodova 1995, Nyerges et al. 2001, Subikova et al. 2002, Karesova et al. 2004, Rakhshandehroo et al. 2005).

4. Commodities
Treatment of scions in warm water did not completely inactivate viruses in cherry, and better

results were obtained by keeping infected plants in chambers at 38°C for several weeks (Jacob 1974). Other result highlighted that the sanitation of virus-infected material can be carried out by dry air thermotherapy for 30 and 65 d at 38°C with further isolation of meristematic tips of various dimensions and their culture on various nutrients (Upadyshev 1996). The guidelines on testing procedures and recommended certification methods for the detection and identification have been summarized (Anonymous 1994). The method used for virus identification was via ELISA (Janeckova and Pluhar 1987).

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Rhacochlaena japonica Ito

(=*Euphranta japonica* (Ito))
Common name(s) Japanese cherry fruit fly
Taxa: Insecta: Diptera: Tephritidae
EPPO A1 list: No. 41 (as *Euphranta japonica*
EU Annex designation: I/A1

Organism
Rhacochlaena japonica is a fruit fly (Diptera Tephritidae) associated with cherries and possibly other *Prunus* species. It is absent from the EU/EPPO region and appears restricted to Japan.

1. Detection
The malaise trap and sweeping have been to survey for the presence of tephritid flies whilst the morphological characteristics of wings were used for identification (Sueyoshi 2005).

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
No information available.

References: 2 retained out of 3 retrieved, none added, 1 used for the summary
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Rhagoletis cingulata (Loew)

Common name(s): Eastern cherry fruit fly, North American cherry fruit fly
Taxa: Insecta: Diptera: Tephritidae
EPPO A1 list: No. 239
EU Annex designation: II/A1

Organism
Rhagoletis cingulata (Lw.) (Diptera: Tephritidae) is distributed in the eastern half of North America, east of Iowa (AliNiazee and Brown 1974) and also in south-eastern and south-central Canada. The principal cultivated hosts of *R. cingulata* are cherries. It differs from *R. indifferens*, which is distributed in west coast areas (AliNiazee and Brown 1974).

1. Detection
Sticky yellow panels showed a good efficiency in catching *R. cingulata*, the panels folded in the middle at an angle of 45° were as considerably more selective than standard vertical flat rectangle, the ammonium acetate alone was the best lure evaluated on panels for *R. cingulata* (Reissig 1976). A high number of captures is obtained with the sticky-coated red spheres of 7.5 cm in diameter in comparison with sphere of other dimensions (Prokopy 1977). The unbaited Rebell trap was the most effective and selective device evaluated for monitoring *R. cingulata* flies (Liburd *et al*. 2001).
In Germany, the first record of *R. cingulata* was carried out in Malaise-trap studies (Merz and Niehuis 2001). Significantly more flies were captured on unbaited Pherocon AM traps hung at 4.6 m than the trap collocated a lower height (Pelz-Stelinski *et al*. 2006). Pherocon (R) AM yellow sticky traps were installed in cherry trees permit to catch two specimens of *R. cingulata* in 2007 at different locations while none were caught in 2008 (Egartner *et al*. 2010).

2. Delimitation
A monitoring programme was started in 2002 in the cherry-growing area of Rhineland-Palatinate, Germany. The dispersal and seasonal abundance of the pest was surveyed by using yellow traps. The data obtained in 3 years revealed that, during this time, the insect had spread throughout the northern parts of the cherry-growing area (Rheinhessen) and also to the south (Lampe *et al*. 2005). In Netherlands pherocon AM yellow sticky pose a good method for monitoring the phenology of *R. cingulata*, provided the host plants in which they are placed are chosen carefully (Smit and Dijkstra 2008).

3. Monitoring
No information available.

4. Commodities
No information available.

References: 16 retained out of 47 retrieved, none added, 9 used for the summary

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Rhagoletis completa Cresson

Common name(s): Walnut husk fly
Taxa: Insect: Diptera: Tephritidae
EU Annex designation: I/A1

Organism

The principal hosts of *Rhagoletis completa* Cresson (Diptera: Tephritidae) are *Juglans* spp. In North America, *J. nigra, J. californica* and *J. hindsii* are attacked; under certain conditions peaches (*Prunus persica*) may be attacked. The range of this insect incluses Mexico and USA, it has been introduced in Italy and Switzerland. In the USA, the pest has one generation per year. Diapausing pupae overwinter in the first layers of the soil and adults emerging in the subsequent summer; larval feeding affects husk tissues, causing shell staining and darkened kernels.

1. Detection

A key was given for the identification of the larvae of *Rhagoletis suavis, R. completa* and *R. juglandis,* which damage the husks of various species of walnut (Steyskal 1973). There are numerous studies that evaluate the trapping methods of adults. The Zoecon Pherocon AM insect traps were most successful in study carried out Missouri (Gibson and Kearby 1978), the commercially available Pherocon AM trap was more effective than sticky food-carton trap baited with ammonium carbonate, the traditional monitoring method (Riedl and Hoying 1980). The combination of the visually attractive fluorescent-yellow rectangle and the ammonia-releasing dry bait ammonium carbonate proved to be a powerful trapping unit with application for monitoring (Riedl and Hoying 1981); moreover, it was highlighted that ammonia odour, generally considered a feeding-type olfactory stimulus, enhanced the response to yellow rectangles and green spheres several fold but at a loss of selectivity (Riedl and Hislop 1985). *R. completa* was recently detected in numerous European countries (Duso 1991b, Merz 1991, Anonymous 1992, Bjelis 2008), in this cases the yellow sticky traps were useful for the monitoring and determine the phenology of fruit fly (Miklavc *et al*. 2010).

2. Delimitation

After the first record, the distribution of *R. completa* was evaluated in reference to infestation level (Duso 1991a, Ciampolini and Trematerra 1992, Duso 1994, Seljak 1999). The yellow rectangular sticky traps were used to know the real distribution of the insect (Mani *et al*. 1994, Bouvet 2009), The GDL C26-2 cross trap with an ammonium-based attractant was more efficient than yellow sticky traps (Verhaeghe *et al*. 2009).

3. Monitoring

Principles for the monitoring of this species have been proposed; Pherocon AM-NB yellow panel traps baited with Trece supercharger lures (ammonium carbonate and placed in high and low canopy positions on the trees) were shown to be the best methods. In addition, highly-placed traps captured more insects as compared to low traps (Opp *et al*. 2000).

4. Commodities

The pest-free period for walnut husk fly *R. completa* was verified by trapping for adults in walnut orchards and roadside tree, the pest-free period guarantee obtain the fruit free from pest and suitable for the export in foreign countries, methyl bromide fumigation was evaluated as a quarantine treatment for use when the pest-free period is not in effect (Yokoyama *et al*. 1992,

Yokoyama and Miller 1994). However, the interceptions of *R. completa* were recorded (Kenis *et al*. 2007).

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Rhagoletis fausta (Osten Sacken)

Common name(s): Black cherry fruit fly
Taxa: Insect: Diptera Tephritidae
EPPO A1 list: No. 241
EU Annex designation: I/A1

Organism

The principal cultivated hosts of *Rhagoletis fausta* (Walsh) are cherries (*Prunus avium* and *P. cerasus*). The range of this species includes Canada and USA. Eggs are laid below the skin of the host fruit and hatch after 3-7 days. The larvae usually feed for 2-5 weeks. Pupation is in the soil under the host plant and this is the normal overwintering stage.

1. Detection

Adults of *Rhagoletis fausta* were captured on spheres 7.5 cm in diameter than on those 3.4 or 1.5 cm in diameter (Prokopy 1977). Sticky yellow panels were generally the most effective; moreover panels folded in the middle at an angle of 45°, were as effective as a standard vertical flat rectangle and considerably more selective (Reissig 1976). The characters for the identification of the main exotic species of the cherry pests have been described (Edland 1990). In a study on the comparison of the more common models of commercial trap, the unbaited Rebell traps offer the best trapping performance (Liburd *et al*. 2001).

2. Delimitation

Information not available.

3. Monitoring

The use of sticky traps to monitor *Rhagoletis fausta* was discussed by Leeper (1978). Subsequently, this led to the use of baited, fluorescent yellow sticky board traps as the preferred method, although these are not considered reliable at low densities. Traps are considered good indicators of first fly emergence, but they are not good indicators of the level of infestation (Compton *et al*. 2005).

4. Commodities

Information not available.

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Prokopy, R. J. 1977. Attraction of Rhagoletis flies (Diptera: Tephritidae) to red spheres of different sizes. Canadian Entomologist 109:593-596.

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Rhagoletis indifferens Curran

Common name(s): Western cherry fruit fly
Taxa: Insecta: Diptera: tephritidae
EPPO A1 list: No. 242
EU Annex designation: I/A1

Organism
Rhagoletis indifferens Curran (Diptera: Tephritidae) principally infests cherries (*Prunus avium*), but also *P. salicina* and other minor cultivated *Prunus* spp. It is distributed in west coast areas of North America as opposed to *R. cingulata* that occupies the eastern half of N America.

1. Detection
There are numerous studies that evaluated the performance of different trap types for the detection of this insect. Traps baited with ammonium carbonate are more efficient than yellow sticky boards or glycine-lye bait pans for determining the presence of different species of *Rhagoletis* (Madsen 1970); a combination of maize protein hydrolysate and maize steep liquor mixed with adhesive (Stikem Special) on double-faced yellow plywood boards attracted more adults than other traps (Banham 1973). Yellow Pherocon AM (apple maggot) traps usually caught more adults of *R. indifferens* than McPhail, Rebell and other traps tested (Burditt 1988). More recently the 10-cm red spheres baited with ammonium carbonate were indicated more efficient in the insect monitoring (Mayer *et al*. 2000). An adult caught on trap allowed the first record in Netherlands (Anonymous 2003); Pherocon (R) AM yellow sticky traps were installed to know the presence of *R. cingulata*, another North American pest of cherry, permitted to exclude the presence of *R. indifferens* in Austria (Egartner *et al*. 2010). A yellow rectangle traps coated with hot melt pressure sensitive adhesive (Alpha Scents, West Linn, OR) showed similar performance than traps coated with a sticky gel (SG) that can leave residues on the hands of users (Yee 2011).

2. Delimitation
The yellow rectangular sticky traps were placed in some regions of Switzerland and around the international airports of Zurich and Geneva, the results showed that *R. indifferens* was caught only in low numbers in a restricted area (Mani *et al*. 1994)

3. Monitoring
The spherical wooden traps were used to monitor the occurrence and phenology of *R. indifferens* (AliNiazee 1981).

4. Commodities
Cherries were randomly sampled and examined from packing houses, and larvae of *R. indifferens* were observed (Hansen *et al*. 2003). Fumigation of harvested sweet cherries with methyl bromide at a rate of 32 g/m^3 for 2 h at a fruit-pulp temperature of 23°C permitted an elevated mortality of larvae (Moffitt *et al*. 1977), while different combinations of methyl bromide rate and temperature were tested. It was thought that treatment of cherries at temperatures below 21°C would aid greatly in maintaining satisfactory quality of the treated fruit and allow greater flexibility in the scheduling and carrying out of commercial fumigations (Moffitt *et al*. 1983). The irradiation would be a potential quarantine treatment for *R. indifferens* larvae in cherries (Burditt and Hungate

1988). A combination of short duration high temperatures under low oxygen, elevated carbon dioxide atmospheric environment were developed to control of *R. indifferens* in sweet cherries; these treatments may provide, with further study, quarantine security in exported sweet cherries where fumigation with methyl bromide is not allowed (Neven and Rehfield-Ray 2006, Neven 2008). Another alternative was for high hydrostatic pressure (HPP) (Neven *et al*. 2007), and the use of radiographic techniques were investigated for their potential to detect internal pests in deciduous tree fruits (Hansen *et al*. 2005).

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Rhagoletis mendax Curran

Common name(s): Blueberry maggot
Taxa: Insecta: Diptera: Tephritidae
EPPO A1 list: No. 243
EU Annex designation: I/A1

Organism

The hosts of *Rhagoletis mendax* are cultivated and wild-harvested species of Ericaceae, especially *Gaylussacia* and *Vaccinium* spp. It is present in North America. Eggs are laid below the skin of the host fruit and hatch after 3-7 days. The larvae usually feed for 2-5 weeks. Pupation is in the soil under the host plant and this is the normal overwintering stage.

1. Detection

The sticky-coated traps showed that small rectangles painted with daylight fluorescent Saturn Yellow captured significantly more flies than any other rectangles tested and other colour, the addition of an odoriferous lure known to attract *Rhagoletis* significantly enhanced *R. mendax* attraction to yellow. The unbaited 7.5-cm red sphere captured a high number of adults (Prokopy and Coli 1978). A study highlighted a different behaviour between the adults such that immature females were captured most frequently with Pherocon traps, mature females and males by spheres (Neilson *et al*. 1984, Teixeira and Polavarapu 2001). Also if other study showed that yellow, green, red, and blue sphere traps (9 cm diameter) were equal to or better than Pherocon AM yellow board traps in attracting blueberry maggot, when if compared the flies per unit area, moreover it confirmed that the both sphere and yellow board traps baited with ammonia were significantly more attractive than unbaited traps (Liburd *et al*. 1998). In a study was evaluated the effect of trap installation (orientation and location) on the level of captures of adult *Rhagoletis mendax* (Geddes *et al*. 1989) also the traps protected from the prevailing wind were more efficient compared with exposed sites (Gaul *et al*. 1995). The addition of the volatiles emitted from its host show that *R. mendax* flies was more attracted this may be exploited to improve monitoring of these important fruit pests (Pelz-Stelinski *et al*. 2005). A short review described the potential methods of diagnostics based on morphological taxonomy and molecular biology for identification of the species of the family Tephritidae (Kinkorova and Pfunder 2005). Real-time PCR is a rapid and efficient method for identification of insect species was proposed to identification of *R. mendax* (Burgher-MacLellan *et al*. 2009).

2. Delimitation

Specific surveys were carried out to know the range of *R. mendax* in Canada, with Pherocon AM trap (Guibord *et al*. 1985, Vincent and Lareau 1989).

3. Monitoring

The insect was monitored in Canada (Straby *et al*. 1981, Anonymous 1983, 1986a, b, Blangez 1989, Anonymous 1995, Farvin 1996, Favrin 1998). To optimize the monitoring, trap numbers and trap spacing were discussed (Wood *et al*. 1983).

4. Commodities

Fumigation with 54% CO for up to 48 h did not adversely affect the quality of the berries and showed a high mortality of *R. mendax* larvae (Prange and Lidster 1992).

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Rhagoletis pomonella (Walsh)

Common name(s): Apple maggot
Taxa: Insecta: Diptera: Tephritidae
EPPO A1 list: No. 41
EU Annex designation: I/A1

Organism

Rhagoletis pomonella (Walsh) is a tephritid fly known also as the apple maggot fly. It is native to North America where it has switched to apples (*Malus pumila*) from *Crataegus* species (hawthorn). It is a serious threat to apple orchards all over the world but is currently largely restricted to North America.

1. Detection

Numerous trials have been carried out to evaluate traps to catch *Rhagoletis pomonella*. Sticky-coated yellow rectangles painted with yellow colour, particularly saturn yellow, were attractive to *R. pomonella* adult (Prokopy 1972). Sticky-coated, 45° angle, apex-down yellow was demonstrated very selective, moreover, unbaited sticky-coated red spheres showed higher efficiency also with mature flies (Prokopy 1975), and 7.5 cm spheres cached more adult than to any other size tested (Prokopy 1977), including unbaited 10 cm red spheres (Duan and Prokopy 1992). In another field test, it was demonstrated that the dark-coloured lures attracted many more than light-coloured ones (Rivard 1972). A yellow gypsy moth trap baited with a 5% soy hydrolysate and 50% ammonium acetate solution also showed a good efficiency (Reissig 1974). The sticky-red spheres baited with a blend of synthetic apple volatiles or ammonium carbonate captured significantly more male and females than unbaited spheres (Agnello et al. 1990, Reynolds and Prokopy 1997). Further work showed that baiting red sticky sphere traps with the volatile blend without ammonium additives created a highly effective and selective device for capturing apple maggot flies (Stenliski and Liburd 2002). Red baited spheres did not trap more adults than red unbaited throughout the entire season, whereas the red spheres did not trap more apple maggot adults than Pherocon AM traps in July, but baited spheres trapped more adults than Pherocon AM traps in the months of August and September (Kroening et al. 1989). Comparison between sticky-red spheres and Pherocon AM indicated that the later trapped more adults, but there was no difference in the number of female trapped (Johnson 1983), whereas the unbaited dark red wooden sticky spheres 8.5 cm in diameter caught more flies than either baited or unbaited yellow sticky rectangle (Drummond et al. 1984). Ladd yellow (a yellow panel 28 by 21.6 cm with 8.5 cm red hemispheres attached to each side) baited with apple volatile attractant or ammonium carbonate were the best traps in other trials (Aliniazee et al. 1987, Jones and Davis 1989). When the traps were positioned in orchards it was important to consider the competition with apple (Rull and Prokopy 2004). In a survey conducted in China in 1989-92 with traps, none of the 14 quarantine fruit flies pests, among which *R. pomonella*, were recorded (Chen and Tseng 1993).
The identification of this family of Diptera is crucial for quarantine measures at ports of entry, where species arrive in poor condition or in larval stage. Molecular methods based on DNA analysis are a promising tool for such diagnostic challenges and have already been used widely for quarantine insects (Kinkorova and Pfunder 2005).

2. Delimitation

After the first record in Oregon, traps placed in an urban grid system covering this and adjacent counties, where apples were grown commercially, caught no adults of this species except one in an apple tree (Anonymous 1980).

3. Monitoring

Surveys were carried out to evaluate the occurrence and population level of *R. pomonella*, the more wide and coordinate monitoring is the national monitoring carried out in Canada, that provide pest distribution information and maps for important pest (Straby et al. 1981, Anonymous 1995, Farvin 1996, Favrin 1998). Yellow sticky traps (Leeper 1978), alone or in association with red spheres trap (Felland et al. 1997, Chen et al. 2002) were a common tool to evaluate the presence of the insect. Pherocon AM Standard traps were useful to determining the timing of sprays (Neilson et al. 1976, Neilson et al. 1981), and the efficiency of different types of trap was tested (Trottier et al. 1975, Bostanian et al. 1993). Other authors suggested that the use Ladd trap with addition of pheromone were useful earlier in the season and when populations were low compared to unbaited traps (Warner and Watson 1991). It is highlighted that the position of trap could be important with respect to obtaining an accurate estimate of populations e.g. the red sphere showed loss of capturing power because of visual competition from red apples towards harvest, but this could be mitigated by adjusting trap positioning, so the red sphere seemed to be a better option for commercial growers, because Ladd traps are more expensive to purchase and more difficult to position (Rull and Prokopy 2003).

4. Commodities

R. pomonella could be introduced with imported fruit, indeed in 1979, USSR forbid the importation of apples from Canada and the USA (Tereshkova 1979). Irradiation seems be an efficient method for postharvest control of quarantine insects, considering that it does not compromise quality of most commodities (Follett et al. 2007).

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Rhagoletis ribicola Doane

Common name(s): Dark currant fly
Taxa: Insecta: Diptera: Tephritidae
EU Annex designation: I/A1

Organism
Rhagoletis ribicola (Diptera Tephritidae) attacks *Ribes* spp., especially gooseberries and red currants, it is present in North America. Eggs are laid below the skin of the host fruit and hatch after 3-7 days. The larvae usually feed for 2-5 weeks. Pupation is in the soil under the host plant and this is the normal overwintering stage. The insect is restricted to North America and is not found elsewhere.

1. Detection
Rhagoletis ribicola was recorded for the first time in Colorado during a trap survey (Kroening et al. 1989). A comparison of different trapping methods showed traps baited with ammonium carbonate to be more efficient than yellow sticky boards or glycine-lye bait pans, but the sticky boards seemed adequate for determining the presence of different species of *Rhagoletis*, among these also *R. ribicola* (Madsen 1970).

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
No information available.

References: 3 retained out of 9 retrieved, none added, 2 used for the summary
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Rhagoletis suavis (Loew)

Common name(s): Walnut husk maggot
Taxa: Insecta: Diptera: Tephritidae
EU Annex designation: I/A1

Organism
Rhagoletis suavis (Diptera Tephritidae) attacks *Juglans* spp. in North America, but it is recorded also on peach. Eggs are laid below the skin of the host fruit and hatch after 3-7 days. The larvae usually feed for 2-5 weeks. Pupation is in the soil under the host plant and this is the normal overwintering stage. Indigenous to North America, this species does not occur in the EU/EPPO region

1. Detection
A key to the larvae of *Rhagoletis suavis, R. completa* and *R. juglandis*, which damage the husks of various species of walnut in North America is available (Steyskal 1973). The trapping techniques for adult of *R. suavis* were discussed, the Zoecon Pherocon AM insect traps were most successful (Gibson and Kearby 1978).

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
No information available.

References: 2 retained out of 66 retrieved, none added, 2 used for the summary

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Rhizoecus hibisci Kawai and Takagi

Common name(s): Root mealybug
Taxa: Insecta: Hemiptera: Pseudococcidae
EPPO A1 listL No. 300
EU Annex designation: I/A2

Organism
Rhizoecus hibisci is a mealybug (Homoptera Pesudococcidae) from Japan, polyphagous on both monocotyledonous and dicotyledonous plants. It attacks the roots and causes non-specific symptoms on plants, such a shriveling and a crinkled appearance. It can be associated with bonsai plants. It has been reported to occur in North America, Asia, and locally within the EU.

1. Detection
The morphological description or identification key for adult female of *Rhizoecus hibisci* was reported from different authors (Kawai and Takagi 1971, Beardsley 1995, Williams 1996).

2. Delimitation
No information available.

3. Monitoring
No information available.

4. Commodities
Rhizoecus hibisci has been intercepted in recent years at quarantine inspections in the USA and in the Netherlands (Williams 1996), in England and Wales (Malumphy and Robinson 2004) on plants from various localities in eastern Asia.

References: 10 retained out of 58 retrieved, none added, 4 used for the summary

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Rhynchophorus ferrugineus (Olivier)

Common name(s): Red palm weevil, Asiatic palm weevil, coconut weevil, red stripe weevil
Taxa: Insecta: Coleopter: Curculionidae
EPPO list A2: No. 339

Organism

Rhynchophorus ferrugineus is a pest of palms (Arecaceae), attacking more than 20 species. The species has been recorded also on *Agave americana*. The red palm weevil is native to Southeast Asia and has spread to many countries across all the continents. The female weevil commences oviposition 1 to 7 days after mating and continues to oviposit up to 25 to 63 days laying about 275 eggs at the base of young leaves or in wounds to the leaves and trunks. The larvae feed on soft fibres and terminal bud tissues. They reach a size of more than 5 cm before pupation. Except just before pupating, they move towards the interior of the palm making tunnels and large cavities. Pupation occurs generally outside the trunk, at the base of the palms. The larva pupates in a cocoon made of brown dried palm fibres. The complete life cycle of the weevil, from egg to adult emergence, takes an average of 3-4 months.

1. Detection

A simplified key for identifying the two palm weevils *R. ferrugineus* and *R. palmarum* is a useful tool for detection (Sacchetti *et al*. 2006). The efficiency of pheromone was demonstrated in numerous field trials. Methyl-5-nonanol (ferrugineol), the most attractive component of the aggregation pheromone of *R. ferrugineus*, was synthesized and showed good results in capturing adult weevils (Gunawardena and Bandarage 1995). Studies on lures of different types and produced by different companies did not identify any lure with a certainly better efficiency (Faleiro *et al*. 2000, Krishnakumar and Maheswari 2003, Sujatha *et al*. 2006). This could depend on the climatic and environmental conditions as well as on distribution of infected palm trees. Similar consideration can be done for the trap colour, although there is weak evidence that red traps showed a better efficiency than white traps (Sansano Javaloyes *et al*. 2008, Al-Saoud *et al*. 2010). There is no evidence for an optimal height to place the traps (Hallett *et al*. 1999, Muthiah and Nair 2006, Mohammadpour and Avand-Faghih 2008, Al-Saoud 2010). The pheromone trap efficiency may be further increased by the addition of food baits (Abraham *et al*. 1999, Faleiro and Chellapan 1999, Muthiah *et al*. 2002, Muthiah *et al*. 2005, Kalleshwaraswamy *et al*. 2006). The importance of insecticidal solution and its periodically replacement to avoid insects from escaping has been highlighted (Faleiro and Satarkar 2002). Traps without insecticide solution but containing funnels were equally efficient to prevent escape of weevils (Hallett *et al*. 1999). A monitoring system based on acoustic sensors can be a possible technique for early detection of red palm weevil larvae in the interior of palms (Potamitis *et al*. 2009, Hussein *et al*. 2010). The performed tests have demonstrated that there is a good correlation between sound intensity around 2.250 Hz and the larvae density (Gutierrez *et al*. 2008). Although some authors suggested some tools to mitigate the problem of the high background noise (Mankin *et al*. 2008), the resolution of the signals emitted by healthy palms is sometimes impossible to discriminate from those of attacked plants (Pinhas *et al*. 2008). The acoustic method was used during the first detection in Spain (Tellez *et al*. 2006). A further possibility for detecting infested palm by red palm weevil is use of trained dogs (Nakash *et al*. 2000).

2. Delimitation

In some regions after the first record, a survey through traps was done to evaluate the distribution

of *R. ferrugineus* (Conti *et al*. 2008, Al-Eryan *et al*. 2010). In urban areas professionals involved in private gardens and public green spaces could provide useful information about incidence and spread of pest, as happened for *R. ferrugineus* in France (Chapin and Chauvel 2008).

3. Monitoring

After the first record that the weevils are present (Ferry 2010), the traps are a useful tool to confirm the weevil presence as has happened in Libya (Al-Eryan *et al*. 2010). Generic surveys on insect pests attacking date palms were carried out in Sultanate of Oman (Al-Zadjali *et al*. 2006) in Hainan Province on coconut trees (Lin *et al*. 2010), and in India on oil palm (Dhileepan 1992). Specific surveys were done to evaluate presence and incidence of *R. ferrugineus* (Vidyasagar *et al*. 2000, Ou *et al*. 2011), to evaluate factors affecting infestation (Alkhazal *et al*. 2009), and to make decision for pest management (Faleiro and Kumar 2008). For monitoring both pheromone traps and direct inspection were used to detect infestation (Faleiro and Kumar 2008).

4. Commodities

Traded palm trees are extensively transported between and within countries, which has afforded the spreading of the *R. ferrugineus* worldwide (Boavida and da Franca 2008, Pinhas *et al*. 2008, Llacer and Jacas 2010). The trade of infested planting material was the cause of introduction from Middle Eastern countries to Africa and Europe (Faleiro 2006) and probably also in many other regions. A safe quarantine treatment against this pest is the use of aluminium phosphide (Llacer and Jacas 2010). A waveguide irradiation chamber at 2.45 GHz. was locally constructed for controlled laboratory experiments to assess the potentiality of using microwave heating for irradiation of weevils (Ali and al-Jabr 2003).

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Rhynchophorus palmarum (Linnaeus)

Common name(s): Palm weevil, South American palm weevil
Taxa: Insecta: Coleoptera: Curculionidae
EPPO A1 list: No. 332
EU Annex designation: I/A1

Organism

The palm weevil *Rhynchophorus palmarum* is a South American species closely-related to the Asian species *Rhynchophorus ferrugineus*, from which it can be discriminated based on morphology. The weevil has a wide host range and occurs in Central and South America and the Caribbean but does not occur in the EU.

1. Detection

Numerous trials were carried out to evaluate the more efficient trap to catch *Rhynchophorus palmarum*. A simple aluminum trap baited with coconut tissue was described which has greased walls to prevent the escape of weevils, compared with the usual split-log trap, more than twice as many weevils were caught (Maharaj 1973). In another experiment the tank trap captured more insects than the trapdoor trap, regarding the baits sugarcane plus molasses or sugarcane only seem the more efficient (Moura et al. 1990). The identification of major component of the male-produced aggregation pheromone, 2-methyl-5(E)-hepten-4-ol (rhynchophorol) can be an important tool for the survey of *R. palmarum* (Jaffe et al. 1993). Buckets or bags with rhynchophorol and insecticide treated sugarcane were the most effective traps, whereas trap color did not influence capture rates. More weevils were captured in bucket traps placed at ground level than at 1.7 or 3.1 m (Oehlschlager et al. 1993). The poor results obtained with traps baited with the pheromone lures alone showed the need of adding a plant kairomone source to the pheromone to obtain higher captures of *R. palmarum* (Tiglia et al. 1998, Alberto Chavez et al. 1999, Duarte et al. 2003), Considering costs, the efficiency of mass trapping of R. palmarum with rhynchophorol may be increased through the use of bucket traps of 6 or 11 L baited with I kg of coconut, sugarcane or cassava. Trapping is made difficult by the requirement for replacement of water and food bait in traps (Osorio-Osorio et al. 2003). A study reported that propylene glycol extends the effective life of trap food bait from 2 weeks to 7 weeks (Oehlschlager 2007). Trials were carried out to evaluate the different release rates of the pheromone in capturing adults (Duarte and Lima 2001).

2. Delimitation

No information available.

3. Monitoring

A survey was carried out in 1990 on the entomofauna of oil palm plantations, signaling the presence of *R. palmarum* (Mexzon and Chinchilla 1991, Arroyo Oquendo et al. 2004). A novel and less expensive trap consisted of tied pieces of sugar cane sprayed with a contact insecticide plus aggregation pheromone lures, they were very reliable for small oil palm plantations (Moura et al. 1998).

4. Commodities

No information available.

References: 23 retained out of 235 retrieved, none added, 14 used for the summary

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Satsuma dwarf virus

Common name(s) SDV,
Taxa: Viruses: Secoviridae: Sadwavirus
EPPO A2 list: No. 279
EU Annex designation: II/A1

Organism
Satsuma dwarf virus is native to Asia and causes a number of symptoms on cultivated citrus, from smaller and deformed leaves to spots on fruits. It is transmitted by grafting but also mechanically. No vector is known to transmit the disease. It is found in Asia but has occurred in the EPPO region (Azeri, 1973).

1. Detection
Satsuma dwarf virus was identified from the typical symptoms produced by sap inoculation of *Phaseolus vulgaris*, blackeye cowpea and sesame (Azeri 1986). A serological method has been used for identification of the virus (Nozu et al. 1986, Shohara 1989), although the more commonly used technique is an enzyme-linked immunosorbent assay (ELISA) (Fidan and Azeri 1990, Hirashima et al. 1991). Enzyme linked immunosorbent assay (DAS-ELISA), SDS-PAGE and electro-blot immunoassays have been developed to evaluate the diversity of satsuma Dwarf virus and related viruses (Iwanami et al. 1993). A simple and rapid immune-chromatographic assay (ICA) to detect Satsuma dwarf virus (SDV) was developed using colloidal gold conjugates of anti-SDV monoclonal antibodies, the analysis is fast, complete in only 15 min, and more sensitive than double antibody sandwich-ELISA. Furthermore, ICA using the anti-SDV monoclonal antibodies could also detect SDV-related viruses (Kusano et al. 2007). The time and part of the plant sampled is important in the successful detection of Satsuma dwarf virus, revealing that the virus can only be detected in young shoots of Satsuma growing under cool temperature (Zhou et al. 1994). To facilitate the efficient detection of SDV, especially from the symptomless hosts, a reverse transcription-polymerase chain reaction (RT-PCR) was designed and optimized (Gao et al. 2006).
Observations in the field and transmission and back transmission tests by sap inoculation from infected white sesame to white sesame were used to first detect the virus in China (Cui et al. 1991); the sap inoculation tests were used for confirmed the first record of Satsuma dwarf virus in mandarins in Turkey as well (Azeri 1973).

2. Delimitation
No information available.

3. Monitoring
A survey was carried out in mandarin trees in China to evaluate the presence of satsuma dwarf virus using the indicator white sesame and ELISA to identify the virus (Zhou et al. 1995). More generic surveys concerning the incidence of virus in citrus trees were carried out in different regions of Korea (Kim et al. 1999, Kim et al. 2001) and within mandarin orchards in Turkey (Onder and Korkmaz 2008).

4. Commodities
Imported trees were possible source of spread in new areas (Zhou et al. 1993). The importance of

detection methods (DAS-ELISA, immune-chromatographic assay (ICA), and RT-PCR) were highlighted in order determine the distribution of SDV-free nursery plants, which is seen as a key control measure (Iwanami 2010). Viral elimination from mother plants has been conducted to produce virus- and viroid-free nursery plants. In citrus, shoot tip culture and semi-micrografting combined with thermotherapy were adopted for virus elimination; however, these methods should be modified for ease or higher efficiency (Ohta et al. 2011).

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Scaphoideus luteolus van Duzee

Common name(s): White-banded leafhopper
Taxa: Insecta: Hemiptera: Cicadellidae
EPPO A2 list: No. 72 (deleted in 1984)
EU Annex designation: II/B

Organism
The adults of *Scaphoideus luteolus* prefer to stay in the inner part of the crown of trees and the eggs are laid in the cork parenchyma of elm bark, within which they overwinter. The eggs hatch in April and May and there are five nymphal instars. The adults are not seen until late June and they are usually found in the inner parts of the crown. Only one generation a year usually occurs. The species is widely distributed in Eastern United States. The species is a vector of elm phloem necrosis phytoplasma (Nielson 1968, Lanier et al. 1988), a highly destructive disease of *Ulmus* spp., a phytoplasma that is widespread in North America but absent in the EPPO region.

1. Detection
No information available.

2. Monitoring
No information available.

3. Delimitation
No information available.

4. Commodities
No information available.

References: 5 retained out of 9 retrieved, 1 added, 2 used for summary

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Scirrhia acicola (Dearness) Siggers

(= *Mycosphaerella* dearnessi Barr)
Common name(s): Brown spot needle blight
Taxa: Fungi: Ascomycota: Mycosphaerellaceae
EPPO A2 list: No. 22
EU Annex designation: II/A1

Scirrhia pini Funk & A.K Parker

(= *Mycosphaerella pini* Rostrup)
Common name(s): Red band needle blight
Taxa: Fungi: Ascomycota: Mycosphaerellaceae
EPPO: not classified
EU Annex designation: II/A2

Organism
Both species are distributed worldwide on *Pinus*, on which they cause ring needle blight (Dothistroma needle blight) and premature needle cast (Anonymous 2008). In Europe, *S. pini* is common, especially at warm locations (Karadzic 1987, Karadzic 1989) but is expanding northwards (Muller et al. 2009), while *S. acicola* has been seldom detected. They can co-occur at the same site (Jankovsky et al. 2009). Reasons for the recent increase in disease incidence are unclear but could be due to increased rainfall in spring and summer coupled with a trend towards warmer springs, optimizing conditions for spore dispersal and infection (Brown and Webber 2008). Both fungi produce a toxin (dothistromin) that is weakly mutagenic and a clastogen (materials that can cause the breakup of chromosomes) rendering it a potential carcinogen. Although the risks to forest workers are considered very low, it is prudent to avoid unnecessary exposure during periods when dothistromin levels are likely to be at their peak (Bradshaw 2004).

1. Detection
The symptoms are similar to, and can be confused with, those of other needle pathogens such as several *Lophodermium* and *Ploioderma* species. Identification can only be made when the conidia mature or by using DNA analysis (Huang et al. 1995, Barnes et al. 2004, Barnes et al. 2008a, Barnes et al. 2008b). Full descriptions of morphological variation of both these fungi in native Central American pine forests are given by Evans (1984). The spores are wind-blown and can also be spread by insects and on forestry equipment, especially shearing tools (Skilling 1974).

2. Delimitation
Spore traps were used in Austria to delimit the infested area. The number of conidia of *S. acicola* in the spore traps was low and the spread of the disease into the pine forests surrounding the town was not detected (Brandstetter and Cech 2003).

3. Monitoring
In Austria, after the eradication of all infested trees, no diseased pines were detected in 2001 and 2002 and few spores were detected in spore traps (Brandstetter and Cech 2003).

4. Commodities

Intercontinental movement is possible in seed lots contaminated with needle debris and in plants for planting. First detection has been often done on ornamental material (plants for planting) (Suto and Ougi 1998).

References: 22 retained out of 102 retrieved, none added, 15 used for the summary

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Scirtothrips aurantii (Faure)

Common name(s): South African citrus thrips
Taxa: Insecta: Thysanoptera: Thipidae
EPPO A2 list: No. 221
EU Annex designation: II/A1

Organism

Usually considered to be a pest of citrus, especially oranges in Southern Africa, this species of thrips has been found on over 50 plant species from a wide range of families. *Scirtothrips aurantii* is native to Africa and all stages feed on epidermal cells of young leaves and the apex of young fruit. They do not feed on mature leaves. Eggs are inserted into young tissues. There are two nymphal stages followed by two pupal stages. Pupation occurs on the ground amongst leaf litter. Breeding is almost continuous although development slows in winter. Adults are probably dispersed downwind but early infestations in citrus orchards may be due to overwintering adults. The potential for natural spread is limited. In international trade, *S. aurantii* could be carried on plants for planting, but interceptions are relatively rare. Only seedlings or cuttings with young growing buds are liable to carry these pests. Only young fruits are attacked so the risk of being carried on harvested fruits is small.

1. Detection

Symptoms of infestation include silvering of the leaf surface, linear thickenings of the leaf lamina, brown frass marking on leaves and fruit, grey to black markings on fruit often forming a ring around the apex; ultimately fruit distortion and early senescence of leaves (Anon, 2005). Considering the small size of the insect, direct visual searching is insufficient to ascertain presence and the electric Berlese method should be used (Anon, 2005).

Members of *Scirtothrips* are distinguished from all other Thripidae by morphological characteristics (Anon, 2005). Identification of *Scirtothrips* spp. is based on male or females adults, both of which are winged (Anon, 2005). A combination of morphological and molecular data have been used to determine relationships between populations of *Scirtothrips* (Hoddle et al., 2008; Morris and Mound, 2004; Timm et al., 2008).

Scirtothrips aurantii is highly attracted to fluorescent yellow and Saturn Yellow sticky traps that have been successfully used to monitor populations and to time treatments (Samways, 1986). Yellow traps were found to be superior to green, red, white and blue variants (Grove et al., 2000a). However, Saturn Yellow sticky traps have not been not commonly used due to their costs, tendency to fade and cumbersome nature (Grout and Richards, 1989). Grout and Richards (1989) used inexpensive non-fluorescent yellow PVC card traps to obtain treatment thresholds.

2. Delimitation

No information found.

3. Monitoring

Samways (1986) found that three fluorescent Saturn Yellow traps per orchard (>5000 trees) were practicable to use, with the traps placed on the shady sides of the trees to reduce fading of the expensive fluorescent yellow material.

In mango orchards Grove et al. (2000b; 2000c) assessed trap catches and visual inspection of fruit

in order to determine when control measures were undertaken. Observations were made from fruit-set until harvest. In each orchard 10 fruit on each of 10 randomly selected trees were visually inspected and the numbers of nymphs and adults noted. Lesions on the fruit were also assessed for severity. Three yellow PVC card traps were used in each orchard. Traps were suspended 1.5-2m high on the northern side of trees diagonally across the orchard, with one in the centre and the other two near opposite corners, approximately 4m apart. Traps were removed weekly and replaced with new ones. Removed traps were transported in clear plastic wrapping and numbers of thrips determined.

In addition to yellow sticky traps and fruit inspections, Grove et al., (2000c) also used dispersal emergence traps to sample mature nymphs and adults. Each trap consisted of a PVC irrigation pipe (130mm in diameter, 100mm in height). The trapping surface was a glass plate with a sticky surface placed on top of the pipe. Traps were placed 300mm from the trunk of three randomly chosen trees, on the northern side. Glass plates were changed weekly and moved to an adjacent tree. Counts were also made of thrips on fruit and in flowers with eggs and emergence holes on new leaves recorded. The yellow sticky traps and counts on fruits were assessed to be effective and practical methods for use by fruit producers to monitor thrips population activity. However, trap catches measure only flying adults (provides an early warning of infestation), whereas counts on fruit determined the presence of both adults and nymphs. Trap catches are also influenced by the number of thrips present on new growth and are more likely to be influenced by weather conditions. On sticky traps *S. aurantii* needs to be distinguished from other thrips species.

Fruit counts are less time consuming than yellow sticky traps. Grove and Pringle (2000) developed a sampling plan consisting of monitoring 10-25 trees and examining not more than 50 fruit per orchard in order to obtain accurate population estimates for pest management purposes.

4. Commodities

No information found.

References: 26 retained out of 130 retrieved, none added, 11 used for the summary

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Scirtothrips citri (Moulton)

Common name(s): California citrus thrips
Taxa: Insecta: Thysanoptera: Thripidae
EPPO A2 list: No. 222
EU Annex designation: II/A1

Organism

Scirtothrips citri is primarily a pest of *Citrus* in southern parts of North America although it has been found infesting more than 50 different plant species. It is of greatest importance on navel oranges and lemons in California. The life history is similar to *S. aurantii*. Development does not occur below 14^{o}C. Eggs are laid in young tissues of leaves, fruit or green twigs; overwintering eggs are laid in the last flush growth of the season. Pupae are found on the ground or in bark crevices. Typically, *S. citri* densities are highest on tree tops and lower in the interiors. The potential for natural spread is relatively limited. In international trade, *S. citri* may be carried on plants for planting but interceptions are relatively rare. Only seedlings or cuttings with young growing buds are liable to carry these pests. Because only young fruit are attacked, the risk of being carried on harvested fruits is small. There is no evidence that *S. citri* has been dispersed beyond its natural range by human activity.

1. Detection

Feeding damage often results in a conspicuous ring of scarred tissue around the apex of young fruit. Most economic damage to fruit occurs in the first 3-6 weeks after petal fall. Heavily scarred fruit show more rapid weight loss than undamaged fruits. Damage is greatest to fruit growing at the outside of the canopy. For *S. citri* the examination of leaves not particularly useful because nymphs are almost exclusively localised to young growing buds, young leaves, sepals and young fruits. Considering the small size of the insect, direct visual search is insufficient and the electric Berlese method should be used (Anon, 2005).

Members of *Scirtothrips* genus are distinguished from all other Thripidae by morphological characteristics (Anon, 2005). Identification of *Scirtothrips* spp. is based on male or females adults, both of which are winged (Anon, 2005). Combinations of morphological and molecular data have been used to determine relationships between populations of *Scirtothrips* (Hoddle et al., 2008).

Methods of detection include yellow sticky traps, ground traps, foliage D-Vac, fruit and foliage counts, pupae papers and emergence traps (Rhodes and Morse, 1989; Grout et al., 1986). *Scirtothrips citri* responds significantly better to fluorescent yellow than to any of the other coloured polyvinyl rectangular traps available, with triangular, elliptical, and rectangular shapes preferred over circular and square ones (Moreno et al., 1984).

Reed and Rich (1975) developed a dispersal and emergence trapping technique where full grown nymphs or prepupae were caught as they dropped to the ground to pupate and adults captured as they emerged. More immatures fell within 0.3m radius around the trunk of the tree and on the north side of the tree, indicating that they were negatively phototactic. Tanigoshi and Moreno (1981) compared traps for monitoring populations of *S. citri*. The PVC-acetate trap was comparable to the Reed-Rich trap in terms of number of thrips captured but had advantages in reduced weight, reduced counting time and allowed for the use of inexpensive, disposable sampling materials.

2. Delimitation

No information found.

3. Monitoring

Monitoring thrips occurring within the canopy of trees is problematic as sweeping is not practical and visual surveys difficult and time consuming.

Rhodes and Morse (1989) assessed various sampling methods (foliage D-Vac, fruit counts, ground traps, pupation papers, and yellow sticky cards) in order to assess treatment thresholds. D-Vac samples were taken twice weekly from 50 adjacent trees. Ground traps monitored late second instars migrating from the trees to pupate and were placed under 5 non-adjacent trees with a single trap place under each tree and the centre of the trap about 30 cm from the tree trunk; the traps were changed twice a week. Pupation papers were used to monitor migrating late second instars and were attached to twigs about 30cm inside the canopy on 20 non-adjacent trees and counted twice weekly. Yellow sticky cards monitored adults and were suspended 1.5m above the ground on the outside of the canopy on five non-adjacent trees; the cards were changed twice weekly. Fruit counts assessed first and second instars and were counted twice a week on 20 randomly selected fruits from five non-adjacent trees. End of season scar counts on 20 adjacent trees were taken prior to harvest on all outside fruit from knee to eye level. Yellow cards and ground traps were found to be too imprecise and the ground traps and D-vac were too labour intensive. Pupation papers provided the best commercially feasible method of monitoring late second instars and early season activity. Fruit counts provide an accurate measure of attack and may be used for threshold-based treatment strategies. Frequent, at least every 3 days, fruit monitoring is essential to detect economic activity levels.

Hare et al., (1989) monitored thrips densities using a combination of foliage counts (in the spring before formation of susceptible fruit and again during the summer after fruit was >2.5cm in diameter) and fruit counts. The number of thrips per 25 distal cm of foliage was monitored on each of 2- 4 terminals per tree, with the number of thrips on each of 10 fruits per tree assessed. Scarring on fruits was quantified according to a scale.

4. Commodities

No information found.

References: 14 retained out of 102 retrieved, 1 added, 8 used for the summary

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Scirtothrips dorsalis Hood

Common name(s): Chilli thrips, yellow tea thrips
Taxa: Insecta: Thysanoptera: Thripidae
EPPO A2 list: No. 223
EU Annex designation: II/A1

Organism

Scirtothrips dorsalis is polyphagous and widespread. Native hosts are probably various species within the Fabaceae, but it is known as a pest on many crops including *Capsicum*, *Citrus*, cotton, onions, roses, strawberry, grapevine and tea. It is only cited as a significant pest of Citrus in Japan and Taiwan. *Scirtothrips dorsalis* is present in tropical Asia, in Oceania, Sub-Saharan Africa, and Central America and has recently been introduced into the USA (Hawaii, Texas and Florida). It has also been found in a tropical glasshouse in the U.K.

The biology is similar to that of other *Scirtothrips* spp. Eggs are inserted into young and soft tissues of leaves, stems and fruit. The first and second instars are found on the green parts of plants from which the second instar seek out some shelterd place (leaf litter or bark crevices) in which to pass through the two resting stages (propupa and pupa). Winged adults are found on the green parts of plants where they feed. The occurrence of *S. dorsalis* typically coincides with the flowering of host plants. As well as the damage caused by feeding, *S. dorsalis* is also known to transmit several plant viruses (Mound and Palmer, 1981). The potential for natural spread is relatively limited. Spread could be facilitated via trade of host plants. Only seedlings or cuttings with young growing buds are liable to carry these pests. Only young fruits are attacked so the risk of being carried on harvested fruits is small.

1. Detection

In common with other members of the genus, this species can cause considerable distortion to young leaves on many of the crops upon which it feeds. Symptoms are silvering of the leaf surface, linear thickenings of the leaf lamina, brown frass markings on leaves and fruit, grey to black markings on fruits often forming a conspicuous ring of scarred tissue around the apex; ultimately fruit distortion and early senescence of leaves results. Thrips are especially difficult to detect in low numbers. Considering the small size of the insect, direct visual search is insufficient and, as with other related species, the electric Berlese method should be used. It should be noted, however, that the Berlese method's efficacy has not been evaluated for *S. dorsalis* (Anon, 2005; Sullivan and Breiter, 2007).

Members of *Scirtothrips* genus are distinguished from all other Thripidae by morphological characteristics (Mound and Palmer, 1981). Identification of *Scirtothrips* spp. is based on male or females adults, both of which are winged (Anon, 2005). A combination of morphological and molecular data has been used to determine relationships between populations of *Scirtothrips* (Hoddle et al., 2008). Toda and Komazaki (2002) have investigated the use of PCR-RFLP to identify thrip species including *S. dorsalis*. However, these methods require that individual insect samples be whole, undamaged and without contaminating substances.

Visual observation of plant damage symptoms coupled with yellow sticky traps can be utilised as a preliminary detection tool. Positive detections can then be followed up by direct plant sampling to capture individuals for taxonomic verification. Other methods include shaking plants to remove thrips, sticky suction traps, emergence traps, removal of plant material and direct counting of

thrips, however these methods may not be suitable for an early detection survey when numbers are low.
Thrips are highly attracted to the colour yellow with yellowish-green traps collecting more adult *S. dorsalis* (Tsuchiya et al., 1995) that other coloured traps. Aliakbarpour and Rawri, (2010) assessed the shaking of mango panicles over a plastic tray, washing the panicle with ethanol , immobilisation of thrips using CO_2 and yellow sticky traps to determine the most effective technique to capture *S. dorsalis*. The CO_2 method was the most effective non-destructive method, although yellow sticky traps were considered the easiest method of detection.
Chu et al., (2006) compared sticky traps, the plastic cup (CC) traps and blue D traps for monitoring *S. dorsalis*. Yellow sticky traps caught more thrips than the CC traps but they also caught a large number of non-target insects. The blue D trap did not consistently capture greater numbers than the CC trap. The sticky traps were less labour intensive, required less component assembly and less expertise in trap placement compared to CC traps.

2. Delimitation
No information found.

3. Monitoring
In monitoring surveys of grape vines in Florida, malformed fruits and foliage were examined during inspections of potential host material (Sullivan and Breiter, 2007). However visual inspections tend to be ineffective if thrips densities are low. Five to 20 leaves from symptomatic plants are collected at random and placed in sealed bags. The foliage is then sent to a laboratory where it is washed in 70% ethanol to remove any thrips. The alcohol and thrips are screened through a mesh diameter of 0.5mm or less and observed under a microscope. Identifications are made by morphological and/or molecular diagonistics. The use of yellow sticky traps is not recommended as the thrip specimens may be damaged, thereby hindering morphological identification.
Silagi and Dixon (2006) describe the sampling of garden centres for *S. dorsalis*. Plants were observed for signs of thrips presence as well as feeding damage. If thrips were present and/or plants exhibited damage, samples were subsequently taken and placed in plastic bags and sent to the laboratory for identification.

4. Commodities
A commodity specific phytosanitary procedure is in place for sampling and identification of *S. dorsalis* in strawberry plants (Anon, 2008). Young growing buds are examined carefully and samples taken to the laboratory for identification.

References: 56 retained out of 126 retrieved, 3 added, 10 used for the summary
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Tsuchiya, M., S. Masui, Kuboyama, N. (1995). Color attraction of yellow tea thrips (*Scirtothrips dorsalis* HOOD). Japanese Journal of Applied Entomology and Zoology **39**(4): 299-303.

Septoria lycopersici Spegazzi var. *malagutii* Ciccarone & Boerema

Common name(s): Septoria leafspot, annular leafspot
Taxa: Fungi: Ascomycota: Mycosphaerellaeae
EPPO: A1 No. No. 142
EU Annex designation: I/A1

Organism
Initially described as *Septoria lycopersici* var. *malagutii* from South America (Piglionica et al. 1978), the fungal pathogen has been redescribed as *Septoria malagutii* after a comparison with specimens of *S. lycopersici var. lycopersici* based on sequence rRNA ITS regions and (TEF)-1 alpha genes. The "var. *malaguti"* conidia proved to be significantly longer and narrower with the conidiogenous cells significantly shorter and narrower than the "var. *lycopersici*" cells. Although the sequences were similar - ITS at 99.6% and TEF-1 alpha at 99.2% - the "var. *lycopersici*" ITS was more similar to several other *Septoria* species than to its "var. *malagutii*" counterpart, suggesting that the latter should recognized as a distinct species (Cline and Rossman 2006).

1 Detection
No information available.

2 Delimitation
No information available.

3 Monitoring
No information available.

4 Commodities
No information available.

References: 3 retained out of 6 retrieved, none added, 2 used for the summary
Cline, E. T. and A. Y. Rossman (2006). "Septoria malagutii sp nov., cause of annular leaf spot of potato." Mycotaxon 98: 125-135.
Piglionica, V., G. Malaguti, et al. (1978). "Septoria disease of potato." Phytopathologia Mediterranea 17(2): 81-89.

Spiroplasma citri Saglio *et al.*

Common name(s): Stubborn, little leaf
Taxa: Bacteria: Tenericutes: Spiroplasmataceae
EPPO (in A2 list, *Circulifer tenellus* reported as vector of *Spiroplasma citri*)
EU Annex designation: II/A2

Organism
Spiroplasma citri, a helical, wall-less prokaryote of the class Mollicutes, is the causative agent of lethal stunting diseases in a number of host plants including citrus and several brassica species. Like other phytopathogenic mollicutes (spiroplasmas and phytoplasmas), it is naturally transmitted by phloem-feeding insects (Berg *et al*. 2001).

1. Detection
A range of serological methods have been developed of which ELISA was the most common (Clark *et al*. 1978, Saillard *et al*. 1978, Hafidi *et al*. 1979, Remah *et al*. 1980, Raju and Nylund 1981). ELISA has been used for detection of the spiroplasma in both plant tissue and the insect vector (Archer *et al*. 1982). The latex agglutination test (LAT) can be used as a rapid and simple qualitative assay for detecting and identifying *S. citri* in culture (Fletcher and Slack 1986). Another detection method uses dot-blot hybridization (Bove *et al*. 1987). To confirm the identification some authors have simultaneously used two different techniques, for example ELISA and dimensional gel electrophoresis (Caglayan and Cnar 1990). In recent years, the serological techniques were augmented by molecular techniques such as dot-immunobinding (Zheng *et al*. 1988) and PCR techniques (Saeed *et al*. 1994, Bove and Garnier 2002, Mahrous *et al*. 2005). Real time PCR techniques have subsequently been developed to improve sensitivity (Yokomi *et al*. 2008).

2. Delimitation
No information available.

3. Monitoring
A survey on leafhopper vector species (Kersting and Sengonca 1992, Kersting and Baspinar 1997) on host or weed species (Sertkaya and Cinar 2002) was carried out to test the presence of *S. citri* by ELISA. ELISA and PCR techniques were used to evaluate the presence of *S. citri* in Tunisia (Najar *et al*. 1998). A monitoring exercise concerning the occurrence and distribution of virus and virus-like diseases highlighted the presence of S*. citri* in citrus growing area in North-West Frontier Province of Pakistan (Arif *et al*. 2005).

4. Commodities
Trade of citrus stock is an important cause of spread of this virus. This fact has emphasised the importance of the production of indexed virus-free mother or foundation stocks, which are included in a budwood certification program (Roistacher 1986).

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Berg, M., U. Melcher, and J. Fletcher. 2001. Characterization of Spiroplasma citri adhesion related protein SARP1, which contains a domain of a novel family designated sarpin. Gene 275:57-64.
Kersting, U. and H. Baspinar. 1997. Occurrence and distribution of Spiroplasma citri and sesame phyllody in sesame in Southern Anatolia. Journal of Turkish Phytopathology 26:1-9.

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Spodoptera eridania (Cramer)

Common name(s): Southern army worm
Taxa: Insecta: Lepidoptera: Noctuidae
EPPO A1 List: No. 196
EU Annex designation: I/A1

Organism

Spodoptera eridania is present in Central and South America and the southern states of the USA. It is a polyphagous generalist feeder that may cause damage to aubergines, *Beta*, *Capsicum*, cassava, cotton, several brassica species, a wide range of legumes, maize and other Poaceae, potatoes, sweet potatoes, tobacco, tomatoes, yams, and many pot plants and vegetables. The larvae feed on the leaves and in severe infestations complete defoliation may occur.

1. Detection

Identification is based on morphological characteristics of the larvae and adults (Crumb 1956, Levy and Habeck 1976, Todd and Poole 1980).

2. Delimitation

No relevant references found.

3. Monitoring

Pheromones have been used to monitor for *S. eridania*. The female-produced sex pheromone for *S. eridania* has been reported to be a mixture of (Z,E) -9,I2-tetradecadien-I-ol acetate (Z,E-9,I2-TDDA) and (Z) -9-tetradecen-l-ol acetate (Z-9.TDA)(Jacobson *et al*. 1970). However, in field trials conducted in Florida it was found that neither Z,E-9,12-TDDA nor Z-9-TDA were attractive alone or in combination to male S. *eridania* and it was concluded that the pheromone identification was incorrect or incomplete (Mitchell and Doolittle 1976). A later study to identify the sex pheromone found that a number of 14-carbon mono-and diunsaturated acetates and a monounsaturated 16-carbon acetate were produced by calling females and field trapping studies indicated that the volatile blend composed of (Z)-9-tetradecen-1-ol acetate (60%), (Z)-9-(E)-12-tetradecadien-1-ol acetate (17%), (Z)-9-(Z)-12 tetradecadien-1-ol acetate (15%), (Z)-9-(E)-11 tetradecadien-1-ol acetate (5%) and (Z)-11-hexadecen-1-ol acetate (3%) was an effective trap bait for males of this species (Teal *et al*. 1985). A pheromone blend of (Z)-9-tetradecen-1-ol acetate (55 78%), (Z,E)9,12-tetradecadien-1-ol acetate (21.23%), (Z,E)-9,11-tetradecadien-1-ol acetate (8.67%) and (Z)-11-hexadecen-1-ol acetate (14.32%) has also been found to be effective for use in traps to monitor for male *S. eridania* (Mitchell and Tumlinson 1994).

4. Commodities

No information

References: 11 retained out of 77 retrieved, 4 added, 7 used for summary

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Spodoptera frugiperda (Cramer)

Common name(s): Southern armyworm
Taxa: Insecta: Lepidoptera: Noctuidae
EPPO A1 List: No. 197
EU Annex designation: I/A1

Organism

Spodoptera frugiperda is a polyphagous species with a preference for the Poaceae. Among North American populations of this species there is a high level of genetic variability as has been demonstrated by restriction fragment length polymorphism (RFLP) analysis of genomic DNA (Lu *et al*. 1992). There are two 'host strains' that are defined by a preference for either large grasses such as corn and sorghum (corn strain) or small grasses such as rice and bermudagrass (rice strain).

1. Detection

Populations have been characterized in the United States using PCR-based methods and haplotypes examined to determine patterns of inbreeding and potential sources of migrants and outbreaks (Pashley *et al*. 1985, Nagoshi and Meagher 2003, Nagoshi *et al*. 2006, Nagoshi *et al*. 2008). Mass migration has been observed using airborne radar (Wolf *et al*. 1990). A polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) technique has been developed to distinguish *S. frugiperda* from six other closely related noctuid species commonly found in pheromone traps (Lewter and Szalanski 2007).

2. Delimitation

No relevant references found.

3. Monitoring

Monitoring for *S. frugiperda* was initially undertaken using traps containing virgin females to attract the male moths (Snow and Copeland 1969, Greene *et al*. 1971) or light traps (Dominguez 1974, Silveira Neto *et al*. 1975). Identification of the female-produced sex pheromone enabled the use of the synthetic pheromone in lures in traps. Originally (*(Z)* -9-tetradecen-l-ol acetate (Z-9.TDA) and *(Z)* -9-dodecen-l-ol acetate (Z-9-DDA)) were reported to be the pheromones of *S. frugiperda*, although use of Z-9-TDA alone in a field bioassay did not attract male moths (Mitchell and Doolittle 1976), but Z-9-DDA was attractive (Mitchell and Doolittle 1976, Tingle and Mitchell 1978, Ward *et al*. 1980). A later study examined the use of a four component blend that consisted of (percentage by weight) (Z)-7-dodecen-1-ol acetate (Z7-12:AC), (0.45%), (Z)-9-dodecen-1-ol acetate (0.25%), (Z)-9-tetradecen-1-ol acetate (Z9-14:AC) (81.61%), and (Z)-11-hexadecen-1-ol acetate (17.69%) and this was found to be highly effective when formulated at 2 mg total pheromone blend in rubber septa, polyvials, or microtubules (Mitchell *et al*. 1985). A two-component pheromone blend consisting (percentage by weight) of 0.58% Z7-12:AC and 99.42% Z9-14: AC on rubber septa dispensers (2 mg total blend) was also highly effective as a lure for *S. frugiperda* males and the authors suggested that the two-component blend could be substituted for the four-component pheromone blend for survey purposes (Mitchell *et al*. 1985). (Tumlinson *et al*. 1986) reported that both (Z)-7-dodecen-1-ol acetate and (Z)-9-tetradecen-1-ol acetate are required for optimum activity and that this blend is a significantly better lure than either virgin females or 25 mg of (Z)-9-dodecen-1-ol acetate in a polyethylene vial.

Variations in the pheromone blend released by female moths from different geographical areas have been determined (Batista-Pereira *et al*. 2006). Differences in the pheromones of the corn and

rice strains have also been observed (Groot *et al*. 2008).

Factors affecting trap catch have been examined. It is reported that wind direction affected the area on the surface of sticky traps where moths landed with significantly more moths captured on the side of the trap downwind from the pheromone source (Tingle and Mitchell 1979). The location of pheromone-baited sticky traps placed in or along the edge of maize fields made no difference in captures of *S. frugiperda* adults when plant height was less than or equal to trap height (Tingle and Mitchell 1979). Traps placed 1.2 m above ground level in groundnut fields captured significantly more moths than those at heights of 0.7 and 0.5 m and traps 25 m inside the fields captured significantly more males than traps at field-edge or 50 m into fields (Tingle and Mitchell 1979).

Trap type affects the number of moths caught and in a comparison of sticky trap types Pherocon 1C and Niutrap traps captured more moths than Delta PBW models (Busoli 1984). Other studies also examined the effect of trap type (Adams *et al*. 1989, Mitchell *et al*. 1989, Pair *et al*. 1989, Malo *et al*. 2001, Meagher and Mitchell 2001, Salas 2003) and trap colour (Mitchell *et al*. 1989, Pair *et al*. 1989, Meagher 2001a)

In a laboratory study, the addition of phenylacetaldehyde to a pheromone lure increased upwind flight of male moths compared with the pheromone lure alone (Meagher and Mitchell 1998). However, in a field study addition of phenylacetaldehyde reduced numbers of moths captured compared to pheromone-baited traps (Meagher 2001b).

4. Commodities

No relevant references found.

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Spodoptera littoralis (Boisduval)

Common name(s): Cotton leafworm, Egyptian cottonworm, Mediterranean brocade moth
Taxa: Insecta: Leidoptera: Noctuidae
EPPO A2 List: No. 120
EU Annex designation: I/A2

Organism

Spodoptera littoralis is one of the most destructive lepidopteran insects within the tropical and subtropical areas, attacking numerous economically important crops all year round (Staneva 2009). It is found in Africa (Algeria, Angola, Benin, Burkina Faso, Burundi, Botswana, Cameroon, Cape Verde, Central African Republic, Chad, Comoros, Congo, Côte d'Ivoire, Egypt, Equatorial Guinea, Eritrea, Gambia, Ghana, Guinea, Kenya, Libya, Madagascar, Malawi, Mali, Mauritania, Mauritius, Morocco, Mozambique, Namibia, Niger, Nigeria, Réunion, Rwanda, Sao Tome and Principe, Senegal, Seychelles, Sierra Leone, Somalia, South Africa, St. Helena, Sudan, Swaziland, Tanzania, Togo, Tunisia, Uganda, Zaire, Zambia, Zimbabwe) Asia (Bahrain, Cyprus, Iran, Iraq, Israel, Jordan, Lebanon, Oman, Saudi Arabia, Syria, Turkey, United Arab Emirates, Yemen) and Europe (widespread in Algeria, Cyprus, Egypt, Israel, Libya, Malta, Morocco, Spain; locally established in Greece, Italy (outdoors in the south and in glasshouses in the north), Portugal (south only) and Tunisia. It found but not established in Denmark, Finland, France, Germany, Netherlands, Switzerland and the UK. It is also reported from Lebanon, Syria and Turkey.

1. Detection

Identification is based on morphological characteristics of the larvae and adults (Blair 1974, Haines 1982). Light traps have found *S. littoralis* in the UK, where it was considered an immigrant (Kennard 1985), and Switzerland where immigration from the South or importation of eggs or larvae on clove and chrysanthemum cuttings or plants from the Canary Island, Israel, Kenya, Italy, France or Spain have been considered to be the source (Haechler 1986).

2. Delimitation

A study examining the presence of *S. littoralis* over the Middle East inferred that this species together with other lepidopteran species could be wind-borne over at least several hundred kilometres (Pedgley and Yathom 1993).

3. Monitoring

Monitoring is undertaken using pheromone or light traps. Synthetic versions of the female-produced sex pheromone are commercially available. The components of the female sex pheromone were identified as tetradecyl acetate, cis-9-tetradecenyl acetate, trans-11-tetradecenyl acetate and cis-9, trans-11-tetradecadienyl acetate (Nesbitt *et al*. 1973). Field studies in Cyprus demonstrated that only cis-9,trans-11-tetradecadienyl acetate was needed to attract male moths and that a loading of 5000 µg remained attractive for up to 40 days (Campion *et al*. 1974). The addition of cis-9-tetradecenyl acetate to cis-9, trans-11-tetradecadienyl acetate reduced trap catches (Campion *et al*. 1974). It is reported that cis-9, trans-11-tetradecadienyl acetate is also produced by male moths and this pheromone may act as an aggregation pheromone (Campion 1975).

Lures containing very pure cis-9,trans-11-tetradecadienyl acetate (92 0r 100% purity) were found to be less attractive than traps containing a virgin female in field studies in Israel and the use of a synergistic compound was suggested (Kehat *et al*. 1976, Kehat 1977). A comprehensive field study

to examine improving the attractiveness of cis-9, trans-11-tetradecadienyl acetate by the addition of secondary pheromone components and related chemicals showed that in comparison with the catches of males in traps baited with cis-9, trans-11-tetradecadienyl acetate alone, catches were increased by the addition of 1-100% tetradecyl acetate in relation to the amounts of cis-9,trans-11-tetradecadienyl acetate (Campion *et al*. 1980). This study also showed that female *S. littoralis* from different geographical areas emitted different pheromone blends (Campion *et al*. 1980).
A study to examine the effect of size, colour and height on trap catch showed that small traps (35 cm in diameter) were as effective as larger traps (65 and 95 cm in diameter), orange traps were more effective than yellow, red, blue or green traps, and traps placed at a height of 1 m were more effective than those placed at 3 and 5 m (Hosny *et al*. 1980).
A study in Italy showed that the funnel trap produced by Agrisense gave higher capture rates than the "Mastrap" produced by Isagro or the "Supergreen" trap produced by Serbios. A hand-made trap, containing the Agrisense and Isagro pheromone lures, gave higher captures than the commercial counterparts. The light trap did not improve on captures of *S. littoralis* in the pheromone traps, but attracted a number of parasitoids (Sannino *et al*. 2001).
A study to examine the efficacy of pheromone and light traps demonstrated that topography had an effect on the type of trap that was most efficient at trapping *S. littoralis*; pheromone traps were more efficient in exposed places and light traps were more efficient than pheromone traps in sheltered areas (Campion 1976). (Hosny *et al*. 1978) reported that pheromone traps were more effective than light traps for catching *S. littoralis* in the Delta region and Middle Egypt. Improved efficiency of pheromone traps in comparison with light traps has been reported by several authors (Nasr *et al*. 1978, Nasr Wissa 1978, Badawi *et al*. 1980).

4. Commodities

Importation of eggs or larvae on clove and chrysanthemum cuttings or plants from the Canary Island, Israel, Kenya, Italy, France or Spain are considered to be a possible source of *S. littoralis* found in Western Switzerland (Haechler 1986). As such, commodity trade can be considered a potential dissemination route for this pest.

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Spodoptera litura (Fabricius)

Common name(s): Cotton leafworm, tobacco cutworm
Taxa: Insecta: Lepidoptera: Noctuidae
EPPO A1 List: No. 42
EU Annex designation: I/A1

Organism

Spodoptera litura is a polyphagous species that attacks crops such as *Colocasia esculenta* (taro), cotton, flax, groundnuts, jute, lucerne, maize, rice, soyabeans, tea, tobacco, aubergines, *Brassica*, *Capsicum*, cucurbit vegetables, *Phaseolus*, potatoes and sweet potatoes. It is a tropical species that is found in Asia, parts of Australia, some Pacific islands and Hawaii. *Spodoptera litura* is not able to overwinter in all areas and it was found that temperatures yielding greater than 0.9 degree days per day above the lower developmental threshold are required for larval survival (Matsuura *et al*. 1991).

1. Detection

An outbreak of *S. litura* was reported in England in 1973 in a specialist nursery where the larvae were found feeding on aquatic plants (Aitkenhead *et al*. 1974). The authors emphasised the difficulty in distinguishing *S. litura* from *S. littoralis* (Aitkenhead *et al*. 1974). Visual identification of the adults of the two species is difficult and dissection of the genitalia is needed to determine the species. An outbreak of *S. litura* was reported in Germany, also on an aquatic plant (Heinicke 1996). Pheromone traps and light traps have been widely used to detect the presence of *S. litura.* Initially 'pheromone traps' used virgin females as the lure to attract male moths (Otake *et al*. 1974). Subsequently, the pheromone for this species was identified (Yushima *et al*. 1974) and pheromone-containing lures are now commercially available.

2. Delimitation

No relevant references found.

3. Monitoring

Monitoring of *S. litura* was initially achieved using light traps or traps containing virgin females. The identification and synthesis of the female-produced sex pheromone Z9, E11-tetradecadienyl acetate and Z9, E12-tetradecadienyl acetate) led to use of traps with a synthetic lure mix (Yushima *et al*. 1974). In a field study a ratio of 9:1 Z9, E11-tetradecadienyl acetate: Z9, E12-tetradecadienyl acetate was shown to be the most effective of the ratios tested and a 1:1 ratio was less effective than Z9, E11-tetradecadienyl acetate alone (Sun *et al*. 2003). Pheromone lure efficiency has been improved by the addition of the plant volatile phenyl acetaldehyde at 0.4 mg per lure, which acts synergistically with the two pheromone components (Shen *et al*. 2009).

A study of trap location demonstrated that pheromone traps were more efficient in open areas with low vegetation when compared with small gardens surrounded by shrubs or under leafy shade trees (Hirano 1976, 1981). Studies have examined factors that influence trap catch including wind speed (Nakamura and Kawasaki 1977, 1984) and type of trap (Dhandapani 1985, Lee 1987, Krishnananda and Satyanarayana 1989, Rao *et al*. 1991a, Kumari and Reddy 1992, Nandagopal and Rajiv 2007). It was found that a greater number of moths were caught in traps with openings on the leeward side (Hirano 1982). Ten traps per hectare have been recommended for monitoring (Lee 1987). Experiments in India demonstrated that male flight pattern changes considerably during the cropping season and thus the optimum height for efficient capture also changed (Rao *et*

al. 1991b). An optimum trap height of 1 metre has been suggested (Rao *et al*. 1991b, Yao *et al*. 2008, Cui *et al*. 2009). However, in a study in a tobacco nursery an optimum trap height of 1.5 metres was suggested (Krishnananda *et al*. 1992). The many different trap types used in the various studies in different crops and geographical areas makes a recommendation for the optimum trap based on these studies difficult.

A comparison of light sources for light traps demonstrated that a mercury vapour lamp of 125 W and an ultraviolet fluorescent lamp of 15 W were the most attractive light sources (Vaishampayan and Verma 1983). Pheromone traps were shown to be more effective than light traps for catching *S. litura* (Nandihalli *et al*. 1990, Shih and Chu 1995, Jiang *et al*. 2009, Prasad *et al*. 2009). A study in Florida that used pheromone traps to monitor for invasive *Spodoptera* spp. including *S. litura* concluded that this task requires high amounts of labour and technical expertise (Meagher *et al*. 2008).

4. Commodities

No relevant references found.

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Squash leaf curl virus

Common name(s): SLCV, curly mottle of watermelon, leaf curl of melon/squash, necromantic mosaic of melon
Taxa: Viruses: Geminiviridae: Begomovirus
EPPO A1 list: No. 224
EU Annex designation: I/A1

Organism
Squash leaf curl virus is mainly found on Cucurbitaceae, which are the hosts on which it has economic importance, but is also recorded on plants of other families. It is present in Asia and Central and North America. It has also recently been recorded for the first time in Egypt, Israel, Palestinian, Jordan. This virus is transmitted efficiently by the whitefly *Bemisia tabaci*.

1. Detection
Among the first techniques proposed, a nucleic acid spot hybridization assay was developed for detection of curcurbit geminiviruses in plant tissue extracts (Polston et al. 1989), whereas enzyme-linked immunosorbent assay (ELISA) techniques also provide a useful tool for the identification of Squash leaf curl virus in *Bemisia tabaci* extracts (Cohen et al. 1989). A dot blot hybridization system using digoxigenin-labeled probes and colorimetric visualization was developed, the colorimetric system was less sensitive than radioactive dot blot hybridization, however the authors consider the method adequate for the detection of viruses from infected plants. This colorimetric system can be carried out in diagnostic laboratories using minimal equipment, suggesting its applicability for use in routine plant virus diagnoses (Harper and Creamer 1995). The molecular hybridization using coat protein gene probe showed high levels of efficiency in the identification of virus (Cai et al. 1994). PCR techniques were developed for identification of two strains of squash leaf curl virus (Wendt et al. 1994). Recently a loop-mediated isothermal amplification (LAMP) assay was employed to develop a simple and efficient system for the detection of squash leaf curl virus (SLCV) in diseased plants, although both the LAMP and the PCR methods were capable of detecting SLCV in infected tissues of squash and melon, the LAMP method would be more useful than the PCR method for detection of SLCV infection in cucurbitaceous plants because it is more rapid, simple, accurate and sensitive (Kuan et al. 2010). The isolation of nucleic acids from the leaves of symptomatic plants, and the subsequent phases of cloning and sequencing, have allowed the identification of squash leaf curl virus in a number of different countries (Idris et al. 2006, Abudy et al. 2010, Ali-Shtayeh et al. 2010, Tahir et al. 2010).

2. Delimitation
No relevant references found.

3. Monitoring
A number of surveys on different crops have been carried out to determine the occurrence and incidence of aphid- and whitefly-transmitted cucurbit viruses (Dodds et al. 1984, Dardon et al. 1994, Palomar et al. 1995, Ramirez Arredondo et al. 1995, Jossey and Babadoost 2006). The techniques used for virus identification were dot-blot hybridization (Al-Musa et al. 2008), RT-PCR analysis of DNA extracts (Kuo et al. 2007), a set of different techniques both serological and molecular were used with a comparative nucleotide sequence analysis to have the certain of identification (El-Dougdoug et al. 2009).

4. Commodities

The trade of infected plants is the main pathway of dissemination of the virus (Rosemeyer et al. 1986), and providing virus- and viroid-free materials is a priority also for the economic consequence (Werkman and Roenhorst 2006).

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Stegophora ulmea (Fries Sydow & Sydow

Common name(s): Black spot of elm, twig blight, elm leaf scab, elm leaf spot
Taxa: Fungi: Ascomycota: Valaceae
EPPO A1 list: No. 315
EU Annex designation: II/A1

Organism
This fungus causes a serious foliar disease in many elm (*Ulmus*) species. The fungus is considered to be native in North America and is widespread, ranging from the Great Plains to the Atlantic Ocean. It has been recorded infecting imported bonsai in Europe and has been found in The Netherlands, where it was eradicated, and possibly in Romania although the record is doubtful.

1. Detection
Numerous features among which conidial morphology, axenic growth, and sporulation have been described (McGranahan and Smalley 1984).

2. Delimitation
No relevant references found.

3. Monitoring
No relevant references found.

4. Commodities
No relevant references found.

References: 4 retained out of 13 references retrieved, none added, 1 used for the summary
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Sternochetus mangiferae (Fabricius)

Common name(s): Mango stone/seed weevil, mango weevil
Taxa: Insecta: Coleoptera: Curculionidae
EPPO A1 list: No. 286
EU Annex designation: II/B

Organism

Sternochetus mangiferae is a specific weevil (Coleoptera Curculionidae) of mango (*Mangifera indica*) seed and there are few outward signs of infestation of the fruit. This weevil has a worldwide distribution although it is absent from the EU/EPPO region although it does pose a threat to mango that is locally cultivated in countries of southern Europe.

1. Detection

External morphological characters as well of adult male and female genitalia of the weevil have been described in detail for identification purposes (Babu et al. 2001a). Methods for the identification in harvested fruits of mango have been suggested (Schotman 1989) and include non-destructive inspection of mango fruit with X-ray imaging (Thomas et al. 1995, Reyes et al. 2000, Maharajan et al. 2005). There are a number of reports detailing the first record of this pest in several Caribbean countries (Anonymous 1984, 1986b, a, c, 1994).

2. Delimitation

Following the first record of *S. mangiferae*, surveying for this pest of mangoes was intensified in Trinidad in 1994. The results suggested that the infestations were limited, with low infestation of fruit and seed (McComie 1996).

3. Monitoring

General surveys of the causes of damage of mango fruit trees have been carried out in a number of studies (Javaid 1986, Babu et al. 2001b, Bellis et al. 2006). Specific surveys on presence and incidence of *S. mangiferae* were carried out in the Philippines (Corey 1987, Karpati 1987) and India (Pande and Dey 1986).

4. Commodities

Mangoes for shipment are frequently infested with the mango seed weevil and this can be an important source spread of this pest (Akamine and Goo 1979, Silfverberg 1982, Kapur et al. 1999, Grove and Beer 2007). Treatments of packaged mangoes for elimination of *S. mangiferae* has been proposed, the most common technique being that of irradiation (Seo et al. 1974, Milne et al. 1977, Akamine and Goo 1979, Joubert and Grove 2000, Follett 2002, Grove et al. 2004, Karnkowski 2004, Follett et al. 2007). Quarantine treatments (heat treatment, cold storage, fumigation, microwaves, irradiation, non-destructive detection X-ray imaging) were all discussed in a review (Grove et al. 2007). The most useful method for non-destructive inspection of fruit is considered to be X-ray analysis (Maharajan et al. 2005).

References: 42 retained out of 108 retrieved, none added, 29 used for the summary

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Strawberry crinkle virus

Common name(s): SCrV, strawberry crinkle
Taxa: Viruses: Rhabdoviridae: Cytorhabdovirus
EU Annex designation: II/A2

Organism
This virus occurs world-wide wherever strawberry aphids of the genus *Chaetosiphon* are found infesting strawberry. It is a primary pathogen on strawberries and nursery material has to be certified as virus-free. It is present throughout Europe.

1. Detection
Indicator clonal plants have been routinely used for detection of strawberry crinkle virus (Crosse 1974, Frazier 1974, Aerts 1978, Bormans and Gilles 1989). On the basis of the characteristics of reactions and symptoms in indicator plants, it is possible to distinguish the cytoplasm associated strawberry crinkle virus from the nucleus associated strawberry latent C virus (Yoshikawa et al. 1986). However, leaf grafting to sensitive indicator plants is a labour-intensive method. The leaflet graft bioassay procedure, utilizing sensitive *Fragaria* clones, was considered the most effective method for detecting a number of viral pathogens, but ELISA and PCR assays are typically more rapid, accurate and sensitive (Zhou and He 2003). Results on the purification of strawberry crinkle virus from strawberries by non-conventional methods (conventional centrifugation combined with ultrafiltration, gel filtration chromatography and zone electrophoresis) showed that the procedure obtains sufficient virus material to facilitate detection methods (Leone et al. 1992). As the concentration of the virus in the plant is low, the purification of the virus particles is difficult and attempts to develop a serological detection method for the virus have been unsuccessful. For this reason, a reverse-transcription polymerase chain reaction (RT-PCR) was developed that indicated that strawberry crinkle virus could not always be detected in strawberry whereas detection in *Physalis pubescens* was much more reliable (Posthuma et al. 2001). Subsequent refinements of the procedure increased the efficiency of the technique and strawberry crinkle virus was also reliably detected by RT-PCR in total RNA extracts from three strawberry plants showing symptoms typical of Strawberry crinkle virus infection, but failed when the intensity of the disease symptoms decreased. A higher performance was obtained using nested PCR (Posthuma et al. 2002). A multiplex reverse transcriptase polymerase chain reaction (RT-PCR) method is described for the simultaneous detection of four aphid-borne strawberry viruses, among these Strawberry crinkle virus, in combination with a plant mRNA specific internal control which can be used as an indicator of the effectiveness of the extraction and RT-PCR (Thompson et al. 2003, Thompson et al. 2004). Further improvements were obtained through the use of a newly developed gel-based RT-PCR and a gel-free AmpliDet RNA assay (Klerks et al. 2004), real-time RT-PCR (TaqMan) (Mumford et al. 2004) and RealTime PCR (Botti and Cardoni 2009, Cubero et al. 2009). These methods were useful for the routine detection of the virus, proving to be rapid, high sensitive, and specific.

2. Delimitation
No information available.

3. Monitoring
Surveys were carried out to evaluate the presence of strawberry virus diseases, including

strawberry crinkle virus (Babovic 1976, Yoshikawa and Inouye 1989, Cieslinska and Zawadzka 1991, Dulic-Markovic Vvana 1992, Tzanetakis et al. 2004, Martin and Tzanetakis 2008). Virus detection was made through leaf grafts onto indicator clones of *Fragaria* (Teliz Ortiz and Trejo Reyes 1989, Karesova et al. 2003), in addition to serological (Hokanson et al. 2000, Martin et al. 2001) and molecular techniques (Babini et al. 2004).

4. Commodities

Infected planting material is responsible of introduction in new geographic areas (Krczal 1979). Use of virus-free material and meristem culture are recommended as control measures (Dolgikh and Vladimirova 1980). Elimination of viruses from strawberries is achieved through thermotherapy in combination with meristem culture (Lines et al. 2006). Strawberry virus-free certification is required, based on a plant indexing method (Anonymous 2008), whilst a molecular protocol based on nested RT-PCR has been developed to be improve routine diagnosis (Autonell et al. 2006).

References: 59 retained out of 110 retrieved, none added, 31 used for the summary

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Strawberry latent C virus

Common name(s): STLCV
Taxa: Viruses (unclassified)
EPPO A1 list: No. 129
EU Annex designation: I/A1

Organism:
The disease is limited to cultivated strawberries and related species, with related weed species infected experimentally.

1. Detection
The pathogen has not been isolated or described morphologically. The organism behaves like a latent virus giving no obvious symptoms on cultivated strawberries except in combination with other virus diseases. It then causes moderate to severe degeneration in the form of extreme stunting, curling and twisting of leaves or an intensification of symptoms attributable to other viruses. Infection can be confirmed by graft-transmission to indicator clones of *Fragaria vesca* or *F. virginiana* (Frazier, 1974).

2. Delimitation
No information.

3. Monitoring
No information.

4. Commodities
No information.

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Strawberry latent ringspot virus

Common name(s) SLRSV, Strawberry latent ringspot
Taxa: Viruses: Secoviridae
EU Annex designation: II/A2

Organism
Strawberry latent ringspot virus (SLRSV) infects a wide range of fruit crops, including strawberry, raspberry, blackcurrants, grapes, olives, amongst others (Bercks *et al*. 1977, Pollini *et al*. 1990, Henriques *et al*. 1992). It can also infect a range of vegetable crops and wild hosts. Often asymptomatic, as suggested by its name, infections can cause decline in some hosts and visible symptoms include leaf mottling. The virus is globally widely distributed and is present throughout much of the EU. Transmission occurs via the nematode vector *Xiphinema diversicaudatum* (Cooper and Sweet 1976), and seed transmission is also widely reported.

1. Detection
Detection of the virus was initially undertaken through the use of indicator plants, such as *Chenopodium quinoa* (Kleinhempel 1970). However, during the 1970s, serological assays were developed that increased the speed of diagnosis with high levels of sensitivity (Thomas 1979, 1980). Subsequently, a number of ELISA / DAS-ELISA techniques have been employed to diagnose the virus (Poggi-Pollini and Giunchedi 1987, Polak *et al*. 1989, Kristek and Polak 1990). Such techniques have been used to detect SLRSV in a number of plant species and tissues, including leaves, fruits, roots and flowers (Henriques *et al*. 1992, Smrcka 1993). In Germany the DAS-ELISA method was used to confirm the presence of the virus in chestnut (Hentsch *et al*. 1996), whilst in the Czech Republic a similar method detected the disease in strawberry and hops (Svoboda 1993, Honetslegrova and Spak 1995) and in Turkey for detection in grapevine and olive (Caglayan 1997, Caglayan *et al*. 2004).
Molecular diagnosis of the disease has become more prevalent in recent years. As such, PCR, RT-PCR and related techniques have been employed to detect SLRSV in olives (Grieco *et al*. 2000, Bertolini *et al*. 2001), strawberry and chestnut (Regner *et al*. 2000), lily (Chang *et al*. 2001) with the advantage of much greater sensitivity when compared with ELISA. Nested PCR procedures have further increased sensitivity over conventional RT-PCR (Pantaleo *et al*. 2001) whilst Co-operational PCR methods also offer greatly enhanced sensitivity (Olmos *et al*. 2002). A novel “Amplidet” RNA assay has also been successfully used (Klerks *et al*. 2001). Methods for the detection of strawberry-infecting virus, including SLRSV, have been reviewed, although little information regarding specific assays was provided (Martin and Tzanetakis 2006) and the methods predominantly used in more recent years have been based on PCR variants (Felix and Clara 2008, Kumari 2009, Ragab *et al*. 2009, Yang *et al*. 2009). Such methods have been effectively used to detect SLRSV in areas previously thought to be free of the virus, for example the USA (Martin *et al*. 2004).

2. Delimitation
No information

3. Monitoring
The occurrence and distribution of SLRSV has been widely monitored, frequently as part of general plant-virus monitoring programmes. Earlier examples identified examined the presence of virus in specific locations, such as nurseries and glasshouses (Hoof and Caron 1975, Sweet 1975). In early cases, diagnosis of the disease was achieved using indicator plants and the true prevalence of the

disease was probably underestimated (Sweet 1980). The sampling of asymptomatic leaf material is often conducted during surveys (Spak 1995, Spak *et al*. 1997), whilst the large survey described by Choueiri *et al*. (2001) collected leaf and branch material from symptomatic trees (peach). A survey of strawberry in the Czech Republic used indicator species for diagnosis followed by DAS-ELISA for leaf samples collected across the country. Sampling procedures for orchard trees as part of a general virus surveys are described by Jarra *et al*. (2001), Saponari *et al*. (2002) and Fadel (2005), whilst some details on sampling of lily have also been described (Bellardi *et al*. 2002) where diagnosis was made using a commercially available serological kit. An extensive survey of strawberry viruses across Europe is reported by Babini *et al*. (Babini *et al*. 2004) that describes the varied sources of samples although does not provide further details. Disappointingly, a number of reports of surveys often omit specific sampling details (e.g. Nyerges *et al*. 2001). As emphasised by Fiore *et al*. (2008) in a survey in Chile, timing of monitoring is important and should coincide with periods when symptoms are most likely to be manifested. Useful details of good surveying practice are also detailed in this reference.

4. Commodities

A number of references include details of surveying commodity products. For example, parsley seed infected by SLRSV was identified by Bellardi *et al*. (1991). Imported bulbs were found to be infected in Israel (Cohen *et al*. 1995) whilst a number of references report on monitoring propagating material as a means of controlling the spread of the virus (Mikhovska *et al*. 1995, Cohen *et al*. 1996, Saponari *et al*. 2002). Other details of phytosanitary procedures appropriate to strawberry are also available (Anonymous 2008, Perez-Jimenez *et al*. 2009)

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Strawberry mild yellow edge virus

Common name(s): SMYEV
Taxa: Viruses: Alphaflexiviridae: Potexvirus
EU Annex designation: II/A2

Organism
Strawberry mild yellow edge disease is probably caused by a virus complex consisting of a potexvirus (strawberry mild yellow edge-associated potexvirus) and a luteovirus originally designated as strawberry mild yellow edge luteovirus. Both viruses have only been found in strawberries. Under natural conditions the viruses are dispersed by the strawberry aphid (*Chaetosiphon fragaefolii*). Movement of the disease also occurs via runners or through the dissemination of propagated material from tissue culture. Seed transmission is not known.

1. Detection
Cultivated strawberries usually remain symptomless but the virus often causes a loss of vigour, stunting, and decreased yield in infected plants. On *Fragaria vesca*, indicator clone symptoms include mottling of young leaves, epinasty, chlorotic flecking, vein necrosis of maturing leaves and premature senescence of older leaves.
Transmission by grafting using the leaflet graft bioassay, or transmission by its aphid vector (*Chaetosiphon fragaefolii*) to sensitive clones of *Fragaria vesca*, is used for detection and identification of the disease. Symptoms usually appear within three weeks of inoculation by aphids or grafting tissue onto indicator clones.
Strawberry mild yellow edge disease can be readily detected by RT-PCR and ELISA and has been detected in all sources of the virus characterised by symptoms on indicator plants (Martin and Tzanetakis (2006). ELISA test kits are available. Reverse transcription polymerase chain reaction (RT-PCR) assays have been developed for detection of the virus (Autonell *et al*., 2006; Cai *et al*, 2009; Chang *et al*., 2007; Cho *et al*, 2011; Hadidi *et al*., 1993; Li *et al*., 2006; Yang *et al*., 2005; Zhang *et al*., 2009). Nucleic acid-based detection techniques have been found to be as sensitive as biological indexing and in some cases virus detection based on AmpliDet RNA and RT-PCR was demonstrated to be more sensitive than the biological tests (Babini *et al*., 2004). TaqMan probes have been designed to increase detection and quantification specificity (Cubero *et al*., 2009). The leaflet graft bioassay procedure is the most effective method for detecting many viral pathogens, but the ELISA test and polymerase chain reaction assays are typically more rapid, accurate/specific and sensitive (Zhou and He, 2003).

2. Delimitation
No information.

3. Monitoring
No information.

4. Commodities
No information.

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Strawberry vein banding virus

Common name(s): SVBV
Taxa: Viruses: Caulimoviridae: Caulimovirus
EPPO A1 list: No. 101
EU Annex designation: 1/A1

Organism:
SVBV is a virus that has only ever been recorded as infecting plants of the genus *Fragaria*. The virus is vectored by a wide range of aphids. The virus is also transmissible through grafting. Symptoms can be variable and include crinkling of the leaves and chlorotic streaks. The disease has a global distribution and is locally present in several EPPO region countries.

1. Detection:
Significant work has been devoted to methods for the detection of SVBV, almost entirely from the diagnostic point of view. Almost all recent methods are molecular in nature, such dot blot hybridization (Stenger *et al*. 1988, Mraz *et al*. 1996, Mraz *et al*. 1999) using radioactive and non-radioactive probes. Polymerase chain reaction (PCR) methods have also been commonly used (Petrzik *et al*. 1998). In many cases detection has been undertaken simultaneously using both PCR and dot-blot methods (Mraz *et al*. 1997, Mraz *et al*. 1999, Franova *et al*. 2001). Nucleic acid sequence based amplification (NASBA) has been developed (Vaskova and Spak 2002) and recently reported methods all adopt a PCR-based approach. ELISA methods have also been used successfully for screening strawberries for the virus (Honetslegrova *et al*. 1995, Hokanson *et al*. 2000, Martin *et al*. 2001) although low levels of SVBC were not detectable (Mraz *et al*. 1997). In addition to immunological and molecular techniques, grafting of material onto strawberry indicator plants has also been commonplace (Babini *et al*. 2004).

2. Delimitation:
No information available

3. Monitoring:
A number of monitoring surveys for SVBC within areas it is known to occur are described in the literature. These include surveying aphid vector species (Karesova *et al*. 2003) and strawberry plants in the Czech Republic (Franova 2001). In the United States, monitoring through randomly sampling plant material and subjecting it to ELISA (Martin *et al*. 2001) or RT-PCR (Martin and Tzanetakis 2008) has been undertaken. A broad-ranging monitoring exercise across five European countries has also been described although details of sampling methodologies are scant (Babini *et al*. 2004).

4. Commodities:
No information available

References: 64 retained out of 78 retrieved, none added, 14 used for the summary

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Strawberry witches' broom agent

(=Strawberry witches' broom phytoplasma)
Common name(s): Witches' broom of strawberry
Taxa: Bacteria: Bacterium-like pathogens
EPPO A1 list: No. 130
EU Annex designation: 1/A1

Organism:
Absent in the EU, this organism attacks plants of the genus *Fragaria* and is currently though to be graft-transmissible only although it has been suggested that phloem-feeding insects may be capable of transmission. Diseased plants fail to bear fruit and have a dwarfed and bushy appearance, a fact that allows infected plants to be readily identified and removed. As a result, this pathogen is of little economic consequence within its native range (North America)(Anonymous 1984). The disease is thought to be of little economic threat to the EPPO region although the susceptibility of European strawberry varieties is not known (Anonymous, 1984).

1. Detection: Infection can be confirmed through grafting and via examination of the phloem for phytoplasmas-type structures using electron microscopy (Anonymous 1984).

2. Delimitation:
No information available

3. Monitoring:
No information available

4. Commodities:
No information available

References: 1 retained of 1 retrieved, none added, 1 used for the summary

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Synchytrium endobioticum (Schilbersky) Percival

Common name(s): Potato wart disease
Taxa: Fungi: Chytridiomycota: Synchitriaceae
EPPO A1 list: No. 82
EU Annex designation: I/A2

Organism

The only known cultivated host of this pathogen is potato, but wild *Solanum* spp. can also become infected and other solanaceous plants, including tomato, can be experimentally infected. Within the EU/EPPO region, the potential for infection in potatoes is the primary concern. *Synchytrium endobioticum* occurs locally in almost all EPPO countries. *Synchytrium endobioticum* is an obligate fungal parasite which does not produce hyphae but sporangia containing 200-300 motile zoospores. Summer sporangia are thin-walled and short lived and are formed in the affected potato tissue and give rise to new zoospore infections. Winter sporangia are thick-walled and are released from decomposing warts into the soil. They can remain viable for at least 30 years and are found at depths of up to 50cm. Favourable conditions for the development of the fungus are cool summers, with an average temperature of 18°C or less, and an annual precipitation of at least 700mm. Spread is by means of zoospores produced initially from the winter sporangia and by summer sporangia during the growing season, but the capacity for natural spread is limited. The fungus may be carried via the international trade of infected potato tubers, soil alone, or accompanying plants from land on which potato wart has occurred in the past. A spore density of 10 spores/g soil gave 100% infection of bait plants, whereas 5 spores / g soil gave 60% infection of potato bait plants.

Due to the limited capacity for natural spread, the disease has been controlled effectively by statutory means in many countries. Strict phytosanitary control and obligatory cultivation of resistant cultivars have allowed for eradication in some countries but eradication can take many years because the fungus survives in soil for decades. Many pathotypes of the fungus exist, defined by their virulence on differential potato cultivars. Pathotype 1 (European race 1) is the most common in the EPPO region and is the only pathotype occurring in most countries.

1. Detection

The typical symptoms of potato wart disease on tubers are the proliferating warts which may vary markedly in form but are primarily spherical to irregular in shape. The infection invariably originates in the eye tissue but may expand to engulf the whole tuber. Wart size can vary from pea-sized to the size of a fist. The fungus affects the tuber initials and tubers but roots are not known to be infected. Early infection of young developing tubers results in their becoming so distorted and spongy as to be scarcely recognisable. In older tubers only the eyes are infected and they develop into characteristic warty, cauliflower-like protuberances. These are initially whitish (or green if exposed to light), but gradually darken and eventually rot and disintegrate. The whole tuber may be replaced by warty proliferations. Symptoms on aerial parts of the plant are not usually apparent, although there may be a reduction in plant vigour. Small greenish warts may form in the position of the aerial buds at the stem bases. Leaves may also be attacked. The disease does not kill the host and in the case of subterranean symptoms, may not be evident until harvest.

Symptoms of potato wart disease may be confused with some other disorders. Identification of the pathotype present is important to determine which resistant varieties of potato should be used.

EPPO standard PM7/28 Diagnostic protocol for *S. endobioticum* describes how to detect and identity *S. endobioticum* and its pathotypes (EPPO 2004).
Two main methods are available for the detection of potato wart disease in soil. The first method is based on a direct analysis of the soil. To be of practical use, any detection methodology would have to detect viable spores down to a level of about 0.1 spores g^1 soil. The procedure recommended by EPPO is a modification of the wet-sieving and flotation technique developed by Pratt (1976). The Hendrickx centrifuge technique was found to have a better extraction recovery, a lower error measurement, lower detection level (down to 0.02 sporangia g-1 soil) and be much less labour intensive than previously recommended methods (Wander *et al*. 2007)). Identification of sporangia is by microscopic examination. Determination of spore viability is difficult and is carried out mainly by microscopy or inoculation on to plants. The second method is based on a bioassay using susceptible potato cultivars (EPPO 2004)) which is reported to detect about 1-5 sporangia g^{-1} soil. The assay requires a 70-100 day growth period after which tubers are examined for the presence of warts. Pathotype identification is possible using the Spieckermann method, the Glynne-Lemmerzahl method and field tests (EPPO 2004)
Van den Boogert *et al*. (2005) developed a real-time PCR assay that was reported to have a detection limit of 10 sporangia per PCR assay. This assay was further developed by Van Gent-Pelzer *et al*. (2010) who developed a TaqMan assay with improved sensitivity and which proved to be reliable for accurate diagnosis of the disease. A conventional PCR method (Niepold and Stachewicz 2004), a microarray method (Abdullahi *et al*. 2005) and a macroarray method (Zhang and Smart 2006) have also been developed but not fully validated.
Connell *et al*. (2009) have investigated the use of two field-compatible nucleic acid-based testing systems (surface plasmon resonance (SPR) and hybridization-induced gold nanoparticle aggregation) as rapid field based detection methods of *S. endobioticum*, both of which provide results within 5 minutes. A custom-built SPR machine is being tested to quantify *S. endobioticum* in field settings.
Surveys have recently detected *S. endobioticum* in Turkey for the first time via visual inspections and soil sampling that lead to large growing areas being placed under quarantine conditions (Basim *et al*. 2005) whilst a survey of tubers indicated that potato wart disease occurs outside the known range in Cana, although few details are provided (Boer 2001).

2. Delimitation

On detection of the pest, the contaminated field is scheduled. A buffer zone, large enough to ensure the protection of the areas surround the contaminated plot, should also be demarcated. Boundaries of a buffer zone may be determined by natural barriers such as meadows, forest, rivers and roads. Partial de-scheduling can be applied subject to bioassay with a susceptible cultivar in two successive years or direct microscopic examination of soil samples in combination with a bioassay. A maximum of 5 sporangia per g soil are tolerated for partial de-scheduling. Complete de-scheduling requires complete elimination of pathogen and requires two successive bioassays with positive and negative controls with highly susceptible potatoes planted in infested and uninfested soil, or a combination of bioassay and direct microscopic examination of soil (EPPO 1999, 2007). Examples from the literature are limited but the eradication programme in Maryland provides an example of a delimiting soil sampling exercise to determine the extent of resting spores present with gardens, determining that detection of spores from what was considered a pest-free area was an isolated case (Putnam and Hampson 1989, Putnam and Sindermann 1994)

3. Monitoring

Synchytrium endobioticum should be considered a notifiable pest. A plot shall be regarded as being contaminated when symptoms of potato wart disease have been found on at least one plant or any viable sporangia are found in soil and the presence of *S. endobioticum* has been confirmed by official services. Monitoring is usually based on general surveillance of the potato production system. Because potato tubers are not allowed to be grown on infected fields and restrictions apply in the buffer zones, inspection of these areas after detection is not relevant except inspection to verify that the phytosanitary measures imposed in these areas are being implemented (EPPO, 2007). (EPPO 2007). Limited details of monitoring for potato wart in the Ukraine are provided by Matskiv *et al*. (1998). This apart, details of monitoring techniques for this pest are largely absent from the literature.

4. Commodities

No information available

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Tachypterellus quadrigibbus (Say)

(=*Anthonomous quadrigibbus* Say)
Common name(s): Apple / Western curculio, large apple curculio
Taxa: Insecta: Coleoptera: Curculionidae
EU Annex designation: II/A1

Organism
Tachypterellus (=*Anthomonous*) *quadrigibbus* is associated with plants of the family Rosaceae and also with *Cornus* spp. Larval development takes place in fruits (Burke and Anderson 1989). This species is univoltine and the period of emergence varies with the climatic condition from mid August to mid September. The adult weevils overwinter on the ground beneath the trees. Its range includes North America and Mexico (Anonymous 2004) and it does not occur in the EU/EPPO region

1. Detection
Information not available.

2. Delimitation
Information not available.

3. Monitoring
Information not available.

4. Commodities
Information not available.

References: 2 retained out of 10 retrieved, none added, 2 used for the summary

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Tatter leaf virus (of Citrus)

Common name(s): CTLV, bud union crease, yellow ring
Taxa: Viruses: Betaflexiviridae: Capillovirus
EPPO A1 list: No. 191
EU Annex designation: I/A1

Organism

All citrus plants are potential hosts of Citrus tatter leaf virus. *Poncirus trifoliate* (trifoliate orange) is immune or highly resistant, but its hybrids can show symptoms after infection. CTLV is sap transmissible to at least 19 non-citrus hosts: *Amaranthus tricolor*, *Catharanthus roseus*, *Chenopodium amaranticolor*, *C. quinoa*, *Cucurbita pepo*, *Dianthus barbatus*, *D. chinensis*, faba beans, *Gomphrena globosa*, *Nicotiana clevelandii*, *N. debneyi*, *N. glutinosa*, peas, *Petunia hybrida*, soyabeans, *Tetragonia tetragonioides*, tomatoes, *Vigna unguiculata* (Nishio et al. 1982).

It can infect a wide range of citrus cultivars. The major method of transmission from citrus to citrus occurs via grafting. Mechanical transmission by knife slashes and leaf-abrasion is easily achieved from infected *Nicotiana clevelandii* to citron (Garnsey 1974), and from citron to citron (Roistacher 1983).

Seed transmission has been observed in *Chenopodium quinoa*, cowpeas and soybean but not in *Fortunella japonica* (Nishio et al. 1982). No natural vector is known. These results suggest that natural transmission occurs only at a very low rate. This virus originates from China and was brought to North America and Korea. It is also present in South Africa and Australia but is absent of EU (Anonymous 1997).

1. Detection

CTLV is often symptomless in citrus plants. Chlorotic leaf symptoms are produced in *Citrus excelsa*, Rusk and Troyer citranges (*Poncirus trifoliata* x *Citrus sinensis*), Swingle citrumelos (*P. trifoliata* x *C. paradisi*) and other *P. trifoliata* hybrids. Leaves of *C. excelsa* may be deformed (so-called tatter leaf), but infected plants often recover after the initial reaction. Stems of citrange (*C. chinensis* x *C. trifoliate*) plants may be deformed and have a zigzag growth pattern associated with chlorotic areas on the stem. Infection in citrange often leads to pitted on their stem.

The most devastating symptom caused by CTLV in commercial citrus is the bud union necrosis or abnormality which can develop when an infected symptomless cultivar is grafted onto trifoliate orange, or trifoliate orange hybrid rootstocks. When infected latent hosts are grafted on rootstocks of *P. trifoliata* or its hybrids, a bud-union crease, showing a yellow to brown line, can be observed in the fields 1 year after grafting when the bark is removed. Affected plants become stunted, chlorotic and over blooming, have early-maturing of fruit, and often die.

Seedlings of Rusk citranges are recommended as indicators for CTLV. Rusk citrange is budded onto virus-free seedlings of rough lemon rootstock and the citrus tissues to be tested are also budded below the citrange bud. The rootstock is cut back 10-14 days later to force development of new sprouts from the Rusk citrange. Optimum temperatures for symptom development are 20-24°C (Miyakawa 1978). Leaf-abrasion inoculation to *Chenopodium quinoa* is also recommended. *C. quinoa* develops chlorotic or necrotic spots on the inoculated leaves, and its upper leaves show vein clearing, twisting and stunting. *Vigna unguiculata* is also used, but symptoms vary markedly depending on virus isolate. ELISA using CTLV antiserum has been utilized (Kawai and Nishio 1990).

PCR-based techniques have been developed (Hailstones et al. 2000), and. showed to be effective in

multiplex assays with several viroids (Ito et al., 2002).

2. Delimitation

No information available.

3. Monitoring

Little information on monitoring efforts for CTVL is available, beyond brief notes of incidences in Korea and North America (Wang et al. 1998, Kim et al. 2001).

4 Commodities

No information available.

References: 22 retained out of 80 retrieved, 3 added, 9 used for the summary

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Tecia solanivora (Povolny)

Common name(s): Guatemalan potato moth
Taxa: Insecta: Lepidoptera: Gelechidae
EPPO A2 list: No. 310

Organism

This moth species is specific to potatoes and originated in Central America but has radiated out across South America and now occurs in the Canary Islands, arriving in 2001 (Anonymous 2005) (Barragan et al. 2004). The pest attacks potatoes growing in the field and also tubers within stores (Povolny 2004). The moth can cause significant economic impacts to the growing crop with yield losses as high as 40% recorded (Povolny 1973) and it has considered one of the more damaging pests of potatoes in South America (Pollet et al. 2003b). The life cycle of the moth has been well described in a number of publications (2005) (Torres W et al. 1997) as have the damage symptoms that it causes (Salas 1987)

1. Detection

The physical appearance of this small, grey moth have been described in a number of articles (Anonymous 2005) (Povolny and Hula 2004) as have the symptoms of damage that are associated with larvae feeding upon tubers (Hilje 1994).

The sex pheromone of *T. solanivora* has been characterized and was initially shown to contain (E)-3-dodecenyl acetate (Nesbitt et al. 1985). Later investigations indicated that the pheromone is a blend of (E)-3-dodecenyl acetate, (Z)-3-dodecenyl acetate, and dodecyl acetate . Synthetic pheromone has been evaluated in the field and has been shown to be capable of catching male moths over a two month period when used in conjunction with plastic traps (Rodriguez V and Lepiz C 1990). Optimal trap distances were found to be 5-10 m, with closer distances causing interference (Rodriguez V et al. 1991). Some limited investigations are available with respect to the efficacy of different traps with and without lures (Julio Ricardo Galindo and Jeannette Amparo Espanol 2004) including a simple sticky trap design.

2. Delimitation

No information available.

3. Monitoring

Pheromone traps have been used evaluate the distribution of *T. solanivora* in Venezuela (Salazar and Torres 1986). Thirty eight traps placed in the main growing areas to determine the widespread presence of the pest. Whilst not a survey as such, compiled reports of damage causes *by T. solanivora* in Colombia revealed its presence in 16 municipalities in 1994 with significant levels of damage apparent in both the field and store (Arias R et al. 1996). Similarly, in Ecuador the severity of the pest was also established through reports of damage to the crop (Pollet 2001). The importance of monitoring has also been emphasised by the fact that the first light of males is highly correlated with weather conditions, enabling prediction of likely infestations prior to their onset (Pollet et al. 2003a).

4. Commodities

Little information is available in this area. However, it has been noticed that failure to destroy infested material in long-term storage was seen as a major factor in the explosion of the population in Ecuador in 2000 (Barragan et al. 2004).

References: 51 retained out of 107 retrieved, none added, 17 used for the summary

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Thecaphora solani (Thirumulachar and O'Brien) Mordue

Common name(s): Potato smut
Taxonomic position: Fungi: Basidiomycota: Glomosporiaceae
EPPO A1 list: No. 4
EU Annex designation: I/A1

Organism
Thecaphora solani is the causal agent of potato smut. Indigenous to the Andean region (present in several countries in South America), it is absent from EPPO region. The main host is potatoes but the solanaceous weed *Datura stramonium* is also attacked. *Thecaphora solani* survives in soil or in tuber debris. Seed tubers transmit the disease (Anonymous 1979).

1. Detection
Because no symptoms are visible above ground, only visual inspections on tubers allow potato smut detection (Jimenez 1985). The pathogen could be identified by molecular analysis in the laboratory (Andrade, Munoz et al. 2004).

2. Delimitation
No information available

3. Monitoring
No information available

4. Commodities
Infected tubers are able transmit and spread the disease (Anonymous 1979; Mordue 1988).

References: 8 retained out of 26 retrieved, 1 added, 4 used for the summary

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Thecodiplosis japonensis Uchida & Inouye

Common name(s): Pine needle gall midge
Taxa: Insecta: Diptera: Cecidomyiidae
EU Annex designation: II/A1

Organism

Thecodiplosis japonensis (Diptera Cecidomyidae) attacks *Pinus spp.,* mainly *P. densiflora* and *P. thunbergii*. Its range includes Japan and Korea. In spring, emerging females lay eggs on developing needles. After hatching, young larvae crawl down to the leaf sheath and feed by sucking sap which induces the formation of galls. The distribution of the insect is confined to the Far East

1. Detection

The best methods for capturing this pest have been evaluated: the suction trap has proven particularly effective for collection of adults when population density was low. Funnel-A traps were also recommended, especially for small plots, and have been demonstrated to be effective in several studies (Woo and Shim 1978, Oh et al. 1979, Lee and Lee 1985).

2. Delimitation

After first record of this species in Korea, a survey was made of the pest's biology, ecology, spread and damage considering also the possible parasitoids that associate with it (Lee 1994).

3. Monitoring

The survey of infestation distribution was carried out in S Korea over different time periods (Anonymous 1976, Lee et al. 1997). The permanent survey sites allowed the evaluation of infestation patterns in both spatial and temporal terms (Chung et al. 1998) and also in relation to fluctuations of its parasites (Jeon and Shin 2002, Jeon et al. 2006).

4. Commodities

No information available.

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Thrips palmi Karny

Common name(s): Palm thrips
Taxa: Insecta: Thysonaptera: Thripidae
EPPO list A1: No. 175.
EU Annex designation: I/A1

Organism

Thrips palmi, the melon thrip, is an important pest of numerous vegetable crops, especially those of the Cucurbitaceae and Solanaceae. It has spread from South-east Asia to most of the rest of Asia, and many Pacific Ocean islands, Africa, Australia, Central and South America, the Caribbean, and Florida. Since 1988 there have been several limited outbreaks in the Netherlands. The adults emerge from the pupae in the soil and move to the leaves or flowers of the plant, where they lay their eggs. The second-stage larva enters the soil, develops there and pupates, completing the life cycle. The specialized mouth-parts are adapted for sap sucking. At 25°C, the life cycle from egg to egg lasts only 17.5 days. *T. palmi* is the vector of several economically important viruses (groundnut bud necrosis tospovirus, watermelon silvery mottle tospovirus, and tomato spotted wilt virus).

1. Detection

Morphological keys have been produced to distinguish *T. palmi* from other native thrips (Strassen 1989, Marullo 1997, Kumar *et al*. 2004, Sovershenova and Levchenko 2004). The larvae can be separated through evaluating their body colour, size and the shape of setae, presence of microtrichia, structure of plaques on integument, marginal processes on abdominal segment IX, and the shape of sclerotized areas. Morphological identification is often a difficult task in the absence of a specialist and is hampered by polymorphism, sex, and stage of development. Molecular techniques can be a useful tool to facilitate the identification. Differences in the mitochondrial cytochrome oxidase I gene (mtCOI) (Asokan *et al*. 2007), direct sequencing and a PCR-RFLP (Brunner *et al*. 2002), DNA barcoding using the Cytochrome oxidase I (COI) gene (Glover *et al*. 2010), PCR-RFLP analyses of the internal transcribed spacer (ITS2) region of ribosomal DNA (Toda and Komazaki 2002), and Real-time PCR (Kox *et al*. 2005) test have been developed to identify economically important thrips species. Specific monoclonal antibodies have also been pproduced (Banks *et al*. 1998). Additionally, the Berlese-funnel technique for thrips extraction from plant material has been developed and tested, indicating that an extraction period of 20 hours is necessary (Casteels *et al*. 2009). The use of sticky traps is an efficient method for the detection of *T. palmi.* Numerous trials have indicated that blue traps have the best trapping ability (Chen Huaping *et al*. 1997, Song *et al*. 1997, Jimenez *et al*. 2004) whilst, conversely, other studies indicated that white sticky traps caught significantly more adults than the other colours examined (Huang 1989, Salas and Mendoza 1996, Babu *et al*. 2004). In one study there was no difference between blue and white using coloured water traps (Freitas and Bueno 1998). Therefore the use of blue traps and, only as a second choice, white trap is strongly suggested. Concerning positioning, traps placed at 50 cm (Huang 1989) or 30 cm above ground level showed the best performance (Jimenez *et al*. 2004). Sticky traps have been used to evaluate the possible presence of this species in Europe, but no specimens of *T. palmi* were detected in surveys carried out across several countries (Kovacs *et al*. 2006, Simala and Milek 2008, Cargnus and Fiori 2009). For direct detection of *T. palmi*, the best technique is leaf beating onto a tray using one leaf of the apical third per plant and 35 plants per field. This method was shown to be superior to other methods (e.g. direct counting of insects on the lower leaf surface) (Bacci *et al*. 2008).

2. Delimitation

After the first record, a survey was carried out to evaluate the distribution of *T. palmi*, showing a progressive expansion in Korea (Hong *et al*. 1998), Cuba (Elizondo *et al*. 2003), and Australia (Layland 1991).

3. Monitoring

Generic surveys of thrips have been carried out in a number of different countries (Hua *et al*. 1997, Cho *et al*. 2000, Arevalo *et al*. 2003). There have been specific surveys of *T. palmi* to describe the species phenology (Osorio and M.C. 2003) or to evaluate the incidence in a particular important crop (Frantz *et al*. 1995, Dharpure 2002) or to understand the host plant range (Kajita *et al*. 1996, Salas 2003).

4. Commodities

Thrips palmi has become one of the most common pests found on internationally traded plant material (Loomans and Vierbergen 1997, Karnkowski 2005). It is commonly detected on products for final consumption (especially orchid cut flowers imported from Thailand for example) (Marullo 1997, Bayart *et al*. 1999, Karnkowski 1999) but was also found in plants for planting (aubergine and balsam pear) (Vierbergen 2001). A survey of thrips occurring in ornamental orchid nurseries and a packhouse in Thailand showed that *T. palmi* was usually present in this protected environment (Kajita *et al*. 1992). Pre-shipment disinfestation treatments are a critical component in the prevention of the spread of *T. palmi* (Hara 2002). Methyl bromide fumigation conditions were evaluated in a number of trials. *Thrips palmi* eggs on broccoli were killed completely in conditions of MB 35g/m^3 for 3 hours at 10°C or MB 26.5g/m^3 for 3 hours at 15°C (Misumi *et al*. 2009), whereas the efficiency of irradiation with gamma rays at a dose of 400 Gy was also demonstrated (Takano *et al*. 2004).

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Tilletia indica Mitra

Common name(s): Karnal or partial bunt of wheat
Taxa: Fungi: Basiomycota: Tilletiaceae
EPPO A1 list: No. 23
EU Annex designation: I/A1

Organism
Tilletia indica is fungal pathogen of wheat (*Triticum* spp.) native to southern Asia, known as karnal blunt of wheat. The disease is primarily found in the Far East although outbreaks have occurred in North and South America.

1. Detection
The detection of *Tilletia indica* is difficult due to the fact that until full maturity there are no obvious visible symptoms on the plant, except for light yellow spots and increased shoot production (Kochanova et al. 2006). A simple technique for the detection of *Tilletia indica* involves soaking seed samples in 0.2% NaOH solution for 24 h at 20°C: seeds infected by the fungus can be identified by the black, diseased portions which contrast with the pale yellow healthy parts (Agarwal and Verma 1983). In absence of bunted grains detectable with a visual inspection, examination of seed washings is essential (Shamshad and Mathur 1989). The detection can be more difficult in seed samples treated with fungicides, the sodium hydroxide seed-soak method was found to give higher counts of infected seed than routine visual inspection. Of the methods used to detect surface-borne teliospores on normal-looking seed, examination of seed washings under a stereomicroscope was found to be the most effective (Agarwal and Mathur 1992). A study was conducted to compare the merits of different techniques for the detection of *T. indica* on wheat. the seed soak method was seen as uneconomical, time-consuming, cumbersome, unreliable and quantitatively inaccurate because many of the teliospores are lost during decantation and drying. However, teliospores are reliably detected by the washing test which is easy, rapid, less time consuming and quantitatively accurate and, as a result, has been adopted by several countries (Varshney 1999). A good efficiency was showed by a combined filter and centrifuge (FC) extraction technique that was developed to assay wheat seed for teliospores of *T. indica* (Castro et al. 1994). A seed immunoblot binding assay (SIBA) was suggested to be useful in routine monitoring of wheat lots for the presence of *T. indica* teliospores (Kumar et al. 1998). An immuno-dipstick test was developed for detection of teliospores of Karnal bunt using crude and DE-52 purified anti-teliospore antibodies (Kesari and Kumar 2003, Kesari et al. 2005).
Immunochemical techniques have been developed for distinguish Karnal bunt from other *Tilletia* species and for accurately and efficiently detecting *T. indica* teliospores in wheat (Kutilek et al. 2001). For example, a PCR assay was developed with a high level of sensitivity (Smith et al. 1996). The technique was further refined (Frederick et al. 1998, Yi et al. 2002), and methods useful for discrimination of *T. indica* and *T. walkeri* were developed using a TaqMan system (Frederick et al. 2000) and a two-step PCR protocol using FRET probes (Tan and Murray 2006). PCR based RAPD profiles could distinguish *T. indica* from other similar species as *T. barclayana* (Mishra et al. 2002). A Real-time fluorescent PCR was developed using a Smart Cycler, which provides the utility of portability for use in the field, rapid cycling times, and multi-channel capability for simultaneously running several different cycling conditions (Frederick and Snyder 2001). A one-tube fluorescent assay for the quarantine detection and identification of *Tilletia indica* and other grass bunts in

wheat were developed (Tana et al. 2009) that could be integrated into the current international diagnostic protocol based on morphology followed by germination of the spores and a molecular protocol to confirm the identity (Tan et al. 2010). The methods used for the isolation of the teliospores of *T. indica* from infected grain is of high importance with size-selective sieving proving to be more than 83% faster than the standard centrifuge seed wash (Peterson et al. 2000). Detection of *T. indica* teliospores in seed can be improved by elimination of contaminating microorganisms with acidic electrolyzed water (Bonde et al. 2003). An indirect ELISA was suggested for the determination of teliospore concentration in infected seed lots employing anti-teliospore antiserum generated against intact teliospores of *Tilletia indica* (Singh et al. 2011).
Image analysis can be a useful tool for the identification of the fungus. For example, image-processing software can automatically locates spores on a given image and calculates perimeter, surface area, number of spines and spine size, maximum and minimum ray radius, aspect ratio and roundness. Principal components analysis (PCA) is subsequently performed on the parameters to obtain a linear separation of spore species (Chesmore et al. 2003).
The protocols based on conventional, serological and biomolecular techniques used for detection and identification of *T. indica* have been summarized and compared (Porta-Puglia and Riccioni 2000, Hernandez-Hernandez et al. 2002). The first record of *T. indica* in the USA used morphometric analysis followed by PCR for diagnosis confirmation (Ykema et al. 1996).

2. Delimitation
No information available.

3. Monitoring
Extensive surveys have been conducted in the wheat-growing states of different countries to determine the incidence of *T. indica* (Singh et al. 1980, 1985, Rai et al. 1988, Anil et al. 1990, Bhutta and Ahmad 1994, Sharma et al. 1998, Bhutta et al. 1999, Beantbir et al. 2001, Rewal et al. 2001, Ehsan Ul et al. 2002, Duhan et al. 2004, Shakoor et al. 2008, Kiran et al. 2010), or the diseases of wheat grain (Paul 1996). A survey was carried out to determine the presence of *T. indica* spores on wheat seeds from various countries collected at the International Centre for Agricultural Research in the Dry Areas (Diekmann 1987). The monitoring was useful in that it helped identify factors associated with development and spread of karnal bunt of wheat (Singh et al. 1996, Beniwal et al. 1997, Borkar and Choudhary 2007).

4. Commodities
The quarantine inspection of imported seed for the risk of introduction of *T. indica* (Shamonin 1980, Kehlenbeck et al. 1997, Valvassori et al. 2004) is of importance. The fungus was identified on wheat imported into the USSR from Mexico (Shinkarenko and Kolesnichenko 1981) or into India (Lambat et al. 1983) and into Syria from different countries (Asaad and Abang 2009). Also, the importance of international agreements and centralized control of germplasm exchange and trade in agricultural products to avoid introduction was highlighted (Oliveira et al. 2002). The techniques used for identification included the blotter test method (Talevi et al. 2004). A model of the risk of entry, establishment, spread, containment, and economic impact of *Tilletia indica* was developed in Australia (Stansbury et al. 2002). Analysis of the interception database proved important in determining likely pathways of introduction based on where, when, and how the disease was intercepted when entering the United States (Marshall et al. 2003). The risk of the introduction and spread within Europe has also been modeled (Anonymous 2010).

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Tomato black ring virus

Common name(s): TBRV, bean ringspot, beet ringspot, potato pseudo-aucuba (and others)
Taxa: Viruses: Secoviridae: Nepovirus
EU Annex designation: II/A2

Organism
TBRV infects a wide range of herbaceous and woody monocotyledonous and dicotyledonous species including many that are important crop plants such as *Vitis vinifera*, tree fruit and small fruit species, sugar beet, potatoes and many vegetables. It appears on these plants as necrotic rings, spots and flecks, systemic chlorotic ringspots, mottle, stunting, leaf malformation and vein yellowing. The nematode *Longidorus elongatus* is a possible vector of the virus (Hubschen *et al*. 2004).

1. Detection
The visual detection of the virus is unsatisfactory and indexing is required (Courcier 1978). The methods of host plant reactions and serological properties have been used to isolate tomato black ring virus on artichoke (Migliori *et al*. 1984). The plant *Nicotiana occidentalis* P-1 has been shown to be a useful test plant for tomato black ring virus (Kryszczuk and Chrzanowska 2000).A number of serological technique have been developed proposed including ELISA (Surguchova *et al*. 1998), sandwich ELISA (Chirkov *et al*. 1991), and DAS-ELISA (Etienne *et al*. 1991). A dot blot hybridization assay has been developed to identify two related nepoviruses, tomato black ring nepovirus and Hungarian latent grapevine chrome mosaic nepovirus (Bretout *et al*. 1989). The serological diagnostic methods and other important factors, such as host range, symptomatology, electron microscopy, purification have been reviewed by Pospieszny and Borodynko (1999). Investigations were carried out to establish optimal time for using ELISA for detection of virus (Kolbanova and Kukharchik 2001) and a variant of this methodology was used to determine the first record of tomato black ring virus in China (Zheng *et al*. 1990).
More recently, mlecular technique have increasingly been used., such as RT-PCR (Palczewska *et al*. 2001, Pospieszny *et al*. 2003, Digiaro *et al*. 2007), a novel highly sensitive isothermal AmpliDet RNA (Klerks *et al*. 2001), multiplex RT-PCR and real-time RT-PCR (Zhang *et al*. 2009) and a TaqMan-based real-time one-step RT-PCR assay (Harper *et al*. 2011).

2. Delimitation
No information available.

3. Monitoring
A survey on the presence *Longidorus* spp. nematode vector of the virus gave positive results in about 10% of the samples of tomato black ring virus and/or raspberry ringspot examined (Stoeen and Bjoernstad 1973). Tomato black ring virus has been identified in generic surveys on several different crops (e.g. crucifers, *Ribes, Rubus*) and in different environments (forest, crop and greenhouse) in several countries (Shukla *et al*. 1974, Shukla and Schmelzer 1975, Sweet 1975, Bremer 1983, Polak *et al*. 1990, Smrcka 1990, Anonymous 1991, Schimanski 1991, Pelet 1992).
The tools used for the detection of virus were commonly ELISA (Fidan 1993, Smrcka 1993, Fidan 1995, Herrera M and Lavin A 1998, Koukharchik *et al*. 2000, Jarrar *et al*. 2001, Nyerges *et al*. 2001, Subikova *et al*. 2002, Cseh *et al*. 2009) and DAS-ELISA (Honetslegrova and Spak 1995, Myrta *et al*. 1995, Spak 1995, Spak *et al*. 1997).

4. Commodities

Techniques to propagate virus-free grapevines from green shoots have been evaluated. Thermotherapy of green shoots proved to be the most successful at low night and high day temperatures in Norway (Anonymous 1973). Another treatment proposed was the use of hot air treatment at 37°C for 3-6 weeks (Kaiser 1980) whilst dry air thermotherapy in conjunction with tissue culture resulted in virus control in up to 80% of plants (Upadyshev 1996). In nurseries and tree plantations the virus was been readily detected (Schimanski *et al*. 1976, Tapio 1985). Tomato black ring virus was isolated from diseased potatoes cv. Anett imported into Kenya (Kaiser *et al*. 1978) and has also been identified in germplasm accessions for numerous plant species imported from different countries (Chalam *et al*. 2005, Chalam *et al*. 2008). The importance of sanitary control of plant material inputs and outputs was highlighted as has been the use both molecular and immuno-serological diagnostic techniques for the detection of this nematode-transmitted viruses (Perez-Jimenez *et al*. 2009).

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Tomato ringspot virus

Common name(s): TomRSV, ringspot and mosaic, Eola rasp leaf (+ several others)
Taxa: Viruses: Secoviridae: Nepovirus
EPPO A2 list: No. 102
EU Annex designation: I/A1

Organism
The name Tomato ringspot virus (TomRSV) indicate viruses occurring in a number of woody and ornamental plants, both dicot and monocot. It has been intercepted on *Pelargonium* imported from North America, where it is vectored by the nematode *Xiphinema americanum*. The virus has a widespread distribution globally and occurs in the EU/EPPO region.

1. Detection
A leaf grafting technique has been developed to obtain higher transmission, shorter incubation periods and more severe initial symptoms (Frazier 1974a), with the selection of virus indicator plant clones (Frazier 1974b). An ELISA test was developed to identify tomato ringspot virus in infected raspberry leaves diluted 1:4000 with healthy raspberry sap and readily identified the virus in leaf, stem, bud, and root samples from infected plants in late autumn. However, detection of the virus in crude red raspberry juice in autumn was unsuccessful by bioassay and highly variable by agar gel serology (Converse 1978). Later the technique was improved in other plant species (Lister et al. 1980, Powell 1990) that developed optimum conditions of reaction and a new incubation medium (Albouy and Poutier 1980). An indirect dot-immunobinding assay (DIA) and a double antibody sandwich (DAS)-DIA show a good efficiency in virus identification (Powell 1987). ELISA and grafting to indicators (Powell et al. 1991) were examined (Gonsalves 1979) as were two serological (ELISA and DAS ELISA) techniques, highlighting the fact that single leaf disks do not provide sufficient Tomato ring spot virus for efficient detection by either ELISA method (Powell 1984). ELISA and dot blot hybridization probes (Powell et al. 1989, Halbrendt et al. 1990) specific to TomRSV infection were developed for virus identification in woody and herbaceous hosts (Hadidi and Powell 1991). The same technique, however, proved unsuccessful for detection of TomRSV RNA from the bark tissue of rootstock. A molecular technique protocol has been developed for the detection of tomato ringspot virus in herbaceous and woody plant tissues and the high sensitivity and specificity of this TomRSV PCR provided researchers with a new and powerful technique to study this important plant virus (Griesbach 1995). Refuter improvements were suggested, including a new colorimetric technique that did not rely on gel electrophoresis or molecular hybridization to detect virus-specific PCR products (Rowhani et al. 1998a). This technique provides a means to apply PCR technology to a large number of samples (Rowhani et al. 1998b). A RT-PCR method for virus detection and identification proved to be less time-consuming and labour-intensive by comparison with the other methods (inoculated test-plants, morphological and serological properties of virus isolates) (Samuitiene et al. 2003b, Zitikaite and Staniulis 2006). Although the efficiency of RT-PCR (Digiaro et al. 2007) and multiplex RT-PCR (Wu et al. 2006) was confirmed, other studies highlighted failures of detection and false positive results. DIG labeled probe hybridization with solid PCR product swas subsequently performed as well as electrophoresis of liquid product in order to reduce false positive result (Zhao et al. 2003). Further to this, a real-time fluorescent RT-PCR one step assay were suggested as reliable, sensitive, quick

and easy detection method (Zhu et al. 2003). However, a nested PCR using 2 rounds PCR amplification with 2 pairs of primer was 100-200 times in sensitivity compared with RT-PCR (Yang et al. 2005). Further techniques suggested include the use of a real-time RT-PCR SYBR Green assay (Stewart et al. 2007) and hybridization capture real-time PCR (Zhao et al. 2007). A single-step multiplex reverse transcription-polymerase chain reaction (m-RT-PCR) for simultaneous detection of virus of stone fruit trees has been described(Youssef and Shalaby 2009) whilst a complementary DNA (cDNA) macroarray has been developed for the simultaneous detection of 12 different potato viruses (Maoka et al. 2010). A novel highly sensitive isothermal AmpliDet RNA has been developed for identification of nematode-transmitted nepoviruses including tomato ring virus; the viruses being identified via a molecular beacon directed to a selected virus specific sequence within the amplicon formed during NASBA (Klerks et al. 2001). A reverse transcriptase-polymerase chain reaction has also been developed to detect TomRSV in vector nematodes (Pinkerton et al. 2002).
Various serological and molecular techniques (ELISA and RT-PCR) were compared (Msikita et al. 2007), showing that the detection limits of DAS-ELISA and RT-PCR methods were about the same, but at least 1000 times lower than that of the semi-nested RT-PCR (Wen et al. 2007). Other molecular techniques, such as a TaqMan assay, also provide reliable diagnostic assays for this pathogen (Osman et al. 2008).

2. Delimitation
No information available.

3. Monitoring
Surveys were carried out to evaluate the presence and incidence of tomato ring spot virus in specific crops (Schooley and Martin 1994, Finn and Martin 1996, Lee et al. 1996, Lockhart and Currier 1996, Azery and Cycek 1997, Khan et al. 1997, Nava et al. 1997, Zouba et al. 1997, Martin 1998, Koukharchik et al. 2000, Moussa et al. 2000, Nome et al. 2000, Herrera M and Madariaga V 2001, Jarrar et al. 2001, Milkus 2001, Rakhshandehroo et al. 2005), and also analysed the presence of the virus in plants, that could be a possible source of introduction into orchards (Powell et al. 1984). Different plant species were analysed for the presence of the tomato ring spot virus using either an ELISA diagnostic test (Ferris and Castello 1988, Powell et al. 1990, Fidan 1993, 1995, Zouba et al. 1997, Martin 1998, Herrera M and Madariaga V 2001, Jarrar et al. 2001, Subikova et al. 2002, Golnaraghi et al. 2004, Navalinskiene and Samuitiene 2004, Medina et al. 2006, Mandic et al. 2007, Jossey and Babadoost 2008, Matus et al. 2008, Massumi et al. 2009, Coneva et al. 2010) graft inoculation in indicator host (Khan et al. 1997, Babini et al. 2004) or DAS-ELISA (Choueiri et al. 2001, Samuitiene et al. 2003a, Salem et al. 2005). A wide survey on different plant species showing symptoms (mosaic, mottle) or without symptoms were analysed by serological tests (double diffusion in agarose gels and ELISA) or electron microscopy to identify a large number of infected plants (Gumedzoe 1993, Navalinskiene and Samuitiene 1999). Commercial kits against for viral detection based on ELISA (Rodriguez et al. 2004), DAS- and indirect-ELISA (Sanchez-Cuevas and Nameth 2002) have been used to evaluate the frequency and distribution of this virus.

4. Commodities
The import of infected material is considered an important route for the introduction of the virus into new areas (Krijthe and Noort 1974, Wood 1989, Bai et al. 2007). There are reports of imported propagation material being analysed with DAS-ELISA test (Ozdemir and Kaya 2008) whilst the procedures of inspection and diagnosis of tomato ringspot have been described for different plant species (Anonymous 1990, Jones 1991, Weidemann 1991). The need of propagating stock

certification has been discussed (Mink et al. 1985, Kanaan-Atallah et al. 2000). Virus free plants are produced by meristem culture and heat treatment. With regard to checking that plants are virus free, a table is given showing seasonal differences in the ease of detection of tomato ring spot virus symptoms on indicator plants and by serology (Paludan and Begtrup 1988). ELISA protocols have been developed to be reliably used in a nursery certification programme (Golino et al. 1992). In addition at this, serological tests and electron microscopy have also been used (Zhu et al. 1995).

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Tomato spotted wilt virus

Common name(s): TSWV, spotted wilt, bronze leaf
Taxa: Viruses: Bunyaviridae: Tospovirus
EPPO list A2: No. 290
EU Annex designation: I/B

Organism

Tomato spotted wilt virus (TSWV) exhibits a worldwide distribution. The virus has a wide host range, mostly herbaceous hosts. Chicory, lettuces, tobacco, tomatoes and various ornamental crops are the main hosts. The virus is mechanically transmissible and is not seed-transmitted. In nature, TSWV is transmitted by several species of thrips. Biologically distinct isolates of TSWV exist in nature and isolates that differ in thrips transmissibility, symptomology and symptom severity have been described. TSWV causes systemic infection in most of the crops it infects and can induce a wide variety of symptoms, which may vary on the same host species, with cultivar, age, and nutritional and environmental conditions of the plant. Further strains of TSWV with different biological properties have been isolated. Infection at early stages of the plant growth causes the most damage and may include severe stunting of the entire plant which often results in death.

1. Detection

The mechanical inoculation of indicator hosts and subsequent visual inspection for local and systemic symptoms is the most suitable method for early detection of tomato spotted wilt virus. The confirmation of TSWV infections, however, is usually achieved using biochemical, serological and, more recently, molecular techniques (Arts *et al*. 1992, Scagliusi *et al*. 1996). Numerous ELISA techniques have been developed: ELISA (Cambra *et al*. 1994, Bo *et al*. 1995), double-antibody sandwich (DAS)-ELISA (Xia *et al*. 1996), Dot-ELISA (Nagata *et al*. 1997), and indirect ELISA (Vasquez and Angarita 1999). Various different ELISAs were evaluated for the detection of tomato spotted wilt tospovirus (TSWV) in plant extracts (Louro 1994). Other techniques include dot-blot hybridization (Saldarelli *et al*. 1996, Eiras *et al*. 2001) and a single-stranded conformational polymorphism (SSCP) assay that could be used to differentiate different virus strains (Finetti Sialer *et al*. 2000).

Numerous approaches have been produced that are based on the polymerase chain reaction (Nolasco *et al*. 1993, Mumford *et al*. 1994, Weekes *et al*. 1996). Various improved techniques have been described including enriching groundnut tissue extracts for TSWV detection by one-tube RT-PCR (Jain *et al*. 1998), a silica capture reverse transcription polymerase chain reaction (SC-RT-PCR), and immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) (Korbin *et al*. 1995). A primer with a quenched fluorophore and a probe complementary to an internal portion of the amplicon has also been developed (Sialer *et al*. 2000).

Triple antibody sandwich (TAS)-ELISA with polyclonal and monoclonal antibodies (PAbs and MAbs) (Roggero *et al*. 1996) were developed. MAbs were used in antigen coated plate ELISA (ACP-ELISA) (Bandla *et al*. 1994). RT-PCR (Tsuda *et al*. 1994) was used to identify TSWV in the insect vector. A monitoring system was developed that uses petunia indicator plants and yellow sticky traps to detect infective thrips and their sources (Ullman *et al*. 1998). Combinations of different techniques were involved in the first report of pathogen in different countries (Ruter and Gitaitis 1993, Pappu *et al*. 1998, Esfandiari *et al*. 2005, Mullis *et al*. 2006).

2. Delimitation

Virus detection in leaves was shown to be reliable if tubers were tested late during storage, and

this proves to be important in avoiding the spreading of virus (Asjes and Blom-Barnhoorn 1997). The identification of the origin of infections is important to avoid spread to other areas (Scheel 1996).

3. Monitoring

National or regional surveys are carried out to evaluate the distribution and incidence of the virus. Usually plants exhibiting the symptoms are collected and the material used for inoculation tests on indicator host, then analysed through ELISA or via molecular tests (Bitterlich and MacDonald 1993, Nagata *et al*. 1995, Hill and Moran 1996, Mertelik *et al*. 1996, Vicchi and Bellardi 1996, Antignus *et al*. 1997, Latham and Jones 1997, Al-Shahwan *et al*. 1998, Gracia *et al*. 1999, Mertelik and Mokra 1999, Ben Moussa *et al*. 2000). Surveys can be extensive, evaluating numerous plant species, or locally intensive, considering important sources of the virus such as ornamental nurseries (Hill and Moran 1996) and ornamental plants (Mertelik and Mokra 1998, Chatzivassiliou *et al*. 2000); finally, specific surveys were conducted to know the incidence on some crops (Fiedorow 1999, Ormeno and Sepulveda 2005).

4. Commodities

The extensive movement of plant material is frequently the source of introductions of the virus into new regions (Kaminska and Korbin 1994, Tomassoli and Barba 1994). A precise test for virus identification is required because, for example, the leaf tests are sometimes unreliable due to unequal distribution of TSWV in, for example, Dahlia (Schadewijk 1996). Thrips, in particular *Frankliniella occidentalis*, contribute the spreading of TSWV on a large scale (Kaminska and Korbin 1994).

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Tomato yellow leaf curl virus

Common name(s): TYLCV
Taxa: Viruses: Geminiviridae: Begomovirus
EPPO A2 list: No. 182
EU Annex designation: II/A2

Organism

The main host of TYLCV is tomatoes. *Datura stramonium* (Jimson weed) and tobacco can be artificially infected. TYLCV is an Old World geminivirus, first described in the Middle East in the 1960s. It remained of limited importance, and presumably of limited geographical distribution, until biotype B of its vector, *Bemisia tabaci,* started to spread dramatically through the EPPO region in the 1980s (Czosnek et al., 1997). In the plant, the virus develops within the phloem and induces cytological changes.

1. Detection

ELISA method were first developed in the early 1990s for diagnosis of this disease (MacIntosh et al. 1992, Abouzid et al. 2002) and, later, ELISA-DAS was implemented for the detection of this virus (Fonseca et al. 2004). Specific DNA probe can serve as a powerful tool in detection of TYLCV that can be discriminated in both plant tissue and in the insect vector (Quinones et al. 1999). A squash-blot method, that does not require treatment of the sample, was showed potential for screening Tomato yellow curl leaf infection in the field (Navot et al. 1989). A PCR assay was also efficient for virus identification in plant tissues and the insect vector (Navot et al. 1992, Di Martino et al. 1993, Mehta et al. 1994, Atzmon et al. 1998, Maxwell et al. 2002). Print-capture PCR (P-PCR), allows direct amplification of DNA from infected plant or whitefly tissues through printing directly on Whatman 3MM paper, without the need of any grinding, incubation, or washing steps prior to the amplification reaction (Navas-Castillo et al. 1998). A further PCR method using degenerate geminivirus primers offers a rapid means of geminivirus detection (Rojas et al. 1993). Specific primers were designed from the sequence of TYLCV-Israel (IL) (Shalaby et al. 1997, Onuki et al. 2004). A single-tube PCR assay gives, with a single reaction, an overview of the species present in the sample and will be useful for screening the causal agents of TYLCD (Davino et al. 2008). A real-time PCR were developed that proved suitable for the detection and quantification of this virus in tomato, pepper and bean plants (Chouchane et al. 2006). A loop-mediated isothermal amplification (LAMP) has also been developed (Fukuta et al. 2003, Fukuta et al. 2005).
Three different molecular techniques (Southern blots, squash blots and polymerase chain reaction amplification) were described by Czosnek et al. (1994). NASH (non-radioactive nucleic acid hybridization) method was optimized to detect TYLCV-Is in tomato (Quinonez et al. 2004) A digoxigenin-labelled DNA probe was used to identify the virus in stem, crown and root sections (Abou Jawdah et al. 1995). A synthetic antigen from a peptide library can be an effective positive control in immunoassays for the detection and identification (Ziegler et al. 1998). A DNA microarray chip was developed for screening 10 major economically important tomato viruses, among which Tomato yellow leaf curl virus, from infected plants using "Combimatrix" platform 40-mer oligonucleotide probes (Tiberini et al. 2010). Trapping of Tomato yellow leaf curl virus (TYLCV) and other plant viruses with a GroEL homologue (Akad et al. 2004) was developed. Several methods were tested to identify and type TYLCV isolates, such as Tomato yellow leaf curl-Sardinia (TYLCV-Sar) and Tomato yellow leaf curl-Israel (TYLCV-Is), from field samples. The RFLP method was seen to be highly reliable due to the easily recognisable pattern produced by the two virus species present in Europe. Dot-blot hybridisation is less expensive for identifying TYLCVs in large

numbers of samples, particularly when a mixture of two probes is used. PAGE of the C2 fragment is the fastest of the methods tested (Accotto et al. 2000). To discriminate between Tomato yellow leaf curl virus isolates a PCR has been developed (Martinez-Culebras et al. 2001, Ueda et al. 2005). The multiplex PCR method was used to discriminate between the TYLCV and TYLCV-Mld, two closely related but distinct viruses (Lefeuvre et al. 2007). Similarly, a real-time TaqMan PCR was developed to detection and differentiation of tomato yellow leaf curl virus (TYLCV) and Tomato yellow leaf curl Sardinia virus (TYLCSV) (Papayiannis et al. 2010)
Critical evaluations have shown that PCR and NAH (nucleic acid hybridization) showed high levels of efficacy, sensitivity, specificity and reproducibility (Martinez et al. 2001). The application of serological techniques for the diagnosis of tomato yellow leaf curl disease were analysed and the results indicated that ELISA and IC-PCR assay are considered to be very practical for diagnosing large numbers of samples. On the other hand, RIPA is considered to be suitable for diagnosis by tomato growers, since it is simple and agile as using a pH test paper (Osaki et al. 2011)
A summary of methods for the detection of TYLCV and TYLCSV has been produced (Accotto and Noris 2007). The PCR method has been used for determining the first record of the virus in some regions (Polston et al. 2002, Akad et al. 2007, Yu et al. 2009, Salati et al. 2010); whereas both TAS-ELISA and PCR were used for the first detection in Reunion Island (Peterschmitt et al. 1999). A set of techniques (inoculated plants as the positive controls, duplex PCR and electrophoretic analysis) was used to confirm TYLCV in the Balearic Islands (Font et al. 2002). The presence of the insect vector in some countries does, however, does not necessarily mean that the virus will also be present (Moriones and Garcia-Andres 2008).

2. Delimitation

In the Netherlands, after the first record, a survey was conducted in all tomato crops in a surrounding area of 40 Km^2. TYLCV was found in 19 of 27 crops. Results of the sequence analyses and surveys suggested that the outbreak resulted from a single introduction of the virus, whilst the insect vector *Bemisia tabaci* accounted for the local spread. Measures taken to eliminate the virus included the removal and subsequent destruction of infected tomato plants as well as the eradication of *B. tabaci*. No TYLCV infections were found during surveys in 2008 (Botermans et al. 2009). Similar actions were undertaken in Jiangsu Province (China), where the survey indicated a serious spreading trend, and eradication was not practicable (Sun et al. 2009).

3. Monitoring

The occurrence of TYLCV has been demonstrated in numerous surveys (Al-Musa and Mansour 1983, Ioannou 1985, Pelet 1992). Numerous surveys were carried out to ascertain the presence, distribution and disease incidence of virus of tomato crops (Nono-Womdim et al. 1996, Ascencio-Ibanez et al. 1999, Navas-Castillo et al. 1999, Alfaro-Fernandez et al. 2010) on more than one crop (Finetti-Sialer et al. 2005). The identification of reservoir weed hosts has also been addressed (Kashina et al. 2002b, Ansari and Tewari 2005, Fanigliulo et al. 2007, Fazeli et al. 2009, Nannini et al. 2009). Some surveys have specifically addressed the presence of the disease in whiteflies (Tahiri et al. 2006), and also the identification of reservoir hosts (Salati et al. 2002). Specific surveys have also addressed the identification of different strains (Martinez Zubiaur et al. 1996, Kashina et al. 2002a). PCR amplification coupled to RFLP allowed both identification and clustering of isolates (Fekih-Hassan et al. 2003). The techniques used were molecular (e.g. PCR (Herrera et al. 1999, Reina et al. 1999, Quinones et al. 2001, Ying and Davis 2001, Sedegui et al. 2002, Lobin et al. 2010), PCR/RFLP and dot blot hybridization (Nannini et al. 2005)), serological (e.g. TAS-ELISA (Al-Shahwan

et al. 2001) DAS-ELISA (Sertkaya and Sertkaya 2005) TAS-ELISA and ELISA (Kashina et al. 2007, Massumi et al. 2009)) or a combination of different techniques (Segundo et al. 2008). All these technique were flanked by morphological analysis (Cardenas-Conejo et al. 2010). DAS-ELISA positive results were obtained with a broad-spectrum reagent combination that is useful to detect a range of begomoviruses. Two probes comprising digoxigenin-labelled DNAs representing the coat protein gene of either TYLCSV or TYLCSV were used to identified both viruses (Crescenzi et al. 2004). Whitefly species and their biotypes, PCR-positive individuals to TYLCV were also considered (Sugiyama et al. 2008). In numerous cases more than one technique was used together as complementary methods to ascertain the identity of the virus (Montasser et al. 1999). A triple antibody sandwich-ELISA was used to detect three viruses in field-collected samples of *Bemisia tabaci* (Konate et al. 1995, Aboul-Ata et al. 2000). A monitoring carried out in numerous countries of the Mediterranean used two simple and reliable multiplex PCR protocols (mPCR) to detect TYLCV and TYLCV-Sardinia viruses, in addition to TYLCV-Mld strain (Anfoka et al. 2008). A worldwide survey of TYLCVs (25 countries) indicated that tomato geminiviruses fall into three main clusters representing viruses from 1) the Mediterranean/Middle East/African region, 2) India, the Far East and Australia, and 3) the Americas (Czosnek and Laterrot 1997).

4. Commodities

A dot-blot hybridization system using digoxigenin-labelled riboprobes and chemiluminescent detection was developed for the diagnosis of infections by different viruses including TYLCV. The system was successfully applied to a sanitary certification purposes. Tomato samples were collected using a systematic sampling method (Saldarelli et al. 1996). The infected plantlets were a source of spread of virus in new region, the Camargue, France during summer 1999, with material coming from Almeria, Spain (Dalmon et al. 2000). The nursery surveys are extremely important in preventing the spread of the virus (Jebbour and Abaha 2002). Imported and exported tomato fruit are not monitored for TYLCV infection because they are not considered to represent a potential risk as a virus source for whiteflies. A survey of tomato fruit imported into Reunion Island indicated that more than 50% of the fruit contained TYLCV as determined by DNA blot analysis. Moreover, it was shown that TYLCV was present at a high titer in tomato fruit, and demonstrated that it can be acquired by whiteflies and subsequently transmitted to healthy tomato plants (Delatte et al. 2003, Falah et al. 2009).

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Toxoptera citricidus (Kirkaldy)

Common name(s): Brown citrus aphid, black citrus aphid, oriental citrus aphid
Taxa: Insecta: Hemiptera: Aphididae
EPPO A2 list: No. 45
EU Annex designation: II/A1

Organism
The brown citrus aphid *Toxoptera citricidus* is specific of all citrus varieties and is considered one of the most serious pests of these species. It infests the stems and new leaves of citrus trees and in addition to feeding damage it is a highly efficient vector of citrus tristeza virus, a virus that can be devastating in certain instances (Michaud 1998). The insect is widely distributed and is known to be present in Africa Asia, the Americas, some Pacific Islands and New Zealand (Michaud 1998)

1. Detection
The taxonomic characters for the identification of *T. citricidus* were described in a key by Stoetzel (1994). The yellow water traps has been used as a useful tool to catch the aphid in a number of studies (Lara et al. 1976, Hermoso de Mendoza et al. 2008). The incidence of *T. citricidus* in Central America was examined with yellow pan traps (Voegtlin and Villalobos 1992). Visual inspection and successively laboratory identification for suspected samples were the techniques used to evaluate the presence of the aphid in Eastern Sicily; the results of this survey suggesting that the aphid had still not arrived in the area at that time (Tumminelli and Pedrotti 2008). Similar results were obtained with collected samples from the trees and yellow water traps in Portugal (Cruz de Boelpaepe and Ferreira 1998).

2. Delimitation
After the first record of *T. citricidus* in Florida, a delimiting survey was carried out to define the actual extent of the infestation and evaluate the sources of the insect (Halbert et al. 1998). A survey carried out in Archipelago of Madeira highlighted the very fast dispersal of *T. citricidus*, which had become one of the most important citrus pests on the islands within six years of first detection (Fernandes and Franquinho Aguiar 2001).

3. Monitoring
The distribution and/or incidence of *T. citricidus* have been widely evaluated with traps (Schwarz et al. 1970, Hidalgo et al. 1998, Carvalho et al. 2002). Similarly, the seasonal abundance and its natural enemies were evaluated in Puerto Rico (Michaud and Browning 1999). In a general survey concerning estimations of alate aphid populations in the Philippines, water-filled yellow Moericke trays placed above ground were used with *T. citricidus* was the more common species trapped (Gavarra and Eastop 1976). An automated optically sensed identification method based on wing beat waveforms was developed for aphids, including *T. citricidus* (Moore and Miller 2002).
The method of sampling is important in order to obtain accurate population estimates; a sampling of 4 young branches (one per quadrant) of the medium stratum from 150 citrus trees is suggested to be a good procedure for monitoring aphids on citrus trees (Primiano et al. 2005). The position of the traps as part of monitoring procedures instigated as part of IPM programmes is seen as essential, a fact that was explored in Brazil by Toledo et al. (2006).

4. Commodities

The fact that some natural enemies of invasive citrus pests were found in Jamaica, none of which were purposefully imported and released, suggests that pest-infested citrus trees were imported into Jamaica without going through appropriate quarantine procedures (Hoy et al. 2007). Michaud (1998) gives an excellent review of the methods employed to monitor and control this pest (some of which are now outmoded) in a wide range of situations, including the commodity context.

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Trioza erytreae (Del Guercio)

Commoon name(s): Citrus psylla
Taxa: Insecta: Hemiptera: Psyllidae
EPPO A1 list: No. 37
EU Annex designation: II/A1

Organism

Trioza erytreae is a plant sap-sucking hemipteran insect (Psyllidae). It is a serious pest of *Citrus* spp. It is the principal vector of the African form of 'citrus greening disease' caused by the bacterium *Liberobacter africanum*. This species is present in many African countries and it is also recorded in Saudi Arabia and Yemen and in the island of Madeira.

1. Detection

Diagnostic characters are provided to distinguish *Trioza erytreae* from *Diaphorina citri*, with which it may be confused and which is also a vector of greening disease (Mead 1977). Horizontal yellow sticky traps collocated at height of 1 m above ground caught more insects compared to traps placed at a greater height (Van Den Berg and Deacon 1989), the most attractive colour seemed be the fluorescent yellow-green (about 530 nm) (Samways 1987a).

2. Delimitation

No information available.

3. Monitoring

A survey to ascertain the distribution of *Trioza erytreae* have been carried out in a number of different countries (Schwarz *et al*. 1970, Bove 1986, Abate 1988, Swai *et al*. 1992, Fernandes and Franquinho Aguiar 2001) by evaluating common parameters such as the signs of attack or insect captured on host plants or trapped in orchards (Tamesse *et al*. 1999). Proposals to improve the method of trapping have been made by several authors (Samways 1987b, Van Den Berg *et al*. 1991).

4. Commodities

No information available.

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Unaspis citri (Comstock)

Common name(s): Citrus snow scale, white louse scale
Taxa: Insecta: Hemiptera: Diaspididae
EPPO A1 list: No. 226
EU Annex designation: II/A1

Organism
Unaspis citri is polyphagous, attacking plant species belonging to 12 genera in 9 families. The main hosts of economic importance are *Citrus* spp., but it is recorded also in other important crops (e.g. bananas, coconuts, and pineapples). This insect usually occurs on the trunk and main limbs of trees under ten years old. Heavy infestations spread to the twigs, leaves and fruit. Although not found in the Europe, the pest is widely distributed across North/Central America, South America, the Caribbean, Oceania and Africa.

1. Detection
A key with illustrations for the recognition of Diaspididae associated to citrus plants in Tucuman (Argentina) was provided by Claps and Teran (2001) who described a range diaspids occurin in Argentina. The morphological features of the scale were compared with those of *Pinnaspis strachani* (Fernandez et al. 1993). The difficulty in identification of this pest was confirmed by the occurrence of inaccurate records of the insect (Charles and Henderson 2002). In a generic survey on the scales, the first record of *U. citri* was done in continental Portugal (Kozar and Franco 1995).

2. Delimitation
No information available.

3. Monitoring
Surveys addressed to the major pest of pineapple in State of Espirito Santo (Brazil) (Culik et al. 2009) or to the cause of decline of Spanish moss (*Tillandsia usneoides*) in Georgia (Tippins and Beshear 1975) indicated the presence of *U. citri*. The biology of *U. citri* in relation to climate was evaluated in a wide-ranging monitoring programme carried out in African countries (Vilardebo 1974).

4. Commodities
No information available.

References: 14 retained out of 89 retrieved, none added, 7 used for the summary

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Venturia nashicola Tanaka & Yamamoto

Common name(s): Japanese pear scab
Taxa: Fungi: Ascomycota: Venturiaceae
EU Annex designation: II/A1

Organism

Venturia nashicola is the causal agent of Japanese pear scab. It attacks various Asian *Pyrus* species (*Pyrus serotina, P. pyrifolia, P. bretschneideri*) and it has also been reported on various wild *Pyrus* spp. such as *P. betulifolia. V. nashicola* causes severe damage to fruit and leaves; the pathogen overwinters as immature pseudothecia in fallen infected leaves on the ground and as dormant mycelia in the inner tissues of bud scales on the tree. Ascospores, produced in the pseudothecia, are considered to be the primary inoculum in spring (Eguchi and Yamagishi 2008). *V. nashicola* is indigenous to eastern Asia and has no history of spread to new areas (Anonymous 1997).

1. Detection

A polymerase chain reaction was developed for the identification of *Venturia nashicola* (Le Cam *et al*. 2001) and the ITS1-5.8S-ITS2 sequences were valuable in another study as they can be used in determining the relationship of the pathogen species from tree-fruit crops with other *Venturia* species (Schnabel *et al*. 1999). Some morphological features of the conidia permit the differentiation of *V. nashicola* from *V. pirina* (Ishii and Yanase 2000). The kinds of liquid culture media, light time, culture temperature, culture time, pH value of culture medium and oscillation frequency are important determinants in the production mycotoxin by *Venturia* spp. (Xu *et al*. 2009).

2. Delimitation

No information available.

3. Monitoring

No information available.

4. Commodities

No information available.

References: 5 retained out of 128 retrieved, 2 added, 7 used for the summary

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Verticillium albo-atrum Reinke & Berthold

Common name: Verticillium wilt
Taxa: Fungi: Ascomycota: Hypocreaceae
EPPO A2 list: No. 85
EU Annex designation: II/A2

Organism

Verticillium albo-atrum is a soil-borne fungal pathogen with no known sexual stage) *V. albo-atrum* has a limited host range and causes yellowing and wilting adversely affecting the host plant's fitness. *V. albo-atrum* is widespread but predominates in temperate regions and survives as a resting mycelium in the soil and on diseased plant remnants, on the soil surface or incorporated into it. Mycelium or conidia are able to infect plants through healthy or wounded roots. Following root invasion, the mycelium passes into the vascular tracts throughout the plant and subsequently induces a wilting condition.

The quarantine significance of *Verticillium* spp. is closely linked to the existence of host-specific strains, and to the ways in which these arise, spread or are selected. These phenomena are interpreted somewhat differently in different countries, and in relation to different hosts.

1. Detection

Morphological, physiological and biochemical characters were investigated the taxonomic relationship among isolates of the genus *Verticillium* (Jun *et al*. 1991). In the initial attempts the discrimination between *Verticillium dahliae* and *V. albo-atrum* proved difficult (Chambers *et al*. 1985). As a result, electrophoretic protein and enzyme patterns, and antigenic structure, proved useful to discriminate these two fungi (Selvaraj and Meyer 1974), differentiating being important as the wrong identification can have consequence on the quarantine measures taken (Walker 1990). Later, a dot-ELISA, double antibody sandwich ELISA (Sundaram *et al*. 1991) and monoclonal antibody-based double-antibody sandwich-ELISA (Van De Koppel and Schots 1995) assays were developed for the identification of *V. albo-atrum* (Lazarovits *et al*. 1987). The molecular techniques appear to successfully discriminate these two pathogenic species and PCR proven to be faster, more sensitive and more accurate than earlier techniques (Nazar *et al*. 1991, Hu *et al*. 1993, Platt *et al*. 1993, Robb and Nazar 1996, Mahuku *et al*. 1999, Heinz and Platt 2000, Radisek and Jakse 2005, Zhang *et al*. 2005. For example, when compared with the traditional plating assay method the results confirmed that when symptoms of verticillium wilt are observed in potato plants in the field, the major verticillium wilt pathogens present in field soils can be rapidly and reliably detected by the PCR assay (Platt and Mahuku 2000). Further development of a molecular detection system, based on DNA array technology, for rapid and efficient detection of *V. albo-atrum* (Lievens *et al*. 2003) or Novel Cleavable Padlock Probe-Based Ligation Detection Assay (van Doorn *et al*. 2009) are also suggested. The multiplex PCR and a nested PCR were facilitated through the availability of new SCAR markers providing a valuable tool for rapid identification of *V. albo-atrum* PG1 and PG2 hop pathotypes (Radisek *et al*. 2004). The amplified fragment length polymorphism (AFLP) technique is useful for the clear separation of *V. albo-atrum* hop isolates according to their level of virulence and provides genetic differentiation among hop *V. albo-atrum* pathotypes (Radisek *et al*. 2003). Another suggested method is the use of MALDI-TOF mass spectrometry, which should prove useful as a rapid and reliable assay for distinguishing different *Verticillium* spp (Tao *et al*. 2009).

2. Delimitation

A survey was carried out to ascertain the distribution of *V. albo-atrum*. Measures of detection and inspection methods were applied to the spread of hops wilt (Gordon *et al*. 1989, Simoncic *et al*. 2001).

3. Monitoring

Specific surveys revealed the incidence of *V. albo-atrum* in different crops (Konstantinova 1974, Ndubizu 1977, Blangez 1989, Howard *et al*. 1991, Martin *et al*. 1991, Huang and Erickson 1995), and the occurrence of different *Verticillum* species has also been considered (Slattery and Eide 1980). The pathogen was observed in generic surveys of the diseases caused by fungi species (Clarke *et al*. 1983, Garcia-E and Teliz Ortiz 1984, Turkensteen 1987, Morales Garcia 1989, Hijano 1991, Fontem 1993, Tesar 1997, Daugaard and Todsen 1998, Kimpinski *et al*. 1998, Gupta *et al*. 2003, Raj *et al*. 2007, Bhutta 2008). The diagnostic technique used was typically ELISA (Clarke *et al*. 1983). To identify the crop areas affected by *Verticillium albo-atrum,* multispectral aerial photography has been used (Misra and Shedha 1990). Pathogenicity tests and amplified fragment length polymorphism (AFLP) molecular analysis determined two groups of *V. albo-atrum* hop isolates (Radisek *et al*. 2005).

4. Commodities

Verticillium albo-atrum has been identified from imported seeds during the process of routine seed health testing for plant quarantine purposes (Christen 1983, Nath *et al*. 1986, Park and Kim 1986, Agarwal *et al*. 2006a, Agarwal *et al*. 2006b, Agarwal *et al*. 2006c, Agarwal *et al*. 2007). This route could be the cause of spread of this pathogen (Sato 1994). In the past a chemical treatment has been evaluated for the control of this pathogen (Easton *et al*. 1972).

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*Verticillium dahlia (*Klebahn)

Taxa: Fungi: Ascomycota: Hypocreaceae
EPPO A2 list: No. 85
EU Annex designation: II/A2

Organism

Verticillium dahlia is a soil borne pathogen that attacks hops and an exceptionally wide range of other annual (e.g. cotton, melons, Solanaceae and various ornamentals) and perennial crops (e.g. olives, pistachios, *Prunus* spp.) and various forest trees. *V. dahliae* is geographically widespread but is most common in tropical and subtropical areas. *V*. *dahliae* naturally occurs at low levels in soils and grows better at temperatures 25-28^{o}C. The fungus can overwinter as mycelium in perennial hosts, plant debris, and vegetative propagative parts.

The quarantine significance of *Verticillium* spp. is closely linked to the existence of host-specific strains, and to the ways in which these arise, spread or are selected. These phenomena are interpreted somewhat differently in different countries, and in relation to different hosts.

1. Detection

The morphology of *Verticillium dahliae* was analyzed in comparison with *V. tricorpus*, a closed species, on semi-selective media (Goud *et al*. 2003). The isolation is a sensitive phase and it was highlighted that the crushing and dry-sieving can improve the isolation of the fungus from soil (Camporota and Rouxel 1977). It was also confirmed that the condition of soil samples (Guo 2000) and type of the selective medium (Yang and Shang 2002) had a significant effect on the identification results. *In vitro* studies indicate pH 9.5 as the optimum condition for the growth, sporulation and maximum glucose coefficient of the fungus. So far, it is recommended to use an alkaline seed-bed when searching for *V. dahlia* on fennel seed (Ghoneem *et al*. 2009). Three quantitative soil assays (dilution plating, wet sieving, and the Andersen sampler technique) were compared for precision, bias, and time required to assay a sample (Nicot and Rouse 1987). Inter-laboratory methods to quantify microsclerotia of *Verticillium dahliae* in soil were compared and the results highlighted that wet plating assays are less accurate than dry plating assays (Termorshuizen *et al*. 1998). An ELISA technique was developed using different antisera (Sundaram *et al*. 1485, Gerik *et al*. 1987, Gunzelmann *et al*. 1991, Xia *et al*. 2001). The first developed serological method highlighted some difficulties in differentiating *V. albo-atrum* and *V. dahliae* (Chambers *et al*. 1985). The wrong identification can have consequences on the decision to quarantine measures and management (Walker 1990). Among the other serological technique were proposed a Dot-Elisa on nitrocellulose membranes (Lazarovits *et al*. 1987), double-antibody sandwich-ELISA (Van De Koppel and Schots 1995, Yucel *et al*. 2005), indirect ELISA (Xu and Chen 2000, Liu *et al*. 2007), indirect competitive ELISA (Plasencia *et al*. 1996), tissue immuno-binding assay (TIBA) (Auger S *et al*. 1995). More recently, molecular techniques have become increasingly important and several PCR methods are available (Robb and Nazar 1996, Mahuku *et al*. 1999, Zhu *et al*. 1999, Platt and Mahuku 2000, Usami *et al*. 2002, Kuchta *et al*. 2008). PCR facilitates the detection in symptomless plants as well as in plants that have recovered from infection by the non-defoliating pathotypes (Mercado-Blanco *et al*. 2002). The mmolecular technique also benefit from better plant and soil DNA extraction methods that improve the detection and quantification of *Verticillium* spp. (Heinz and Platt 2000). PCR-amplified intergenic sequences may provide sensitive probes for detection and differentiation of very closely related species (Nazar *et al*. 1991). Other

authors demonstrated that PCR-based assays are faster, more sensitive and more accurate than earlier procedures. ((Hu *et al*. 1993, Morera *et al*. 2005).
Random amplified polymorphic DNA (RAPD) technique was used for the specific detection of *Verticillium dahliae* (Li *et al*. 1370, Uchiyama 1996, Li *et al*. 1999). Random amplified fragment length polymorphism is also useful for the diagnosis of fungi of the *Verticillium* spp. genus (Radisek *et al*. 2001). Other suggested molecular techniques include a nested-PCR that is useful to detect both defoliating and non-defoliating pathotypes (Saito *et al*. 2001, Mercado-Blanco *et al*. 2003, Perez-Artes *et al*. 2003), and a real-time PCR that offers the opportunity for the quantification of soil-borne pathogens (Schena *et al*. 2003, Gayoso *et al*. 2007). A triplex PCR system was optimized to the detection of *Botrytis cinerea, Colletotrichum gloeosporioides* and *Verticillium dahliae* simultaneously at an early stage (Wang *et al*. 2010). Further molecular techniques include DNA array technology (Lievens *et al*. 2003) and a Padlock probes - multiplex detection (Szemes *et al*. 2005). A multiplex-nested-PCR assay was demonstrated to be clearly superior in detecting the pathogen when compared to conventional isolation procedures. Moreover, the method allowed the detection of *V. dahliae* infections in asymptomatic plants that had produced false negatives when checked by microbiological isolation procedures (Collado-Romero *et al*. 2009). A PAGE was developed to identify the defoliated strain of *Verticillium dahliae* (Fu *et al*. 2001) whilst a MALDI-TOF-MS protocol should also prove useful as a rapid and reliable assay for distinguishing different *Verticillium* spp. (Tao *et al*. 2009).

2. Delimitation
No information available.

3. Monitoring
The incidence of the *Verticillium dahliae* was evaluated in a surveys of different crops in numerous countries and regions (Karaca *et al*. 1971, Matovu 1973, McKeen and Thorpe 1973, Saydam and Copcu 1973, Sheppard and Viswanathan 1974, Mamluk and Skaria 1979, Thanassoulopoulos *et al*. 1979, Kitazawa and Suzui 1980, Blanco-Lopez *et al*. 1984, Shen 1985, Celetti and Platt 1987, Turkensteen and Eduardo Nieto 1987, Turkensteen 1988, Yang and Wei 1988, Kruger 1989, El-Ahmad and Mouselli 1990, Infantino and Porta-Puglia 1990, Onan *et al*. 1992, Chitzanidis 1995, Heppner and Heitefuss 1995, Kimpinski *et al*. 1998, Yakutkin and Tavolzhanskri 1999, Davis *et al*. 2001, Pomar *et al*. 2001, Armengol *et al*. 2004, Ozan and Maden 2004, Dervis and Bicici 2005, Sanogo and Carpenter 2006, Harding and Wicks 2007, Rodriguez *et al*. 2009). The monitoring could prove to be useful in ascertaining the cultivars most susceptible to the pathogen (Slattery and Eide 1980, Dervis *et al*. 2010). The identification of the level of pathogenicity of the serotypes of *Verticillium dahliae* was evaluated using both molecular technique (AFLP) and standard vegetative compatibility group tester strains (Bender and Shoemaker 1984, Strunnikova and Muromtsev 1987, Ligoxigakis and Vakalounakis 1994, Bhat *et al*. 2003, Radisek *et al*. 2003, Duan *et al*. 2004, Ciccarese *et al*. 2005, Omer *et al*. 2008, Dervis *et al*. 2009a, Dervis *et al*. 2009b). The weeds growing in olive orchards were demonstrated to be of potentially significant importance in the survival and increase of inoculum, affecting disease incidence (Thanassoulopoulos *et al*. 1981, Ligoxigakis *et al*. 2002). Plant parts and soil are most commonly used to identify the pathogen but the fungus was also detected in irrigation water, a source of dissemination of the disease, by a sensitive nested PCR assay (Rodriguez *et al*. 2007). The climatic and agricultural factors that favour the disease caused by *V. dahliae* have been evaluated (Al-Ahmad 1988, Rodriguez *et al*. 2008, Javier Lopez-Escudero *et al*. 2010). The effects of several factors (medium used for plating, the amount of sieved soil inoculated to plates and the length of time of incubating plates) on

estimation of *Verticillium dahliae* in soil by the wet-sieving method have been studied whilst bait methods using eggplants (*Solanum melongena*) or antirrhinums (*Antirrhinum majus*) proved ineffective for estimating *V. dahliae* in naturally infested soil (Harris *et al*. 1993). Here, morphological characteristics of the fungal mycelium were useful to identify the pathogen (Sanei *et al*. 2004).
Molecular techniques (RFLPs, RAPDs, and DNA fingerprints, ISSR markers) were useful to evaluate the diversity and the origin of the pathogen (Dobinson *et al*. 1998, Bellahcene *et al*. 2005, Abdel-Azize *et al*. 2006, Song *et al*. 2011). A test was carried out to quantify microsclerotia of *Verticillium dahliae* in soil (Goud and Termorshuizen 2003).
Severity level of cotton verticillium wilt can be effectively estimated utilizing high spatial resolution image and partial least squares regression analysis and the result presents an important reference approach for further monitoring crop pests (Jing *et al*. 2010).

4. Commodities
Verticillium dahliae has been found in imported seeds during routine seed health testing for plant quarantine purposes (Han *et al*. 1986, Park and Kim 1986, du Toit *et al*. 2005, Rampersad 2010. Imported plants and cut flowers constitute another significant route of spread of the pathogen (Teliz Ortiz *et al*. 1986, Thanassoulopoulos 1993, Silva *et al*. 2005). The utility of testing for material free from *Verticillium dahliae* and, for example, the safety of micropropagated mint shoots for certified planting stock programs has been discussed (Wang and Reed 2003).
It is concluded that second-hand machinery should be cleaned thoroughly prior to importation and inspected carefully by quarantine officers on arrival, and the efficacy and feasibility of fumigation could be considered (Allen 1995). A reduction in the fraction of *V. dahliae* infected plants could result from cold storage (Shaw *et al*. 2002). dsRNA technology may help in identifying virus-diseased olive trees (Martelli *et al*. 1995), since the usual plating method is time-consuming and not always reliable, especially due to the frequent presence of symptomless plants (Mascarello *et al*. 2001, Mercado-Blanco *et al*. 2001).

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Xanthomonas campestris pv. *citri* (Hasse) Dye

(*Xanthomonas axonopodis* pv. *citri* (Hasse) Vauterin et al.)
Common name(s): Citrus canker, bacterial canker of citrus, Asiatic canker (+ several others)
Taxa: Bacteria: Proteobacteria: Xanthomonadaceae
EPPO A1 list: No. 1
EU Annex designation: II/A1

Organism
Several pathotypes have been recognized within *X. a. citri.* Different types of bacterial canker occur and are induced by variants of the same causal agent, primarily distinguished by their geographical origin and host range. Pathotype A has the widest host range and a global distribution and causes Asiatic citrus canker (CBC-A). Pathotype B is restricted to lemon, although Mexican lime, sour orange and pummelo are also susceptible (CBC-B). Pathotype C is restricted to Mexican lime (*C. aurantifolia*) (CBC-C).
Citrus is the main host of economic importance. Natural infections are known to occur only on *Citrus* spp., hybrids and cultivars and on *Poncirus trifoliata*, *Fortunella* spp. (*F. japonica*, F. *margarita*), *Severinia buxifolia* and *Swinglea glutinosa*. In general, grapefruits (*C. paradisi*), limes (*C. aurantiifolia*) and *Poncirus trifoliata* are highly susceptible. Sour oranges (*C. aurantium*), lemons (*C. limon*) and oranges (*C. sinensis*) are moderately susceptible. Mandarins (*C. reticulata*) are moderately resistant. Other members of the Rutaceae, including members of the sub-families Aurantioideae, Rutoideae and Toddalioideae, are susceptible to artificial infection by *X. campestris* pv. *citri*. One non-rutaceous host, *Lansium domesticum* (Meliaceae), has been reported. Cancrosis B strains have a similar host range to cancrosis A strains, but affect certain hosts much less severely (Stall & Civerolo, 1991). Canker C and D strains affect only limes (*C. aurantiifolia*).
The pathogen causes necrotic lesions on leaves, stems and fruits. Severe infections can cause defoliation, badly blemished fruits, premature fruit drop, twig dieback and general tree decline. The disease is not systemic and causes local lesions only. The disease is present in Asia, Africa, North America, South America and Oceania, but absent in the EU and EPPO region.
The primary inoculum sources for spring infections are lesions on shoots and leaves resulting from infections the previous autumn and in which the pathogen overwinters. The bacterium survives in leaf, shoot and fruit lesions that develop during the spring. Current season lesions are sources of bacteria for secondary infections. During wet, warm weather in the spring and early summer, the bacterium oozes out of overwintering lesions when free moisture is present. Young, actively growing leaves and shoots are infected. Infection occurs through natural openings (e.g. stomatal pores) and wounds. The bacterium multiplies in the intercellular spaces while the host cells divide, producing scab-like lesions. The pathogen has been reported to survive for various periods of time in association with citrus and non-citrus hosts, in infected plant tissue debris and in the soil.
Symptoms of the disease are visible on all above-ground parts of susceptible hosts, particularly young, actively growing leaves, twigs, stems, trunks, thorns and fruit. Lesions first appear as pin-point spots that become small, slightly raised pustules or blister-like eruptions. Initially, these appear on the lower leaf surface about 7 days after infection. Subsequently, the blisters become visible on the upper leaf surface. The young lesions are usually translucent due to water-soaking of the tissue. Lesions are initially circular but may develop irregularly. Lesions are light-coloured at first and become tan or brown. As lesions develop, the epidermis ruptures and the lesions become spongy or corky. The lesions finally become crater-like with a raised margin and sunken centre. The centre of large, old lesions may crack and/or drop out (see EPPO Data Sheets on Quarantine Pests, *Xanthomonas axonopodis* pv. *Citri*).

1. Detection
Standard methods and protocols for the identification of *X. a. citri* are described in detail in the “EPPO Diagnostic protocols for regulated pests” PM 7/44 (1).
In this instruction the following procedures for identification on plants with symptoms as well as in asymptomatic samples are outlined:

- Screening tests using PCR and ELISA
- Direct isolation
- Pathogenicity tests
- Attached leaf assay
- Detached leaf assay
- Indirect ELISA
- Molecular identification
- RFLP analysis
- Genomic fingerprinting
- Fatty acid profiling (FAP)

(Anonymous, 2005)

The literature on the detection of the pathogen is vast and still increasing. Considering this fact it seems feasible to provide only some procedures for the identification of *X. citri* reflecting the literature search findings. Nucleic acid sequence based amplification (NASBA) is a method of amplifying RNA, for the detection of RNA viruses and human pathogenic bacteria. Recently, NASBA has also been employed for the detection of plant diseases caused by viruses and quarantine bacteria. A major citrus pathogen, *Xanthomonas citri* subsp. *citri* (Xcc), causal agent of citrus bacterial canker, is being studied in depth due to its economic importance, with recent focus concentrating on its viability and survival under different stress conditions and control treatments. In this work, a NASBA protocol using primers for gumD mRNA has been developed to assess the viability of this pathogen under different bacteriocidal treatments. This method is rapid, specific and sensitive, and is able to detect viable bacterial cells, using a hybridization device which allows the visualization of the results in only 30 min (Scuderi et al., 2010).

Bacterial sensitivity to different various phages was examined by electro-orientation spectroscopy, fluorometry, and electron microscopy. The strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Yersinia pestis*, *Mycobacterium smegmatis*, and *Xanthomonas campestris* were used. The fluorescence intensity of a membranotropic agent in the ANS - cell - phage system was shown to depend on the interaction of a bacterial virus and a microorganism. Fluorometric data correlated with electro-orientation spectroscopic findings. An analysis of the low-frequency site makes it possible to determine phage adsorption on the bacterial surface. The changes in electro-orientation effects at high frequencies suggest that there are barrier dysfunctions in the external membranes and that there is cellular phage reproduction. Whether fluorometry and electro-orientation spectroscopy can be further used for rapid identification of microorganisms by using phages is discussed (Zhilenkov et al.1999).

The detecting technique with the bacteriophage XCP1 of citrus bacterial canker disease in the nonsymptomatic tissues is reported first in this paper. On the basis of the principle of bacteriophage absorption and multiplication, the double-layer agar method was used for determinating the average number of the free bacteriophage plaques to detect the nonsymptomatic citrus tissues. The sensitivity of detection is 103-4 CFU/ml and 12-15 hours are required for all the procedures. The determination standard of the absorption detection is P < Pck - 3SD called positive reaction "+", and P .gtoreq. Pck - 3SD called negative reaction "-". The determination standard of the multiplication detection is II P > I P + 3SD and IIP > IIPck + 3SD called positive reaction "+", and IIP .gtoreq. IP + 3SD and IIP .ltoreq. IIPck + 3SD called negative reaction "-". The 80 samples from 12 counties and cities of Sichuan province [China] were detected with the bacteriophage absorption and multiplication detection techniques, which proved that the techniques were of high sensitivity, specificity, accuracy and rapidity (Wang et al. 1990).

Cellular fatty acids of 80 strains of 9 pathovars of *X. campestris* were analysed by gas-liquid chromatography and mass spectrometry. Forty eight fatty acids were identified, the most important being the 16:0 (averaging at least 4.5% of the total), the cis- and trans -9 16:1 (over 14.4%) and the iso and anteiso 15:0 (over 30%). Other major fatty acids (each averaging over 1% of total) were the saturated 14:0 and 15:0, the hydroxy-substituted iso 3-OH 11:0, 3-OH 12:0 and iso 3-OH 13:0, and the branch-chained iso 11:0,iso 16:0, iso 17:1, iso 17:0 and anteiso 17:0. Of 33 minor fatty acids detected and identified, only 7 have previously been reported in *Xanthomonas*. Significant differences (P<0.01) in mean percentages of 5 major fatty acids

and 4 (chemical) class totals were detected among pathovars, which statistically separated into 3 groups by rank analysis. *X. campestris* pv. Dieffenbachiae was in a group by itself; pathovars campestris, citri (pathotypes A and B), manihotis, phaseoli, pruni and vesicatoria were in a second group, and glycines, begonia [begoniae] and citri (pathotype E) were in a third (Wells et al., 1993).
Techniques of isolation of *Xanthomonas* spp. from plant material and the soil are described. Particular reference is made to *Xanthomonas albilineans*, the cause of leaf scald of sugar cane, which because of difficulties in isolation, its slow growth on artificial media and the ease with which isolation plates become overgrown by saprophytic bacteria or fungi has presented special problems. Special reference is also made to suitable selective media for the isolation of *Xanthomonas campestris*, which previously have been difficult to find. The classification of *Xanthomonas*, description of the genus, pigmentation, colony form and extracellular polysaccharide slime production, variation between species in nutritional and biochemical properties, bacteriophage relationships, nucleic acid hybridization, serology and electrophoresis are then discussed (Hayward, A. C., 1979).

2. Delimitation
No information available.

3. Monitoring
The EPPO quarantine procedures recommend the following methods for inspection:

- Examination of plants or fresh fruits of Rutaceae moving in trade (visual inspections)
- Growing season nursery inspection in the country of origin (visual inspections)
- Area survey in the country of origin (visual inspection, isolation, Immunofluorescence)
- Testing suspected presence *of X. c. citri* in an EPPO country (visual inspection, isolation, pathogenicity test, indirect ELISA, Immunofluorescence and/or genomic fingerprinting)

As for the survey EPPO recommends to determine the presence or absence of *X. c. citri* in citrus groves due to carrying out surveys in spring, summer or autumn following growth flushes, by examining leaves, fruits and branches on a suitable number of trees. In the absence of symptoms leave samples are to be taken and analysed in the Lab using isolation on artificial nutrient media and/or detached leaves, ELISE and IF (Anonymous, 1990). A survey for citrus canker was undertaken in August 1986 in at the Christmas Island (Shivas, 1987).

4. Commodities
The EPPO specific quarantine requirement recommends all EPPO countries to require that imported plants or fresh fruits of Rutaceae should be found free from symptoms of infection by *X.c. citri*. The Import of planting material from countries where the disease occurs is prohibited. The imported fruit must be free from leaves and peduncles and the production place must have been found free after inspection (Anonymous, O., 1990).

References: 76 retained out of 2024 retrieved, none added, 8 used for summary

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Xanthomonas campestris pv. oryzae (Ishiyama) Dye.

(=*Xanthomonas oryzae* pv. Oryzae (Ishiyama) Swings et al.)
Common name(s): Bacterial blight, Kresek disease
Taxa: Bacteria: Proteobacteria: Xanthomonadaceae
EPPO A1 list: No. 2
EU Annex designation: II/A1

Organism
The pathogen is widely distributed globally. It is reported from Asia, from some African countries including Burkina Faso, Cameroon, Gabon, Mali, Niger, Senegal and Togo. It also occurs in North America, Central- and South America as well as Australia. *Xanthomonas oryzae* pv. *oryzae* is absent in the EU, but reported from Russias far east and the Ukraine as representatives of the EPPO region.
The principal host of the pathogen is rice, but also a number of wild or minor cultivated Poaceae (*Leersia* spp., *Leptochloa* spp., *Oryza* spp., *Paspalum scrobiculatum*, *Zizania*, *Zoysia* spp.) are attacked.
The symptoms of an *X. oryzae* infection are: after planting on young plants leaves emerge pale-green to grey-green water-soaked streaks near the leaf tip and margins. These lesions coalesce and become yellowish-white with wavy edges. Eventually, the whole leaf may be affected, becomes whitish or greyish and then dies. Leaf sheaths and culms of the more susceptible cultivars may be attacked.
The bacterium penetrates the host by way of hydathodes and wounds on the roots or leaves, but may also enter via stomata. Subsequently there will be a build-up of bacteria which exudes onto the leaf surface and re-enter the plant through the hydathodes. Once inside the vascular system, the bacterium multiplies and moves in both directions. Spread takes place in wind and rain, but primarily due to irrigation water. Potential inoculum sources include infected planting material, volunteer rice plants, infected straw and weed hosts.
Many different pathotypes of the bacterium exist (Anonymous, 200).

1. Detection
Standard methods and protocols for the identification of *X. oryzae* pv. *oryzae* are described in detail in the “EPPO Diagnostic protocols for regulated pests” PM 7/80 (1).
In this instruction the following procedures for identification on plants with symptoms as well as in asymptomatic samples are outlined:

Isolation
- Direct plating from symptomatic and asymptomatic leaves collected from the field
- Direct isolation from seeds

Rapid screening tests
- Detection from asymptomatic leaves by PCR
- Direct detection from seeds by BIO-PCR

Identification
- Fatty acid profiles
- PCR
- ELISA
- Pathogenicity tests

(Anonymous, 2007)

The literature on the detection of the pathogen is extensive and still increasing. Considering this fact it seems feasible to provide only some procedures for the identification of *X. oryzae* pv. *oryzae* refelecting the literature search findings.
A combined molecular diagnostic and DNA fingerprinting PCR technique for *Xanthomonas oryzae* pv. *oryzae* pathogens from rice has been developed in Africa by this study, using four primer pairs designed from Xoo (NC_007705.1), Xoc (NZ_AAQN01000001.1), Pf (AB021381.1) and Pss (NC_007005.1) complete genome sequence (Onasanya et al., 2010).
To establish the novel molecular quantitative assays for quantification of bacterial population of *Xanthomonas oryzae* pv. *oryzae* (Xoo) in planta, real-time quantitative polymerase chain reaction (RTQ-PCR) assay based on SYBR Green I technology was developed to target lipA and purH for the quantification of in planta growth of Xoo. The changes in bacterial population density in planta measured by RTQ-PCR assay is similar to those assessed by bacterial plate counting. There is no significant difference between the two primer sets evaluated for RTQ-PCR. Bacterial accumulation within rice showing no disease symptoms was observed at 3 day post-inoculation (dpi), then the bacterial density within rice increased significantly 5 dpi with rising bacterial leaf blight, and bacterial numbers reached a peak and maintained a high population 9-14 dpi when the plants displayed severe disease symptoms. Such a relation between bacterial population density in planta and host plant disease progression might be associated with quorum sensing of the pathogen. The results of this study illustrate that RTQ-PCR can be successfully used to directly and accurately quantify Xoo within leaf tissues of rice. Furthermore, bacterial target gene copies-total DNA amount-bacterial population-host disease progression, a hypothetical model of the pathogen assessment, has been proposed for accurate monitoring of bacterial infection process of rice by Xoo, which might be applicable to the molecular quantification of other bacterial and fungal diseases of rice (Sun et al., 2007).
Scanning electron-microscopy observations made on the leaves of Italian ryegrass and maize, inoculated with *Xanthomonas campestris* pvs *graminis*, *oryzae* and *oryzicola* clearly showed a difference in the distribution pattern among the different pathovars tested. Pvs *oryzae* and *oryzicola* could be detected on the leaf trichomes of ryegrass, while pv. *graminis* was not. On maize leaves, the attachment of pvs *ozyzae* and *oryzicola* to trichomes was much more pronounced than on ryegrass. In addition, pv. oryzae spread further into leaves of maize than did pvs *graminis* and *oryzicola* and could be detected in masses until at least the sixth day after inoculation. These observations suggest that non-host plants such as maize could function as alternate inoculum sources of pv. *oryzae* for nearby rice plants (De Cleene, 1989).
Bacteriophage isolation and a direct immunofluorescent technique were used to detect *Xanthomonas campestris* pv. *oryzae* in rice seed. Direct immunofluorescence was the most sensitive technique, enabling the detection of 10superscript 2 c.f.u./ml. Only seeds collected in the dry season were free of the pathogen (Unnamalai et al., 1988).

2. Delimitation
No information available.

3. Monitoring
It is reported of several surveys carried out in order to investigate the disease, their distribution and severity in surveyed each area (Wakimoto et al., 1981; Jones et al., 1991; Saha & Dutta, 2007; Kumar et al., 1999).

Aiming to determine the relationship between rice grain yield and pest injuries in 3 rice crop irrigated areas surveys were conducted in Vietnam (Hoang Dinh et al., 2001).
A survey was conducted during the crop year 2002 in Punjab, Sindh, Balochistan, NWFP and Azad Jammu & Kashmir to study the latest situation. Resulting from the investigations it was observed that if plant produces panicles, the sterility percentage increases as well as the number of immature grains. Grains from diseased plants are easily broken during milling. When there is heavy infection, no grain formation takes place (Akhtar et al. 2003).

4. Commodities

In this report the importance of international agreements and centralized control of the germplasm exchange and trade in agricultural products to avoid introduction of harmful quarantine organisms is emphasized. Introduction of harmful organisms via germplasm exchange into Brazil is reported, such as *Ditylenchus dipsaci* on potato, banana bunchy top nanovirus, *Tilletia indica* on wheat, and *Xanthomonas oryzae* pv. *oryzae* on rice (Oliveira et al., 2002).

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Xanthomonas campestris pv. *oryzicola* (Fang *et al*.) Dye

(=*Xanthomonas oryzae* pv. *oryzicola* (Fang *et al*.) Swings *et al*.)
Common name(s): Bacterial leaf streak of rice, leaf streak of rice
EPPO A1 list: No. 3
EU Annex designation: II/A1

Organism
The pathogen is absent within the EPPO and EU region. It is distributed within the tropical areas of Asia. X. *oryzae pv. oryzicola* is found in Madagascar, Nigeria and Senegal. Also it is reported from Australia (Northern Territory).

Organism
The principal host of both pathovars is rice. *Oryza sativa* subsp. *japonica* is usually more resistant than subsp. *indica* to pv. *oryzicola*. The two bacteria also attack a number of wild or minor cultivated Poaceae (*Leersia* spp., *Leptochloa* spp., *Oryza* spp., *Paspalum scrobiculatum*, *Zizania*, *Zoysia* spp.), including poaceous weeds
Symptoms of an infection with *Xanthomonas oryzae* pv. *oryzicola* on leaves are narrow, dark-greenish, water-soaked, interveinal streaks of various lengths, initially restricted to the leaf blades. The lesions enlarge, turn yellowish-orange to brown depending on cultivar and eventually coalesce. Tiny amber droplets of bacterial exudate are often present on the lesions. In its advanced stages, the disease is difficult to distinguish from that caused by *X. oryzae* pv. *oryzae* but lesion margins remain linear rather than wavy as in the latter case. Direct observation of the bacterium may be necessary for confirmation. Damage is often associated with lepidopterous leaf rollers, leaf-folders and hispa beetles, since bacteria readily enter the damaged tissue resulting from insect infestation.
The bacterium enters the leaf through stomata or wounds. Spread within a crop occurs by mechanical contact and in rain and irrigation water; under favourable warm wet conditions, rapid and severe disease development can occur. The role of seed transmission in perpetuation of the disease is recognized, but the part played by weeds is little understood. The bacterium can persist from season to season on infected leaves and leaf debris, but is unable to survive in non-sterile soil (Anonymous, 1997).

1. Detection
Standard methods and protocols for the identification of *X. oryzae* pv. *oryzicola* are described in detail in the “EPPO Diagnostic protocols for regulated pests” PM 7/80 (1).
In this instruction the following procedures for identification on plants with symptoms as well as in asymptomatic samples are outlined:

i. Isolation
    a. Direct plating from symptomatic and asymptomatic leaves collected from the field
    b. Direct isolation from seeds
ii. Rapid screening tests
    a. Detection from asymptomatic leaves by PCR
    b. Direct detection from seeds by BIO-PCR
iii. Identification
    a. Fatty acid profiles
    b. PCR
    c. ELISA

d. Pathogenicity tests

(Anonymous, 2007)

The literature on the detection of the pathogen is extensive and still increasing. Considering this fact it seems feasible to provide only some procedures for the identification of *X. oryzae* pv. *oryzae* reflecting the literature search findings.

The polyclonal antiserum raised against *Xanthomonas oryzae* pv. *oryzicola* in New Zealand white rabbit was used to detect the pathogen in leaves and seeds of rice. The titre value of polyclonal antiserum raised against the pathogen was measured by indirect ELISA. The antiserum raised against X. *oryzae* pv. *oryzicola* (1:100 dilution) could detect the bacterium in seeds and leaves, but it did not react with X. oryzae pv. oryzae-infected leaves. Thus, the antiserum is very specific to X. *oryzae* pv. *oryzicola* alone (Varanavasiappan et al., 2006).

A computational genomics pipeline was used to compare sequenced genomes of *Xanthomonas* spp. and to rapidly identify unique regions for development of highly specific diagnostic markers. A suite of diagnostic primers was selected to monitor diverse loci and to distinguish the rice bacterial blight and bacterial leaf streak pathogens, *Xanthomonas oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*, respectively. A subset of these primers was combined into a multiplex polymerise chain reaction set that accurately distinguished the two rice pathogens in a survey of a geographically diverse collection of *X. oryzae* pv. *oryzae*, X. *oryzae* pv. *oryzicola*, other xanthomonads, and several genera of plant-pathogenic and plant- or seed-associated bacteria. This computational approach for identification of unique loci through whole-genome comparisons is a powerful tool that can be applied to other plant pathogens to expedite development of diagnostic primers (Lang et al., 2010).

A pair of specific primers was designed and used for detection of *X. oryzae* pv. *oryzicola* (Xooc), the rice bacterial leaf streak pathogen. The results showed that all tested Xooc strains (31 isolates) were specific detected, 15 strains of *Xanthomonas oryzae* pv. *oryzae* and other tested bacteria strains had not any amplification signal. The detection limit was 20 cells. Xooc was successfully detected from the seeds harvest from the natural infected and artificially inoculated rice plants (Zhang et al. 2008).

2. Delimitation

No information available.

3. Monitoring

No information available

4. Commodities

No information available.

References: 29 retained out of 169 retrieved, 2 added, 5 used for summary

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Zhang, H., Y. Jiang, et al. (2008). Specific detection of Xanthomonas oryzae pv. oryzicola by PCR techniques. Acta Phytopathologica Sinica 38(1): 1-5.

Xanthomonas campestris pv. *phaseoli* (Smith) Dye

(=*Xanthomonas axonopodis* pv phaseoli (Smith) Vauterin et al.
Common name(s): Bacterial blight of bean, common blight of bean, fuscous blight of bean
Taxa: Bacteria: Proteobacteria: Xanthomonadaceae
EPPO A2 list: No. 60/61
EU Annex designation: II/A2

Organism

The principal host is *Phaseolus vulgaris*, but other legume species are naturally infected, including P. lunatus, *Vigna aconitifolia* and *V. Radiate*, *P. coccineus*, *P. acutifolius* and *Lupinus polyphyllus* are hosts only by artificial inoculation. The pathogen is distributed all over the globe and is also present in the EU and EPPO region.

The bacterium enters the leaves via stomata or wounds, and subsequently invades the intercellular spaces, causing a gradual dissolution of the middle lamella. The stem is entered in three ways: via the stomata of the hypocotyl and epicotyl; through the vascular system of the leaf; or from infected cotyledons. The seed is penetrated via the vascular system of the pedicel and funiculus. The micropyle also serves as a point of entry into the seed. The pathogen either remains in the seedcoat or passes to the cotyledons when the seed germinates, and so infection of the young plant results.

The bacterium can remain viable for several years beneath the seedcoat. Other inoculum sources include infected plant debris in the soil and alternate host plants on which the pathogen can overwinter. A single source of inoculum in a crop may contaminate an area of more than 8 m around it; thus, one diseased plant in 10 000 is sufficient to cause a severe epidemic.

Dissemination in the field occurs in wind-driven rain, and insects. Overhead sprinkler irrigation may provide a means for spread. The disease is severe under conditions of high rainfall and humidity, with maximum development around 28°C.

The main symptoms caused by *X. campestris* pv. *phaseoli* are lesions on seedlings, plants, and pods, which are water soaked areas accompanied by halos. Spots often enlarge and the surrounding tissue is discoloured or sunken in. Plants often exhibit a characteristic wilting during the heat of the day, with recovery of turgidity at night. The extent of symptom expression varies with the different growing stages (Anonymous 1997).

1. Detection

Common bacterial blight of bean is caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) and *Xanthomonas axonopodis* pv. *phaseoli* var. Fuscans (Xapf). These seedborne, quarantined pathogens can cause up to 40% yield loss in susceptible cultivars and also reduce seed quality, especially if seed is produced under humid conditions. Seed health testing is one of the most significant control steps to protect against contamination of seeds for domestic planting and international seed trade. Based on sequence information from RAPD fragments generated by Xap-specific primers, we developed a realtime PCR for detection and quantification of Xap and Xapf. Primer and probe specificity was tested against DNA of several Xanthomonas species and pathovars of X. axonopodis. Several close related *Xanthomonas* strains could not be amplified using this PCR assay. The detection limit of the TaqMan assay for purified DNA and cells was 20 fg and 20 CFU per 25 µl PCR reaction mixture, respectively. This assay may be useful as a rapid, highly sensitive and specific detection method to ensure seed quality control and meet phytosanitary regulations. This is also the first real-time PCR assay developed for Xap and Xapf (HE et al. 1999).

Using pathogen-free seed is an important factor in disease management. In this study, capability of several detection methods including Indirect ELISA, Direct PCR, Bio-PCR and Ic-PCR were compared for monitoring *Xanthomonas axonopodis* pv. *phaseoli* in bean seeds collected from Markazi province fields. The extractions of seed samples soaked in washing buffer and distilled water separately were used for Indirect ELISA and PCR amplification with species specific pairs of primer X4c\X4e. In Ic-PCR, seed extracts were loaded in wells which were coated with immunoglobulin and the sample DNA after boiling was subjected to PCR. For Bio-PCR assay, aliquots of the extracts were plated onto semi-selective modified nutrient broth yeast extract agar. The growing colonies were suspended in sterile distilled water after boiling was applied for PCR amplification. The results indicated that sensitivity of Indirect ELISA was low and at least 105 cfu/ml was needed as a detection threshold. The results for direct PCR were not reproducible and Ic-PCR was found an expensive method. The Bio-PCR technique was considered as a reliable and specific method which was able to detect as little as 1 cfu/ml in seed extracts plated on semi-selective media. Comparing the results indicated that the Bio-PCR assay is suitable for sensitive and routine testing of seed samples of beans for the presence of *X. axonopodis* pv. *phaseoli* (Akhavan et al. 2009).
Xanthomonas axonopodis pv. *phaseoli* detection on artificially inoculated bean seeds was investigated. The method of the International Seed Federation – ISF (2006) was used. It included bacteria extraction from seeds and isolation on semiselective media with pathogenicity test of the investigated isolates. ELISA and PCR were used for verification of results. The results showed that the semiselective media MT (Milk Tween Agar) and XCP1 (Xanthomonas campestris pv. phaseoli Agar) were very suitable for isolation of *X. axonopodis* pv. *phaseoli*. Pathogenicity was confirmed on young bean plants. ELISA test and PCR confirmed that all investigated isolates and reisolates belong to the bacterium *X. axonopodis* pv. *phaseoli*. (Balaž et al., 2008)
The efficiency of a semi-selective medium developed for *Xanthomonas axonopidis* pv. *malvacearum* (Xam), was verified with some modifications, in detecting the presence of *X. axonopodis* pv. *phaseoli* (Xap) in bean seed. Seed samples naturally infected with the disease were collected from the State of Paraná. The bacterial growth developed around the infected seed in the semi-selective medium after 12 days after incubation. The recovery frequency of bacterial colonies in relation to nutrient agar varied between 30 and 112%, depending upon the isolate. Efficiency of tolylfluanid to remove the bacterium has been evaluated. The bacteria was recovered from the untreated seeds, but not from the seeds treated in a solution of tolylfluanid (1.20g/L water), and placed in plates containing semi-selective medium. In the glasshouse, seeds treated with tolylfluanid, did not produce any symptoms of the disease till 30 days after seeding, whereas untreated seeds yielded 9.75% plants with typical disease symptoms. In an another experiment, untreated seeds yielded 7,08% and 11.67% diseased plants, whereas treated seeds yielded 0,5% and 2.4% diseased plants, 26 and 46 days after planting, respectively. The disease from the bean seed was also verified in laboratory and glasshouse (Lopes et al., 2008).

2. Delimitation
No information avaialable

3. Monitoring
Surveys to detect *Xanthomonas campestris* pv. *phaseoli* were conducted in several countries mostly to answer questions concerning improvement of detection methods, crop management and plant protection. Field surveys were conducted in 3 major *Phaseolus vulgaris* growing areas of Ethiopia. Correspondence analysis was used to characterize differences in disease severity between regions and seasons, and to determine associations between geographic areas and

cropping systems, areas and diseases, and cropping systems and diseases (Habtu et al., 1996).
Samples of dry bean plants with common bacterial blight symptoms, caused *by Xanthomans campestris* pv. *phaseoli*, and with wildfire symptoms, caused by *Pseudomanas syringae* pv. *tabaci* were collected during the 1986 and 1987 rainy seasons. Seventy five samples from the 1986 crop and fifty seven samples from the 1987 crop were submitted to isolation in order to recover these bacteria. Samples from the 1986 and 1987 crops had respectively 84.0 and 75.4% infections with *X. campestris* pv. *phaseoli* and samples from the 1986 had 4.0% infection with P. syringae pv tabaci. A wide incidence of *X. campestris* pv. *phaseoli* was observed in dry bean fields in Parana state, Brazil (Maringoni & Komori 1989).
Aerial infra-red false-colour photographs were used to assess the incidence of seed-borne bacterial blight caused by *Xanthomonas phaseoli* in 34 *Phaseolus vulgaris* fields in SW Ontario in 1968. In some fields up to twice the number of infected plants were detected by infra-red photography as were found by ground surveys carried out the same day. The incidence of blight was correlated with the pedigree of the seed used in planting the fields, ranging from zero in some fields of Breeder seed to more than 50% in some fields of Commercial seed (Wallen & Jackson 1971).

4. Commodities

Rravnikar et al. (1996) report about their results in erradicating different pathogenes (*Xanthomonas campestris* pv. *phaseoli* and bean common mosaic potyvirus as well as cucumber mosaic cucumovirus) from bean seeds. For the elimination from cultivar Starozagorski, dry heat treatment of seeds and thermotherapy of seedlings in combination with meristem culture were used. Following this procedure, bacterial contamination of cultures was reduced to 4%.
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References: 70 retained out of 245 retrieved, none added, 9 used for the summary

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Xanthomonas campestris pv. *pruni* (Smith) Dye

(=*Xanthomonas arboricola* pv. *pruni* (Smith) Vauterin et al.)
Common name(s): bacterial canker/leaf spot/shot-hole of stone fruit, black spot of plum
Taxa: Bacteria: Proteobacteria: Xanthomonadaceae
EPPO A2 list: No. 62
EU Annex designation: II/A2

Organism

X. campestris pv. *pruni* attacks only *Prunus* spp., and particularly the fruit crops almonds, peaches, cherries, plums, apricots and P. salicina. Other exotic or ornamental species of Prunus attacked include P. davidiana and P. laurocerasus.

The pathogene is distributed all over the globe. It is also present within the EU and EPPO region.

Infection is first apparent on the lower surface as small, pale-green to yellow, circular or irregular areas with a light-tan centre. These spots soon become evident on the upper surface as they enlarge, becoming angular and darkening to deep-purple, brown or black. The immediately surrounding tissue may become yellow. The diseased areas drop out, shot hole effect. Bacterial ooze may be associated with the spots.

On peach*, X. campestris* pv. *pruni* overwinters primarily in the intercellular spaces of the cortex, phloem and xylem parenchyma towards the tips of twigs produced during the preceding season. On plum and apricot, summer cankers formed in one season continue developing the following spring, so providing a source of inoculum at this time. Plum buds and fallen leaves have also been reported as overwintering sites. In the spring, before host division starts, the bacteria in the intercellular spaces multiply and cause the epidermis to rupture, so initiating a visible lesion referred to as a spring canker. Inoculum from these cankers is disseminated in rain and wind and infects new leaf growth via stomata. Lesions developing on the leaf exude bacteria which bring about secondary infections. Following foliage infection, summer cankers develop in the green tissue of the shoot, but usually become sealed off by a periderm layer and, as cankers tend to dry out during the course of summer, the viability of bacteria therein is largely reduced; thus, except in certain localities, summer cankers in plum and peach are of no importance as overwintering sites for the bacterium, or in initiating infections the following spring (Anonymous, 1997).

1. Detection

Standard methods and protocols for the identification of *X. campestris* pv. *pruni* are described in detail in the “EPPO Diagnostic protocols for regulated pests” PM 7/64 (1).

In this instruction the following procedures for identification on plants with symptoms as well as in asymptomatic samples are outlined:

Detection on symptomless plant material

a. Sample size
b. Extraction
c. Direct isolation
d. Immunofluorescence (IF)

Identification

a. Biochemical tests
b. Protein profiling
c. Fatty acid profiling (FAME)
d. Pathogenicity tests

e. Inoculation of plants

(Anonymous, 2006)

The literature on the detection of the pathogen is extensive and still increasing. Considering this fact it seems feasible to provide only some procedures for the identification of *X.* campestris pv. pruni reflecting the literature search findings.

In this study *Xanthomonas arboricola* pv. *pruni*, along with other main pathogens of the stone fruit trees, a review of detection methods is presented. As there is a general lack of updated standardized protocols for the detection of most of these bacteria, the most appropriate media for their isolation are reported along with serological reagents, PCR and real-time PCR protocols with comments on their accuracy for the analysis of these pathogens in plant samples. Serological techniques are not: very useful for these pathogens due to the current lack of specific antibodies commercially available. As to molecular methods, it is surprising to find so many PCR protocols for *Agrobacterium* species, very few and unspecific for the Pseudomonas species pathogenic to stone fruit trees, and several recent PCR protocols for *X. arboricola* pv. *pruni*. (Lopez et al., 2010).

The bacterium can undergo an epiphytic phase and/or be latent and can be transmitted by plant material, but currently, only visual inspections are used to certify plants as being *X. arboricola* pv. *pruni* free. A novel and highly sensitive real-time TaqMan PCR detection protocol was designed based on a sequence of a gene for a putative protein related to an ABC transporter ATP-binding system in *X. arboricola* pv. *pruni*. Pathogen detection can be completed within a few hours with a sensitivity of 10(2) CFU ml(-1), thus surpassing the sensitivity of the existing conventional PCR. Specificity was assessed for *X. arboricola* pv. *pruni* strains from different origins as well as for closely related *Xanthomonas* species, non-*Xanthomonas* species, saprophytic bacteria, and healthy Prunus samples. The efficiency of the developed protocol was evaluated with field samples of 14 Prunus species and rootstocks. For symptomatic leaf samples, the protocol was very efficient even when washed tissues of the leaves were directly amplified without any previous DNA extraction. For samples of 117 asymptomatic leaves and 285 buds, the protocol was more efficient after a simple DNA extraction, and *X. arboricola* pv. *pruni* was detected in 9.4% and 9.1% of the 402 samples analyzed, respectively, demonstrating its frequent epiphytic or endophytic phase. This newly developed real-time PCR protocol can be used as a quantitative assay, offers a reliable and sensitive test for *X. arboricola* pv. *pruni*, and is suitable as a screening test for symptomatic as well as asymptomatic plant material (Palacio-Bielsa et al. 2011).

Young leaves of peach seedlings were detached and surface sterilized with 70% ethanol. Inoculum (106 [cfu] per ml) of *X. campestris* pv. *pruni* was infiltrated with a needle-less syringe at several sites (approximately 70 cfu per site) on the abaxial side. The leaves were incubated on 0.5% water agar for 2 wk at 25 C under a 16-hr photoperiod. Leaves remained healthy and disease symptoms developed at the inoculation sites. The number of lesions produced at each site was directly proportional to the amount of inoculum infiltrated (Randhawa & Civerolo, 1060).

Aarrouf et al. (2008) report about studies implementing immunofluorescence and cytohistochemical investigations. Using a commercial immunodetection kit, Xap cells were specifically identified in tissues from infected leaves and fruits.

2. Monitoring

No information available.

3. Delimitation

No information available.

4. Commodities

No information available.

References: 41 retained out of 490 retrieved, 6 used for the summary

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Randhawa, P. S. and E. L. Civerolo (1060). A Detached-Leaf Bioassy for Xanthomonas-Campestris athovar Pruni. Phytopathology 75(9): 1060-1063.

Xanthomonas campestris pv. *vesicatoria* (Doidge) Dye

(=*Xanthomonas axonopodis* pv. *vesicatoria* Vauterin et al.)
Taxa: Bacteria: Proteobacteria: Xanthomonadaceae
EPPO A2 list: No. 157
EU Annex designation: II/A 2

Organism
The principal hosts are tomatoes and *Capsicum*. Various other Solanaceae, mainly weeds, have been recorded as incidental hosts: *Datura* spp., *Hyoscyamus* spp., *Lycium* spp., *Nicotiana rustica*, *Physalis* spp., Solanum spp. (e.g. on fruits of potato).
X. vesicatoria occurs widely in tomato- and *Capsicum*-growing areas in the warmer parts all over the world. It is, however, absent from glasshouse production in cooler areas, at least in Europe. It is widely distributed in the EPPO region and found also in the EU.
Symptoms on fruits of tomato are: superficial corky spots or scabs, with water-soaked margins, oval or irregular in shape, 2-10 mm in diameter. *Capsicum* fruits rarely show symptoms, but may drop if infected early. On leaves of tomato or *Capsicum*, lesions appear as irregular water-soaked areas, at first green, later becoming brown and necrotic. *X. vesicatoria* survives from one crop to another mainly on seed, but also in infected debris, e.g. stalks. It may be able to survive in the soil to some extent, possibly in the rhizosphere of non-host plants (Bashan et al., 1982a). Solanaceous weeds may act as alternate hosts.
In glasshouses, seed-borne infection is the only important consideration. Spread is primarily by rain-splash or by overhead irrigation, but handling of young plants is also important. Leaves are infected through stomata, and fruits through small wounds, e.g. abrasions, insect punctures. Only young fruits are infected. The bacterium can multiply epiphytically on young plants in the absence of symptoms. On *Capsicum*, it can multiply as a slime on the surface of young fruits. The disease is favoured by heavy rainfall, high humidity and temperatures above 30°C. The bacterium can survive on tomato and Capsicum seeds for periods of at least 10 years (Anonymous, 1997).

1. Detection
Sharon et al. (1982) describes a method, which enabled the detection of 101-102 cells of the pathogens in tomato and pepper (*Capsicum annuum*) seed respectively. The method is based on the enrichment of the compatible pathogen inside a detached host leaf when placed on a water agar medium. It proved better than the diagnostic growth media method commonly used and permitted detection in symptomless plants.
The usefulness of enzyme-linked immunosorbent assay on nitocellulose membranes (dot-ELISA) for diagnosis and identification of plant pathogenic bacteria was tested in this study. Five pathovars of *Xanthomonas campestris* and two antisera, one produced against pv. *vesicatoria* and the other against pv. translucens, were used in a model system. A 10-min incubation of the bacterial cells, dot blotted on membranes, in diluted sera, followed by either alkaline phosphatase conjugated protein A or goat antirabbit globulin, resulted in a specific reaction between the homologous serum and bacteria. Populations of 1000-2000 cfu per spot (ca. 0.3 cm2) could be detected with these reagents. The streptavidin-biotinylated peroxidase complex produced a definitive reaction with as few as 800 cfu, but cross-reactions became evident at the higher cell concentrations among all five pathovars in tests with both antisera. Cell-free extracts, obtained by centrifugation of boiled bacteria, reacted similarly to live cells. Unrelated bacteria did not react with either antiserum.
Extracts of lesions from tomato and pepper leaves infected with *X. campestris* pv. *vesicatoria* reacted positively with the antiserum produced against this pathovar but not that produced with pv. *translucens*. Samples of supernatants from boiled lesions reacted with similar intensity as those from homogenized tissues (Lazarovits et al. 1987).

2. Delimitation
No information available

3. Monitoring

The activities categorized under Monitoring are mostly detection surveys carried out to estimate the extent and distribution of the pathogen. In the following only more recent sources are cited.

According to Kornev et al. (2010) a survey was conducted to determine the distribution of Black spot on tomatoes in European part of Russia at 2004-2008. The disease was present everywhere, and rate of infected plants was up to 70%. Genetic analysis showed the presence of two pathogen species - *X. vesicatoria* and *X. gardneri in* Russian Federation. A detection survey was carried out also in Tanzania in 1997 and 1998. Field surveys during the rainy seasons of showed that bacterial spot could be found in tomato and sweet pepper fields in all the mainland vegetable regions of the northern and southern highlands in, but not in Zanzibar. In fields of tomato surveyed (59 in 1997, 50 in 1998) in which bacterial spot was observed, disease incidence varied greatly between years and fields (from <5% to >90%). In pepper, incidence was never greater than 5% (10 fields surveyed in 1997, three in 1998). *Xanthomonas campestris* pv. *vesicatoria* suspected from symptoms on tomato and sweet pepper fruit was confirmed by isolation on semi-selective media, followed by biochemical tests for Xanthomonas and pathogenicity tests on tomato. Using dilution plating, Xcv was found in five of 26 samples of farmers' saved seed, was absent from 12 commercial tomato seed lots and present in two of three commercial pepper seed lots sampled (Black et al,. 2001).

4. Commodities

A study was undertaken to evaluate the effect of heat treatment (70 degrees C for 96 h) on germination and seed structure, as well as to determine the efficiency of heat therapy to prevent bacterial growth and disease establishment. Two experiments were carried out using tomato seeds inoculated by vacuum with *X. campestris* pv. *vesicatoria* [*X. vesicatoria*] strain ENA 4463 adjusted to 10sup7sup cells ml sup-1/sup followed by air-drying. In the first assay, four treatments were designed as follows: (1) inoculated seeds; (2) inoculation followed by heat treatment (70 degrees C/96 h); and two controls, uninoculated seeds without (3) and with (4) heat treatment. In the second assay, three treatments (1, 2 and 3) were compared. Samples from different treatments were evaluated for the physiological status of the seeds and presence of the bacteria. The latter was tested by recovering the bacteria by vacuum extraction, isolation in semi-selective medium and visualizations through scanning electron microscopy (SEM). The efficiency of the heat treatment as a method to control the disease ranged from 99.96 to 100% for the second and first assay, respectively. Furthermore, there was no significant effect of the heat on seed germination. However, under SEM, heat-treated seed showed some superficial structural changes, characterized by a greater number of broken trichomes, and a deposition of trichomes randomly or organized in plates over the seed surface and morphological changes in the bacterial cell shape (Silva et al., 2002).

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Silva, A. M. S., M. G. F. Carmo, et al. (2002). Dry heat therapy treatment of tomato seeds: eradication efficiency of Xanthomonas campestris pv. vesicatoria and effects on seed structure. Fitopatologia Brasileira 27(6): 586-593.

Xanthomonas fragariae Kennedy & King

Taxa: Bacteria: Proteobacteria: Xanthomonadaceae
EPPO A2 list: No. 135
EU Annex designation: II/A2

Organism

Xanthomonas fragariae is the causal agent of bacterial angular leaf spot of strawberry. It is a potentially serious disease which was first discovered in the USA in 1962 and spread to Europe probably with planting material. It was first reported in Italy in 1973 and it is widespread in nurseries in many countries and has been responsible for important production losses in Europe (Lopez, Aramburu *et al*. 1985; Bosshard and Schwindt 1997). *Xanthomonas fragariae* is easily transmitted via asymptomatic plants with latent infections. Residues of infected leaves left in or on soil and crown infections on runners used for planting are sources of inoculum for primary infections. In infected residues, bacteria survive from one crop to the next. Symptoms appear under favourable conditions as well as after cold storage. The disease is generally not destructive but heavy yield losses may occur. The use of healthy planting material and avoidance of conditions favouring disease are the main control methods. EPPO summarizes important information in a Data Sheet for this quarantine pest (Anonymous 1986).

1. Detection

Symptoms (small angular water-soaked spots) appear initially only on the lower leaf surface and become apparent on the upper surface about 2 weeks later after lesions increase. Moreover, international movement of latently infected plants is the main cause of introduction and symptom expression depends on specific environmental conditions. The presence of angular leaf spot may be detected by field inspection but definitive diagnosis should always be obtained through laboratory analysis. Visual inspection is not useful for detecting plants infected systemically.
Many authors have worked on *Xanthomonas fragariae* detection methods. EPPO, in a diagnostic protocol for this quarantine organism (Anonymous 2006), gives detailed information of the different methods. Biochemical and physiological identification, serological tests, and molecular methods are used for identification on plants with symptoms or in asymptomatic samples. Molecular methods have been developed have been available for several years and now facilitate detection in symptomless strawberry plants (Moltmann and Zimmermann 2005), and provide more rapid diagnosis (Stoger and Ruppitsch 2004; Turechek and Hartung 2007; Vandroemme, Baeyen *et al*. 2008; Young, Marney *et al*. 2011). Real time PCR assays allow specific detection to avoid confusion with *Xanthomonas arboricola* pv. *fragariae* (Weller, Beresford-Jones *et al*. 2007). Yang published a review of *Xanthomonas fragariae* detection methods and their improvements (Yang, Liu *et al*. 2010). For several quarantine bacteria (one of which is *Xanthomonas fragariae*) standardized and validated testing schemes and methods have been developed in the EU (Janse 2005).

2. Delimitation

After the first report of *Xanthomonas fragariae* in Italy, outbreak spread was evaluated by biochemical, physiological and pathogenicity tests performed in different provinces on various cultivars (Scortichini and Rossi 1994). The disease's progress was studied in experimental strawberry field plots by Roberts *et al.* (Roberts, Berger *et al*. 1997)

3. Monitoring

Analysis of detection method performances under different tolerances may provide guidance to manage disease below unacceptable levels (Turechek, Hartung *et al*. 2008). Outbreak sites were kept under official monitoring when *Xanthomonas fragariae* was detected in the United Kingdom (Matthews-Berry and Reed 2009).

4. Commodities

Disease spread across the world has mainly been due to movement of symptomless infected strawberry plants; EPPO describes the phytosanitary procedures by which consignments of *Fragaria* spp. plants for planting are subjected to import control (Anonymous 2008).

References: 40 retained out of 145 retrieved, 2 added, 17 used for the summary

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Anonymous (2006). EPPO Standards-Diagnostics- PM7/65 (1) Xanthomonas fragariae. EPPO Bulletin 36: 135-144.

Anonymous (2008). Draft commodity-specific phytosanitary procedure consignment inspection of Fragaria plants for planting. Bulletin OEPP 38(3): 396-406.

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Vandroemme, J., S. Baeyen, *et al*. (2008). Sensitive real-time PCR detection of Xanthomonas fragariae in strawberry plants. Plant Pathology 57(3): 438-444.

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Yang, W., X. Liu, *et al*. (2010). Research progress of detection technology for Xanthomonas fragariae." Plant Quarantine (Shanghai) 24(3): 40-45.

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Xiphinema americanum Cobb *sensu lato*

Common name(s): American dagger nematode
Taxa: Nematoda: Dorylaimida: Longidoridae
EPPO A1 list: No. 150
EU Annex designation: II/A1

Organism
Xiphinema americanum is a polyphagous soil nematode indigenous to the Americas. It has an extremely wide host range and is present throughout much of the Americas, Asia and Oceania. It is absent from the EPPO region.

1. Detection
Xiphinema americanum, including larval stages, is redescribed from topotypes, based on the morphometric characters of females. A study of hundreds of populations classified by various authors as *X. americanum sensu lato* has led the present authors to recognize 25 species which can be subdivided into 6 groups classified mainly on the shape of the lip region and tail (Lamberti and Bleve-Zacheo 1979). The further analysis of a collection of nematodes of *X. americanum* has resulted in the description of five new species (Lamberti and Golden 1986). Morphological traits were generated to identify *Xiphinema americanum sensu lato* (Griesbach and Maggenti 1990, Cho and Robbins 1991, Prior *et al*. 2010), also for the juvenile stages (Halbrendt and Brown 1992). The methods for extracting nematodes from plant tissues are important preliminary steps to identification (Tacconi and Ambrogioni 1995).

2. Delimitation
No information available.

3. Monitoring
A generic survey was carried out to evaluate the occurrence and distribution of longidorid nematodes in Latin America (Doucet *et al*. 1998) or specific on the genus *Xiphinema* were carried out in Guyane and Martinique (Swart and Queneherve 1998).

4. Commodities
The increase of trade constitutes a high phytosanitary risk of introduction of plant parasitic nematodes (Queneherve and Berg 2005) to areas outside their current ranges. The importance of testing soils for the presence from nematodes under certain certification schemes and for export crops has been highlighted (Ward and Hockland 1996). Plant passport and other documentation are provided where control is obligatory (Silvestro and Tacconi 1998).

References: 18 retained out of 30 retrieved, none added, 12 used for the summary

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Queneherve, P. and E. v. d. Berg. 2005. List of the phytoparasitic nematodes (Tylenchida and Dorylaimida) of the French overseas departments (Guadeloupe, Martinique and Guyane) and regulatory considerations. Bulletin OEPP/EPPO Bulletin 35:519-530.

Silvestro, D. d. and R. Tacconi. 1998. Nematodes: passport and certification of fruit crops. Informatore Fitopatologico 48:25-29.

Swart, A. and P. Queneherve. 1998. The genus Xiphinema (Nematoda: Longidoridae) in Guyane and Martinique. Fundamental & Applied Nematology 21:581-604.

Tacconi, R. and L. Ambrogioni. 1995. Quarantine nematodes. Editrice "Lo Scarabeo", Bologna.

Ward, M. G. and S. Hockland. 1996. Nematodes and plant health: Legislation and sampling strategies in decision making for nematode management. Pesticide Science 47:77-80.

Xiphinema californicum Lamberti & Bleve-Zacheo

Common name(s):
Taxa: Nematoda: Dorylaimida: Longidoridae
EPPO A1 list: No. 261
EU Annex designation: I/A1

Organism
Xiphinema californicum is a polyphagous soil nematode occurring in the Americas.

1. Detection
Xiphinema californicum has been described and compared with other species of the genus (Lamberti and Bleve-Zacheo 1979); further morphological characters (e.g. total length, distance of vulva from anterior end, spear length, body diameter at vulva, tail length, anal body diameter, and length and diameter of hyaline tail tip) have also described in another publications (Georgi 1988, Lamberti *et al*. 1988). Observations have also been undertaken on the juvenile stages (Halbrendt and Brown 1992).

2. Delimitation
No information available.

3. Monitoring
The presence of *Xiphinema californicum* was recorded in a number of surveys through the collection of soil and root samples (Lownsbery and Lownsbery 1985, Alkemade and Loof 1990, Magunacelaya *et al*. 2004, Erum and Shahina 2010). The nematodes were examined and analysed statistically by canonical discriminant analysis (CDA) with 11 measurements and body ratios used in the analysis and identification of the species present (Cho and Robbins 1991).

4. Commodities
The possible presence of *X. californicum* in soils of nursery stone fruits has been highlighted, and control measures proposed and discussed (Tacconi and Talame 1995). Obligatory regulations were foreseen as necessary for propagation material and to provide controls on numerous organisms among which *X. californicum* was one (Silvestro and Tacconi 1998).

References: 16 retained out of 35 retrieved, none added, 11 used for the summary

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Silvestro, D. d. and R. Tacconi. 1998. Nematodes: passport and certification of fruit crops. Informatore Fitopatologico 48:25-29.

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Xylella fastidiosa Wells *et al.*

Common name(s): Pierce's disease, Californian vine disease, leaf scorch, phony disease (and others)
Taxa: Bacteria: Proteobacteria: Xanthomonadaceae
EPPO A1 list: Nos 137 & 166
EU Annex designation: I/A1

Organism
Symptoms caused by *X. fastidiosa* were first observed in grapevines and have since been reported on many fruit-tree and ornamental species, including peach, almond, elm, oak, plane, mulberry, maple and citrus. In citrus, the disease mostly affects oranges. The citrus strains seem more serologically related to the peach strains than the grapevine strains. *X. fastidiosa* is present and proliferates only in xylem vessels in roots, stems and leaves. The vessels are ultimately blocked by bacterial aggregates and by tyloses (outgrowths of the parenchyma) and gums formed by the plant. Natural transmission occurs via insects that feed on xylem sap, such as sharpshooter leafhoppers. The bacterium is efficiently acquired by vector insects with no latent period and persists in infective adult insects indefinitely. Most of the important vectors in North and South America do not occur in Europe. *X. fastidiosa* has been found in seeds of sweet oranges and to be translocated to seedlings after the germination of infested seeds (Pria Junior *et al*., 2003).The bacterium overwinters in the xylem of the host plant as well as in weeds (EPPO, 2004). Wet winters promote survival of high vector populations and favour disease spread in regions with dry summers.

1. Detection
Inspection of crops suspected to be infected with *X. fastidiosa* is fundamental for detecting early symptoms of infection. ELISA and PCR tests are then utilised for rapid and reliable identification of the pathogen (Anonymous, 2004). There are seasonal fluctuations in the detection of infected specimens. In citrus recovery of the pathogen from roots and stems is best during two periods: midsummer (June – August) and midwinter (December – February). On citrus it takes 9-12 months incubation before symptoms appear in nursery trees.
On citrus symptoms are more obvious on 3-6 year old trees. Foliar symptoms are very similar to nutrient deficiency and other diseases. Affected trees show leaves with chlorotic yellow spots, resembling zinc deficiency; the lower surface shows slightly raised brownish necrotic spots. Fruit are much smaller than normal and very firm. Flower and fruit set occur at the same time on healthy and affected trees but fruit remain small and ripen earlier. The growth rate of affected trees is greatly reduced and twigs and branches may wilt. The plants do not usually die, nor do the roots show any apparent symptoms.
Anonymous (2004) and Janse (2010) describe the diagnostic protocols for the detection and identification of *X. fastidiosa*. The bacterium can be detected microscopically in vessels in cross-sections of petioles and by electron microscopy, SDS page or indirect immunofluorescence microscopy (Alves *et al*., 2004; Bazzi *et al*., 1994; Carbajal *et al*., 2004). Methods such as grafting on to susceptible indicator plants are available and may have their place in certification schemes in which woody indicators are routinely used. An improved bioassay (faster, better symptom expression) on *Nicotiana tabacum* is available for strains associated with almond leaf scorch disease and Pierce's disease of grapevine (Francis *et al*., 2008). *X. fastidiosa* can also be isolated on suitable selective media, but the bacterium is slow growing.
ELISA and PCR are used for routine detection of the pathogen, with leaves taken from symptomatic

trees (Anonymous, 2004). However both detection methods are limited by low titre or patchy distribution of the bacterium within a host plant. Serological methods are less sensitive than culture but are the easiest means of detection and identification by ELISA or use of fluorescent antibodies. Membrane entrapment immunofluorescence (MEIF) and dot immunoblotting assay (DIBA) have proved useful for the preliminary detection of *X. fastidiosa* in citrus (Hartung *et al*, 1994; Lee *et al*., 1992), but they are not conclusive for the completion of the diagnostic process.
DNA hybridisation probes and PCR primers specific to *X. fastidiosa* have been developed (Minsavage *et al*., 1994). Application PCR is limited in part by the presence of PCR inhibitors, however, inhibition can be overcome and sensitivity increased by culturing bacteria on agar media prior to PCR (termed BIO-PCR). However, *X. fastidiosa* grows slowly, requiring 10-14 days for visible colonies to appear. In response, Fatmi *et al*. (2005) developed an agar-absorption BIO-PCR method for detecting *X. fastidiosa* in grape and citrus plants. Real-time Taq-Man PCR and loop-mediated isothermal amplification (LAMP) methods have also been developed by Francis *et al*., (2006) and Harper *et al*., (2010) which provide a high level of specificity with the potential for diagnosis in the field. Immunocapture-PCR (IC-PCR) and Immuno-PCR (I-PCR) assays have been found to provide quick and very sensitive methods for screening *X fastidiosa*, with the advantage of not requiring any concentration or DNA purification steps while still allowing an accurate diagnosis of the bacterium (Peroni *et al*., 2008). Nested PCR has also been used to detect the bacterium in insect vectors (Bextine *et al*., 2004; Ciapina *et al*., 2004; Hail *et al*., 2010).
Recently multiplexed lateral flow microarray assays have been developed for the detection of *X. fastidiosa* in citrus, which provide highly sensitive and specific assays (Cary *et al*., 2011). The USDA is also developing micro-arrays (Yao *et al*., 2006).

2. Delimitation
No information.

3. Monitoring
Surveys of commercial groves have used random stratified sampling, with samples proportional to the size of the strata (Ayres *et al*., 2001). The stratified factors used were: variety, zone and plant age (Ayres *et al*., 2001).
In Brazil surveys of citrus groves involved the systematic sampling of groves every 5km within a sub-region (Laranjeira *et al*., 2008). In each farm, the groves were randomly selected based on property size and they were geo-referenced as a four-sided polygon. Each polygon was evaluated by walking through in a W-shaped path and inspecting individual trees for typical CVC symptoms in the leaves and/or fruits. At least 1% of the plants of each area were evaluated (Laranjeira *et al*., 2008).

4. Commodities
No information.

References: 161 retained out of 698 retrieved, 4 added, 21 used for the summary

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Xylophilus ampelinus (Panagopoulos) Willems et al.

Common name(s): Bacterial blight, Canker of grapevine
Taxa: Bacteria: Proteobacteria: Burkholderiales
EPPO A2 list: No. 133
EU Annex designation: II/A2

Organism
The only known host of *Xylophilus ampelinus*, bacterial blight, is *Vitis vinifera*. The disease was originally described in Greece (Crete) and was named *Xanthomonas ampelina*. It was transferred to the new genus *Xylophilus* (Willems *et al*. 1987) on the basis of DNA and RNA studies (Anonymous 2009) and there are numerous studies that concern the systematic position of the organism. Bacterial necrosis of grapevines is characterized by typical symptoms such as cankers on stems and petioles, by necrotic foliar spots and by bud death. *Xylophilus ampelinus* is known to occur in many European countries and in Asia, in South Africa and in South America.

1. Detection
The efficiency of an indirect ELISA has been evaluated in comparison with immunofluorescence (Aramburu *et al*. 1988). Indirect ELISA and DAS-ELISA have shown similar levels of sensitivity, but problems occur due to the cross reactitivity with other bacteria (Lopez *et al*. 1987a). TAS-ELISA, another serological technique, was developed for the rapid detection of *Xylophilus ampelinus* (Goszczynska *et al*. 2001).
A PCR was developed for *Xylophilus ampelinus* identification testing four primers (Manceau *et al*. 2000), this technique was subsequently improved obtaining more specificity and sensitivity (Manceau *et al*. 2005). Other molecular techniques, that offer a high efficiency, are nested PCR (Botha *et al*. 2001) and real-time PCR (Dreo *et al*. 2007).
The first detection of bacteria occurs through the observations of symptoms in the field (longitudinal necrotic, progressive scion dieback). *Xylophilus ampelinus* was isolated from affected tissues and identified through its morphological, cultural, physiological and biochemical characteristics. The inoculation into leaves and shoots of potted grapevine cuttings is undertaken to obtain the re-isolation of the organism (Grasso *et al*. 1979, Garau *et al*. 1987). In recent years molecular tools were added to the roster of other techniques that are available (Dreo *et al*. 2005).

2. Delimitation
No information available.

3. Monitoring
Specific survey to determine the distribution of *Xylophilus ampelinus* was carried out in Spain (Lopez *et al*. 1987b) and Slovenia (Seljak *et al*. 2005), evaluating the climatic factors that are favourable to the organism.

4. Commodities
No information available.

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GLOSSARY OF TERMS

ELISA. Enzyme-linked immunosorbent assay. A means of detecting a specific protein using antibodies.

DASI-ELISA. Double Antibody Sandwich Indirect-ELISA

FISH Fluorescent *In Situ* Hybridization

IC-PCR. Immuno-Capture PCR

IEF. Iso-Electric Focussing. A method whereby gels are run and protein spots generated based on their isoelectric point (often followed by separation in a second dimension based on molecular weight).

IFAS. Immuno-Fluorescent Antibody Staining.

ITS-RFLP. The use of Restriction Fragment Length Polymorphism (RFLP) of the Internal Transcribed Spacer (ITS)

LAMP. Loop Mediated Isothermal Amplification; is a novel approach to nucleic acid amplification which uses a single temperature.

MALDI-TOF. Matrix-Assisted Laser Desorption/Ionization – Time of Flight Mass Spectrometry

MB. Methyl Bromide, a widely used fumigant (though currently being phased-out)

MLO. Mycoplasma-Like Organism (mycoplasma = bacteria lacking cell walls)

Morphometric analysis. Analysis of the relative dimensions of animal parts

mtCOI. Mitochondrial Cytochrome Oxidease 1 gene. Sequencing of regions of this gene is commonly used to identify and differentiated species (e.g. whiteflies and thrips).

PCR – Polymerase Chain Reaction

PFA. Pest Free Area

PGLC. Curie Point Pyrolysis Gas-Liquid Chromatography

qRT-PCR. Quantitative Real-Time PCR

RAPD. Random Amplified Polymorphic DNA

rDNA. Ribosomal DNA

RT-PCR. Real-Time PCR

SDS-PAGE. Sodium Dodecyl Sulphate-Polyacrylamide Electrophoresis. A means of separating proteins.

SPR. Surface Plasmon Resonance. A procedure that facilitates “lab on a chip” diagnostic tests.