



***REPORT***

Rome, Italy  
16-20 March 2015

# **Tenth Session of the Commission on Phytosanitary Measures 16-20 March, 2015**



**Food and Agriculture Organisation of the United Nations**

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**Annex 7 to ISPM 27** (*Diagnostic protocols for regulated pests*) on *Potato spindle tuber viroid* (adopted by the Standards Committee on behalf of the CPM).

## TENTH SESSION OF THE COMMISSION ON PHYTOSANITARY MEASURES

*16th - 20th March 2015*

### 1. Opening of the Session

- [1] Following a moment of silence to commemorate the passing of Bureau Member, Dr Mohamed Refaat Rasmy, the Chairperson of the Commission on Phytosanitary Measures (CPM), Ms Kyu-Ock Yim, opened the meeting.
- [2] FAO Deputy-Director-General Ms Helena Semedo welcomed members of the CPM to FAO. She reminded CPs that over \$1 trillion worth of agricultural products are traded internationally each year, with food accounting for more than 80 per cent of the total. Ms Semedo stressed the need to increase efforts to protect food security and the environment, to ensure trade is safe from pests of plants and that a failure to monitor the spread of plant pests and diseases could have disastrous consequences on agricultural production and food security for millions of poor farmers. She described how the IPPC is visibly integrated into FAO's strategic framework which outlines the organization's vision, strategic objectives, and desired outcomes in terms of hunger eradication and agriculture development. In concluding, she welcomed progress in ePhyto and reiterated the uniqueness of the IPPC as the only international standard setting organization for plants and plant products as well as its importance in FAO.
- [3] The Minister of Agriculture, Food and Rural Affairs of the Republic of Korea, Mr Dong-pil Lee made his remarks via video message. The Minister recognised the importance of the Commission's work at all levels including in helping developing countries trade and protect their environments through the IPPC standards. He thanked the current Chairperson for her work and wished members a successful meeting.
- [4] The Officer in Charge of the IPPC thanked those present for their continuous support to international plant health. He noted that there were still many challenges facing the IPPC and plant protection in general, but that the CPM had an opportunity this year to begin addressing those challenges globally if it decided to support the effort to establish an International Year of Plant Health.
- [5] A list of participants is presented in Appendix 03.

### 2. Adoption of the Agenda

- [6] The Chairperson detailed changes to the agenda<sup>1</sup> and the order in which items would be addressed. Following a proposal by some CPs, CPM agreed to add a point on strategic issues on pest diagnosis under the AOB point of the agenda.

- [7] The CPM:

- (1) *adopted* the Agenda and noted the Documents list (Appendix 01 and 02).

#### 2.1 EU statement of competence

- [8] The CPM:

- (2) *noted* the Statement of Competencies and Voting Rights<sup>2</sup> submitted by the European Union (EU) and its 28 member states.

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<sup>1</sup> CPM 2015/08; CPM 2015/CRP/01. All CPM-10 (2015) documents are available at (<https://www.ippc.int/en/core-activities/governance/cpm/>).

<sup>2</sup> CPM 2015/INF/14

### 3. Election of the Rapporteur

[9] The CPM:

- (3) *elected* Ms Olga Lavrentjeva as Rapporteur.

### 4. Establishment of the Credentials Committee

[10] The IPPC Secretariat explained that a Credentials Committee was needed to conform with FAO rules. It would be composed of seven members, one per FAO region, as well as one CPM Bureau member.

[11] The Committee would be assisted by the FAO Legal Office in determining the validity of contracting parties' (CP) credentials.

[12] The CPM:

- (4) *elected* a Credentials Committee to conform to FAO rules.  
(5) *elected* Marc Gilkey (United States) as the Chairperson of the Credentials Committee. The Credentials Committee accepted a total of 114 credentials. The number to establish a quorum for the Commission was set at 92.

### 5. Report by the Chairperson of the Commission on Phytosanitary Measures

[13] The CPM Chairperson referred to her report<sup>3</sup> and presented additional comments. She also announced the appointment of the new IPPC Secretary Mr Jingyuan Xia and briefly explained the appointment had been made in line with FAO regulations. She emphasized the importance of raising awareness about the IPPC, the vital importance of plant health and thanked the Bureau members and Secretariat for their collaborative efforts.

[14] The CPM:

- (6) *noted* the report.

[15] The CPM Chairperson invited former IPPC Secretary Mr Yukio Yokoi to address the CPM. He expressed his thanks for the support received from the CPM, other international organisations, the Bureau and the Secretariat during his period of office and underlined his desire to continue to support the work of the CPM in the future.

[16] CPs thanked Mr Yokoi for his work and accomplishments.

### 6. Report by the IPPC Secretariat

[17] The Officer-in-Charge (OiC), IPPC Secretariat, introduced the 2014 annual report<sup>4</sup> noting that the IPPC Secretariat had seen and would be seeing many changes, including a new Secretary, as well as possible new activities such as implementation, ePhyto, and an effort to establish an International Year of Plant Health. He highlighted the main goals for the future and the major achievements from the past year.

[18] Some CPs underlined the need for the annual plan to be made available not only in English and in a timely manner to ensure their effective participation in meetings.

[19] In response, the OiC reaffirmed the Secretariat's commitment to have all official documents available in the six official languages as early as possible. He acknowledged the concerns of members, recognised the importance of the issue and explained that resource constraints had not always made it possible to provide the necessary translations as early as was needed.

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<sup>3</sup> CPM 2015/INF/05

<sup>4</sup> CPM 2015/INF/01

[20] The CPM:

- (7) *noted* the IPPC Secretariat annual report on the progress undertaken on the CPM work programme in 2014.

## 7. Governance

[21] Some CPs commented on the manner in which the new IPPC Secretary had been appointed and underlined the need to see a transparent and open procedure for selection in the future.

### 7.1 IPPC Secretariat Enhancement Evaluation

[22] The CPM Chairperson introduced the topic of the IPPC Secretariat Enhancement Evaluation<sup>5</sup> and invited Mr Nico van Opstal, lead of the evaluation team to briefly present the results of the team's work.

[23] Some CPs stated that further time was required to complete a detailed analysis of the evaluation report<sup>6</sup> and requested the CPM to develop a process to collect and consider the comments from contracting parties, Bureau and the Secretariat. There was appreciation for the work of the evaluation team, which had completed the report in a relatively short time frame, and support for some of the recommendations.

[24] Some CPs raised issues and concerns in the report's recommendations including Governance, the frequency of CPM meetings, the role of the Strategic Planning Group (SPG), the Finance Committee and article 14 issues.

[25] In response to questions, the representative from the evaluation team confirmed that the report was aligned with the terms of reference established concerning the conclusions of the previous 2007 evaluation. He further confirmed that in recommending a reduction in the number of meetings, there was no intention to create additional work for the Bureau. He clarified that suggestions regarding staffing and legal enhancement were also aimed at supporting the work of the Secretariat.

[26] In response to a CP request on the process to present comments to the Organization on the evaluation report, the FAO Legal Representative stated that as the IPPC is a statutory body with functional autonomy within FAO, it does not have direct reporting lines to the governing bodies of the Organization. Nevertheless, the CPM could still report to Council through the Committee on Agriculture (which meets next year) or, more appropriately, through the Programme Committee (whose next Session will take place in the autumn). A small group (Chile, Canada, EU, France, US Japan, with representation from the Bureau and the Secretariat) met to determine how best to respond to the report.

[27] The CPM:

- (8) *noted* the evaluation.
- (9) *invited* members, regional plant protection organizations (RPPOs) and the Secretariat to provide comments on the report by 15 May 2015, and
- (10) *authorized* the Bureau to:
- a. *review* comments and input received at its June 2015 meeting;
  - b. *engage* with the new Secretary of the IPPC and FAO as the Organization also considers the evaluation and its recommendations;

<sup>5</sup> CPM 2015/16. The full Enhancement evaluation report is available at: <https://www.ippc.int/en/publications/8074/>

<sup>6</sup> CPM 2015/INF/13; CPM 2015/CRP/09

- c. *formulate* a proposal for endorsement by CPM-11 (2016) regarding a plan for implementing the recommendations of the IPPC Secretariat Enhancement Evaluation and present this to the SPG in October 2015 for review;
- d. *initiate* more immediate actions regarding those recommendations which are considered operationally and economically feasible by the Bureau and inform SPG 2015 on those actions;
- e. *develop* a practical mechanism for CPM to monitor and track FAO and Secretariat efforts at implementing the agreed recommendations in the evaluation report.

## 7.2 Summary of the Strategic Planning Group Report

- [28] The Chairperson of the SPG October 2014, Mr Peter Thomson, presented the SPG report<sup>7</sup>.
- [29] CPs commented on the highly participatory nature of the meeting and the innovative proposals put forward. Mr Thompson noted the strong presence of developing countries at this meeting.
- [30] A concern was raised on the selection process for members of the group as it was felt they may not speak for national plant protection organisations (NPPOs), and also did not necessarily report back to them.
- [31] The Secretariat supported the broader nature of the group and acknowledged the value of nominations taking place through NPPOs.
- [32] The CPM:
- (11) *noted* the report.
  - (12) *noted* the narratives developed for the themes identified by the 2014 SPG, understanding that these narratives will serve as the basis for future SPG discussion on strategic directions that the IPPC should consider.
  - (13) *agreed* to provide comments on the narratives as well as identify and describe other significant future trends to the Bureau member from their respective region by May 15 2015 for further discussion at SPG 2015.
  - (14) *agreed* to consider and discuss the proposed seven themes for the development of the new IPPC Strategic Framework (2020-2029).
  - (15) *agreed* that the IPPC Strategic Framework (2020-2029) should be developed with the following themes in mind:
    - i. Technology, innovation and data
    - ii. Resource mobilization
    - iii. Advocacy and awareness through strong communication
    - iv. Implementation, participation and collaboration
    - v. The IPPC is a center of excellence and innovation
    - vi. The IPPC contribution to food security, environmental protection and economic prosperity
    - vii. Simplify regulatory environment for the complexities of future global trade

## 7.3 Abolishment of the Caribbean Plant Protection Commission

- [33] The Secretariat introduced the paper<sup>8</sup>.

<sup>7</sup> CPM 2015/24 and CPM 2015/INF/03

<sup>8</sup> CPM 2015/21

- [34] Dominica, on behalf of the Caribbean, thanked FAO and the IPPC Secretariat for all the technical and legal assistance, and financial support received. They acknowledged the importance of a functional RPPO and stated their desire to establish such a body at the earliest opportunity.
- [35] Support was expressed for an active RPPO in the region with assistance provided by the IPPC Secretariat and FAO legal services.

## 8. International Standard Setting

### 8.1 Report on the activities of the Standards Committee

- [36] The Standards Committee (SC) Chairperson summarized the activities undertaken by the SC since CPM-9 (2014)<sup>9</sup>. She acknowledged the work done by many experts, including those involved with the SC, technical panels, expert working groups, diagnostic protocols drafting groups and the IPPC Secretariat staff in preparing draft ISPMs for adoption by CPM. She urged CPM members to continue to nominate and support experts to be involved in standard setting activities.
- [37] She stated that formal objections had been received on two drafts. The formal objection on the draft ISPM on *International movement of wood* (2006-029) raised a question on the concept of a standard. The SC chair sought the views of CPM members in particular on the format and content of commodity standards and raised the issue of the scope of a commodity standard (see also discussions under 8.2).
- [38] For phytosanitary treatments, she was grateful for the work done at the expert consultations which had led to the joint FAO/IAEA division of nuclear techniques in food and agriculture to conduct research on population differences in fruit flies. Although the SC had presented four draft ISPMs for a vote, she hoped an agreement could be reached through consensus.
- [39] Finally, she reflected on the successful operation of the technical panels since their inception. She pointed out that phytosanitary treatments present options for countries to use and are based on measures that have been implemented by countries and are recommended only after thorough evaluation of efficacy data. Diagnostic protocols, which may be challenging to implement in some cases, include methods that are considered reliable and reproducible.
- [40] She concluded thanking the SC for the interesting discussions and support throughout her mandate as SC Chairperson. The SC May 2015 would be her last meeting as the SC Chairperson.
- [41] The CPM:
- (16) *noted* the update on the 2014 activities of the SC and thanked the SC Chairperson, the SC members, technical experts and others involved in the standard setting process.

### 8.2 Adoption of International Standards for Phytosanitary Measures

- [42] The Secretariat introduced the papers<sup>10</sup> on the draft International Standards for Phytosanitary Measures (ISPMs) proposed for adoption.
- [43] The Secretariat informed the CPM that formal objections 14 days prior to the CPM-10 (2015) session had been received for the following two ISPMs:
- [44] *International movement of growing media in association with plants for planting* (2005-004) (CPM 2015/06\_02) and *International movement of wood* (2006-029) (CPM 2015/06\_03). These draft ISPMs will be returned to the SC for their consideration. Details on the formal objections were presented separately<sup>11</sup>.

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<sup>9</sup> CPM 2015/18

<sup>10</sup> CPM 2015/06 and Attachments 01-09; CPM 2015/CRP/06

<sup>11</sup> CPM 2015/INF/15

- [45] A CP found that the content of the draft ISPM on *International movement of wood* (2006-029) was not consistent with current standards, which brought up the issue in general of the content of a commodity standard. It was suggested that the SC examine this issue and develop criteria for the content of commodity standard and their mode of development.
- [46] One CP underlined the importance of commodity standards such as ISPM 15 (*Regulation of wood packaging material in international trade*). They hoped that issues related to commodity standards be addressed as soon as possible, specifically those concerns related to the draft ISPMs on the *International movement of wood* (2006-029) that had received a formal objection prior to this CPM. As this CP was concerned that the SC would not have time fully to consider and discuss this issue, they suggested the CPM authorize the creation of a working group to consider the issue in order to enable the continued development of commodity standards.
- [47] The CPM agreed that the concept of a commodity standard should be determined and a small group was convened in the margins of the CPM with Argentina, Australia, Canada, EU, Japan, New Zealand, Sudan and United States.
- [48] The group reported back to the CPM with terms of reference<sup>12</sup> for the working group to discuss the concept of a commodity standard (see Appendix 04). It was noted that discussion papers would be welcomed for the working group to consider. Concerns were raised on the participation of industry in the working group, and the Secretariat explained that industry representatives would be invited to participate only as “invited experts” and would not be part of the decision making process.
- [49] The Secretariat introduced a paper<sup>13</sup>, requested by the Bureau, that recognized all the contributions to the standard setting process by contracting parties, organizations and experts for standards adopted at this CPM. (Appendix 5)
- [50] Lastly, the Secretariat informed the CPM that the explanatory document on ISPM 15 (*Regulation of wood packaging material in international trade*) has been revised and the updated version posted on the IPP<sup>14</sup>.
- [51] As some standards had been presented to the CPM for adoption on previous occasions but received formal objections, they were presented for adoption at CPM-10 (2015) by a vote. This was the case for the draft ISPM on *Determination of host status of fruit to fruit fly (Tephritidae)* (2006-031) and three draft cold treatments to be included as annexes to ISPM 28.
- [52] Several CPs expressed the need to adopt standards by consensus and that there should be improved communication with the country submitting the formal objection in order to try to resolve the issues raised. They also stated that standards should be based on science and that objections should be discussed in technical terms.
- [53] One CP<sup>15</sup> stated there were serious shortcomings in the draft ISPM on *Determination of host status of fruit to fruit fly (Tephritidae)* (2006-031) that resulted from the substitution of the term “semi-natural host” for the term “conditional host”. They felt the proposed draft only provided guidance to scientists on how to conduct trials to determine whether certain species of fruits (or vegetables) were hosts for fruit flies. They also found the draft failed to provide guidance to the phytosanitary community about conditions under which traded commodities should be subject to regulatory actions. Use of terminology in this standard could conflict with or have implications for a future broad concept standard on host status.

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<sup>12</sup> CPM 2015/CRP/08

<sup>13</sup> CPM 2015/CRP/07

<sup>14</sup> Explanatory documents for ISPMs are available at: <https://www.ippc.int/en/core-activities/standards-setting/explanatory-documents-international-standards-phytosanitary-measures/>

<sup>15</sup> CPM 2015/CRP/04

- [54] They further noted that this draft had been substantially modified by the SC in November 2014 and that contracting parties had had no opportunity to review the draft before it was presented to CPM-10 (2014).
- [55] The Chairperson, acknowledging that the CPM preferred not to vote on these standards, sought agreement from the CPM to adopt these ISPMs through consensus.
- [56] The three phytosanitary cold treatments, originally presented to CPM-10 (2015) for adoption by a vote, were represented to the CPM for adoption by consensus.
- [57] The draft ISPM on *Determination of host status of fruit to fruit fly (Tephritidae)* (2006-031), also originally presented to the CPM for adoption by a vote, was also represented to CPM for adoption by consensus, however one CP continued to express technical concerns on this standard. In order to address these concerns, the CPM agreed by consensus not to vote on this standard and returned it to the SC.
- [58] The CPM Chairperson recalled that the Standard setting procedure would be reviewed in the SC-7 meeting in May 2015 and that all the points mentioned in the discussions should be forwarded for the group to consider.
- [59] The CPM:
- (17) *agreed* to return the draft ISPM on *Determination of host status of fruit to fruit fly (Tephritidae)* (2006-031) as contained in CPM 2015/06\_01 to the Standards Committee for further consideration.
  - (18) *adopted* Annex 3 to ISPM 26 (Establishment of pest free areas for fruit flies (Tephritidae)) on Phytosanitary procedures for fruit fly (Tephritidae) management (2005-010) (Appendix 13).
  - (19) *adopted* the 2013 amendments to ISPM 5 *Glossary of Phytosanitary Terms* (1994-001) (Appendix 13).
  - (20) *adopted* Annex 16 to ISPM 28 (*Phytosanitary treatments for regulated pests on regulated articles*) on *Cold treatment for Bactrocera tryoni on Citrus sinensis* (2007-206E) (Appendix 13)
  - (21) *adopted* Annex 17 to ISPM 28 (*Phytosanitary treatments for regulated pests on regulated articles*) on *Cold treatment for Bactrocera tryoni on Citrus reticulata x C. sinensis* (2007-206F) (Appendix 13)
  - (22) *adopted* Annex 18 to ISPM 28 (*Phytosanitary treatments for regulated pests on regulated articles*) on *Cold treatment for Bactrocera tryoni on Citrus limon* (2007-206G) (Appendix 13)
  - (23) *adopted* Annex 19 to ISPM 28 (*Phytosanitary treatments for regulated pests on regulated articles*) on *Irradiation for Dysmicoccus neobrevipes, Planococcus lilacinus and Planococcus minor* (2012-011) (Appendix 13)
  - (24) *noted* that the Standards Committee adopted on behalf of CPM the following three diagnostic protocols as Annexes to ISPM 27 (*Diagnostic protocols for regulated pests*):
    - *Phyllosticta citricarpa* (McAlpine) Aa on fruit
    - *Xanthomonas citri* subsp. *citri*
    - *Potato spindle tuber viroid*.
  - (25) *invited* contracting parties to provide their comments on “Review of the standard setting procedure” to their SC members by 27 March 2015.
  - (26) *reviewed and agreed* to Terms of Reference for a working group to discuss the concept of a commodity standard (Appendix 4).
  - (27) *acknowledged* the contributions of the members of the Standards Committee (SC) who have left the SC since CPM-9 (2014) or will leave the SC after the SC-7 meeting in May 2015 (Detailed list in the Appendix 5):

### 8.3 Noting translation adjustments to International Standards for Phytosanitary Measures adopted at CPM-9 (2014)

- [60] The Secretariat introduced the paper<sup>16</sup> noting that the Language Review Groups (LRGs) for Chinese, French and Spanish had reviewed the ISPMs adopted at CPM-9 (2014) in collaboration with FAO translation services. It was noted that there was no new LRG Coordinator for Russian and currently no LRG for Arabic.
- [61] CPM was informed that an LRG for Arabic was being formed.
- [62] The CPM:
- (28) noted that Appendix 1 to ISPM 12 (*Electronic phytosanitary certificates, information on standard XML schemas, and exchange mechanisms*), Annex 2 to ISPM 26 (*Control measures for an outbreak within a fruit fly-pest free area*), Phytosanitary Treatment 15 (*Vapour heat treatment for Bactrocera cucurbitae on Cucumis melo var. reticulatus*) and Diagnostic Protocol 4 (*Tilletia indica* Mitra) have been reviewed by the Chinese, French and Spanish LRGs and FAO translation services.
  - (29) noted that no LRG for Arabic had been established and encouraged contracting parties who use Arabic to form an LRG.
  - (30) noted that as no new LRG Coordinator for Russian has been selected, ISPMs adopted at this CPM were not reviewed by the LRG in Russian.
  - (31) encouraged contracting parties that use Russian to nominate a coordinator, inform the Secretariat and reactivate their LRG.
  - (32) urged its members who participate in LRGs to ensure that the deadlines for the CPM adopted LRG process are followed and due dates respected.
  - (33) agreed that once the Secretariat has applied the changes as indicated in track changes in the Attachments 1 to 11 to CPM 2015/07, the previous versions of the ISPMs are revoked and replaced by the newly noted versions.
  - (34) thanked the LRG Coordinators Mr Liu HUI (Chinese), Mr Lucien K. KOUAMÉ (French) and Ms Beatriz MELCHO (Spanish).

### 8.4 Proposed ink amendments to correct inconsistencies in the use of terms in adopted standards

#### *ISPM 5 Glossary of phytosanitary terms*

- [63] The Secretariat introduced the paper on the proposed ink amendments to correct internal inconsistencies in ISPM 5 *Glossary of phytosanitary terms*<sup>17</sup> in relation to the qualifier “as a commodity class”.

#### *Phytosanitary status – consistency across standards*

- [64] The Secretariat introduced the proposed ink amendments to replace phytosanitary status with more accurate terms in ISPM 1 Phytosanitary principles for the protection of plants and the application of phytosanitary measures in international trade, ISPM 7 Phytosanitary certification system, ISPM 12 Phytosanitary certificates, ISPM 11 Pest risk analysis for quarantine pests, ISPM 21 Pest risk analysis for non-quarantine pests, ISPM 22 Requirements for the establishment of areas of low pest prevalence, ISPM 23 Guidelines for inspection, ISPM 24 Guidelines for the determination and recognition of equivalence of phytosanitary measures, ISPM 26 Establishment of pest free areas for fruit flies

<sup>16</sup> CPM 2015/07

<sup>17</sup> CPM 2015/09

(Tephritidae), ISPM 29 Recognition of pest free areas and areas of low pest prevalence and ISPM 30 Establishment of areas of low pest prevalence for fruit flies (Tephritidae)<sup>18</sup>.

[65] The CPM:

- (35) *noted* the ink amendments presented in Table A.1 of document CPM 2015/09 and *asked* the Secretariat to incorporate them into ISPM 5 (*Glossary of phytosanitary terms*).
- (36) *noted* the ink amendments to replace *phytosanitary status* presented in Table A.1-A.6 of document CPM 2015/11 and *asked* the Secretariat to incorporate them into the relevant ISPMs.
- (37) *noted* that the ink amendments to ISPM 1, ISPM 5, ISPM 7, ISPM 11, ISPM 12, ISPM 21, ISPM 22, ISPM 23, ISPM 24, ISPM 26, ISPM 29 and ISPM 30 will be translated and applied to language versions of the standards as resources permit.
- (38) *agreed* that once the Secretariat has applied these ink amendments, the previous versions of the standards are revoked and replaced by the newly noted version.

## 8.5 Revocation and replacement of old versions of ISPMs

[66] The Secretariat introduced the paper outlining the proposed mechanism to ensure older versions of ISPMs were replaced by the latest versions of ISPMs and revoked when revisions were adopted or noted by the CPM<sup>19</sup>. This mechanism would imply that, when the revision of an ISPM was presented to CPM, consequential changes to references to this ISPM in other ISPMs would also be presented as ink amendments if necessary. Upon adoption of the revised ISPM, the CPM would be requested to revoke the previous version of the ISPM and replace it with the newly adopted revision.

[67] He noted that, based on an in-depth analysis, ink amendments (including modifications to cross-references to old versions of ISPMs) needed to be applied to some existing ISPMs in order to allow old versions of ISPMs to be revoked. These ink amendments were presented, in English only, in attachment 1 of the paper. The ink amendments would be translated and applied to the language versions of the ISPMs as resources became available.

[68] He further clarified, that once the Secretariat had applied all the proposed changes, all previous versions of ISPMs (in all languages) would be revoked and replaced by the newly adopted or noted versions. This also included previous versions of ISPM 5 and ISPM 26, following the adoption of revised versions during this session of the CPM under agenda item 8.2. It also included previous versions of ISPMs for which this session of the CPM had noted ink amendments under agenda item 8.4.

[69] Lastly, he stated that Appendix 2 of ISPM 27 and Appendix 1 of ISPM 28 were proposed for deletion to help streamline the publication of these standards and their annexes in the six official FAO languages.

[70] Some CPs welcomed the revocation of previous versions of ISPMs. They noted that ink amendments had been presented to CPM in three documents and in two agenda items and suggested that to ensure greater transparency in future these amendments should be presented together.

[71] The CPM:

- (39) *adopted* the elimination of Appendix 2 to ISPM 27 and Appendix 1 to ISPM 28 (which will be maintained separately by the IPPC Secretariat and posted on the IPP until it can be replaced by a database) and *noted* that ISPM 27 and ISPM 28 will have minor adjustments to reflect the removal of these two appendices.
- (40) *noted* ink amendments (Attachment 1 to CPM 2015/05).

<sup>18</sup> CPM 2015/11, Tables A1-A6

<sup>19</sup> CPM 2015/05

- (41) *agreed* that once the Secretariat has applied the changes mentioned above, all previous versions of ISPMs are revoked and replaced by the newly adopted or noted versions.

## 8.6 The development of a Framework for standards and implementation – update

- [72] The Secretariat introduced the paper<sup>20</sup> on the development of a Framework for standards and implementation which also included the standards portion of the IPPC Framework for Standards and Implementation” (Annex I to the paper), listing standards and gaps where standards may be needed.
- [73] Some CPs suggested an addition to the recommendations so that the Framework for Standards and Implementation could become a valuable tool to identifying gaps and priorities for the IPPC work programme. They also requested that the Standards Committee ensure that the *Criteria for justification and prioritization of proposed topics* corresponded to the current focus on implementation.
- [74] The CPM:
- (42) *Requested* the Secretariat to consider possible interactions with the Codex Alimentarius and the World Organization for Animal Health (OIE) on the identified areas of common interest as it relates to the Standards Setting work programme when relevant.
- (43) *Agreed* to reserve time at CPM for discussions on concepts and implementation issues related to draft or adopted standards, especially high priority issues considering the Framework for standards and implementation.
- (44) *Requested* the Secretariat to continue to develop the Framework for standards and implementation and ensure that this has a broader application, not only for gap analysis but also allow contracting parties to see what guidance is already available or missing.
- (45) *Noted* the draft standards portion of the IPPC Framework for Standards and Implementation presented in Annex 1 to CPM 2015/19, noting that the full Framework for Standards and Implementation will be presented to CPM-11 (2016) for adoption.
- (46) *Adopted* the *Criteria for justification and prioritization of proposed topics* (Appendix 6).
- (47) *Agreed*, that once adopted, the *Framework for Standards and Implementation* is used as basis for planning of the IPPC Secretariat’s work programme.

## 8.7 Topics for IPPC standards

### 8.7.1 Adjustments to the List of topics for IPPC standards

- [75] The Secretariat introduced the *List of topics for IPPC standards*<sup>21</sup>. He recalled that the Standards Committee modifies subjects and that these modifications, approved by the SC since the last CPM, were therefore presented to this CPM session only for noting.
- [76] The Secretariat noted that a topic had been proposed by the Technical Panel for Forest Quarantine because it was believed there was a technical error in the Annex 1 of ISPM 15.
- [77] A CP suggested that the call for topics scheduled for 2015 be delayed until the Framework for Standards and Implementation had been adopted. If this was not possible, it was recommended that the topics be reviewed against the Framework for Standards and Implementation.
- [78] Some CPs supported maintaining the call for topics in 2015 as a possibility to collect topics for the future work of the IPPC Secretariat, and to understand which standards are important to countries, noting that the prioritization would then be done referencing the Framework.

<sup>20</sup> CPM 2015/19

<sup>21</sup> CPM 2015/10; *List of topics for IPPC standards* is available in language at <https://www.ippc.int/core-activities/standards-setting/list-topics-ippc-standards>

- [79] One CP suggested delaying the work on the draft standard on Minimizing pest movement by sea containers (2008-001) requesting a CPM-11 (2016) Special Topics session on this issue.
- [80] CPM requested a small working group be formed comprising the EU, New Zealand and United States that would meet during CPM to discuss options for addressing the issue of a draft ISPM on Minimizing pest movement by sea containers (2008-001).
- [81] The SC Chairperson reported on the discussions of this small group. She informed CPM that the group recognized that despite the considerable work done so far on the topic there were still differences in opinion amongst CPM members about how to progress. The group was very supportive of the proposal to hold a Special Topic session on sea containers to highlight the risks and to enhance understanding of the complex issues related to the topic, with the purpose of facilitating the further drafting of the standard. She recommended that this should be organized as a special topic session at CPM-11 (2016).
- [82] The group further proposed that the Secretariat should continue with the call for experts for the Expert Working Group on Sea Containers. These experts should be invited to attend the special topic session at CPM-11 (2016) to hear CPM members' views. Pending the outcome of the special topics session, an Expert Working Group on Sea Containers could take place in 2016, hosted by the United States.
- [83] One CP pointed out that with the adoption of the three cold treatments (see discussions under 8.2), the drafting of the standards on Requirements for the use of phytosanitary treatments as phytosanitary measures<sup>22</sup> should also be sped up. The CP further pointed out, that due to the enormous volume of grain involved in international trade, the drafting on the International movement of grain (2013-018) should also be expedited
- [84] Regarding the CPM paper on adjustments to the *List of Topics for IPPC standards*, a CP suggested that in the future, easy reference to the explanations on why adjustments to the list are proposed be presented in the paper.
- [85] The CPM:
- (48) *adopted* the addition of the following topic:
- *Revision of dielectric heating section (Annex 1 (Approved treatments associated with wood packaging material) to ISPM 15 (Regulation of wood packaging material in international trade)).*
- (49) *noted* the deletion of the TPPT subject:
- *Sulfuryl fluoride fumigation of wood packaging material (2007-101)*
- (50) *noted* the consequent addition of the following TPPT subjects:
- *Sulfuryl fluoride fumigation of insects in debarked wood (2007-101A)*
  - *Sulfuryl fluoride fumigation of nematodes and insects in debarked wood (2007-101B).*
- (51) *adopted* the new priority 2 for the following topics:
- *Safe handling and disposal of waste with potential pest risk generated during international voyages (2008-004)*
  - *International movement of wood products and handicrafts made from wood (2008-008)*

<sup>22</sup> *Requirements for the use of chemical treatments as a phytosanitary measure (2014-003); Requirements for the use of fumigation as a phytosanitary measure (2014-004); Requirements for the use of temperature treatments as a phytosanitary measure (2014-005); Requirements for the use of modified atmosphere treatments as a phytosanitary measure (2014-006)*

- *Guidance on pest risk management* (2014-001)
- *Authorization of entities other than national plant protection organizations to perform phytosanitary actions* (2014-002)

(52) *adopted* the new priority 3 for the following topic:

- *Minimizing pest movement by air containers and aircrafts* (2008-002)
- *Requirements for the use of irradiation as a phytosanitary measure* (Revision to ISPM 18) (2014-007)

(53) *adopted* the new priority 4 for the following topics:

- *Use of specific import authorization* (Annex to ISPM 20: *Guidelines for a phytosanitary import regulatory system*) (2008-006).
- Revision of ISPM 4 (*Requirements for the establishment of pest free areas*) (2009-002)

(54) *noted* the revised titles for the following topics, subjects and terms:

**Topics:**

- *International movement of growing media in association with plants for planting* (2005-004)
- *Authorization of entities other than national plant protection organizations to perform phytosanitary actions* (2014-002)
- *Use of specific import authorization* (Annex to ISPM 20: *Guidelines for a phytosanitary import regulatory system*) (2008-006)

**DPs:**

- Genus *Anastrepha* (2004-015)
- *Xiphinema americanum sensu lato* (2004-025)
- Genus *Liriomyza* (2006-017)

**Terms:**

- *contaminating pest, contamination* (2012-001)
- *bark (as a commodity)* (2013-005)

(55) *noted* the addition of the term *endangered area* (2014-009) and the deletion of the terms *pest list* (2012-014) and *commodity pest list* (2013-013)

(56) *requested* the Secretariat to update the CPM adopted *List of topics for IPPC standards* accordingly, and post the updated version on the IPP.

(57) *agreed* to have a call for topics in 2015 and invited CPs to propose priorities that may fill gaps identified by the Framework for standards and implementation (the standards portion).

(58) *noted* there would be a special topics session held at CPM-11 (2016) to hear CPs' views on sea containers and that work on the topic *Minimizing pest movement by sea containers* (2008-001) would be delayed pending the outcome of the special topics session.

## 9. Implementation

### 9.1 Status of ISPM 15 Symbol Registration

[86] The FAO Legal Officer updated the CPM on the Secretariat's efforts to facilitate the ISPM 15 symbol registration process<sup>23</sup>. In 2014, the IPPC Secretariat initiated new registrations for 17 countries which

<sup>23</sup> CPM 2015/12

were identified as the first group based on the prioritization criteria. In addition, in order to raise awareness about the importance of protecting the symbol and assist NPPOs in their interaction with their respective government, a letter was sent to the responsible Minister in each country explaining the purpose of registration and highlighting the need for political and financial support in registering or renewing the registration. Another letter was also sent to NPPOs providing information on the reimbursement procedures for compensating the costs of registration renewals done in 2013. In addition, the Secretariat informed the CPM of the work plan for 2015.

[87] The CPM:

- (59) *noted* the progress made in 2014 and the work plan for 2015 with regard to registration of the ISPM 15 symbol.
- (60) *encouraged* contracting parties to continuously support the process of registration of the ISPM 15 symbol, including renewals of registrations that are due to expire.
- (61) *encouraged* contracting parties to reimburse the IPPC Secretariat for registration and registration renewal costs as soon as practically possible.

## 9.2 Implementation Programme on Surveillance and the Implementation, Review and Support System (IRSS)

[88] The Secretariat presented the principal conclusions of the Open-ended Working Group (OEWG) on Implementation<sup>24</sup> which took place in August 2014 in Rome. The conclusions were that the pilot implementation programme should focus broadly on surveillance and cover all ISPMs related to the topic. The programme should be three years in duration at which point it would be reviewed. In addition, the OEWG recommended that at the same time as the pilot Implementation Programme on Surveillance (IPS) is on-going, efforts should be made to identify the next priority topic for the implementation programme to follow the IPS. The OEWG suggested a process in this regard.

[89] With regard to the IRSS, there was a general agreement that it is integrated into both the work programme of the IPPC Secretariat and the proposed IPS strategic work programme at various levels. The IRSS will be an important mechanism in defining the future implementation priorities as well as providing key strategic and analytical support to various activities outlined in the pilot programme. The conduct of case studies, preparation of technical papers among other products will be key contributions to the International Year of Plant Health as well as to the proposed IPPC flagship publication on the State of Plant Health in the World. The IRSS will also be instrumental for the review and monitoring of the IPS.

[90] Some CPs expressed support for the pilot implementation programme. Some CPs indicated that the current proposal could be an effective starting point but that detail, priorities and steps for coordination and implementation were required. As a result it was suggested that the Secretariat collaborate with experts to identify and prioritize work activities for inclusion under the IPS.

[91] Some CPs noted that the implementation programme had two functions, the first to undertake activities that improve surveillance, and the definition of a country's pest status, and second to pilot processes for implementing the Convention and its standards. The CP felt it was important that lessons learned from the IPS were captured and applied to ensure other implementation programmes were efficient and effective, and the right topics were prioritized.

[92] The CPM:

- (62) *acknowledged* the efforts of contracting parties who participated in the OEWG on Implementation, in particular the efforts of the participants from New Zealand who also did considerable work before the meeting.

<sup>24</sup> CPM 2015/23 Rev.02; CPM2015/INF/17; CPM 2015/CRP/10

- (63) *approved* the “*Strategic work plan in for the implementation programme on surveillance*” (Appendix 12).
- (64) *requested* the Secretariat to select and collaborate with experts to:
- (a) *Identify* relevant activities and their linkages using the content of CPM2015/23 Rev. 02, Annex 2: “*Activities to take place within 3 years of the implementation plan on surveillance*” as a possible basis.
  - (b) *Provide* advice on priority activities taking into account funding availability.
  - (c) *Provide* advice on the timing of proposed activities and the opportunities to cooperate with contracting parties, RPPOs and other organizations.
  - (d) *Provide* advice on options for contracting party commitment and involvement in the programme's activities including in-kind contributions, expertise, and financial support.
  - (e) *Provide* advice on resource mobilisation strategies to support the programme.
  - (f) *Provide* advice on topics for future implementation programmes based on lessons learned, including criteria for setting priorities (topics and activities).
  - (g) *Provide* advice to integrate relevant CPM work areas and collaborating with other subsidiary bodies as needed on the implementation work plans that are developed.
  - (h) *Report* to CPM-11 (2016) through the Bureau on the progress of activities under the approved “*Strategic work plan for the implementation programme on surveillance*” and invite comments for possible adjustments.
- (65) *delegated* to the IPPC Secretariat the management of the implementation programme on surveillance under the oversight of the Bureau.
- (66) *urged* contracting parties and RPPOs to commit to increased emphasis on plant pest surveillance, and
- (67) *urged* contracting parties to contribute resources and motivate others to contribute resources to ensure that the IPPC pilot programme, the Implementation Programme on Surveillance, is a success and has the expected impact.

### 9.3 ePhyto Update

- [93] Mr Peter Thompson (New Zealand) presented the CPM with the latest information on developments concerning the work of the ePhyto Steering Group<sup>25</sup>. He described the activities undertaken by, or with the support of, the steering group that included: awareness raising via a new website; a series of fact sheets and frequently asked questions; presentations in regional workshops, as well as a side session of CPM-10.
- [94] He explained that the steering group, members of the Secretariat capacity development team, Bureau members and a representative from OIRSA had prepared an STDF proposal for a USD 1 200 000 project to build the capacity of contracting parties to exchange phytosanitary certificates electronically. This was in addition to forming a smaller technical sub-group which had developed the specifications for the eventual ePhyto hub.
- [95] Many CPs expressed strong support for the development of an ePhyto hub and there was also support for the development of a pilot project based on a generic national system. Several CPs offered their support and experience of national ePhyto systems. The Republic of Korea proposed that the ePhyto global symposium would be held in the Republic of Korea in November 2015. Issues regarding resource mobilisation, costs, security, governance and hosting of the system were discussed.

<sup>25</sup> CPM 2015/26

[96] In response to some of the questions raised Mr Thompson outlined the basic needs that countries would require to establish a national system starting with a simple web based platform with basic functionality.

[97] Describing elements in the high-level design of the system, he spoke of possible security considerations including encryption and said that a hub could perhaps be hosted with the International Computing Centre (UNICC) in order to have the same protections that the UN enjoys.

[98] The CPM:

(68) *noted* the activities of the ePhyto Steering group (ESG) and the IPPC Secretariat.

(69) *noted* the ePhyto materials now on the IPP ,

(70) *confirmed* its full support for the submission of the STDF proposal on ePhyto for the activities outlined above to enable contracting parties to provide phytosanitary assurances in trade in an innovative, cost effective and globally harmonized way.

(71) *supported* the Secretariat to implement the project, subject to the outcome of the STDF proposal decision.

(72) *supported* the development of a hub for ePhyto and provide additional resources needed to proceed with the development and pilot of the hub and generic national system.

(73) *supported* the continued work of the ePhyto Steering Group under the oversight of the CPM Bureau.

(74) *encouraged* the ePhyto Steering Group and Secretariat to urgently continue its work in this area including:

a. Participation in the management of the submitted STDF project and associated activities

b. Developing business rules and other requirements to implement the hub

(75) *requested* the CPM Bureau report back to CPM-11 (2016) on progress.

(76) *invited* the Bureau to consider how to further develop administrative and legal aspects, a management structure for the hub, a cost recovery system for the use of the hub and report to the CPM-11 (2016).

## 10. International Plant Protection Convention Financial Report, Budget and Resource mobilization

[99] The Secretariat presented the paper<sup>26</sup>. Some CPs expressed concerns about some of the decisions in the paper as they felt it was too early to make these decisions without an agreement on the International Year of plant Health.

[100] The CPM:

(77) *acknowledged and thanked* the following contracting parties for their contributions to the operations of the IPPC Secretariat: Australia, Canada, the European Union, France, Japan, the Republic of Korea, the Republic of South Africa, Switzerland, Sweden, the Netherlands, the United Kingdom and the United States of America.

[101] The CPM was presented with the 2014 financial report<sup>27</sup>. The Secretariat noted that numerous activities were successfully carried out during the year with limited financial resources. Nevertheless, as the work program of the Secretariat is incrementally increasing each year, extra-budgetary support

<sup>26</sup> CPM 2015/02 Rev.01

<sup>27</sup> CPM 2015/27

is needed to sustain the work program in future years. This was successfully accomplished in 2014 and will remain so in 2015, a key concern is sustainability of resources in 2016 and beyond.

[102] The CPM:

- (78) *noted* the 2014 Financial report of the IPPC Secretariat.
- (79) *adopted* the 2014 Financial report for the Special trust fund of the IPPC (Multi-donor) (Table 3). See Appendix 11.
- (80) *encouraged* contracting parties to contribute to the Special trust fund of the IPPC (Multi-donor).
- (81) *thanked* contacting parties which contributed to the IPPC Secretariat's work programme in 2014.

## 11. Capacity Development

### 11.1 CDC Evaluation – update

[103] The Secretariat presented the paper<sup>28</sup> and informed the CPM that regrettably, the evaluation of the CDC had not been completed in time for CPM-10 (2015). As a result, the CPM was invited to discuss possible next steps in the evaluation and how to address the results. It was suggested to take into consideration the materials developed in the evaluation exercise so far.

[104] One CP felt it was important for a review to be undertaken before CPM could be asked to decide on the future structure of the CDC.

[105] The CPM:

- (82) *supported* the option to extend the current mandate of the CDC for one year and have a different person produce an evaluation report for consideration at the Bureau meeting in June 2015. The Bureau should then present the outcome of this report along with the enhancement evaluation report to CPM-11 (2016) for a decision.

## 12. National Reporting Obligations

[106] The Secretariat presented the report and noted that 2014 had been a year of considerable national reporting obligations (NRO) activity. While contracting parties continue to meet and update their NROs, the NRO Advisory Group (NROAG) met in July to elaborate the NRO Programme and work plan. During this process the NROAG declared July 2014 to March 2015 as the “IPPC Year of IPPC Official Contact Points (OCPs)”. The Secretariat has been very active in ensuring the OCPs are current and updated when appropriate. The NRO Update is also distributed monthly to inform OCPs of changes, updates and good practices related to NROs. This has been well received.

[107] The Secretariat summarized the NRO Programme<sup>29</sup> submitted for CPM consideration and noted the NRO work plan is still in the process of finalization due to the number of stakeholders involved and will be presented to CPM-11 (2016) for their consideration. Based on the advice received from the NROAG, who provided guidance on activities and priorities (available in the report of the NROAG<sup>30</sup>), during 2015 countries will continue to meet their NROs while the Secretariat will focus on:

- a. the finalization of the NRO work plan
- b. building on the successes of the “Year of OCP” in 2014
- c. support the Year of “Organization of NPPO”
- d. improve the IPP website for NRO usability and functionality, and

<sup>28</sup> CPM 2015/25 and CPM 2015/INF/17

<sup>29</sup> CPM 2015/22

<sup>30</sup> <https://www.ippc.int/en/publications/report-first-meeting-nroag-draft/>

- e. the development of NRO-related on-line training modules as and when resources are available.

[108] Some CPs expressed their support for the progress made on the development of the NRO programme and there was also support in principle for the proposed programme and procedures when seen in the context of the IPPC Secretariat Enhancement and possible integration with other IPPC programmes.

[109] CPs also addressed issues of prioritization of the different aspects of the programme, the visibility of superseded decisions. The need to address issues regarding public versus bilateral obligations was also stated.

[110] Concerns were expressed about the feasibility of online courses for countries with poor internet connection.

[111] Some CPs suggested that quality control guidance should be discussed by the CPM and not only at Bureau level.

[112] In response to another question, the Secretariat pointed out that contracting parties are not being forced to change their contact point but are requested to update the information where the details are out-of-date or incorrect.

[113] The CPM:

- (83) *provisionally endorsed* the IPPC NRO Programme and IPPC NROs procedures (presented in Appendix 1 and 2), and agreed that past CPM decisions relating to NRO activities of the IPPC Information Exchange programme are superseded by the revised NRO Programme and NRO procedures.
- (84) *agreed* that the Secretariat will undertake basic quality control on information uploaded by the contracting parties and that this will be based on “the NRO Quality Control guidelines” which are to be produced through the NROAG for approval by the CPM in 2016.
- (85) *agreed* that the NRO work plan will be presented to CPM-11 (2016) with clear deliverables, priorities and expected resources needs (financial and human).

### 13. Communications

#### 13.1 Communications Work Plan

[114] The Secretariat presented the IPPC Communications Work Plan<sup>31</sup>, taking into account the IPPC Communication needs assessment conducted in early 2014.

[115] The Secretariat summarized some of the communication efforts being undertaken, including the establishment of an editorial team on communication within the Secretariat, the IPPC newsletter, the redesign of the International Phytosanitary Portal (IPP –www.ippc.int) and the proposal for an International Year of Plant Health (IYPH).

[116] CPs stated that strong communications were vital for the long term success of the IPPC and paramount to improving the profile of plant health on national, regional and international levels.

Several CPs commented on the plan and generally felt that although it provided some foundational work to drive internal and external communications; it could have been more detailed at this stage, with greater emphasis on transforming intentions into actions and less research into additional topics in the field of communications.

[117] The CPM:

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<sup>31</sup> CPM 2015/04 Rev. 01

- (86) *provisionally endorsed* the IPPC Communications Work Plan until a new, more detailed Communications Work Plan is presented to CPM-11 (2016). The new plan should include more concrete communication actions including details on the IYPH and the development of advocacy material for resource mobilization. The revised draft Communications Work Plan will be submitted to the SPG and the Bureau before going back to the CPM.

### 13.2 Proposal for an International Year of Plant Health

- [118] Mr Ralf Lopian (Finland) presented the proposal<sup>32</sup> for an International Year of Plant Health (IYPH), as requested by CPM-9 (2014) and confirmed that Finland would be willing to act as a champion for proclamation of the IYPH.

- [119] The proposal was strongly supported by many CPs as a pivotal initiative to raise awareness on plant health worldwide. CPs regarded the IYPH as an important step to address future pest risk challenges. Many CPs expressed their full support to Finland for the proclamation of an IYPO in 2020.

- [120] Turkey explained that they will hold the Presidency of the G20 in 2015 and at the Antalya summit in November high level messages regarding the IYPH could be communicated to ministers to seek their support.

- [121] Some CPs expressed the need for the initiative to have a detailed work programme with precise and clearly focussed objectives. It was suggested that a Steering Group be established to present a detailed work programme and a clarified set of objectives to CPM-11 (2016).

- [122] The CPM:

- (87) *decided* to pursue an IYPH for 2020.
- (88) *requested* the CPM Bureau and the Financial Committee to form a small steering committee to continue with detailed planning of an IYPH and present a detailed work-programme for the planning of the IYPH 2020 to CPM-11 (2016).
- (89) *requested* the IPPC Secretariat to report to FAO Council and Conference on the CPM intention to petition for and organize an IYPH in 2020 and start internal consultations with other FAO units.
- (90) *welcomed* the proposal by Finland that they would propose to FAO Conference that an IYPH be held in 2020.
- (91) *requested* contracting parties to inform their permanent representative to FAO as well as relevant authorities responsible for UN affairs about their support for an IYPH 2020.
- (92) *invited* contracting parties to pledge financial or in-kind support for an IYPH at CPM-11 (2016).
- (93) *requested* the IPPC Secretariat to liaise with Turkey regarding IYPH and the G20 summit to be held in Antalya in November 2015.

## 14. Liaison and partnership and cooperation of the IPPC Secretariat with relevant organizations

### 14.1 Activities with international organizations

- [123] The Secretariat introduced the paper<sup>33</sup> and confirmed that activities with international organizations had been presented in line with the CPM-9 (2014) decision, to reflect those organizations with which the IPPC Secretariat has a partnership and those that the Secretariat liaises and cooperates with. In addition, activities with organizations where the IPPC Secretariat participates as a member or partner

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<sup>32</sup> CPM 2015/14

<sup>33</sup> CPM 2015/17

were presented separately. The IPPC Secretariat continues to engage with organizations that have a shared mandate and the June 2015 CPM Bureau meeting will discuss developing memorandums of cooperation between the IPPC Secretariat and possibly the WTO-SPS Secretariat and with the WCO Secretariat. Some CPs suggested exploring cooperation with the International Organization for Biological Control.

[124] The CPM:

(94) *noted* the activities of the IPPC Secretariat in relation to international organizations

#### **14.2 Report of the 26th Technical Consultation among regional plant protection organizations**

[125] A representative from OIRSA introduced their paper<sup>34</sup> and presented the report<sup>35</sup> of the 26<sup>th</sup> session of the Technical Consultation among RPPOs which was held in Antigua, Guatemala from 10-14 November 2014. He thanked the IPPC Secretariat for their support and reviewed highlights of the coordinated activities of the RPPOs to help implement the IPPC. It was stressed that e-Phyto was a high priority for RPPOs.

[126] Several CPs requested that, in future, greater detail be provided in the CPM paper which should highlight the main issues discussed at the TC among RPPOs so that CPs could better understand the substantive issues discussed.

[127] The CPM:

(95) *noted* the report of the 26<sup>th</sup> Technical Consultation among RPPOs.

#### **14.3 Reports from selected international organizations**

[128] The Bureau had invited the SPS Committee Secretariat and the CBD Secretariat to make oral presentations.

*Oral reports were presented by the following organizations:*

*Report by the Secretariat of the SPS Committee*

[129] The representative from the WTO-SPS gave a brief presentation on the activities of the organization as detailed in their report<sup>36</sup>. He highlighted and updated the CPM on the most important aspects of SPS's work including the specific trade concerns and the adoption of the new WTO trade facilitation agreement (TFA) aimed at simplifying trade procedures, increasing transparency and reducing bureaucracy in trade.

[130] He noted that the new TFA does not diminish WTO Members' existing right to take science-based measures to protect human, animal or plant life or health within their territories. He urged the NPPOs to make sure that they are involved in the TFA implementation discussions.

[131] CPs raised questions and comments on specific trade concerns (STCs), ePhyto and a request for more capacity development activities in francophone countries in Africa.

[132] One CP also requested further collaboration with the IPPC Secretariat to clarify issues concerning SPS obligations and NRO activities as well as additional obligations, if any, under the TFA.

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<sup>34</sup> CPM 2015/20

<sup>35</sup> Report of the 26<sup>th</sup> TC among RPPOs:

[https://www.ippc.int/static/media/files/publications/en/2015/01/29/tc\\_rppo\\_-\\_26th\\_final\\_report.pdf](https://www.ippc.int/static/media/files/publications/en/2015/01/29/tc_rppo_-_26th_final_report.pdf)

<sup>36</sup> CPM 2015/INF/07

- [133] In response, the representative from the WTO-SPS Secretariat described activities in various regions, explained the importance of meeting the reporting obligations to various organizations and that the WTO and IPPC dispute settlement systems were two different mechanisms.
- [134] He further stated that submitting WTO notifications did not necessarily mean that the IPPC reporting obligations were met as these are different obligations under two separate agreements.
- [135] Responding to concerns from CPs, the IPPC Secretariat noted that the WTO and IPPC dispute settlement processes were originally designed to complement each other. Recent changes in the WTO dispute settlement system have meant that there is more overlap than in the past. The Secretariat stated that CPM-9 (2014) had agreed a recommendation that the IPPC needed to be more proactive in providing support to WTO members before Specific Trade Concerns (STCs) arose. He added that there was a need to find a mutually acceptable way of doing this and the IPPC Secretariat had identified this aspect as an area for more formal collaboration between the IPPC and SPS Secretariats.

#### ***Report by the CBD Secretariat***

- [136] The representative from the Secretariat of the Convention on Biological Diversity (CBD) gave a brief presentation on the activities of the organization as detailed in their report<sup>37</sup>. She highlighted and updated the CPM on the most important aspects of the decisions made that may be relevant to the work of the IPPC by the Conference of the Parties at its twelfth meeting, and the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety at its seventh meeting, held in October 2014 in Pyeongchang, Republic of Korea.
- [137] In particular she highlighted that voluntary guidance on “Devising and Implementing Measures to address the Risks Associates with the Introduction of Alien Species as Pets, Aquarium and Terrarium Species, and Live Bait and Live Food” is additional guidance to CPs.
- [138] She also noted the ongoing work of the CBD in relation to efforts to help achieve the Aichi Biodiversity Target 9 on invasive alien species, collaboration through the Liaison Groups on Invasive Alien Species and for Biodiversity Related Conventions which now includes the IPPC, sharing information, application of international standards and guidance relevant to the management of invasive alien species, including the species recognized as pests and capacity development in management of invasive alien species and living modified organisms.
- [139] She emphasized the importance of collaboration between the CBD<sup>38</sup> national focal points and the IPPC<sup>39</sup> national contact points and provided information on how to find their contact information on the internet.

#### ***Written reports or statements were presented by the following international and regional organizations:***

- Report<sup>40</sup> on activities carried out by the Inter-American Institute for Cooperation on Agriculture (IICA).
  - Statement<sup>41</sup> from the International Atomic Energy Agency (IAEA), through its Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.
- [140] A written report<sup>42</sup> was also presented by the Secretariat for the Standards and Trade Development Facility (STDF) of which the IPPC Secretariat and FAO are partners.

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<sup>37</sup> CPM 2015/INF/09 Rev. 01

<sup>38</sup> CBD National focal points: <http://www.cbd.int/doc/lists/nfp-cbd.pdf>

<sup>39</sup> IPPC National contact points: <https://www.ippc.int/en/countries/>

<sup>40</sup> CPM 2015/INF/11

<sup>41</sup> CPM 2015/CRP/02

<sup>42</sup> CPM 2015/INF/12

## 15. Recommendations

### 15.1 Criteria for CPM Recommendations

- [141] The CPM was presented with a proposal<sup>43</sup> to define the criteria for CPM recommendations.
- [142] At the request of the CPM, a small group of contracting parties met and considered the proposal, as well as interventions made during the Plenary<sup>44</sup>. They proposed modifications to both the Process for adopting CPM recommendations and the Criteria.
- [143] CPs expressed their appreciation for the constructive discussion in the small group and the willingness to find consensus.
- [144] Whilst CPs were able to agree on the proposal to modify the process for adopting CPM recommendations they felt more time was required to reflect on the need for and content of possible criteria.
- [145] The CPM:
- (96) *adopted* the revised process for adopting CPM recommendations (Appendix 7)
  - (97) *agreed* to delay adopting criteria for CPM recommendations until CPM-11 (2016)

### 15.2 Adoption of CPM Recommendations

- [146] The CPM received a proposal<sup>45</sup> for a CPM Recommendation on sea containers which was developed by a group of experts from Argentina, Denmark, Gabon, Japan, the Netherlands and USA, and circulated for comments.
- [147] Comments were considered by the Secretariat and the draft CPM Recommendation revised. The Bureau then further revised the draft which was presented to CPM.
- [148] Proposed changes were presented<sup>46</sup> and the CPM agreed.
- [149] The CPM:
- (98) *encouraged* the IPPC secretariat:
    - a. *to work with* International Maritime Organization (IMO), the International Labour Organization (ILO) and United Nations Economic Commission for Europe (UNECE) to raise awareness amongst their members of the risks arising from the international movement of sea containers and the benefit of ensuring that sea containers are clean,
    - b. *to explore* the possibilities and the finances needed to develop a brochure and poster addressed in particular to exporters, consignors, consignees, packing and transport operators, to issues related to the risk of pest movement with sea containers,
  - (99) *requested* the IPPC Secretariat to write to the Secretariats to the Convention on Biodiversity (CBD) and the World Animal Health Organization (OIE) requesting they endorse the CPM Recommendation on Sea Containers with the aim of minimizing the movement of pests with sea containers and to consider developing, in parallel, their own recommendations regarding organisms of their concern with similar involvement of their members and industry.

<sup>43</sup> CPM 2015/03; CPM 2015/INF/16

<sup>44</sup> CPM 2015/CRP/12

<sup>45</sup> CPM 2015/15

<sup>46</sup> CPM 2015/INF/17

(100) *adopted* the CPM Recommendation on Sea Containers as presented in Appendix 8.

### 15.3 Proposal for CPM recommendation on pest diagnosis

[150] The EU proposed a CPM recommendation on the importance of pest diagnosis be developed<sup>47</sup> and presented a draft text for information<sup>48</sup> (see also agenda item 20). CPs indicated they would like to provide comments on the draft and this was agreed; the recommendation would be processed through the established process. CPs were requested to submit their comments to the EU by 15 May 2015.

[151] The CPM:

(101) *agreed* to develop a CPM recommendation on pest diagnosis following the process established by the CPM.

## 16. Dispute Settlement

### 16.1 Report on SBDS activities

[152] The Chairperson of the SBDS presented a verbal report and noted that there had been two changes in SBDS membership, but they had, nevertheless, managed to progress on a number of tasks resulting from the CPM recommendations adopted at CPM-9. She noted that this work would continue in 2015 but could only be finalised after the completion of the phytosanitary dispute between the Republic of South Africa and European Union, as some of the revisions would be based on lessons learnt in that process. The CPM Chairperson acknowledged with gratitude the in-kind contributions provided by Japan to this activity.

[153] The Chairperson of the SBDS confirmed that the SBDS had held a teleconference coordination meeting in March 2015 and that a full meeting of the SBDS was planned for June 2015 after more progress has been made in the South Africa / EU dispute.

[154] The CPM:

(102) *noted* the report by the SBDS.

### 16.2 Dispute avoidance and settlement cases

[155] The Secretariat presented the paper<sup>49</sup> and informed the CPM that there had been a substantial increase in the consultations on dispute avoidance and settlement options from FAO member countries. He stated that these enquiries were resulting from FAO field projects that were requiring input from the Secretariat.

[156] He noted that a positive consequence of these activities was the awareness training within FAO and the FAO regions that was beginning to take place. The Secretariat would be required to provide further input into these projects and this would be based on the assistance contracting parties request.

[157] He confirmed that the phytosanitary dispute between the Republic of South Africa and the European Union was progressing and that a second call for independent experts for the IPPC Citrus Black Spot Expert Committee had been made with a deadline for nominations on 29 March 2015. He confirmed that the SBDS would be providing oversight on this process.

[158] The CPM:

(103) *noted* the dispute settlement support that the Secretariat is providing.

(104) *noted* the developments, and support from the Secretariat in the dispute over Citrus black Spot between the Republic of South Africa and the European Union.

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<sup>47</sup> CPM 2015/28

<sup>48</sup> CPM 2015/CRP/03

<sup>49</sup> CPM 2015/29

## 17. Contracting Parties Reports of Successes and Challenges of Implementation<sup>50</sup>

### *Implementation of the ISPM 15*

- [159] Canada and NAPPO presented the paper<sup>51</sup> and spoke of the benefits of ISPM 15. They stated that due to the large volumes of wood packaging moving in international trade, the level of non-compliance continued to present a significant pest risk to forests.
- [160] Canada proposed that the IPPC Secretariat, NAPPO and other interested RPPOs work to organize an international workshop to discuss the challenges of implementation; recommendations to improve ISPM 15 and to explore opportunities for cooperative approaches for enforcement. Some CPs and RPPOs supported this proposal.
- [161] CPs shared the concern regarding non-compliance and supported continued collaboration on the implementation of ISPM 15.

### *Report from the APPPC ePhyto Working Group*

- [162] The Representative of the APPPC presented the paper<sup>52</sup> and reported on the workshop held on Building Understanding and Preparedness for Electronic Phytosanitary Certification in Bangkok, Thailand in October, 2014.

## 18. Special Topics Session

- [163] The following special topics<sup>53</sup> were presented:

***EPPO programme on diagnostics*** - Serving the needs of EPPO plant pest diagnostic laboratories  
EPPO Secretariat: Françoise Petter, Madeleine McMullen, Baldissera Giovani.

***New treatment technologies for phytosanitary applications***  
Ron A. Sequeira - USDA APHIS PPQ Science and Technology.

***Risk based surveillance systems***  
Professor Mark Burgman - Centre of Excellence for Biosecurity Risk Analysis.

- [164] All the presentations were well received and CPs were encouraged to study the presentations, which will be made available on the IPP<sup>54</sup>. Contracting parties were also invited to liaise with fellow members and organisations to further their understanding of the topics presented.

## 19. Membership and potential replacements for CPM Subsidiary bodies

- [165] The Secretary introduced the paper for the regional nominations<sup>55</sup> and urged regions to consider establishing a more permanent process for selecting their nominations from their regions to allow the IPPC Secretariat to be able to liaise with a contact point in the region that would have an understanding of the selection process for each region.

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<sup>50</sup> CPM 2015/INF/02

<sup>51</sup> CPM 2015/INF/10

<sup>52</sup> CPM 2015/INF/08

<sup>53</sup> CPM 2015/INF/06

<sup>54</sup> <http://www.phytosanitary.info/activity/cpm-10-special-topics-session>

<sup>55</sup> CPM 2015/INF/11

## CPM Bureau

[166] The Secretariat introduced the paper<sup>56</sup> regarding the unexpected changes to the CPM Bureau. He noted the sudden death of the CPM Bureau member from the Near East region, Mr Mohamed Refaat Rasmy Abdelhamid (Egypt) and the resignation of the CPM Vice-chairperson from the Southwest Pacific region, Mr Peter Thomson (New Zealand).

[167] The CPM:

- (105) *elected* Ms Lois Ransom (Australia) as both the new CPM Bureau member for the Southwest Pacific region and CPM Vice-chairperson for the remainder of Mr Peter Thomson's (New Zealand) term which ends at CPM-11 (2016)
- (106) *elected* Mr Khidir Gebriel Musa Edres (Sudan) as the CPM Bureau member for the Near East region for the remainder of Mr Mohamed Refaat Rasmy Abdelhamid's (Egypt) term which ends at CPM-11 (2016).
- (107) *noted* the current membership and the potential replacements for the CPM Bureau as presented in Appendix 9 of this report.
- (108) *agreed* to review in the Bureau the current procedures and general rules for nominations.

## Standards Committee

[168] The Secretariat presented the paper<sup>57</sup>.

[169] The CPM:

- (109) *noted* the current membership and the potential replacements for the SC as presented in Appendix 10 of the this report, and
- (110) *confirmed* new members and potential replacements for the SC as presented in Appendix 10 of this report.

## Subsidiary Body on Dispute Settlement

[170] The CPM:

- (111) *noted* the current membership and the potential replacements for the SBDS as presented in Appendix 10 of this report.
- (112) *confirmed* new members and potential replacements for the SBDS as presented in Appendix 10 of this report.

## 20. Any other business

[171] Some CPs presented a paper on strategic issues associated with pest diagnosis which included proposed recommendations for the CPM. A few CPs requested more time to consider the paper presented and the EU was advised to propose this topic to CPM-11 (2016) as an agenda item (see also discussions under agenda item 15.3).

## 21. Date and venue of the next session

[172] CPM-11 (2016) was provisionally scheduled for 4-8 April 2016 in Rome<sup>58</sup>.

## 22. Adoption of the Report

[173] The CPM:

- (113) *adopted* the report.

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<sup>56</sup> CPM 2015/30

<sup>57</sup> CPM 2015/13

<sup>58</sup> CPM 2015/CRP/05

## 23. Acknowledgements

- [174] The CPM recognized the contributions of Mr John Hedley (New Zealand) for his lifetime commitment to the goals of the International Plant Protection Convention.
- [175] In addition, the CPM recognized the major contributions of Ms Jane Chard (United Kingdom) for her sustained efforts in standard setting in particular as the most recent SC Chairperson.
- [176] Many other SC members who were leaving the SC, the SBDS and the Bureau were also recognized.
- [177] As this would be the last CPM for IPPC Secretariat staff member Ms Ana Peralta, the CPM expressed thanks for her major contribution to the goals of the IPPC in particular her work in the area of capacity development.

## APPENDIX 01 – Detailed Agenda

- 1. Opening of the Session**
- 2. Adoption of the Agenda**
  - 2.1 EU Statement of Competence
- 3. Election of the Rapporteur**
- 4. Establishment of the Credentials Committee**
- 5. Report by the Chairperson of the Commission on Phytosanitary Measures (CPM)**
- 6. Report of the activities of the IPPC Secretariat**
- 7. Governance**
  - 7.1 IPPC Secretariat Enhancement Evaluation - update
  - 7.2 Summary of the Strategic Planning Group report
  - 7.3 Abolishment of the Caribbean Plant Protection Commission
- 8. International Standard Setting**
  - 8.1 Report on the activities of the Standards Committee
  - 8.2 Adoption of International Standards for Phytosanitary Measures
  - 8.3 Noting translation adjustments to International Standards for Phytosanitary Measures adopted at CPM-9 (2014)
  - 8.4 Proposed ink amendments to correct inconsistencies in the use of terms in adopted standards
  - 8.5 Revocation and replacement of old versions of ISPMs
  - 8.6 The development of a Framework for standards and implementation - update
  - 8.7 Topics for IPPC standards
    - 8.7.1 Adjustments to the *List of topics for IPPC standard*
- 9. Implementation**
  - 9.1 Status of ISPM 15 Symbol Registration
  - 9.2 Implementation Programme on Surveillance and the Implementation, Review and Support System (IRSS) - update
  - 9.3 ePhyto - update
- 10. International Plant Protection Convention Financial Report, Budget and Resource mobilization**
- 11. Capacity Development**
  - 11.1 CDC Evaluation - update
- 12. National Reporting Obligations**
  - 12.1 NRO Programme
- 13. Communications**
  - 13.1 Communications Work Plan
  - 13.2 Proposal for an International Year of Plant Health
- 14. Liaison and Partnership and Cooperation of the IPPC with relevant organizations**
  - 14.1 Activities with international organizations
  - 14.2 Report of the 26th Technical Consultation among Regional Plant Protection Organizations
  - 14.3 Reports from selected international organizations
- 15. Recommendations**
  - 15.1 Criteria for CPM Recommendations
  - 15.2 Adoption of CPM Recommendations
- 16. Dispute Settlement**
  - 16.1 Report on SBDS activities
  - 16.2 Dispute avoidance and settlement cases
- 17. Contracting Parties Reports of Successes and Challenges of Implementation**
- 18. Special Topics Session**

- 19. Membership and potential replacements for CPM subsidiary bodies**
- 20. Any other business**
- 21. Date and venue of the next Session**
- 22. Adoption of the Report**

## APPENDIX 02 – List of Documents

### Pre-Session documents

Document number	Agenda item	Document Title	Available Languages
CPM 2015/01	02	Provisional Agenda	EN/ES/FR/AR
CPM 2015/02 Rev 01	10	Resource Mobilization	EN/FR/ES/RU/AR/ZH
CPM 2015/03	15.1	Possible criteria for the CPM recommendations	EN/FR/ES/RU/AR/ZH
CPM 2015/04 Rev.01	13.1	Communications Work Plan	EN/FR/ES/RU/AR/ZH
CPM 2015/05	08.5	Revocation and replacement of old versions of ISPMs	EN/FR/ES/RU/AR/ZH
CPM 2015/06	08.2	Adoption of International Standards for Phytosanitary Measures (+ 9 annexes)	EN/FR/ES/RU/AR/ZH
CPM 2015/06_01	08.2	Draft ISPM on Determination of host status of fruit to fruit flies (Tephritidae)	EN/FR/ES/RU/AR/ZH
CPM 2015/06_02	08.2	International movement of growing media in association with plants for planting	EN/FR/ES/RU/AR/ZH
CPM 2015/06_03	08.2	International movement of wood	EN/FR/ES/RU/AR/ZH
CPM 2015/06_04	08.2	Draft ISPM on Phytosanitary procedures for fruit fly (Tephritidae) management	EN/FR/ES/RU/AR/ZH
CPM 2015/06_05 (Rev.01 RU only)	08.2	Draft ISPM - Amendments to ISPM 5 (Glossary of phytosanitary terms)	EN/FR/ES/RU/AR/ZH
CPM 2015/06_06	08.2	Cold treatment on <i>Bactrocera tryoni</i> on <i>Citrus sinensis</i>	EN/FR/ES/RU/AR/ZH
CPM 2015/06_07	08.2	Cold treatment on <i>Bactrocera tryoni</i> on <i>Citrus reticulata</i> x <i>C. sinensis</i>	EN/FR/ES/RU/AR/ZH
CPM 2015/06_08	08.2	Cold treatment on <i>Bactrocera tryoni</i> on <i>Citrus limon</i>	EN/FR/ES/RU/AR/ZH
CPM 2015/06_09	08.2	Irradiation for <i>Dysmicoccus neobrevipes</i> , <i>Planococcus lilacinus</i> and <i>Planococcus minor</i>	EN/FR/ES/RU/AR/ZH
CPM 2015/07 (Rev 01 EN only)	08.3	Noting translation adjustments to International Standards for Phytosanitary Measures adopted at CPM-9 (2014)	EN/FR/ES/RU/AR/ZH
CPM 2015/08 Rev 01 (Rev 03 EN only)	02	Provisional Detailed Agenda	EN/FR/ES/RU/AR/ZH
CPM 2015/09	08.4	Proposed ink amendments to correct inconsistencies in the use of terms in adopted standards - ISPM 5 (Glossary of phytosanitary terms) for correction of inconsistencies	EN/FR/ES/RU/AR/ZH
CPM 2015/10	08.7.1	Adjustments to the List of topics for IPPC standards	EN/FR/ES/RU/AR/ZH
CPM 2015/11	08.4	Proposed ink amendments to correct inconsistencies in the use of terms in adopted standards - phytosanitary status	EN/FR/ES/RU/AR/ZH
CPM 2015/12 Rev.01	09.1	Status of ISPM 15 Symbol Registration	EN/FR/ES/RU/AR/ZH
CPM 2015/13	19	Membership and Potential Replacements for CPM Subsidiary Bodies	EN/FR/ES/RU/AR/ZH
CPM 2015/14	13.2	Proposal for an International Year of Plant Health	EN/FR/ES/RU/AR/ZH

Document number	Agenda item	Document Title	Available Languages
CPM 2015/15	15.2	Proposal for a CPM Recommendation on Sea Containers - Rationale for developing and adopting a CPM Recommendation on Sea Containers	EN/FR/ES/RU/AR/ZH
CPM 2015/16	07.1	IPPC Enhancement Evaluation - update	EN/FR/ES/RU/AR/ZH
CPM 2015/17	14	Partnerships, liaison and cooperation of the IPPC with relevant international organizations	EN/FR/ES/RU/AR/ZH
CPM 2015/18	08.1	Report on activities of the Standards Committee - 2014	EN/FR/ES/RU/AR/ZH
CPM 2015/19	08.6	The development of a Framework for standards and implementation - update	EN/FR/ES/RU/AR/ZH
CPM 2015/20	14.2	Report of the 26th Technical Consultation among Regional Plant Protection Organizations	EN/FR/ES/RU/AR/ZH
CPM 2015/21	07.3	Abolishment of the Caribbean Plant Protection Commission (CPPC)	EN/FR/ES/RU/AR/ZH
CPM 2015/22	12.1	NRO Programme	EN/FR/ES/RU/AR/ZH
CPM 2015/23 (Rev 02 EN only)	09.2	Implementation Programme on Surveillance and the Implementation, Review and Support System (IRSS) - update	EN/FR/ES/RU/AR/ZH
CPM 2015/24	07.2	Summary of the Strategic Planning Group report	EN/FR/ES/RU/AR/ZH
CPM 2015/25	11.1	CDC Evaluation - update	EN/FR/ES/RU/AR/ZH
CPM 2015/26	09.3	ePhyto update	EN/FR/ES/RU/AR/ZH
CPM 2015/27	10	International Plant Protection Convention Financial Report, Budget and Resource mobilization - IPPC 2014 Financial Report	EN/FR/ES/RU/AR/ZH
CPM 2015/28	15	Recommendations - Proposed recommendation on the importance of pest diagnosis	EN/FR/ES/RU/AR/ZH
CPM 2015/29	16.2	Dispute avoidance and settlement cases	EN/FR/ES/RU/AR/ZH
CPM 2015/30	19	Membership and potential replacements for CPM subsidiary bodies - Election of the members of the CPM Bureau	EN/FR/ES/RU/AR/ZH

**Information papers**

<b>Document number</b>	<b>Agenda item</b>	<b>Document Title</b>	<b>Available Languages</b>
CPM 2015/INF/01	06	Report of the activities of the IPPC Secretariat: 2014 Highlights	EN/FR/ES/RU/AR/ZH
CPM 2015/INF/02	17	Contracting Parties Reports of Successes and Challenges of Implementation	EN/FR/ES/RU/AR/ZH
CPM 2015/INF/03	7.2	Summary of the Strategic Planning Group report	EN/FR/ES/RU/AR/ZH
CPM 2015/INF/04	N/A	Capacity Development pre-CPM training session, CPM-10 side sessions and CPM-10 Market Places	ENGLISH ONLY
CPM 2015/INF/05	05	Report by the Chairperson of the Commission on Phytosanitary Measures	EN/FR/ES/RU/AR/ZH
CPM 2015/INF/06	18	Special Topics Session	ENGLISH ONLY
CPM 2015/INF/07	14.3	Reports from selected international organizations: Report of activities of the SPS Committee and other relevant WTO activities in 2014	EN/FR/ES
CPM 2015/INF/08	17	Contracting Parties Reports of Successes and Challenges of Implementation - Report from the APPPC ePhyto Working Group to CPM10	ENGLISH ONLY
CPM 2015/INF/09 (Rev 01 EN only)	14.3	Reports from selected international organizations: Report of the Secretariat of the Convention on Biological Diversity (CBD)	ENGLISH ONLY
CPM 2015/INF/10	17	Contracting Parties Reports of Successes and Challenges of Implementation - Implementation of the ISPM 15	EN/FR/ES
CPM 2015/INF/11	14.3	Reports from selected international organizations - Report on activities carried out by the Inter-American Institute for Cooperation on Agriculture (IICA)	EN/ ES
CPM 2015/INF/14	02.1	EU Statement of Competence	ENGLISH ONLY
CPM 2015/INF/13	07.1	IPPC Secretariat Enhancement Evaluation – update: Preliminary Views and Ideas for Going Forward	ENGLISH ONLY
CPM 2015/INF/15	08.2	Adoption of International Standards for Phytosanitary Measures - Formal Objections to draft ISPMs presented for adoption by CPM-10 (2015)	ENGLISH ONLY
CPM 2015/INF/12	14.3	Reports from selected international organizations - STDF Overview	ENGLISH ONLY
CPM 2015/INF/16	15.1	Criteria for CPM Recommendations - Comments from COSAVE	ENGLISH ONLY
CPM 2015/INF/17	09.2; 09.3; 11.1; 15.2	Statements from the European Union and its 28 Member States regarding various CPM-10 Agenda items	ENGLISH ONLY

**APPENDIX 03 – Participants list****MEMBER COUNTRIES  
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## **APPENDIX 04 – Terms of reference for a Working Group to discuss the concept of a commodity standard**

### **Background**

The Commission on Phytosanitary Measures (CPM) – 10 in 2015 has identified the need to have further and in depth discussions and analysis about the concept of a commodity standard.

### **Process**

A small group will meet and complete the tasks outlined below. The report of this meeting will be presented to the Strategic Planning Group (SPG) in 2015 that will provide written input on strategic aspects to the Standards Committee (SC) November 2015. The SC will make recommendations to the CPM - 11 (2016).

The IPPC Secretariat will issue a call for discussion papers to contracting parties, National Plant Protection Organizations (NPPOs), Regional Plant Protection Organizations (RPPOs) and relevant international organizations with a deadline of 12 June 2015.

### **Scope**

Consider the concept and content of a commodity standard and the process for development.

### **Tasks**

The working group will:

- discuss the concept of a commodity standard within the context of the suite of IPPC standards and the Framework for Standards and Implementation
- discuss and propose the purpose, content and format of commodity standards
- consider and propose a process for the development of a commodity standard, including, if relevant, how to consult with stakeholders from industry and other relevant international organizations
- analyse and propose a system to maintain and update commodity standards.

### **Members and expertise**

Approximately 6-10 experts will be selected by the CPM Bureau.

Experts should have a combined knowledge of the IPPC Standard Setting process and developing and setting phytosanitary regulations (in particular where industry stakeholders are engaged).

In addition, a few invited experts from industry will be invited.

### **Date and venue**

The meeting is tentatively scheduled to be held from 20 to 24 July 2015 and hosted by the European and Mediterranean Plant Protection Organization (EPPO) in Edinburgh, Scotland, UK.

The work of this group will be supported by the IPPC Secretariat.

## APPENDIX 05 – Acknowledgement of contributions to the Standards Setting Process

[178] During its tenth session the CPM acknowledged the contributions of the members who had contributed to the Standards Setting process in relation to CPM-10 (2015) adopted ISPMs.

*Standards Committee (SC) who have left the SC since CPM-9 (2014) or will leave the SC after the SC-7 meeting in May 2015*

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[179] *The CPM:*

[180] *acknowledged* the contributions of contracting parties, RPPOs and other organizations and, in particular, individual experts for their efforts (specific roles are noted in parenthesis) in the development of the following ISPMs adopted at CPM-10 (2015).

**Annex 3 on Phytosanitary procedures for fruit fly (*Tephritidae*) management (2005-010) to ISPM 26 (Establishment of pest free areas for fruit flies (*Tephritidae*))** developed by the Technical Panel on Pest Free Areas and Systems Approaches for Fruit Flies:

**Australia:** Mr Robert DUTHIE (TPFF member)

**Brazil:**

- Hosted the 2011 TPFF meeting
- Mr Odilson RIBEIRO E SILVA (TPFF Steward)
- Mr Aldo MALAVASI (TPFF member)

**Chile:** Mr Jaime Gonzalez (TPFF member)

**FAO/IAEA:**

- Hosted the 2009 and 2010 TPFF meeting
- Mr Rui CARDOSO-PEREIRA (TPFF member)

**Japan:** Mr Kenji TSURUTA (TPFF member)

**Jordan:** Ms Mary BAHDOSHEH (TPFF member)

**Israel:** Mr David OPATOWSKI (Steward)

**Malaysia:** Mr Keng Hong TAN (TPFF member)

**Mexico:** Mr José Luis ZAVALA LÓPEZ (TPFF member)

**Viet Nam:** Ms Thanh Huong HA (Assistant Steward)

**Mexico:**

- Ms Ana Lilia MONTEALEGRE LARA (TPFF Steward)
- Mr Martin ALUJA (Invited expert to 2010 TPFF meeting)

**North American Plant Protection Organization (NAPPO):** Mr Walther ENKERLIN (TPFF member)

**South Africa:** Mr Jan Hendrik VENTER (TPFF member)

**Suriname:** Ms Alies VAN SAUERS-MULLER (TPFF member)

**United States of America:**

- Ms Julie ALIAGA (TPFF Steward, TPFF Assistant Steward)
- Mr Kevin M. HOFFMAN (Invited expert to 2011 TPFF meeting).

**Amendments to ISPM 5 *Glossary of Phytosanitary Terms* (1994-001)** developed by the Technical panel for the Glossary:

**China:** Ms Hong NING (TPG member)

**Denmark:** Mr Ebbe NORDBO (TPG Assistant steward)

**Egypt:** Mr Shaza Roushdy OMAR (TPG member)

**European Plant Protection Organization (EPPO):**

- Mr Andrei ORLINSKI (TPG member)
- Mr Ian SMITH (Invited Expert)

**France:** Ms Laurence BOUHOT-DELDUC (TPG member)

**New Zealand:** Mr John HEDLEY (TPG Steward, TPG member)

**United States of America:** Ms Stephanie BLOEM (TPG member)

**Uruguay:** Ms Beatriz MELCHO (TPG member)

**Annexes (Phytosanitary Treatments) to ISPM 28** (*Phytosanitary treatments for regulated pests on regulated articles*) developed by the Technical Panel on Phytosanitary Treatments (2004-005):

**PT 16** *Cold treatment for Bactrocera tryoni on Citrus sinensis* (2007-206E)

**Argentina:** Mr Eduardo WILLINK (Treatment lead)

**Australia:**

- Submitted treatment
- Mr Bart ROSSEL (TPPT Steward)
- Mr David PORRITT (TPPT Steward)
- Mr Andrew JESSUP (TPPT member)

**China:** Mr Yuejin WANG (TPPT member)

**Indonesia:** Mr Antarjo DIKIN (TPPT member)

**IAEA/FAO:** Mr Andrew PARKER (Invited expert)

**Indonesia:** hosted the 2014 TPPT meeting

**Japan:**

- hosting 2010 and 2012 TPPT meetings
- Mr Mitsusada MIZOBUCHI (TPPT member)

**Jordan:** Mr Mohammad KATBEH BADER (TPPT member)

**New Zealand:** Mr Michael ORMSBY (TPPT member)

**Republic of Korea:** Mr. Min-Goo PARK (TPPT member)

**South Africa:** Ms Alice BAXTER (Treatment lead)

**Thailand:** hosting the 2007 TPPT meeting

**United Kingdom:**

- Ms Jane CHARD (TPPT Steward)
- Mr Ray CANNON (TPPT member)

**United States of America:**

- Mr Scott MYERS (Assistant treatment lead)
- Mr Patrick GOMES (TPPT member)
- Mr Guy HALLMAN (TPPT member)
- Mr Scott WOOD (TPPT member)
- Mr Larry ZETTLER (TPPT member)

**PT 17** *Cold treatment for Bactrocera tryoni on Citrus reticulata x C. sinensis (2007-206F)*

**Argentina:** Mr Eduardo WILLINK (Treatment lead)

**Australia:**

- submitted treatment
- Mr Bart ROSSEL (TPPT Steward)
- Mr David PORRITT (TPPT Steward)
- Mr Andrew JESSUP (TPPT member)

**China:** Mr Yuejin WANG (China)

**Indonesia:**

- hosted the 2014 TPPT meeting
- Mr Antarjo DIKIN (TPPT member)

**IAEA/FAO:** Mr Andrew PARKER (Invited expert)

**Japan:**

- Hosted 2010 and 2012 TPPT meetings
- Mr Mitsusada MIZOBUCHI (TPPT member)

**Jordan:** Mr Mohammad KATBEH BADER (TPPT member)

**New Zealand:** Mr Michael ORMSBY (TPPT member)

**Republic of Korea:** Mr Min-Goo PARK (TPPT member)

**South Africa:** Ms Alice BAXTER (Treatment lead)

**Thailand:** hosted the 2007 TPPT meeting

**United Kingdom:**

- Ms Jane CHARD (TPPT Steward)
- Mr Ray CANNON (TPPT member)

**United States of America:**

- Mr Scott MYERS (Assistant treatment lead)
- Mr Patrick GOMES (TPPT member)
- Mr Guy HALLMAN (TPPT member)
- Mr Scott WOOD (TPPT member)
- Mr Larry ZETTLER (TPPT member)

**PT 18** *Cold treatment for Bactrocera tryoni on Citrus limon (2007-206G)*

**Argentina:** Mr Eduardo WILLINK (Treatment lead)

**Australia:**

- submitted treatment
- Mr Bart ROSSEL (TPPT Steward)

- Mr David PORRITT (TPPT Steward)
- Mr Andrew JESSUP (TPPT member)

**China:** Mr Yuejin WANG (China)

**Indonesia:**

- Hosted the 2014 TPPT meeting
- Mr Antarjo DIKIN (TPPT member)

**IAEA/FAO:** Mr Andrew PARKER (Invited expert)

**Japan:**

- Hosted 2010 and 2012 TPPT meetings
- Mr Mitsusada MIZOBUCHI (TPPT member)

**Jordan:** Mr Mohammad KATBEH BADER (TPPT member)

**New Zealand:** Mr Michael ORMSBY (TPPT member)

**Republic of Korea:** Mr Min-Goo PARK (TPPT member)

**South Africa:** Ms Alice BAXTER (Treatment lead)

**Thailand:** Hosted the 2007 TPPT meeting

**United Kingdom:**

- Ms Jane CHARD (TPPT Steward)
- Mr Ray CANNON (TPPT member)

**United States of America:**

- Mr Scott MYERS (Assistant treatment lead)
- Mr Patrick GOMES (TPPT member)
- Mr Guy HALLMAN (TPPT member)
- Mr Scott WOOD (TPPT member)
- Mr Larry ZETTLER (TPPT member)

**PT 19** *Irradiation for* *Dysmicoccus neobrevipes*, *Planococcus lilacinus* and *Planococcus minor* (2012-011)

**Argentina:** Mr Eduardo WILLINK (TPPT member)

**Australia:**

- Mr Andrew JESSUP (TPPT member)
- Mr Bart ROSSEL (TPPT Steward)

**China:** Mr Yuejin WANG (TPPT member)

**IAEA/FAO:** Mr Andrew PARKER (Treatment lead, Invited expert)

**Indonesia:** Hosted the 2014 TPPT meeting

**Japan:** Hosted the 2012 TPPT meeting

**Jordan:** Mr Mohammad KATBEH BADER (TPPT member)

**New Zealand:** Mr Michael ORMSBY (TPPT member)

**United States of America:**

- Mr Guy HALLMAN (Assistant treatment lead)
- Mr Patrick GOMES (TPPT member)
- Mr Scott WOOD (TPPT member)

**Vietnam:** submitted treatment

**Annexes (diagnostic protocols) to ISPM 27** (*Diagnostic protocols for regulated pests*) by the Technical Panel on Diagnostic Protocols (2004-002):

**DP 5: *Phyllosticta citricarpa* (McAlpine) Aa on fruit (2004-023)****Australia:**

- Mr Mallik MALIPATIL (TPDP member)
- Mr Brendan RODONI (TPDP member)

**Canada:** Mr Delano JAMES (TPDP member)

**China:** Ms Liping YIN (TPDP member)

**Brazil:** Mr Marcel B. SPÓSITO (Scientific contribution)

**European Plant Protection Organization (EPPO):** Hosted the 2012 TPDP meeting

**France:** Ms Géraldine ANTHOINE (TPDP member)

**Germany /EPPO:**

- Hosted the 2008 TPDP meeting
- Mr Jens-Georg UNGER (TPDP Steward)

**Greece:** Ms Irene VLOUTOGLOU (Lead author)

**Malaysia:** Mr Keng-Yeang LUM (TPDP member)

**Netherlands:**

- Mr Johannes de GRUYTER (Discipline lead)
- Mr Johan MEFFERT (Co-author)
- Mr Peter J.M. BONANTS (Scientific contribution)

**New Zealand:**

- Mr Robert TAYLOR (TPDP member)
- Mr Gerard CLOVER (TPDP member)

**United Kingdom:** Ms Jane CHARD (TPDP Steward)

**United States of America:**

- Hosted the 2010 TPDP meeting
- Mr Norman B. BARR (TPDP member)
- Mr Lavern W. TIMMER (Scientific contribution)

**Uruguay:**

- Ms Ana Lía TERRA (TPDP member)
- Ms Beatriz MELCHO (TPDP Assistant Steward)
- Mr Luis E Diaz MORALES (Co-author)

**South Africa:**

- Ms Esther VAN DEN BERG (TPDP member)
- Ms Mariette TRUTER (Scientific contribution)

**DP 6: *Xanthomonas citri* subsp. *citri* (2004-011)**

**Argentina:** Ms Rita LANFRACHINI (Co-author)

**Australia:**

- Mr Brendan RODONI (TPDP member)
- Mr Mallik MALIPATIL (TPDP member)

**Canada:** Mr Delano JAMES (TPDP member)

**China:** Ms Liping YIN (TPDP member)

**European Plant Protection Organization (EPPO):** Hosted the 2012 TPDP meeting

**France:** Ms Géraldine ANTHOINE (TPDP member)

**Germany:** Mr Jens-Georg UNGER (TPDP member)

**Malaysia:** Mr Keng-Yeang LUM (TPDP member)

**Netherlands:** Mr Johannes de GRUYTER (TPDP member)

**New Zealand:**

- Mr Robert TAYLOR (TPDP member and Discipline lead)
- Mr Gerard CLOVER (TPDP member)

**United Kingdom:** Ms Jane CHARD (TPDP Steward)

**United States of America:**

- Mr Ed CIVEROLO (Co-author)
- Mr Norman B. BARR (TPDP member)

**Uruguay:**

- Ms Beatriz MELCHO (TPDP Assistant Steward)
- Ms Ana Lía TERRA (TPDP member)
- Mr Enrique Francisco Verdier ROSSI (Lead author)

**South Africa:** Ms Esther VAN DEN BERG (TPDP member)

**Spain:**

- Ms María M. López GONZÁLEZ (Co-author)
- Mr Jaime CUBERO (Scientific contribution)

#### **DP 7: *Potato spindle tuber viroid* (2006-022)**

**Australia:**

- Mr Mallik MALIPATIL (TPDP member)
- Mr Brendan RODONI (TPDP member)

**Canada:**

- Mr Delano JAMES (TPDP member and discipline lead)
- Mr Huimin XU (Co-author)
- China: Ms Liping YIN (TPDP member)

**Denmark:** Mr Steen L. NIELSEN (Scientific contribution)

**European Plant Protection Organization (EPPO):** Hosted the 2012 and 2014 TPDP meeting

**France :** Ms Géraldine ANTHOINE (TPDP member)

**Germany:**

- Mr L. SEIGNER (Scientific contribution)
- Mr S. WINTER (Scientific contribution)
- Mr M. WASSENEGGER (Scientific contribution)

**Malaysia:** Mr Keng-Yeang LUM (TPDP member)

**Netherlands**

- Mr Johannes de GRUYTER (TPDP member)
- Mr H. KOENRAADT (Scientific contribution)
- Ms Johanna ROENHORST (Co-author)
- Mr J.Th.J. VERHOEVEN (Scientific contribution)

**New Zealand:**

- Mr Gerard CLOVER (Discipline lead)
- Mr Robert TAYLOR (TPDP member)

**United Kingdom:**

- Mr Colin JEFFRIES (Lead author)
- Ms Jane CHARD (TPDP Steward)

- Mr A. FOX (Scientific contribution)
- Ms T. JAMES (Scientific contribution)
- Mr W. MONGER (Scientific contribution)
- Mr V. MULHOLLAND (Scientific contribution)

**United States of America:**

- Mr Jorge ABAD (Co-author)
- Mr Norman B. BARR (TPDP member)

**Uruguay:**

- Ms Beatriz MELCHO (TPDP Assistant Steward)
- Ms Ana Lía TERRA (TPDP member)
- Ms Ana ETCHERVERS (Co-author)

**Spain:** Ms Nuria DURAN-VILA (Co-author)

## APPENDIX 06 – Criteria for justification and prioritization of proposed topics

*Adopted by CPM-10 (2015)*

Priority will be given to topics with the largest global impact.

### **Core criteria** (must provide information)

1. Contribution to the purpose of the IPPC as described in article I.1
2. Linkage to IPPC Strategic Objectives (SOs) and Organizational results demonstrated
3. Feasibility of implementation at the global level (includes ease of implementation, technical complexity, capacity of NPPOs to implement, relevance for more than one region).
4. Clear identification of the problems that need to be resolved through the development of the standard.
5. Availability of, or possibility to collect, information in support of the proposed standard (e.g. scientific, historical, technical information, experience).
6. Supporting criteria (provide information as appropriate)

### **Practical**

1. Feasibility of adopting the proposed standard within a reasonable time frame.
2. Stage of development of the proposed standard (is a standard on the same topic already widely used by NPPOs, RPPOs or a relevant international organization).
3. Availability of expertise needed to develop the proposed standard.

### **Economic**

1. Estimated value of the plants protected.
2. Estimated value of trade affected by the proposed standard (e.g. volume of trade, value of trade, the percentage of Gross Domestic Product of this trade) if appropriate.
3. Estimated value of new trade opportunities provided by the approval of the proposed standard.
4. Potential benefits in terms of pest control or quarantine activities.

### **Environmental**

1. Utility to reduce the potential negative environmental consequences of certain phytosanitary measures, for example reduction in global emissions for the protection of the ozone layer.
2. Utility in the management of non indigenous species which are pests of plants (such as some invasive alien species).
3. Contribution to the protection of the environment, through the protection of wild flora, and their habitats and ecosystems, and of agricultural biodiversity.

### **Strategic**

1. Extent of support for the proposed standard (e.g. one or more NPPOs or RPPOs have requested it, or one or more RPPOs have adopted a standard on the same topic).
2. Frequency with which the issue addressed by the proposed standard emerges as a source of trade disruption (e.g. disputes or need for repeated bilateral discussions, number of times per year trade is disrupted).
3. Relevance and utility to developing countries.
4. Coverage (application to a wide range of countries/pests/commodities).

5. Complements other standards (e.g. potential for the standard to be used as part of a systems approach for one pest, complement treatments for other pests).
6. Foundation standards to address fundamental concepts (e.g. treatment efficacy, inspection methodology).
7. Expected standard longevity (e.g. future trade needs, suggested use of easily outdated technology or products).
8. Urgent need for the standard.

## APPENDIX 07 – Process for developing and adopting CPM Recommendations

*[Adopted by CPM-9 (2014), revised by CPM-10 (2015)]<sup>59</sup>*

[1] The Process for developing and adopting CPM Recommendations is as follows:

- (1) A contracting party (CP) or the IPPC Secretariat may propose a topic for a CPM Recommendation and present it to the CPM. An initial draft of the proposed recommendation and the rationale or justification for its need should be presented to the CPM for consideration.
- (2) The need for a new CPM Recommendation should be discussed and agreed by the CPM.
- (3) A draft or, if necessary, a revised draft CPM Recommendation should then be prepared by the IPPC Secretariat (or where appropriate by the CP making the proposal) by 15 May and circulated for comments along with the rationale or justification for its need for a period of three months.
- (4) Comments should be submitted and compiled using the IPPC Online Comment System (OCS) and compiled comments will be published on the IPP.
- (5) The IPPC Secretariat will revise draft CPM Recommendations based on comments received, and then submit the revised draft to the CPM Bureau for consideration of comments, revision if necessary and recommendation to the CPM for adoption
- (6) The draft CPM Recommendation is submitted to the CPM for adoption.
- (7) If the draft CPM Recommendation is not adopted and needs further review or revision, the CPM may decide to send it to an appropriate CPM body or group for further revision. The revised CPM Recommendation is then sent to the next CPM for consideration and adoption.
- (8) Adopted CPM Recommendations are numbered and formatted by the IPPC Secretariat and posted on the IPP.

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<sup>59</sup> Because the Process for adopting CPM recommendations which was used in documents CPM 2015/03 and CPM\_2015\_CRP\_12 was a version slightly different from the Process for developing and adopting CPM recommendations as adopted by CPM-9 (2014), the IPPC Secretariat has edited and merged the two versions (version adopted by CPM-9 (2014) and version revised by CPM-10 (2015)) into one.

## APPENDIX 08 – CPM Recommendation on Sea Containers

*CPM-10 - 2015*

### Background

Surveys carried out in some countries have indicated that sea containers (also known as Cargo Transport Units (CTUs)) to a varying degree may carry contamination, in particular in the form of interior and exterior presence of seeds, snails, slugs, soil, spiders and other biosecurity risk items that may pose a pest risk.

The packing of sea containers with cargo is the most likely stage in the sea container supply chain at which contamination can occur. Operators' procedures for cleanliness and cleaning of sea containers, for handling of containers and cargo, need therefore to take into account the risk of contamination at the packing stage.

To that end, the International Maritime Organization (IMO), the International Labour Organization (ILO) and United Nations Economic Commission for Europe (UNECE), with the support from the IPPC Expert Working Group on Sea Containers, have revised their joint Code of Practice for Packing of Cargo Transport Units to incorporate several elements of phytosanitary importance such as the references to sea container cleaning in chapter 8, annex 5 and, in particular, annex 6, Minimizing the risk of recontamination. This was recognized and appreciated by CPM-9 (2014).

The present recommendation proposes actions to be taken by NPPOs, the IPPC Secretariat and other international organizations.

### Recommendation

Sea containers moved internationally should be as clean as possible, in order to minimize the movement of pests.

Thus the CPM *encourages* NPPOs to:

- *recognize* the risk of pests and regulated articles that can be moved with sea containers
- *communicate* to those involved in packing of sea containers or in the movement of sea containers in and out of their country information about the risk of pest movement with sea containers
- *support* the implementation of the relevant parts of the Code of Practice for Packing of Cargo Transport Units<sup>60</sup> (International Maritime Organization (IMO), International Labour Organization (ILO) and United Nations Economic Commission for Europe (UNECE))
- *gather* information on pest movement via the sea containers themselves, rather than with the cargo moved within sea containers and to share such information, when and if, serious trends arise, and
- *analyse* the possible pest risk and, where justified and practical, *take proportionate action* to mitigate risk.

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<sup>60</sup> Link to the Code of Practice for Packing of Cargo Transport Units (ILO/IMO/UNECE):  
<https://www.ippc.int/publications/code-practice-packing-cargo-transport-units-ctu-code-imoilounece>

## APPENDIX 09 – CPM Bureau Memberships

### *Annex 3A - Current CPM Bureau memberships.*

*Updated 2015-03-19 after it was approved by the CPM*

*Rows indicating vacant positions are shaded in grey.*

Region	Country	Name	Nominated/ Re-nominated	Current term/duration	End of current term
Africa	Cote D'Ivoire	Mr Lucien KOUAME KONAN	CPM-2 (2012) CPM-9 (2014)	2nd Term/2 years	2016
Asia	Republic of Korea	Ms Kyu-Ock YIM	CPM-5 (2010) CPM-7 (2012) CPM-9 (2014)	3rd term / 2 years	2016
Europe	Netherlands	Mr Cornelis Antonius Maria VAN ALPHEN	CPM-9 (2014)	1st term / 2 years	2016
Latin America and Caribbean	Argentina	Mr Diego QUIROGA	CPM-9 (2014)	1st term / 2 years	2016
Near East	Sudan	Mr Khidir Gebriel MUSA EDRES	Proposed at CPM-10 (2015) to replace Mr Mohamed Refaat Rasmy Abdelhamid (Egypt)	Replacement term	2016
North America	USA	Mr John GREIFER	CPM-5 (2010) CPM-7 (2012) CPM-9 (2014)	3rd term / 2 years	2016
Southwest Pacific	Australia	Ms Lois RANSOM	Proposed at CPM-10 (2015) to replace Mr Peter Thomson (New Zealand)	Replacement term	2016

**Annex 3B - Current CPM Bureau replacement memberships***(as of 2015-03-18)**Rows indicating vacant positions are shaded in grey.*

<b>Region</b>	<b>Country</b>	<b>Name</b>	<b>Nominated/ Re-nominated</b>	<b>Current term/duration</b>	<b>End of current term</b>
Africa	Eritrea	Mr Mesghena TEKLEAB	CPM-9 (2014)	1st term/ 2 years	2016
Asia	Japan	Mr Masato FUKUSHIMA	CPM-9 (2014)	1st term/ 2 years	2016
Europe	France	Ms Emmanuelle SOUBEYRAN	CPM-10 (2015)	1st term/ 2 years	2017
Latin America and Caribbean	Mexico	Mr Francisco Javier TRUJILLO ARRIAGA	CPM-9 (2014)	1st term/ 2 years	2016
Near East		VACANT			
North America	Canada	Mr Gregory WOLFF	CPM-9 (2014)	1st term/ 2 years	2016
Southwest Pacific	Australia	Mr. Kim RITMAN	CPM-10 (2015)	1st term/ 2 years	2017

## APPENDIX 10 – SC and SBDS Memberships and Potential Replacements

### 1. Standards Committee Membership and Potential Replacements

*Updated 2015-03-19 after it was approved by CPM*

*This refers to the document CPM 2015/13*

#### Annex 1A - Standards Committee Membership

FAO region	Country	Name	Nominated/ Re-nominated	Current term/duration	End of current term
Africa	Ghana	Ms Ruth WOODE	CPM-8 (2013)	1st term / 3 years	2016
	Algeria	Ms Nadia HADJERES	CPM-10 (2015)	1st term / 3 years	2018
	Kenya	Ms Esther KIMANI	CPM-9 (2014)	1st term / 3 years	2017
	Cameroon	Ms Alice Ntoboh Siben NDIKONTAR	CPM-10 (2015)	1st term / 3 years	2018
Asia	China	Mr Lifeng WU	CPM-10 (2015)	1st term / 3 years	2018
	India	Mr D.D.K. SHARMA	CPM-8 (2013)	1st term / 3 years	2016
	Thailand	Mrs Walaikorn RATTANADECHAKUL	CPM-10 (2015)	1st term / 3 years	2018
	Vietnam	Ms. Thanh Huong HA	CPM-7 (2012) CPM-10 (2015)	2nd term / 3 years	2018
Europe	Netherlands	Mr Nicolaas Maria HORN	CPM-9 (2014)	1st term / 3 years	2017
	Norway	Ms. Hilde Kristin PAULSEN	CPM-7 (2012) CPM-10 (2015)	2nd term / 3 years	2018
	Poland	Mr Piotr WLODARCZYK	CPM-7 (2012) CPM-10 (2015)	2nd term / 3 years	2018
	France	Ms Laurence BOUHOT- DELDUC	CPM-10 (2015)	1st term / 3 years	2018
Latin America and Caribbean	Argentina	Mr Ezequiel FERRO	CPM-8 (2013)	1st term / 3 years	2016
	Chile	Mr Álvaro SEPÚLVEDA	CPM-10 (2015)	1st term / 3 years	2018
	Costa Rica	Mr Guillermo SIBAJA CHINCHILLA	Replacement member for Ms Maria Soledad CASTRO DOROCHESSI CPM-5 (2010) CPM-8 (2013)	2nd term/3 years	2016
	Mexico	Ms. Ana Lilia MONTEALEGRE LARA	CPM-7 (2012) CPM-10 (2015)	2nd term/3 years	2018
Near East	Jordan	Ms Fida'a Ali RAWABDEH	Replacement for Mr Mohammad Reza ASGHARI	2nd term/ 3 years	2016

FAO region	Country	Name	Nominated/ Re-nominated	Current term/duration	End of current term
			CPM-8(2013)		
	Iran	Ms Maryam JALILI MOGHADAM <sup>61</sup>	CPM-10 (2015)	1st term / 3 years	2018
	Sudan	Mr Kamaleldin ABDELMAHMOUD AMEIN BAKR <sup>62</sup>	CPM-10 (2015)	1st term / 3 years	2018
	Yemen	Mr Gamil Anwar Mohammed RAMADHAN	CPM-8(2013)	1st term/3 years	2016
North America	Canada	Ms. Marie-Claude FOREST	CPM-3 (2008) CPM-6 (2011) CPM-9 (2014)	3rd term / 3 years	2017
	USA	Ms. Marina ZLOTINA <sup>63</sup>	CPM-10 (2015)	1st term / 3 years	2018
Southwest Pacific	Australia	Mr Jan Bart ROSSEL	CPM-6 (2011) CPM-9 (2014)	2nd term/3 years	2017
	Papua New Guinea	Mr Pere KOKOA	CPM-10 (2015)	1st term / 3 years	2018
	New Zealand	Mr Stephen BUTCHER	CPM-10 (2015)	1st term / 3 years	2018

<sup>61</sup> To also replace Mr Basim Mustafa KHALIL (Iraq) at the 2015 May SC meeting

<sup>62</sup> To also replace Mr Khidir Gebriel MUSA EDRES (Sudan) at the 2015 May SC meeting

<sup>63</sup> To also replace Ms Julie ALIAGA (USA) at the 2015 May SC meeting

**Annex 1B-Standards Committee Potential Replacements**

FAO region	Order	Country	Name	Nominated Re-nominated	/ Current term/duration	End current term	of
Africa	1	Nigeria	Mr Moses Adegboyega ADEWUMI	CPM-8 (2013)	1st term / 3 years	2016	
	2	Zambia	Mr Kenneth MSISKA	CPM-10 (2015)	1st term / 3 years	2018	
Asia	1	Indonesia	Mr HERMAWAN	CPM-9 (2014)	1st term/ 3 years	2017	
	2	Japan	Mr Masahiro SAI	CPM-10 (2015)	1st term / 3 years	2018	
Europe	1	United Kingdom	Mr Samuel BISHOP	CPM-10 (2015)	1st term/3 years	2018	
	2		VACANT				
Latin America and Caribbean	1	Trinidad and Tobago	Mr Anthony St. HILL	CPM-8 (2013)	1st term / 3 years	2016	
	2	Panama	Ms Judith Ivette VARGAS AZCÁRRAGA	CPM-9 (2014)	1st term / 3 years	2017	
Near East	1	Egypt	Ms Shaza OMAR	CPM-10 (2015)	1st term/ 3 years	2018	
	2	Oman	Mr Suleiman AL TOUBI	CPM-10 (2015)	1st term/ 3 years	2018	
North America	To replace Canada	Canada	Mr Brian DOUBLE	CPM-9 (2014)	1st term/ 3 years	2017	
	To replace USA	USA	Mr John GREIFER	CPM-10 (2015)	1st term/ 3 years	2018	
Southwest Pacific	To replace Australia or New Zealand		VACANT				
	To replace Pacific Island's representative	Samoa	Lupeomanu Pelenato FONOTI	CPM-10 (2015)	1st term/ 3 years	2018	

## 2. Subsidiary Body on Dispute Settlement: Membership and Potential Replacements

### Annex 2A - Subsidiary Body on Dispute Settlement Membership

FAO region	Country	Name	Nominated / Re-nominated	Current term / Duration	End of current term
Africa	Gabon	Ms Seraphine MINKO	CPM-10 (2015)	1st term / 2 years	2017
Asia	Bangladesh	Mr Mohamed AHSAN ULLAH	CPM-10 (2015)	1st term / 2 years	2017
Europe	Netherlands	Ms Mennie GERRITSEN-WIERLARD	CPM-7 (2012) CPM-9 (2014)	2nd term / 2 years	2016
Latin America and Caribbean	Panama	Mr Luis BENAVIDES	CPM-8 (2013) CPM-10 (2015)	2nd term / 2 years	2017
Near East	Yemen	Mr Abdulah AL SAYANI	CPM-9 (2014)	1st term / 2 years	2016
North America	Canada	Mr Steve CÔTÉ	CPM-7 (2012) CPM-9 (2014)	2nd term / 2 years	2016
Southwest Pacific	Samoa	Ms Talei FIDOW	CPM-9 (2014)	1st term / 2 years	2016

### Annex 2B-Subsidiary Body on Dispute Settlement Potential Replacements

FAO region	Country	Name	Nominated / Re-nominated	Current term / Duration	End of current term
Africa	Mozambique	Ms Antonia VAZ TAMBOLANE	CPM-10 (2015)	1st term / 2 years	2017
Asia	Japan	Mr Manabu SUZUKI	CPM-10 (2015)	1st term / 2 years	2017
Europe	France	Mr Benjamin GENTON	CPM-7 (2012) CPM-9 (2014)	2nd term / 2 years	2016
Latin America and Caribbean	Argentina	Ms María Julia PALACIN	CPM-10 (2015)	1st term / 2 years	2017
Near East	Oman	Mr Sulaiman MAHFOUDH AL-TOUBI	CPM-5 (2010) CPM-7 (2012) CPM-9 (2014)	3rd term / 2 years	2016
North America	USA	Mr John GREIFER	CPM-10 (2015)	1st term / 2 years	2017
Southwest Pacific	New Zealand	Mr Peter THOMSON	CPM-8 (2013) CPM-10 (2015)	2nd term / 2 years	2017

## APPENDIX 11 – Financial report for the Special Trust Fund for IPPC

**Table 3. Special Trust Fund of the IPPC (Multi-donor) Contributions vs. Expenditures (2012-2014) (in USD)- detailed breakdown**

<b>Contributions</b>	<b>2004-2011*</b>	<b>2012</b>	<b>2013</b>	<b>2014</b>
Australia		-	-	139,695
Japan		-	28,500	28,500
New Zealand		30,000	80,000	-
Republic of Korea		100,000	100,000	100,000
USA		-	175,000	-
Canada		-	-	337,255
Netherlands		-	-	50,000
Sweden		-	-	70,000
Other		3,143	936	2,751
<b>Total</b>	<b>2,421,027</b>	<b>133,143</b>	<b>384,436</b>	<b>728,201</b>

<b>Expenditures</b>	<b>2004-2011*</b>	<b>2012</b>	<b>2013</b>	<b>2014</b>
Professional and General service staff		7,588	193,650	240,328
Consultants		110,622	148,154	81,381
Travel		95,330	118,258	90,316
Contracts		1,433	-	92,626
Other		38,313	25,327	46,548
<b>Total</b>	<b>1,398,633</b>	<b>253,286</b>	<b>485,389</b>	<b>551,199</b>

<b>Balance</b>	<b>1,022,394</b>	<b>902,251</b>	<b>801,298</b>	<b>978,300</b>
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\*Detailed contributions breakdown is only shown for the 2012-2014 period

## APPENDIX 12 – Strategic work plan in for the implementation programme on surveillance

- [1] CPM 9<sup>64</sup> requested the Secretariat to work with an Open-ended Working Group (OEWG) on Implementation and the Bureau to establish the required mechanisms to focus on the implementation of the Convention, and ensure the work of the IPPC Secretariat and CPM bodies are coordinated to work together to deliver a coherent programme of work.
- [2] The Secretariat convened an OEWG on Implementation<sup>65</sup> in which representatives from NPPOs from a number of contracting parties attended as well as representatives from each of the following CPM Bodies: Bureau, Capacity Development Committee (CDC), Standards Committee (SC) and Subsidiary Body on Dispute Settlement (SBDS) as well as a representative from the National Reporting Obligations Advisory Group (NROAG). The OEWG discussed at length the issues of implementation and the challenges the Secretariat would face to develop and put in place such a programme. The principal conclusions are as follows:
- (1) The pilot implementation programme should focus broadly on surveillance and cover all ISPMs related to the topic. The programme should be 3 years in duration at which point it would be reviewed.
  - (2) The Secretariat should, at the same time as the pilot Implementation Programme on Surveillance (IPS) is ongoing, begin to identify the next priority topic for the implementation programme to follow the IPS. The OEWG suggested a process in this regard as follows:
    - Each implementation programme should be able to be linked to an obligation, responsibility or right set out in the International Plant Protection Convention.
    - The prioritization process should be an analytical process led by the Secretariat, with active input from contracting parties and RPPOs. The Implementation Review and Support System (IRSS) would play a key role in this phase.
    - Only 1-2 priorities would be proposed to the CPM at a time in the form of a high-level description of the work plan for future implementation programmes that would facilitate rapid decision-making. The description would consist of the following main elements:
      - (1) Situation analysis
      - (2) High-level goal
      - (3) Objective of the programme
      - (4) Scope of the programme
      - (5) Potential activities to take place within programme
      - (6) Indicators of success
      - (7) Risks (factors that might cause the programme not to succeed)
    - In year 1, CPM could approve at least one of the priorities and then delegate (i) the development of a detailed work plan to the Secretariat (with selected experts as needed) and (ii) guidance on operational management to the Bureau. In year 2, a summary version of the work plan would be available to inform CPM.
- [3] The OEWG prepared a proposed Strategic Work Plan for the IPS following the elements outlined above and it is presented in Annex 1 to this paper. The Secretariat did further work on the proposal to

<sup>64</sup> CPM 9 Final report: <https://www.ippc.int/publications/cpm-9-final-report-updated-version-posted-23-september-2014>

<sup>65</sup> OEWG Implementation Report: [https://www.ippc.int/sites/default/files/documents/20140911/final-report\\_oewg-implementation\\_10-09-2014\\_201409111203--159.83%20KB.pdf](https://www.ippc.int/sites/default/files/documents/20140911/final-report_oewg-implementation_10-09-2014_201409111203--159.83%20KB.pdf)

identify the tasks that could be undertaken over the next three years for the IPS. Activities to take place within first three years of the IPS is presented in Annex 2.

- [4] Recognising that the Implementation Programme requires the Secretariat and the respective subsidiary bodies to be closely integrated, Senior staff from the IPPC Secretariat met in November 2014 to discuss possible structures for the IPPC Secretariat that would help successfully support the IPS. The Secretariat agreed to support implementation working more closely through the units but recognised that there is ongoing work that will run concurrently as not all the Secretariat activities are related to surveillance.
- [5] The outcome of the OEWG was circulated to the SPG, subsidiary bodies and the CDC and received widespread support. The CDC in particular identified elements of the proposed IPS strategic work plan that could be supported and aligned Secretariat's capacity development work plan to support this initiative. At the Framework for Standards meeting,<sup>66</sup> participants identified standards that are in the pipeline for review and others that could be placed as a priority to also align with the IPS. The NROAG<sup>67</sup> meeting also discussed its role and their possible contributions to the activities in the IPS, some of these are outlined in the strategic work plan.
- [6] The strategic work plan for the IPS also contemplates efforts that would contribute to other IPPC initiatives such as the International Year for Plant Health<sup>68</sup> and the overall IPPC advocacy and communications work plan. Some activities outlined in the strategic work plan are activities already being undertaken or expected to be undertaken by the various units of the Secretariat. This strategic work plan brings together these efforts in a more cohesive way and will help achieve a more precise set of goals and objectives.
- [7] The IRSS is integrated into both the work programme of the IPPC Secretariat and the proposed IPS strategic work programme at various levels. The IRSS will be instrumental as a mechanism to define the future implementation priorities as well as providing key strategic and analytical support to various activities outlined in this pilot programme. The conduct of studies and preparation of technical papers will be a key contributions to the year of plant health as well as to the proposed IPPC flagship publication on the State of Plant Health in the World. The IRSS will also be instrumental for the review and monitoring of the IPS.
- [8] The report of the Implementation Review Response (IRR)<sup>69</sup> is posted on the IRSS webpage. The recommendations contained in this report are presented in Annex 3 to this paper and they support the direction for the establishment of implementation programmes and the need for cohesive, cross cutting integration of the structures of the IPPC Secretariat in terms of work programmes and operations to ensure success. Some recommendations also align to the findings of the recent IPPC Enhancement Evaluation (See CPM 2015/16).
- [9] The OEWG agreed with CPM 9 (2014) that the results and impact of the pilot programme should be reviewed at an appropriate time to determine if the IPS should be continued. A monitoring and evaluation component will be introduced into the implementation programmes to help manage and measure the success of such programmes. Efforts to introduce a monitoring and evaluation component into the work of the Secretariat is already being considered by the Secretariat. The IRSS will play a principal role in this monitoring and evaluation component.

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<sup>66</sup> Framework for standards report, August, 2014:

[https://www.ippc.int/sites/default/files/documents/20141007/2014-08\\_report\\_frameworkstds\\_2014-10-07\\_201410070809--833.67%20KB.pdf](https://www.ippc.int/sites/default/files/documents/20141007/2014-08_report_frameworkstds_2014-10-07_201410070809--833.67%20KB.pdf)

<sup>67</sup> NROAG report, July 2014: [https://www.ippc.int/sites/default/files/documents/20141104/report\\_nroag-07-2014\\_2014-10-28\\_201411041210--2.01%20MB.pdf](https://www.ippc.int/sites/default/files/documents/20141104/report_nroag-07-2014_2014-10-28_201411041210--2.01%20MB.pdf):

<sup>68</sup> IYPH paper CPM 10: to be posted

<sup>69</sup> IRR report on IRSS webpage: to be posted

- [10] The activities outlined in the strategic work plan for the IPS are indicative and can be scaled up or down depending on the resources available. Resources from a number of projects will be channelled to support the activities. Project formulation and resource mobilisation to support the IPS will also be prioritised.
- [11] The IPPC Secretariat currently manages several trust funds and a portion of these trust funds could be used to support the initiation of a the strategic work plan for the IPS. As stated above, the approximate total annual cost of IPS and IRSS work programme is USD 859 000 (for 3 years it is USD 2 577 000). Some trust funds currently in place, primarily GCP/GLO/391/EC, GCP/GLO/551/SWI and MTF/GLO/122/MUL could provide support to the first year of the strategic work plan for IPS but other resources would be needed to be sourced in order to sustain it through the three year timeframe.
- [12] The CPM is requested to:
- *acknowledge* the efforts of contracting parties who participated in the OEWG on Implementation, in particular the efforts of the participants from New Zealand who also did considerable work done prior to the meeting.
  - *approve* the strategic work plan for the implementation programme on surveillance and associated activities to take place in the first three year as presented in Annex 1 and 2 of this paper
  - *delegate* to the IPPC Secretariat the oversight and management of the implementation programme on surveillance under the oversight of the Bureau; and,
  - note the recommendations outlined in the Implementation Review Response report (See annex 3 to this paper)
  - *encourage* the IPPC Secretariat, the Bureau and CPM Subsidiary bodies to consider the recommendations contained in the Implementation Review Response, particularly in relation to their work programmes and in relation to the implementation programme on surveillance.
  - *urge* contracting parties to contribute resources to ensure that the IPPC pilot programme, the Implementation Programme on Surveillance, is a success and has the expected impact.

## Annex 1

### Proposed strategic work plan for the Implementation Programme on Surveillance

#### A. SITUATION ANALYSIS

Many contracting parties do not know their pest situation because of a lack of understanding of the ISPM, or the lack of human and financial resources and other factors.

This programme, the implementation programme on surveillance (IPS), is intended to help contracting parties know what pests are present nationally in order to facilitate trade, conduct pest risk analysis (PRAs), protect plant health, produce a list of regulated pests, and determine the status of pests in their country, region, and world. The IPPC is the international agreement in place (IPPC) to help address these issues, and surveillance is a one of the foundational elements that needs to be addressed. Through years of consultation and analysis, it has been demonstrated that many contracting parties have challenges knowing the status of pests in their countries.

#### B. HIGH-LEVEL GOAL

Functional national surveillance programmes that improve the national knowledge of pest status, so as to meet the goal of the IPPC to prevent the spread and introduction of pests.

#### C. OBJECTIVE OF THE IMPLEMENTATION PROGRAMME

To facilitate the practical implementation of surveillance based on IPPC standards to contribute to the prevention of the spread and introduction of plant pests and enable more countries to share

information on pest status in order to support food security, facilitate trade, and protect the environment.

The purpose of establishing a pilot implementation programme is to enable the IPPC Secretariat, CPM and contracting parties to test a new approach for improving the implementation of the IPPC and its' standards in a simple, carefully-planned and coordinated way.

#### **D. SCOPE OF THE IMPLEMENTATION PROGRAMME ON SURVEILLANCE**

This will be a pilot of a global programme. It will develop tools and resources that can be used by all contracting parties. Some workshops may be delivered at a regional level. At national level, implementation of specific programmes in their country can be initiated by the contracting party.

Duration: 3 years from the time of having resources have been secured. As this is a pilot programme, it will engage in a limited number of selected activities.

Contracting parties wishing to participate should:

- have surveillance as part of NPPO or RPPO priorities
- express a desire to participate at the commencement of the IPS and
- demonstrate commitment to participate actively

#### **E. POTENTIAL ACTIVITIES TO TAKE PLACE WITHIN THE IPS**

##### **NPPO Management**

- 1) Country-level evaluation of implementation of ISPM 6 (Guidelines for surveillance). The global programme develops tools and guidance for the evaluation; contracting parties conduct and report on the evaluation; global programme encourages and monitors and analyzes the extent of contracting party delivery.
- 2) Sustainable resourcing (human, financial and infrastructure resources of national programmes) (development of planning tools, resource mobilization materials, management training).

##### **Advocacy and communications**

- 3) Advocacy activities to demonstrate the value of pest surveillance, outline national responsibilities, support institutional development of surveillance capacities, explain policies and show the resources needed (eg. compile evidence, case studies, best practices and success stories)
- 4) Regional workshops to share experiences

##### **Technical**

- 5) Support regional initiatives for the development of systems for data collection and management as well as training on how to use the data
- 6) Enhance information exchange mechanisms on pest status between contracting parties
- 7) Interacting with national and regional expert through networks to share information on pest status (including e-groups)
- 8) Technical manuals and guidelines
  - a) Guidance to help achieve a common understanding of general surveillance (how to use the information and understand the multiple uses)
  - b) Guidance on the collection and validation of information at the country level (how to do general surveillance)
  - c) Guidance on specific surveillance including delimitation and trace-back
  - d) How to manage NPPO relationship with RPPOs and other groups (universities, private sector etc.) to collect, manage and validate information.
- 9) Improvement and alignment of ISPMs related to surveillance

**Policy**

- 10) Support NPPOs to engage with relevant resources to support the development/updating of national legislative/policy/regulations

**F. GLOBAL INDICATORS OF SUCCESS OF THE IPS**

After three years, there should be:

- Improved pest reporting with an increase in the number of contracting parties with updated pest lists
- Improved quality of pest reports
- Improved access to information on pest status of other countries
- National legislation that is more suited to support surveillance
- Improved level of implementation perceived in national-level evaluations
- Database systems improved
- Databases for surveillance are in use by more contracting parties
- Capacities to deliver surveillance improved
- More high-level authorities convinced of importance of surveillance
- Improved diagnostic capacities
- More resources being applied to surveillance
- Evidence of timely and appropriate responses to pest outbreaks
- Country feedback shows that surveillance programme has improved
- Country feedback shows that other countries' surveillance programmes have improved
- Impact on market access for developing countries
- Increase in number of contracting parties with updated pest lists
- Large number of success stories from contracting parties

Where available baseline information should be used to measure success. Also consider longer term impact/indicators.

**G. Factors that might cause the IPS not to succeed**

- no awareness at decision-maker level to make time, resources etc available to do surveillance and participate in programme
- contracting parties hesitant to provide pest information because of trade concerns
- CPM not able to decide on priorities for work programme
- lack of funding (at the national, regional and global level)
- civil conflict, political instability, natural disasters
- instability of human resources and organization
- limited cooperation and coordination between national stakeholders
- lack of alignment between IPPC and RPPOs and others
- inability to promote the value of the IPS (including availability of information)
- complexity of the issue giving rise to management and communication failure.

## ANNEX 2

**ACTIVITIES TO TAKE PLACE WITHIN THREE YEARS OF THE IMPLEMENTATION PROGRAMME ON SURVEILLANCE**

*Legend: Implementation Review and Support System (IRSS); Capacity Development (CD); Standards Setting (StdSet); Regional Plant Protection Organizations (RPPOs); National Plant Protection Organizations (NPPOs); National Reporting Obligations (NRO);*

Programme area	Activity area	Scope of activities	Key implementers	Timeline	Results to/Impacts: links	Funding (USD)
<b>NPPO Management</b>	1. National -level evaluation of implementation of ISPM 6 ( <i>Guidelines for surveillance</i> ) (global programme encourages, monitors and analyses the extent of contracting party delivery)	(global programme develops tools and guidance for the evaluation; contracting parties conduct and report on the evaluation)	<b>IRSS, CD, StdSet, RPPOs, NPPOs</b>	<b>Year 1</b>	IRSS; CD work programme; State of plant protection in the world; Year of Plant Health; RPPO work programmes; NRO and NPPO work programmes.	120 000
	2. Sustainable resourcing of national programmes (human, financial and infrastructure resources)	(planning tools, resource mobilization resources, management training)	<b>CD, RPPOs, NPPOs</b>	<b>Year 1 and 2</b>	CD work programme; State of plant protection in the world; Year of Plant Health; RPPO work programmes; NPPO work programmes	120 000
<b>Advocacy and communications</b>	1. Advocacy activity on the value of pest surveillance and national responsibilities, support for institutional development of surveillance capacities, policies and the resources needed	(compile evidence, case studies, best practices, success stories)	<b>IRSS, IPPC, Advocacy, RPPOs, NPPOs, External partners</b>	<b>Year 1-3</b>	IRSS; CD work programme; State of plant protection in the world; Year of Plant Health; RPPO work programmes; NRO and NPPO work programmes.	900 000
	2. Regional workshops to share experiences	Organize and conduct targeted workshops in FAO regions based on evidence, case studies, best practices and success stories. (1 workshop per year)	<b>IRSS, CD, NRO, StdSet, RPPOs and NPPOs, External Partners</b>	<b>Year 2-3</b>	IRSS; CD work programme; State of plant protection in the world; Year of Plant Health; RPPO work programmes; NRO and NPPO work programmes.	220 000

Programme area	Activity area	Scope of activities	Key implementers	Timeline	Results to/Impacts:	links	Funding (USD)
Technical	1. Support regional initiatives for development of systems for data collection, and management;	Review, develop or collaborate and provide training on how to use them	NRO, CD, RPPOs, NPPOs and External Partners	Year 1-3	NRO; CD work programme; IRSS; State of plant protection in the world; Year of Plant Health; RPPO work programmes; NPPO work programmes.		102 000
	2. Enhance information exchange mechanisms on pest status between contracting parties	Activities to be determined after situation analysis	NRO, CD, RPPOs, NPPOs, IRSS	Year 1-3	NRO; CD work programme; IRSS; State of plant protection in the world; Year of Plant Health; RPPO and NPPO work programmes.		58 000
	3. Developing national and regional expert networks to share information on pest status (including e-groups)	Activities to be determined after situation analysis	NRO, CD, RPPOs, NPPOs and External partners, IRSS	Year 1-3	NRO; CD work programme; IRSS; State of plant protection in the world; Year of Plant Health; RPPO and NPPO work programmes.		45 000
	4. Technical manuals and guidelines	Guidelines for common understanding of general surveillance (how to use the information – understand the multiple uses)	StdSet, CD, RPPOs, NPPOs, IRSS and External Partners	Year 2-3	CD work programme; SS; NRO; State of plant protection in the world; Year of Plant Health; RPPO and NPPO work programmes		88 000
		Guidance on collection and validation of information at country level (how to do general surveillance)	CD, RPPOs, NPPOs, IRSS and External Partners	Year 2-3			88 000
		Guidance on specific surveillance including delimitation and trace-back	CD, RPPOs, NPPOs, IRSS and External Partners	Year 2-3			88 000
		How to manage NPPO relationship with RPPOs and other groups (universities, private sector etc.) to collect, manage and validate information.	RPPOs, NPPOs, CD, StdSet, IRSS and External Partners	Year 2-3			88 000

Programme area	Activity area	Scope of activities	Key implementers	Timeline	Results to/Impacts:	links	Funding (USD)
	5. Improvement and alignment of ISPMs related to surveillance	Review of ISPMs that address issues related to surveillance (In pipeline 4, 6 & 8 as well as those not yet added to the IPPC list of topics: 17 & 19)	StdSet, CD, RPPOs, NPPOs, IRSS and External Partners	Year 1-3	SS and CD work programme; NRO; State of plant protection in the world; Year of Plant Health; RPPO and NPPO work programmes		450 000
<b>Policy</b>	1.Support NPPOs to engage with relevant resources to support development / updating of national legislative / policy / regulations	Review status at country level, identify relevant interventions, Prioritise interventions, develop and disseminate them	CD, StdSet, NRO, RPPOs, NPPOs, IRSS, and External Partners e.g. FAO-LEGA	Year 1.5 - 3	IRSS; CD work programme; IPPC advocacy and communications work programme; State of plant protection in the world; Year of Plant Health; RPPO and NPPO work programmes.		210 000
<b>ESTIMATED COST OF A 3 YEAR IMPLEMENTATION AND IRSS WORK PROGRAMME</b>							<b>2 577 000</b>

**ANNEX 3****RECOMMENDATIONS FROM THE IMPLEMENTATION REVIEW REPORT****Recommendation 1:**

It is strongly recommended to undertake a regular monitoring of the fulfilment of reporting obligations by contracting parties. Annual reports, including the identification of contracting parties not honouring their reporting obligations, should be provided to the CPM.

**Recommendation 2:**

It is recommended to develop a cross-cutting information exchange policy and work-programme in consultation with the standard development and implementation clusters within the IPPC Secretariat.

**Recommendation 3:**

Future implementation review activities should continue to choose certain topics as focal themes.

**Recommendation 4:**

The implementation review of the next phase of the IRSS should focus on investigating the relevance and impact of diagnostic and taxonomic services for the implementation IPPC and ISPM provisions.

**Recommendation 5:**

The CPM should consider merging IPPC capacity development activities with the IRSS into one programme aimed at improving the implementation of IPPC and ISPMs. The CPM should also consider to establish a subsidiary body on implementation issues aimed at supervising all CPM activities directed towards implementation issues.

**Recommendation 6:**

The CPM and the IPPC Secretariat should investigate on how they can improve their respective working procedures in order to incorporate crosscutting implementation issues into the implementation and development of their work programme.

**Recommendation 7:**

In order to avoid questionnaire fatigue and confusing answers the CPM and the IPPC Secretariat should develop a quality control system for IRR questionnaires and limit the overall amount of questionnaires sent to contracting parties to an sustainable level.

**Recommendation 8:**

The IPPC Secretariat and the CPM should attribute special attention to the implementation of IPPC and ISPM provisions in the Near-East region. Implementation assistance to the Near-East region countries and NEPPO should be considered to improve implementation in this FAO region.

**Recommendation 9:**

A global symposium or workshop should address the topic of small farmer involvement in NPPO activities.

**Recommendation 10:**

The CPM should consider revising ISPM 13 with regard to incorporating a standardized notification format. Such a notification format maybe incorporated into the electronic phytosanitary certification system. The CPM should also consider to intensify efforts concerning the reporting of phytosanitary requirements.

**Recommendation 11:**

The CPM should consider revising ISPM 19 with a view to provide clearer guidance on the establishment of lists of regulated pests and their publication of the IPP.

**APPENDIX 13 – ISPMs adopted by CPM-10**

- Annex 3 to ISPM 26 (*Establishment of pest free areas for fruit flies (Tephritidae)*) on Phytosanitary procedures for fruit fly (Tephritidae) management (2005-010)
- Amendments to ISPM 5 *Glossary of Phytosanitary Terms* (1994-001)
- Annex 16 to ISPM 28 (*Phytosanitary treatments for regulated pests on regulated articles*) on Cold treatment for *Bactrocera tryoni* on *Citrus sinensis* (2007-206E)
- Annex 17 to ISPM 28 (*Phytosanitary treatments for regulated pests on regulated articles*) on Cold treatment for *Bactrocera tryoni* on *Citrus reticulata* x *C. sinensis* (2007-206F)
- Annex 18 to ISPM 28 (*Phytosanitary treatments for regulated pests on regulated articles*) on Cold treatment for *Bactrocera tryoni* on *Citrus limon* (2007-206G)
- Annex 19 to ISPM 28 (*Phytosanitary treatments for regulated pests on regulated articles*) on Irradiation for *Dysmicoccus neobrevipes*, *Planococcus lilacinus* and *Planococcus minor* (2012-011)
- Annex 5 to ISPM 27 (*Diagnostic protocols for regulated pests*) on *Phyllosticta citricarpa* (McAlpine) Aa on fruit (adopted by the Standards Committee on behalf of the CPM)
- Annex 6 to ISPM 27 (*Diagnostic protocols for regulated pests*) on *Xanthomonas citri* subsp. *citri* (adopted by the Standards Committee on behalf of the CPM)
- Annex 7 to ISPM 27 (*Diagnostic protocols for regulated pests*) on *Potato spindle tuber viroid* (adopted by the Standards Committee on behalf of the CPM).

ISPM 26



**INTERNATIONAL STANDARDS FOR  
PHYTOSANITARY MEASURES**

**ISPM 26**

**ESTABLISHMENT OF PEST FREE AREAS FOR  
FRUIT FLIES (TEPHRITIDAE)**

Produced by the Secretariat of the International Plant Protection Convention  
*Adopted 2015; published 2015*



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## Publication history

*This is not an official part of the standard*

2004-04 ICPM-6 added topic *Pest free areas and systems approached for fruit flies* (2004-027)

2004-09 TPFF developed the draft text

2004-11 SC approved Specification No. 27 *Pest free areas for fruit flies*

2005-04 SC revised draft and approved for member consultation

2005-06 Member consultation

2005-09 TPFF revised draft text

2005-11 SC approved draft to be submitted for adoption

2006-04 CPM-1 revised and adopted standard

**ISPM 26.** 2006. *Establishment of pest free areas for fruit flies* (Tephritidae). Rome, IPPC, FAO.

2006-04 CPM-1 added topic *Trapping procedures for fruit flies* (2006-037)

2006-05 SC approved Specification 35 *Trapping procedures for fruit flies of the family Tephritidae*

2007-12 TPFF developed draft text cooperated with IAEA

2008-05 SC approved draft for member consultation

2008-06 Member consultation

2009-05 SC revised draft and proposed as Appendix to ISPM 26

2009-05 SC-7 revised draft

2009-11 SC revised draft

2010-03 CPM-5 reviewed draft and returned to SC with guidance for modifications

2010-04 SC reviewed draft and returned to TPFF

2010-10 TPFF revised draft

2010-11 SC approved draft to be submitted for adoption

2011-03 CPM-6 revised and adopted Appendix 1

2009-11 SC introduced topic *Establishment and maintenance of regulated areas upon outbreak detection in fruit fly free areas* (2009-007)

2010-03 CPM-5 added topic (2009-007)

2010-11 SC approved draft specification for member consultation

2011-02 Member consultation

2011-05 SC revised and approved Specification 53

2011-08 TPFF developed draft text

2012-04 SC revised and approved draft for member consultation

2012-06 Member consultation

2013-03 TPG reviewed comments

2013-05 SC-7 approved for Substantial concerns commenting period

2013-10 Substantial concerns commenting period

2013-11 SC approved draft to be submitted for adoption

2014-04 CPM-9 adopted Annex 2.

.

2014-07 Secretariat corrected error in table of contents

2005-11 SC recommended topic: *Suppression and eradication procedures for fruit flies* (2005-010) to be added to the work programme

2006-04 CPM-1 (2006) added topic (2005-010)

2006-11 SC approved Specification 39

2009-09 TPFF drafted text

2011-01 TPFF recommended draft ISPM *Phytopathological Procedures for Fruit Fly (Tephritidae) Management* (2005-010) to SC as an annex to ISPM 26

2011-05 SC noted TPFF recommendation

2012-04 SC reviewed draft ISPM and returned it to steward for redrafting

2012-12 Steward revised draft in consultation with TPFF

2013-05 SC revised in meeting and approved for MC

2013-07 MC

2014-02 Steward revised draft ISPM

2014-05 SC-7 revised and approved for SCCP

2014-07 SCCP

2014-11 Steward revised draft after SCCP

2014-11 SC revised and approved for CPM adoption

2015-03 CPM adopted Annex 3

2015-04 IPPC Secretariat incorporated ink amendments following revoking of standards procedure.

Publication history last modified: 2015-04

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## Adoption

This standard was adopted by the First Session of the Commission on Phytosanitary Measures in April 2006. Revision of Appendix 1 on Fruit fly trapping was adopted by the Sixth Session of the Commission on Phytosanitary Measures in March 2011. Annex 2 was adopted by the Ninth Session of the Commission on Phytosanitary Measures in April 2014. Annex 3 was adopted by the Tenth Session of the Commission on Phytosanitary Measures in March 2015.

## INTRODUCTION

### Scope

This standard provides guidelines for the establishment of pest free areas for fruit flies (Tephritidae) of economic importance, and for the maintenance of their pest free status.

### References

IPPC. 1997. *International Plant Protection Convention*. Rome, IPPC, FAO.

The present standard also refers to other International Standards for Phytosanitary Measures (ISPMs). ISPMs are available on the IPP at <https://www.ippc.int/core-activities/standards-setting/ispms>.

### Definitions

Definitions of phytosanitary terms used in the present standard can be found in ISPM 5 (*Glossary of phytosanitary terms*).

### Outline of Requirements

The general requirements for establishing a fruit fly-pest free area (FF-PFA) include:

- the preparation of a public awareness programme
- the management elements of the system (documentation and review systems, record-keeping)
- supervision activities.

The major elements of the FF-PFA are:

- the characterization of the FF-PFA
- the establishment and maintenance of the FF-PFA.

These elements include the surveillance activities of trapping and fruit sampling, and official control on the movement of regulated articles. Guidance on surveillance and fruit sampling activities is provided in Appendixes 1 and 2.

Additional elements include: corrective action planning, suspension, loss of pest free status and reinstatement (if possible) of the FF-PFA. Corrective action planning is described in Annex 1.

## BACKGROUND

Fruit flies are a very important group of pests for many countries due to their potential to cause damage in fruits and to their potential to restrict access to international markets for plant products that can host fruit flies. The high probability of introduction of fruit flies associated with a wide range of hosts results in restrictions imposed by many importing countries to accept fruits from areas in which these pests are established. For these reasons, there is a need for an ISPM that provides specific guidance for the establishment and maintenance of pest free areas for fruit flies.

A pest free area is “an area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained” (ISPM 5). Areas initially free from fruit flies may remain naturally free from fruit flies due to the presence of barriers or climate conditions, and/or maintained free through movement restrictions and related measures (though fruit flies have the potential to establish there) or may be made free by an eradication programme (ISPM 9 (*Guidelines for pest eradication programmes*)). ISPM 4 (*Requirements for the establishment of pest free areas*) describes different types of pest free areas and provides general guidance on the establishment of pest free areas. However, a need for additional guidance on establishment and maintenance of pest free areas specifically for fruit flies (fruit fly-pest free areas, FF-PFA) was recognized. This standard describes additional requirements for establishment and maintenance of FF-PFAs. The target pests for which this standard was developed include insects of the order Diptera, family Tephritidae, of the genera *Anastrepha*, *Bactrocera*, *Ceratitis*, *Dacus*, *Rhagoletis* and *Toxotrypana*.

The establishment and maintenance of an FF-PFA implies that no other phytosanitary measures specific for the target species are required for host commodities within the PFA.

## REQUIREMENTS

### 1. General Requirements

The concepts and provisions of ISPM 4 apply to the establishment and maintenance of pest free areas for all pests including fruit flies and therefore ISPM 4 should be referred to in conjunction with this standard.

Phytosanitary measures and specific procedures as further described in this standard may be required for the establishment and maintenance of FF-PFA. The decision to establish a formal FF-PFA may be made based on the technical factors provided in this standard. They include components such as pest biology, size of the area, pest population levels and dispersal pathway, ecological conditions, geographical isolation and availability of methods for pest eradication.

FF-PFAs may be established in accordance with this ISPM under a variety of different situations. Some of them require the application of the full range of elements provided by this standard; others require only the application of some of these elements.

In areas where the fruit flies concerned are not capable of establishment because of climatic, geographical or other reasons, there should be no records of presence and it may be reasonable to conclude that the pest is absent (ISPM 8 (*Determination of pest status in an area*)). If, however, the fruit flies are detected and can cause economic damage during a season (Article VII.3 of the IPPC), corrective actions should be applied in order to allow the maintenance of a FF-PFA.

In areas where the fruit flies are capable of establishment and known to be absent, general surveillance in accordance with ISPM 8 is normally sufficient for the purpose of delimiting and establishing a pest free area. Where appropriate, import requirements and/or domestic movement restrictions against the introduction of the relevant fruit fly species into the area may be required to maintain the area free from the pest.

## 1.1 Public awareness

A public awareness programme is most important in areas where the risk of introduction is higher. An important factor in the establishment and maintenance of FF-PFAs is the support and participation of the public (especially the local community) close to the FF-PFA and individuals that travel to or through the area, including parties with direct and indirect interests. The public and stakeholders should be informed through different forms of media (written, radio, TV) of the importance of establishing and maintaining the pest free status of the area, and of avoiding the introduction or re-introduction of potentially infested host material. This may contribute to and improve compliance with the phytosanitary measures for the FF-PFA. The public awareness and phytosanitary education programme should be ongoing and may include information on:

- permanent or random checkpoints
- posting signs at entry points and transit corridors
- disposal bins for host material
- leaflets or brochures with information on the pest and the pest free area
- publications (e.g. print, electronic media)
- systems to regulate fruit movement
- non-commercial hosts
- security of the traps
- penalties for non-compliance, where applicable.

## 1.2 Documentation and record-keeping

The phytosanitary measures used for the establishment and maintenance of FF-PFA should be adequately documented as part of phytosanitary procedures. They should be reviewed and updated regularly, including corrective actions, if required (see also ISPM 4).

The records of surveys, detections, occurrences or outbreaks and results of other operational procedures should be retained for at least 24 months. Such records should be made available to the NPPO of the importing country on request.

## 1.3 Supervision activities

The FF-PFA programme, including regulatory control, surveillance procedures (for example trapping, fruit sampling) and corrective action planning should comply with officially approved procedures.

Such procedures should include official delegation of responsibility assigned to key personnel, for example:

- a person with defined authority and responsibility to ensure that the systems/procedures are implemented and maintained appropriately
- entomologist(s) with responsibility for the authoritative identification of fruit flies to species level.

The effectiveness of the programme should be monitored periodically by the NPPO of the exporting country, through review of documentation and procedures.

## 2. Specific Requirements

### 2.1 Characterization of the FF-PFA

The determining characteristics of the FF-PFA include:

- the target fruit fly species and its distribution within or adjacent to the area
- commercial and non-commercial host species

- delimitation of the area (detailed maps or global positioning system (GPS) coordinates showing the boundaries, natural barriers, entry points and host area locations, and, where necessary, buffer zones)
- climate, for example rainfall, relative humidity, temperature, prevailing wind speed and direction.

Further guidance on establishing and describing a PFA is provided in ISPM 4.

## **2.2 Establishment of the FF-PFA**

The following should be developed and implemented:

- surveillance activities for establishment of the FF-PFA
- delimitation of the FF-PFA
- phytosanitary measures related to movement of host material or regulated articles
- pest suppression and eradication techniques as appropriate.

The establishment of buffer zones may also be necessary (as described in section 2.2.1) and it may be useful to collect additional technical information during the establishment of the FF-PFA.

### **2.2.1 Buffer zone**

In areas where geographic isolation is not considered adequate to prevent introduction to or reinfestation of a PFA or where there are no other means of preventing fruit fly movement to the PFA, a buffer zone should be established. Factors that should be considered in the establishment and effectiveness of a buffer zone include:

- pest suppression techniques which may be used to reduce the fruit fly population, including:
  - use of selective insecticide-bait
  - spraying
  - sterile insect technique
  - male annihilation technique
  - biological control
  - mechanical control, etc.
- host availability, cropping systems, natural vegetation
- climatic conditions
- the geography of the area
- capacity for natural spread through identified pathways
- the ability to implement a system to monitor the effectiveness of buffer zone establishment (e.g. trapping network).

### **2.2.2 Surveillance activities prior to establishment**

A regular survey programme should be established and implemented. Trapping is the preferred option to determine fruit fly absence or presence in an area for lure/bait responsive species. However, fruit sampling activities may sometimes be required to complement the trapping programme in cases where trapping is less effective, for example when species are less responsive to specific lures.

Prior to the establishment of a FF-PFA, surveillance should be undertaken for a period determined by the climatic characteristics of the area, and as technically appropriate for at least 12 consecutive months in the FF-PFA in all relevant areas of commercial and non-commercial host plants to demonstrate that the pest is not present in the area. There should be no populations detected during the surveillance activities prior to establishment. A single adult detection, depending on its status (in accordance with ISPM 8), may not disqualify an area from subsequent designation as an FF-PFA. For qualifying the area as a pest free area, there should be no detection of an immature specimen, two or

more fertile adults, or an inseminated female of the target species during the survey period. There are different trapping and fruit sampling regimes for different fruit fly species. Surveys should be conducted using the guidelines in Appendixes 1 and 2. These guidelines may be revised as trap, lure and fruit sampling efficiencies improve.

### **2.2.2.1 Trapping procedures**

This section contains general information on trapping procedures for target fruit fly species. Trapping conditions may vary depending on, for example, the target fruit fly and environmental conditions. More information is provided in Appendix 1. When planning for trapping, the following should be considered.

#### **Trap type and lures**

Several types of traps and lures have been developed over decades to survey fruit fly populations. Fly catches differ depending on the types of lure used. The type of trap chosen for a survey depends on the target fruit fly species and the nature of the attractant. The most widely used traps include Jackson, McPhail, Steiner, open bottom dry trap (OBDT), yellow panel traps, which may use specific attractants (para-pheromone or pheromone lures that are male specific), or food or host odours (liquid protein or dry synthetic). Liquid protein is used to catch a wide range of different fruit fly species and capture both females and males, with a slightly higher percentage of females captured. However identification of the fruit flies can be difficult due to decomposition within the liquid bait. In traps such as McPhail, ethylene glycol may be added to delay decomposition. Dry synthetic protein baits are female biased, capture less non-target organisms and, when used in dry traps, may prevent premature decomposition of captured specimens.

#### **Trap density**

Trap density (number of traps per unit area) is a critical factor for effective fruit fly surveys and it should be designed based on target fruit fly species, trap efficiency, cultivation practices, and other biotic and abiotic factors. Density may change depending on the programme phase, with different densities required during the establishment of FF-PFA and the maintenance phase. Trap density also depends on the risk associated with potential pathways for entry into the designated PFA.

#### **Trap deployment (determination of the specific location of the traps)**

In a FF-PFA programme, an extensive trapping network should be deployed over the entire area. The trapping network layout will depend on the characteristics of the area, host distribution and the biology of the fruit fly of concern. One of the most important features of trap placement is the selection of a proper location and trap site within the host plant. The application of GPS and geographic information systems (GIS) are useful tools for management of a trapping network.

Trap location should take into consideration the presence of the preferred hosts (primary, secondary and occasional hosts) of the target species. Because the pest is associated with maturing fruit, the location including rotation of traps should follow the sequence of fruit maturity in host plants. Consideration should be given to commercial management practices in the area where host trees are selected. For example, the regular application of insecticides (and/or other chemicals) to selected host trees may have a false-negative effect on the trapping programme.

#### **Trap servicing**

The frequency of trap servicing (maintaining and refreshing the traps) during the period of trapping should depend on the:

- longevity of baits (attractant persistency)
- retention capacity
- rate of catch
- season of fruit fly activity
- placement of the traps

- biology of the species
- environmental conditions.

### **Trap inspection (checking the traps for fruit flies)**

The frequency of regular inspection during the period of trapping should depend on:

- expected fruit fly activity (biology of the species)
- response of the target fruit fly in relation to host status at different times of the year
- relative number of target and non-target fruit flies expected to be caught in a trap
- type of trap used
- physical condition of the flies in the trap (and whether they can be identified).

In certain traps, specimens may degrade quickly making identification difficult or impossible unless the traps are checked frequently.

### **Identification capability**

NPPOs should have in place, or have ready access to, adequate infrastructure and trained personnel to identify detected specimens of the target species in an expeditious manner, preferably within 48 hours. Continuous access to expertise may be necessary during the establishment phase or when implementing corrective actions.

### **2.2.2.2 Fruit sampling procedures**

Fruit sampling may be used as a surveillance method in combination with trapping where trapping is less effective. It should be noted that fruit sampling is particularly effective in small-scale delimiting surveys in an outbreak area. However, it is labour-intensive, time consuming and expensive due to the destruction of fruit. It is important that fruit samples should be held in suitable condition to maintain the viability of all immature stages of fruit fly in infested fruit for identification purpose.

### **Host preference**

Fruit sampling should take into consideration the presence of primary, secondary and occasional hosts of the target species. Fruit sampling should also take into account the maturity of fruit, apparent signs of infestation in fruit, and commercial practices (e.g. application of insecticides) in the area.

### **Focusing on high-risk areas**

Fruit sampling should be targeted on areas likely to have presence of infested fruits such as:

- urban areas
- abandoned orchards
- rejected fruit at packing facilities
- fruit markets
- sites with a high concentration of primary hosts
- entrance points into the FF-PFA, where appropriate.

The sequence of hosts that are likely to be infested by the target fruit fly species in the area should be used as fruit sampling areas.

### **Sample size and selection**

Factors to be considered include:

- the required level of confidence
- the availability of primary host material in the field
- fruits with symptoms on trees, fallen or rejected fruit (for example at packing facilities), where appropriate.

### **Procedures for processing sampled fruit for inspection**

Fruit samples collected in the field should be brought to a facility for holding, fruit dissection, pest recovery and identification. Fruit should be labelled, transported and held in a secure manner to avoid mixing fruits from different samples.

### **Identification capability**

NPPOs should have in place, or have ready access to, adequate infrastructure and trained personnel to identify fruit fly immature stages and emerged adults of the target species in an expeditious manner.

### **2.2.3 Controls on the movement of regulated articles**

Movement controls of regulated articles should be implemented to prevent the entry of target pests into the FF-PFA. These controls depend on the assessed risks (after identification of likely pathways and regulated articles) and may include:

- listing of the target fruit fly species on a quarantine pest list
- regulation of the pathways and articles that require control to maintain the FF-PFA
- domestic restrictions to control the movement of regulated articles into the FF-PFA
- inspection of regulated articles, examination of relevant documentation as appropriate and, where necessary for cases of non-compliance, the application of appropriate phytosanitary measures (e.g. treatment, refusal or destruction).

### **2.2.4 Additional technical information for establishment of a FF-PFA**

Additional information may be useful during the establishment phase of FF-PFAs. This includes:

- historical records of detection, biology and population dynamics of the target pest(s), and survey activities for the designated target pest(s) in the FF-PFA
- the results of phytosanitary measures taken as part of actions following detections of fruit flies in the FF-PFA
- records of the commercial production of host crops in the area, an estimate of non-commercial production and the presence of wild host material
- lists of the other fruit fly species of economic importance that may be present in the FF-PFA.

### **2.2.5 Domestic declaration of pest freedom**

The NPPO should verify the fruit fly free status of the area (in accordance with ISPM 8) specifically by confirming compliance with the procedures set up in accordance with this standard (surveillance and controls). The NPPO should declare and notify the establishment of the FF-PFA, as appropriate.

In order to be able to verify the fruit fly free status in the area and for purposes of internal management, the continuing FF-PFA status should be checked after the PFA has been established and any phytosanitary measures for the maintenance of the FF-PFA have been put in place.

## **2.3 Maintenance of the FF-PFA**

In order to maintain the FF-PFA status, the NPPO should continue to monitor the operation of the surveillance and control activities, continuously verifying the pest free status.

### **2.3.1 Surveillance for maintenance of the FF-PFA**

After verifying and declaring the FF-PFA, the official surveillance programme should be continued at a level assessed as being necessary for maintenance of the FF-PFA. Regular technical reports of the survey activities should be generated (for example monthly). Requirements for this are essentially the same as for establishment of the FF-PFA (see section 2.2) but with differences in density and trap locations dependent upon the assessed level of risk of introduction of the target species.

### 2.3.2 Controls on the movement of regulated articles

These are the same as for establishment of the FF-PFA (provided in section 2.2.3).

### 2.3.3 Corrective actions (including response to an outbreak)

The NPPO should have prepared plans for corrective actions that may be implemented if the target pest(s) is detected in the FF-PFA or in host material from that area (detailed guidelines are provided in Annex 1), or if faulty procedures are found. This plan should include components or systems to cover:

- outbreak declaration according to criteria in ISPM 8 and notification
- delimiting surveillance (trapping and fruit sampling) to determine the infested area under corrective actions
- implementation of control measures
- further surveillance
- criteria for the reinstatement of freedom of the area affected by the outbreak
- responses to interceptions.

A corrective action plan should be initiated as soon as possible and in any case within 72 hours of the detection (of an adult or immature stage of the target pest).

## 2.4 Suspension, reinstatement or loss of a FF-PFA status

### 2.4.1 Suspension

The status of the FF-PFA or the affected part within the FF-PFA should be suspended when an outbreak of the target fruit fly occurs or based on one of the following triggers: detection of an immature specimen of the target fruit fly, two or more fertile adults as demonstrated by scientific evidence, or an inseminated female within a defined period and distance. Suspension may also be applied if procedures are found to be faulty (for example inadequate trapping, host movement controls or treatments).

If the criteria for an outbreak are met, this should result in the implementation of the corrective action plan as specified in this standard and immediate notification to interested importing countries' NPPOs (see ISPM 17 (*Pest reporting*)). The whole or part of the FF-PFA may be suspended or revoked. In most cases a suspension radius will delimit the affected part of the FF-PFA. The radius will depend on the biology and ecology of the target fruit fly. The same radius will generally apply for all FF-PFAs for a given target species unless scientific evidence supports any proposed deviation. Where a suspension is put in place, the criteria for lifting the suspension should be made clear. Interested importing countries' NPPOs should be informed of any change in FF-PFA status.

### 2.4.2 Reinstatement

Reinstatement should be based on requirements for establishment with the following conditions:

- no further detection of the target pest species for a period determined by the biology of the species and the prevailing environmental conditions<sup>1</sup>, as confirmed by surveillance, or
- in the case of a fault in the procedures, only when the fault has been corrected.

### 2.4.3 Loss of FF-PFA status

If the control measures are not effective and the pest becomes established in the whole area (the area recognized as pest free), the status of the FF-PFA should be lost. In order to achieve again the FF-PFA, the procedures of establishment and maintenance outlined in this standard should be followed.

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<sup>1</sup> The period starts from the last detection. For some species, no further detection should occur for at least three life cycles; however the required period should be based on scientific information including that provided by the surveillance systems in place.

This annex is a prescriptive part of the standard.

## **ANNEX 1: Guidelines on corrective action plans**

The detection of a single fruit fly (adult or immature) of the target species in the FF-PFA should trigger enforcement of a corrective action plan.

In case of an outbreak, the objective of the corrective action plan is to ensure eradication of the pest to enable reinstatement of pest status in the affected area into the FF-PFA.

The corrective action plan should be prepared taking into account the biology of the target fruit fly species, the geography of the FF-PFA area, climatic conditions and host distribution within the area.

The elements required for implementation of a corrective action plan include:

- legal framework under which the corrective action plan can be applied
- criteria for the declaration of an outbreak
- time scales for the initial response
- technical criteria for delimiting trapping, fruit sampling, application of the eradication actions and establishment of regulatory measures
- availability of sufficient operational resources
- identification capability
- effective communication within the NPPO and with the NPPO(s) of the importing country(ies), including provision of contact details of all parties involved.

### **Actions to apply the corrective action plan**

#### *(1) Determination of the pest status of the detection (actionable or non-actionable)*

- (1.1) If the detection is a transient non-actionable occurrence (ISPM 8), no further action is required.
- (1.2) If the detection of a target pest may be actionable, a delimiting survey, which includes additional traps, and usually fruit sampling as well as an increased trap inspection rate, should be implemented immediately after the detection to assess whether the detection represents an outbreak, which will determine necessary responsive actions. If a population is present, this action is also used to determine the size of the affected area.

#### *(2) Suspension of FF-PFA status*

If after detection it is determined that an outbreak has occurred or any of the triggers specified in section 2.4.1 is reached, the FF-PFA status in the affected area should be suspended. The affected area may be limited to parts of the FF-PFA or may be the whole FF-PFA.

#### *(3) Implementation of control measures in the affected area*

As per ISPM 9, specific corrective or eradication actions should be implemented immediately in the affected area(s) and adequately communicated to the community. Eradication actions may include:

- selective insecticide-bait treatments
- sterile fly release
- total harvest of fruit in the trees
- male annihilation technique
- destruction of infested fruit
- soil treatment (chemical or physical)
- insecticide application.

Phytosanitary measures should be immediately enforced for control of movement of regulated articles that can host fruit flies. These measures may include cancellation of shipments of fruit commodities from the affected area and as appropriate, fruit disinfestation and the operation of road blocks to

prevent the movement of infested fruit from the affected area to the rest of the pest free area. Other measures could be adopted if agreed by the importing country, for example treatment, increased surveys, supplementary trapping.

(4) *Criteria for reinstatement of a FF-PFA after an outbreak and actions to be taken*

The criteria for determining that eradication has been successful are specified in section 2.4.2 and should be included in the corrective action plan for the target fruit fly. The time period will depend on the biology of the species and the prevailing environmental conditions. Once the criteria have been fulfilled the following actions should be taken:

- notification of NPPOs of importing countries
- reinstatement of normal surveillance levels
- reinstatement of the FF-PFA.

(5) *Notification of relevant agencies*

Relevant NPPOs and other agencies should be kept informed of any change in FF-PFA status as appropriate, and IPPC pest reporting obligations observed (ISPM 17).

This annex was adopted by the Ninth Session of the Commission on Phytosanitary Measures in April 2014.  
This annex is a prescriptive part of the standard.

## **ANNEX 2: Control measures for an outbreak within a fruit fly-pest free area (2014)**

### **BACKGROUND**

A fruit fly (Tephritidae) outbreak detected in a fruit fly-pest free area (FF-PFA) may pose a risk for those importing countries where the fruit fly species is considered a quarantine pest. This annex describes control measures to be taken in a fruit fly eradication area established within an FF-PFA in the event of an outbreak.

Corrective actions and other phytosanitary measures that may be used in an eradication area within an FF-PFA are covered by this standard.

The eradication area and the related control measures are established with the intent to eradicate the target fruit fly species and restore FF-PFA status, to protect the surrounding FF-PFA, and to meet the phytosanitary import requirements of the importing country, where applicable. In particular, control measures are needed because movements of regulated articles from and through an eradication area pose a potential risk of spreading the target fruit fly species.

### **1. Establishment of an Eradication Area**

The national plant protection organization (NPPO) of the exporting country should declare an outbreak in accordance with this and other relevant international standards for phytosanitary measures. When a target fruit fly species outbreak is detected within an FF-PFA, an eradication area should be established based on a technical evaluation. The free status of the eradication area should be suspended. If control measures cannot be applied to establish an eradication area, then the status of the FF-PFA should be revoked in accordance with this standard.

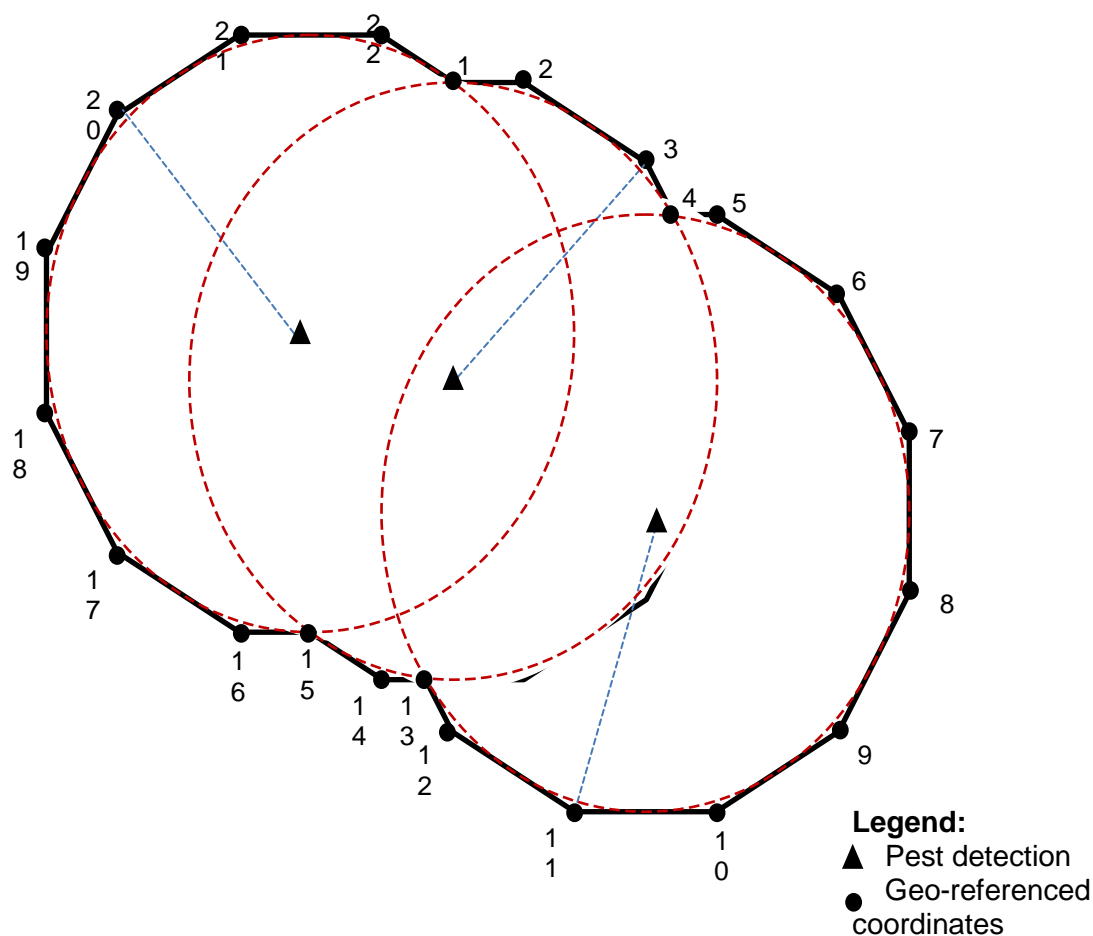
The eradication area should cover the infested area. In addition, a buffer zone should be established in accordance with this standard, and as determined by delimiting surveys, taking into account the natural dispersal capability of the target fruit fly species, its relevant biological characteristics, and other geographic and environmental factors.

A circle delimiting the minimum size of the eradication area should be drawn, centred on the actual target fruit fly species detection and with a radius large enough to comply with the above considerations, as determined by the NPPO of the exporting country. In the case of several pest detections, several (possibly overlapping) circles should be drawn accordingly, as illustrated in Figure 1.

If necessary for the practical implementation of the eradication area, the NPPO of the exporting country may decide to adjust the eradication area to correspond to administrative boundaries or topography, or to approximate the circle with a polygon.

A georeferencing device (e.g. global positioning system (GPS)) or map with geographical coordinates may be used for delimiting and enabling recognition of the eradication area. Signposts may be placed along boundaries and on roads to alert the public, and notices may be published to facilitate public awareness.

The NPPO of the exporting country should inform the NPPO of the importing country when a fruit fly outbreak is confirmed and an eradication area is established within an FF-PFA.



**Figure 1:** Example of delimiting circles and approximating polygons to determine the eradication area around three pest detections.

## 2. Control Measures

Each stage of the production chain (e.g. growing, sorting, packing, transporting, dispatching) may lead to spread of the target fruit fly species from the eradication area into the FF-PFA. This statement does not apply to any facilities located in the FF-PFA and handling only host fruit from the FF-PFA. Appropriate control measures should be applied to manage the pest risk for the surrounding FF-PFA and the importing country.

Control measures in use in other fruit fly-infested areas may be implemented in the eradication area.

Control measures may be audited by the NPPO of the importing country, in accordance with the NPPO of the exporting country's requirements.

Control measures applied at each stage of the production chain are described in the following sections.

### 2.1 Production

During the production period, within the eradication area, the NPPO of the exporting country may require control measures to avoid infestation, such as fruit bagging, fruit stripping (i.e. removal of unwanted fruits from trees), protein bait sprays, sterile insect technique, parasitoid releases, field sanitation, male annihilation technique, bait stations or netting.

## 2.2 Movement of regulated articles

Movement of regulated articles (e.g. soil, host plants, host fruit) into, from, through or within the eradication area should comply with control measures to prevent the spread of the target fruit fly species and should be accompanied by the necessary documentation to indicate the articles' origin and destination. This also pertains to moving regulated articles for phytosanitary certification.

## 2.3 Packing and packing facilities

Fruit packing facilities may be located within or outside the eradication area and may pack host fruit grown in or outside the eradication area. Control measures preventing spread of the target fruit fly species should be taken into account in each case.

The NPPO of the exporting country should:

- register the facility
- require control measures to prevent the target fruit fly species from entering or escaping the facility, as appropriate
- require and approve methods of physical separation of different host fruit lots (e.g. by using insect-proof packaging) to avoid cross-contamination
- require appropriate measures to maintain segregation of host fruits originating from areas of different pest status (e.g. separate locations for reception, processing, storage and dispatch)
- require appropriate measures regarding the handling and movement of host fruit through the facility to prevent mixing of fruit from areas of different pest status (e.g. flowcharts, signs and staff training)
- require and approve methods of disposal of rejected host fruit from the eradication area
- monitor the target fruit fly species at the facility and, if relevant, in the adjacent FF-PFA
- verify the packing material is insect proof and clean
- require appropriate control measures to eradicate target fruit fly species from the facility when they are detected
- audit the facility.

## 2.4 Storage and storage facilities

Fruit storage facilities may be located within or outside the eradication area. Such facilities should be registered with the NPPO of the exporting country and comply with the control measures to prevent the spread of the target fruit fly species; for example, they should:

- maintain distinction and separation between host fruit originating from the eradication area and from the FF-PFA
- use an approved method of disposal of host fruit from the eradication area that has been rejected as a result of inspection or quality control activities
- monitor for the target fruit fly species at the facility and if relevant, in the adjacent FF-PFA
- take appropriate control measures to eradicate the target fruit fly species from the facility when detected.

## 2.5 Processing and processing facilities

If the processing facility is located within the eradication area, host fruit destined for processing (such as juicing, canning and puréeing) does not pose additional fruit fly risk to the area.

If the facility is located outside the eradication area, the NPPO of the exporting country should require measures within the facility to prevent the escape of the target fruit fly species, through insect-proof reception, storage and processing areas.

Monitoring for the target fruit fly species may be conducted at the facility and, if relevant, in the adjacent FF-PFA. Appropriate control measures should be taken to eradicate target fruit fly species from the facility when they are detected.

Approved disposal of rejected host fruit and plant waste from the eradication area should be required by the NPPO of the exporting country. Rejected host fruit should be disposed of in such a way that the target fruit fly species are rendered non-viable.

## **2.6 Treatment and treatment facilities**

Treatment facilities should be registered by the NPPO of the exporting country.

Post-harvest treatment (e.g. cold treatment, heat treatment, fumigation, irradiation), or in some cases pre-harvest treatment (e.g. bait spray, fruit bagging), may be required for host fruit moving into an FF-PFA or being exported to countries where the target fruit fly species is regulated as quarantine pest.

Control measures preventing the escape of the target fruit fly species may be required for treatment facilities located within the FF-PFA, if treating regulated articles from the eradication area. The NPPO of the exporting country may require physical isolation within the facility.

The NPPO of the exporting country should approve the method of disposal of rejected host fruit from the eradication area to reduce the risk of spread of the target fruit fly species. Disposal methods may include double bagging followed by deep burial or incineration.

## **2.7 Sale inside the eradication area**

Host fruit sold within the eradication area may be at risk of infestation if exposed before being sold (e.g. placed on display in an open air market) and may therefore need to be physically protected, when feasible, to avoid spread of the target fruit fly species while on display and being stored.

## **3. Documentation and Record-Keeping**

The control measures, including corrective actions, used in the eradication area should be adequately documented, reviewed and updated (see also ISPM 4). Such documents should be made available to the NPPO of the importing country on request.

## **4. Termination of Control Measures in the Eradication Area**

Eradication of the target fruit fly species in the eradication area should meet the requirements for reinstatement of an FF-PFA status after an outbreak, according to this standard. The declaration of eradication should be based on no further detections of the target fruit fly species for a period determined by its biology and prevailing environmental conditions, as confirmed by surveillance referred to in this standard.<sup>2</sup>

The control measures should remain in force until eradication is declared. If eradication is successful, the particular control measures in the eradication area may be terminated and the FF-PFA status should be reinstated. If eradication is unsuccessful, the FF-PFA delimitation should be modified accordingly. The NPPO of the importing country should be notified as appropriate.

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<sup>2</sup> The period starts from the last detection. For some species, no further detection should occur for at least three life cycles; however, the required period should be based on scientific information, including that provided by the surveillance systems in place.

This annex was adopted by the Tenth Session of the Commission on Phytosanitary Measures in March 2015.  
This annex is a prescriptive part of the standard.

### **ANNEX 3: Phytosanitary procedures for fruit fly (Tephritidae) management (2015)**

This annex provides guidelines for the application of phytosanitary procedures for fruit fly management.

Various phytosanitary procedures are used for fruit fly suppression, containment, eradication and exclusion. These procedures may be applied to establish and maintain fruit fly-pest free areas (FF-PFAs) (this standard) and areas of low pest prevalence for fruit flies (FF-ALPPs) (ISPM 30 (*Establishment of areas of low pest prevalence for fruit flies (Tephritidae)*)), as well as to develop systems approaches for fruit flies (ISPM 35 (*Systems approach for pest risk management of fruit flies (Tephritidae)*)).

The phytosanitary procedures include mechanical and cultural controls, insecticide bait application technique (BAT), bait stations, male annihilation technique (MAT), mass trapping, sterile insect technique (SIT), biological control, and controls on the movement of regulated articles. Many of these procedures can be environmentally friendly alternatives to insecticide application for managing fruit flies.

#### **1. Objectives of Fruit Fly Management Strategies**

The four strategies used to manage target fruit fly populations are suppression, containment, eradication and exclusion. One or more of these strategies can be used depending on the circumstances and objectives. The corresponding phytosanitary procedures used for fruit fly management should take into account the phytosanitary import requirements of the importing country, fruit fly status in the target area, hosts, host phenology and host susceptibility, pest biology, and economic and technical feasibility of the available phytosanitary procedures, as relevant.

##### **1.1 Suppression**

Suppression strategies may be applied for purposes such as to:

- reduce a target fruit fly population to below an acceptable level
- establish an FF-ALPP (ISPM 22 (*Requirements for the establishment of areas of low pest prevalence*); ISPM 30)
- implement a corrective action in an FF-ALPP when the specified level of low pest prevalence has been exceeded (ISPM 22; ISPM 30)
- reduce a target fruit fly population in order to achieve a specified pest population level that can be used as part of a systems approach (ISPM 14 (*The use of integrated measures in a systems approach for pest risk management*); ISPM 35)
- precede, as part of a process, target fruit fly population eradication in order to establish an FF-PFA (ISPM 4).

##### **1.2 Containment**

Containment strategies may be applied for purposes such as to:

- prevent the spread of a target fruit fly from an infested area to an adjacent FF-PFA
- contain an incursion of a target fruit fly into non-infested areas
- protect, as a temporary measure, individual areas where target fruit flies have been eradicated as part of an ongoing eradication programme in a larger area.

##### **1.3 Eradication**

Eradication strategies may be applied for purposes such as to:

- eliminate a fruit fly population in order to establish an FF-PFA (ISPM 4)

- eliminate an incursion of a quarantine fruit fly before establishment can occur (this may be part of a corrective action plan in an FF-PFA if the target fruit fly species is detected).

## **1.4 Exclusion**

Exclusion strategies may be applied to prevent the introduction of a fruit fly into an FF-PFA.

## **2. Requirements for the Application of the Phytosanitary Procedures**

The following requirements should be considered when applying phytosanitary procedures for fruit fly management:

### **2.1 Fruit fly identification capabilities**

Accurate identification of the target fruit fly species should be ensured so that the appropriate strategies and phytosanitary procedures can be selected and applied. National plant protection organizations (NPPOs) should have access to trained personnel to identify detected specimens of adult and, where possible, immature stages of the target fruit fly species in an expeditious manner (ISPM 6 (*Guidelines for surveillance*)).

### **2.2 Knowledge of fruit fly biology**

The biology of the target fruit fly species should be known in order to determine the appropriate strategy to address its management and select the phytosanitary procedures that will be applied. Basic information on the target fruit fly species may include life cycle, hosts, host sequence, host distribution and abundance, dispersal capacity, geographical distribution and population dynamics. The climatic conditions may also affect the strategy adopted.

### **2.3 Area delimitation**

The area in which the phytosanitary procedures will be applied should be delimited. Geographical characteristics and host distribution within this area should be known.

### **2.4 Stakeholder participation**

Successful implementation of fruit fly phytosanitary procedures requires active and coordinated participation of interested and affected groups, including government, local communities and industry.

### **2.5 Public awareness**

An ongoing public awareness programme should be put in place to inform interested and affected groups about the pest risk and phytosanitary procedures that will be implemented as part of the fruit fly management strategy. Such a programme is most important in areas where the risk of introduction of the target fruit fly species is high. For the success of the management programme it is important to have the support and participation of the public (especially the local community) within the management programme area and of individuals who travel to or through the area.

### **2.6 Operational plans**

An official operational plan that specifies the required phytosanitary procedures should be developed. This operational plan may include specific requirements for the application of phytosanitary procedures and describe the roles and responsibilities of the interested and affected groups (ISPM 4; ISPM 22).

## **3. Phytosanitary Procedures Used in Fruit Fly Management Strategies**

Fruit fly management strategies may involve the use of more than one phytosanitary procedure.

Phytosanitary procedures may be applied in an area, at a place of production or at a production site; during the pre- or post-harvest period; at the packing house; or during shipment or distribution of the commodity. Pest free areas, places of production and production sites may require the establishment

and maintenance of an appropriate buffer zone. Appropriate phytosanitary procedures may be applied in the buffer zone if necessary (this standard and ISPM 10 (*Requirements for the establishment of pest free places of production and pest free production sites*)).

### 3.1 Mechanical and cultural controls

Mechanical and cultural control procedures may be applied in order to reduce the level of fruit fly populations. These controls include phytosanitary procedures such as orchard and field sanitation, fruit stripping, pruning, host plant removal or netting, fruit bagging, host-free periods, use of resistant varieties, trap cropping, ploughing and ground swamping.

The effectiveness of field sanitation increases when the collection and disposal of fallen fruit are focused on the preferred hosts and are done continuously on an area-wide basis. For good results, collection and disposal should be done before, during and after harvest.

Fruit that remains on the host plants after harvest, fruit rejected because of poor quality during harvest and packing, and fruit on host plants present in the surrounding area should be collected and safely disposed of (e.g. by deep burial).

Elimination or maintaining a low level of vegetation at the place of production will facilitate collection of fallen fruit. In addition, when vegetation is kept low fallen fruit with larvae may be more exposed to direct sunlight and natural enemies, which will contribute to fruit fly larvae mortality.

Bagging of fruit and use of exclusion netting can prevent fruit fly infestation of the fruit. Where used, bagging or exclusion netting should be carried out before the fruit becomes susceptible to fruit fly infestation.

The pupae of many fruit flies can be targeted by disturbing the soil medium in which they pupate. This can be done by ground swamping (causing pupae anoxia) or ploughing (causing physical damage, desiccation to the pupae and exposing them to natural enemies).

### 3.2 Insecticide bait application technique

BAT uses an appropriate insecticide mixed together with a food bait. Commonly used food baits include attractants such as hydrolysed protein, high-fructose syrup and molasses, used alone or in combination. This technique is an effective control of adult fruit fly populations and reduces the negative impacts on non-target insects and the environment.

Insecticide bait applications should start in time to target maturing adults and to prevent the infestation of fruit. For fruit protection this may be up to three months before the beginning of the harvesting season for fruit intended for export or on detection of the first adult flies or larvae in the field or urban area. Maturing adults should be targeted as this is when protein demands are at their highest. The number of and intervals between applications will depend on the characteristics of the target fruit fly species (biology, abundance, behaviour, distribution, life cycle, etc.), host phenology and weather conditions.

Insecticide baits can be applied from the ground or from the air.

#### 3.2.1 Ground application

Ground application of insecticide bait is usually used for relatively small production areas, such as individual orchards, or in urban areas.

The insecticide bait should generally be applied on or inside the middle-to-top part of the canopy of host and shelter plants, but specific application should relate to the height of the host plant. For low-growing host plants (e.g. cucurbits, tomatoes, peppers), the insecticide bait should be applied on taller plants surrounding the cultivated area that serve as shelter and a source of food. In FF-PFAs, as part of an emergency action plan to eliminate an outbreak, the insecticide bait can also be applied to non-host plants or other appropriate surfaces around the detection site.

### 3.2.2 Aerial application

Aerial application of insecticide bait may be used on large production areas and in areas where hosts are scattered in patches over large areas of land. Aerial spraying may be more cost-effective than ground spraying for large-scale programmes, and a more uniform coverage of bait in the target area may be achieved. In some countries, however, aerial spraying may be subject to restrictions due to environmental considerations.

Once the treatment area is selected, it may be defined using a georeferencing device and recorded in digitized maps using geographical information systems (GIS) software in order to ensure the efficient application of bait sprays and reduce the environmental impact.

To treat the target area, insecticide bait applications may not need to be applied as full coverage but only in some swathes, such as every second or third swath. The altitude and speed of aerial application should be adjusted to conditions such as bait viscosity and nozzle specifications, wind velocity, temperature, cloud cover and topography of the terrain.

### 3.3 Bait stations

Lure and kill devices known as “bait stations” may be a more environmentally-friendly control procedure for fruit fly suppression than BAT. Bait stations consist of an attractant and a killing agent that may be contained in a device or directly applied to an appropriate surface. Unlike traps, bait stations do not retain the attracted fruit flies.

Bait stations are suitable for use in, for example, commercial fruit production operations, area-wide fruit fly management programmes, public areas and, in many cases, organic groves. Bait stations may be used in fruit fly pest free areas for population suppression of localized and well-isolated outbreaks. In infested areas known to be fruit fly reservoirs and sources of incursions into FF-ALPPs and FF-PFAs, bait stations should be deployed at high densities.

It is recommended that the attractant used in the bait station be female-biased, thereby directly reducing the overall fruit infestation.

### 3.4 Male annihilation technique

MAT involves the use of a high density of bait stations consisting of a male lure combined with an insecticide to reduce the male population of target fruit flies to such a low level that mating is unlikely to occur (FAO, 2007).

MAT may be used for the control of those fruit fly species of the genera *Bactrocera* and *Dacus* that are attracted to male lures (cuelure or methyl eugenol). Methyl eugenol is more effective than cuelure for male annihilation of species attracted to these lures.

### 3.5 Mass trapping

Mass trapping uses trapping systems at high density to suppress fruit fly populations. In general, mass trapping procedures are the same as for traps used for survey purposes (Appendix 1). Traps should be deployed at the place of production early in the season when the first adult flies move into the field and populations are still at low levels and should be serviced appropriately.

Trap density should be based on such factors as fruit fly density, physiological stage of the fruit fly, efficacy of the attractant and killing agent, phenology of the host and host density. The timing, layout and deployment of traps should be based on the target fruit fly species and host ecological data.

### 3.6 Sterile insect technique

Sterile insect technique (SIT) is a species-specific environmentally-friendly technique that can provide effective control of target fruit fly populations (FAO, 2007).

SIT is effective only at low population levels of the target species and may be used for:

- suppression, where SIT may be a stand-alone phytosanitary procedure or combined with other phytosanitary procedures to achieve and maintain low population levels
- containment, where SIT may be particularly effective in areas that are largely pest free (such as buffer zones) but that are subjected to regular pest entries from adjacent infested areas
- eradication, where SIT may be applied when population levels are low to eradicate the remaining population
- exclusion, where SIT may be applied in endangered areas that are subject to high pest pressure from neighbouring areas.

### 3.6.1 Sterile fruit fly release

Sterile fruit flies may be released from the ground or from the air. Release intervals should be adjusted according to the longevity of the insect. Sterile fruit flies are generally released once or twice per week but the frequency of release may be influenced by circumstances such as pupae supply, staggered adult fly emergence and unfavourable weather. To establish sterile fruit fly release density, the quality of the sterile fruit flies, the level of the wild population and the desired sterile : wild fruit fly ratio should be considered.

After release of the sterile fruit flies, trapping and identification of the sterile and wild flies should be performed in order to evaluate the effectiveness of the release procedure and also to prevent unnecessary corrective actions. Released sterile flies should be recaptured in the same traps that are used for detection of the wild population as this provides feedback on whether the desired sterile fruit fly density and sterile : wild fly ratio were attained (FAO, 2007).

Ground release may be used when aerial release is neither cost-effective nor efficient (i.e. discontinuous distribution or relatively small area), or where additional releases are required to provide a higher density of fruit flies for a particular reason (e.g. in areas where a specified level of pest prevalence is exceeded).

Aerial release is more cost-effective than ground release for large-scale programmes and it provides a more uniform sterile fruit fly distribution than ground release, which may clump sterile fruit flies in localized sites or along release routes. Once the release area is selected, it may be defined using a georeferencing device and recorded in digitized maps using GIS software: this will help ensure the efficient distribution of sterile flies. The most common methods for aerial release are chilled adult and paper bag systems (FAO, 2007).

To determine the release altitude, several factors should be considered, including wind velocity, temperature, cloud cover, topography of the terrain, vegetation cover, and whether the target area is urban or rural. Release altitudes range from 200 to 600 m above ground level. However, lower release altitudes should be preferred, especially in areas subjected to strong winds (to prevent excessive sterile fruit fly or bag drift) and in areas where predation by birds is high and frequent. Release in the early morning, when winds and temperature are moderate, is preferable.

### 3.6.2 Sterile fruit fly quality control

Routine and periodic quality control tests should be carried out to determine the effect of mass rearing, irradiation, handling, shipment duration, holding and releasing on the performance of the sterile fruit flies, according to desired quality parameters (FAO/IAEA/USDA, 2014).

## 3.7 Biological control

Classic biological control may be used to reduce fruit fly populations. For further suppression, inundative release may be used. During inundative release, large numbers of natural enemies, typically parasitoids, are mass reared and released during critical periods to reduce pest populations. The use of biological control by inundation is limited to those biological control agents for which mass-rearing technology is available. The mass-reared natural enemies should be of high quality so that suppression

of the target fruit fly population can be effectively achieved. The release of the biological control agents should be directed towards marginal and difficult to access areas that have high host density and that are known to be fruit fly reservoirs and sources of infestation for commercial fruit production or urban areas.

### **3.8 Controls on the movement of regulated articles**

For FF-PFAs, and under certain circumstances for FF-ALPPs, controls on the movement of regulated articles should be implemented to prevent the entry or spread of target fruit fly species.

## **4. Materials Used in the Phytosanitary Procedures**

The materials used in the phytosanitary procedures should perform effectively and reliably at an acceptable level for an appropriate period of time. The devices and equipment should maintain their integrity for the intended duration that they are deployed in the field. The attractants and chemicals should be certified or bio-assayed for an acceptable level of performance.

## **5. Verification and Documentation**

The NPPO should verify the effectiveness of the chosen strategies (suppression, containment, eradication and exclusion) and relevant phytosanitary procedures. The main phytosanitary procedure used for verification is adult and larval surveillance, as described in ISPM 6.

NPPOs should ensure that records of information supporting all stages of the suppression, containment, eradication and exclusion strategies are kept for at least two years.

## **6. References**

- FAO.** 2007. *Guidance for packing, shipping, holding and release of sterile flies in area-wide fruit fly control programmes*, ed. W. Enkerlin. Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture. FAO Plant Production and Protection Paper 190. Rome. 145 + vii pp.
- FAO/IAEA/USDA.** 2014. *Product quality control for sterile mass-reared and released tephritid fruit flies*. Version 6.0. Vienna, International Atomic Energy Agency. 164 pp.

This appendix was adopted by the Sixth Session of the Commission on Phytosanitary Measures in March 2011.  
This appendix is for reference purposes only and is not a prescriptive part of the standard.

## APPENDIX 1: Fruit fly trapping (2011)

This appendix provides detailed information for trapping procedures for fruit fly species (Tephritidae) of economic importance under different pest statuses. Specific traps, in combination with attractants, and killing and preserving agents, should be used depending on the technical feasibility, the species of fruit fly and the pest status of the areas, which can be either an infested area, an area of low pest prevalence (FF-ALPP), or a pest free area (FF-PFA). It describes the most widely used traps, including materials such as trapping devices and attractants, and trapping densities, as well as procedures including evaluation, data recording and analysis.

### 1. Pest status and survey types

There are five pest statuses where surveys may be applied:

- A. Pest present without control. The pest is present but not subject to any control measures.
- B. Pest present under suppression. The pest is present and subject to control measures. Includes FF-ALPP.
- C. Pest present under eradication. The pest is present and subject to control measures. Includes FF-ALPP.
- D. Pest absent and FF-PFA being maintained. The pest is absent (e.g. eradicated, no pest records, no longer present) and measures to maintain pest absence are applied.
- E. Pest transient. Pest under surveillance and actionable, under eradication.

The three types of surveys and corresponding objectives are:

- **monitoring surveys**, applied to verify the characteristics of the pest population
- **delimiting surveys**, applied to establish the boundaries of an area considered to be infested by or free from the pest
- **detection surveys**, applied to determine if the pest is present in an area.

Monitoring surveys are necessary to verify the characteristics of the pest population before the initiation or during the application of suppression and eradication measures to verify the population levels and to evaluate the efficacy of the control measures. These are necessary for situations A, B and C. Delimiting surveys are applied to determine the boundaries of an area considered to be infested by or free from the pest such as boundaries of an established FF-ALPP (situation B) (ISPM 30) and as part of a corrective action plan when the pest exceeds the established low prevalence levels or in an FF-PFA (situation E) as part of a corrective action plan when a detection occurs. Detection surveys are to determine if the pest is present in an area, that is to demonstrate pest absence (situation D) and to detect a possible entry of the pest into the FF-PFA (pest transient actionable) (ISPM 8).

Additional information on how or when specific types of surveys should be applied can be found in other standards dealing with specific topics such as pest status, eradication, pest free areas or areas of low pest prevalence.

### 2. Trapping scenarios

As the pest status may change over time, the type of survey needed may also change:

- Pest present. Starting from an established population with no control (situation A), phytosanitary measures may be applied, and potentially lead toward an FF-ALPP (situation B and C) or an FF-PFA (situation D).
- Pest absent. Starting from an FF-PFA (situation D), the pest status is either maintained or a detection occurs (situation E), where measures would be applied aimed at restoring the FF-PFA.

### 3. Trapping materials

The effective use of traps relies on the proper combination of trap, attractant and killing agent to attract, capture, kill and preserve the target fruit fly species for effective identification, counting data collection and analysis. Traps for fruit fly surveys use the following materials as appropriate:

- a trapping device
- attractants (pheromones, parapheromones and food attractants)
- killing agents in wet and dry traps (with physical or chemical action)
- preservation agents (wet or dry).

#### 3.1 Attractants

Some fruit fly species of economic importance and the attractants commonly used to capture them are presented in Table 1. Presence or absence of a species from this table does not indicate that pest risk analysis has been performed and in no way is it indicative of the regulatory status of a fruit fly species.

**Table 1.** A number of fruit fly species of economic importance and commonly used attractants

Scientific name	Attractant
<i>Anastrepha fraterculus</i> (Wiedemann) <sup>4</sup>	Protein attractant (PA)
<i>Anastrepha grandis</i> (Macquart)	PA
<i>Anastrepha ludens</i> (Loew)	PA, 2C-1 <sup>1</sup>
<i>Anastrepha obliqua</i> (Macquart)	PA, 2C-1 <sup>1</sup>
<i>Anastrepha serpentina</i> (Wiedemann)	PA
<i>Anastrepha striata</i> (Schiner)	PA
<i>Anastrepha suspensa</i> (Loew)	PA, 2C-1 <sup>1</sup>
<i>Bactrocera carambolae</i> (Drew & Hancock)	Methyl eugenol (ME)
<i>Bactrocera caryeae</i> (Kapoor)	ME
<i>Bactrocera correcta</i> (Bezzi)	ME
<i>Bactrocera dorsalis</i> (Hendel) <sup>4</sup>	ME
<i>Bactrocera invadens</i> (Drew, Tsuruta, & White)	ME, 3C <sup>2</sup>
<i>Bactrocera kandiensis</i> (Drew & Hancock)	ME
<i>Bactrocera musae</i> (Tryon)	ME
<i>Bactrocera occipitalis</i> (Bezzi)	ME
<i>Bactrocera papayae</i> (Drew & Hancock)	ME
<i>Bactrocera philippinensis</i> (Drew & Hancock)□	ME
<i>Bactrocera umbrosa</i> (Fabricius)	ME
<i>Bactrocera zonata</i> (Saunders)	ME, 3C <sup>2</sup> , ammonium acetate (AA)
<i>Bactrocera cucurbitae</i> (Coquillett)	Cuelure (CUE), 3C <sup>2</sup> , AA
<i>Bactrocera neohumeralis</i> (Hardy)	CUE
<i>Bactrocera tau</i> (Walker)	CUE
<i>Bactrocera tryoni</i> (Froggatt)	CUE
<i>Bactrocera citri</i> (Chen) ( <i>B. minax</i> , Enderlein)	PA
<i>Bactrocera cucumis</i> (French)	PA
<i>Bactrocera jarvisi</i> (Tryon)	PA
<i>Bactrocera latifrons</i> (Hendel)	PA
<i>Bactrocera oleae</i> (Gmelin)	PA, ammonium bicarbonate (AC), spiroketal (SK)
<i>Bactrocera tsuneonis</i> (Miyake)	PA

Scientific name	Attractant
<i>Ceratitis capitata</i> (Wiedemann)	Trimedlure (TML), Capilure (CE), PA, 3C <sup>2</sup> , 2C-2 <sup>3</sup>
<i>Ceratitis cosyra</i> (Walker)	PA, 3C <sup>2</sup> , 2C-2 <sup>3</sup>
<i>Ceratitis rosa</i> (Karsch)	TML, PA, 3C <sup>2</sup> , 2C-2 <sup>3</sup>
<i>Dacus ciliatus</i> (Loew)	PA, 3C <sup>2</sup> , AA
<i>Myiopardalis pardalina</i> (Bigot)	PA
<i>Rhagoletis cerasi</i> (Linnaeus)	Ammonium salts (AS), AA, AC
<i>Rhagoletis cingulata</i> (Loew)	AS, AA, AC
<i>Rhagoletis indifferens</i> (Curran)	AA, AC
<i>Rhagoletis pomonella</i> (Walsh)	butyl hexanoate (BuH), AS
<i>Toxotrypana curvicauda</i> (Gerstaecker)□	2-methyl-vinylpyrazine (MVP)

<sup>1</sup> Two-component (2C-1) synthetic food attractant of ammonium acetate and putrescine, mainly for female captures.

<sup>2</sup> Three-component (3C) synthetic food attractant, mainly for female captures (ammonium acetate, putrescine, trimethylamine).

<sup>3</sup> Two-component (2C-2) synthetic food attractant of ammonium acetate and trimethylamine, mainly for female captures.

<sup>4</sup> Taxonomic status of some listed members of the *Bactrocera dorsalis* complex and of *Anastrepha fraterculus* is uncertain.

### 3.1.1 Male-specific attractants

The most widely used attractants are pheromone or parapheromones that are male specific. The parapheromone trimedlure (TML) captures species of the genus *Ceratitis* (including *C. capitata* and *C. rosa*). The parapheromone methyl eugenol (ME) captures a large number of species of the genus *Bactrocera* (including *B. carambolae*, *B. dorsalis*, *B. invadens*, *B. musae*, *B. philippinensis* and *B. zonata*). The pheromone spiroketal captures *B. oleae*. The parapheromone cuelure (CUE) captures a large number of other *Bactrocera* species, including *B. cucurbitae* and *B. tryoni*. Parapheromones are generally highly volatile and can be used with a variety of traps (examples are listed in Table 2a). Controlled-release formulations exist for TML, CUE and ME, providing a longer-lasting attractant for field use. It is important to be aware that some inherent environmental conditions may affect the longevity of pheromone and parapheromone attractants.

### 3.1.2 Female-biased attractants

Female-specific pheromones/parapheromones are not usually commercially available (except, for example, 2-methyl-vinylpyrazine). Therefore, the female-biased attractants (natural, synthetic, liquid or dry) that are commonly used are based on food or host odours (Table 2b). Historically, liquid protein attractants (PA) have been used to capture a wide range of different fruit fly species. Liquid protein attractants capture both females and males. These liquid attractants are generally less sensitive than the parapheromones. In addition, liquid attractants capture high numbers of non-target insects and require more frequent servicing.

Several food-based synthetic attractants have been developed using ammonia and its derivatives. This may reduce the number of non-target insects captured. For example, for capturing *C. capitata* a synthetic food attractant consisting of three components (ammonium acetate, putrescine and trimethylamine) is used. For capturing of *Anastrepha* species the trimethylamine component may be removed. A synthetic attractant lasts approximately 4–10 weeks depending on climatic conditions. It captures few non-target insects and significantly fewer male fruit flies, making this attractant suited for use in sterile fruit fly release programmes. New synthetic food attractant technologies are available for use, including the long-lasting three-component and two-component mixtures contained in the same patch, as well as the three components incorporated in a single cone-shaped plug (Tables 1 and 3).

In addition, because food-foraging female and male fruit flies respond to synthetic food attractants at the sexually immature adult stage, these attractant types are capable of detecting female fruit flies earlier and at lower population levels than liquid protein attractants.

**Table 2a.** Attractants and traps for male fruit fly surveys

Fruit fly species	Attractant and trap (see below for abbreviations)																										
	TML/CE											ME								CUE							
	CC	CH	ET	JT	LT	MM	ST	SE	TP	YP	VARs+	CH	ET	JT	LT	MM	ST	TP	YP	CH	ET	JT	LT	MM	ST	TP	YP
<i>Anastrepha fraterculus</i>																											
<i>Anastrepha ludens</i>																											
<i>Anastrepha obliqua</i>																											
<i>Anastrepha striata</i>																											
<i>Anastrepha suspensa</i>																											
<i>Bactrocera carambolae</i>												x	x	x	x	x	x	x	x								
<i>Bactrocera caryeae</i>												x	x	x	x	x	x	x	x								
<i>Bactrocera citri</i> ( <i>B. minax</i> )																											
<i>Bactrocera correcta</i>												x	x	x	x	x	x	x	x								
<i>Bactrocera cucumis</i>																											
<i>Bactrocera cucurbitae</i>																				x	x	x	x	x	x	x	x
<i>Bactrocera dorsalis</i>												x	x	x	x	x	x	x	x								
<i>Bactrocera invadens</i>												x	x	x	x	x	x	x	x								
<i>Bactrocera kandiensis</i>												x	x	x	x	x	x	x	x								
<i>Bactrocera latifrons</i>																											
<i>Bactrocera occipitalis</i>												x	x	x	x	x	x	x	x								
<i>Bactrocera oleae</i>																											
<i>Bactrocera papayae</i>												x	x	x	x	x	x	x	x								
<i>Bactrocera philippinensis</i>												x	x	x	x	x	x	x	x								
<i>Bactrocera tau</i>																				x	x	x	x	x	x	x	x
<i>Bactrocera tryoni</i>																				x	x	x	x	x	x	x	x
<i>Bactrocera tsuneonis</i>																											
<i>Bactrocera umbrosa</i>												x	x	x	x	x	x	x	x								
<i>Bactrocera zonata</i>												x	x	x	x	x	x	x	x								
<i>Ceratitis capitata</i>		x	x	x	x	x	x	x	x	x	x																
<i>Ceratitis cosyra</i>																											
<i>Ceratitis rosa</i>		x	x	x	x	x	x	x	x	x	x																
<i>Dacus ciliatus</i>																											
<i>Myiopardalis pardalina</i>																											
<i>Rhagoletis cerasi</i>																											

Fruit fly species	Attractant and trap (see below for abbreviations)																										
	TML/CE											ME								CUE							
	CC	CH	ET	JT	LT	MM	ST	SE	TP	YP	VARs+	CH	ET	JT	LT	MM	ST	TP	YP	CH	ET	JT	LT	MM	ST	TP	YP
<i>Rhagoletis cingulata</i>																											
<i>Rhagoletis indifferens</i>																											
<i>Rhagoletis pomonella</i>																											
<i>Toxotrypana curvicauda</i>																											

**Attractant abbreviations**

TML Trimedlure  
CE Capilure  
ME Methyl eugenol  
CUE Cuelure

**Trap abbreviations**

CC Cook and Cunningham (C&C) trap  
CH ChamP trap  
ET Easy trap  
JT Jackson trap  
LT Lynfield trap  
MM Maghreb-Med or Morocco trap  
ST Steiner trap  
SE Sensus trap  
TP Tephri trap  
VARs+ Modified funnel trap  
YP Yellow panel trap

**Table 2b.** Attractants and traps for female-biased fruit fly surveys

Fruit fly species	Attractant and trap (see below for abbreviations)																									
	3C							2C-2					2C-1	PA			SK+AC		AS (AA, AC)				BuH			MVP
	ET	SE	MLT	OBDT	LT	MM	TP	ET	MLT	LT	MM	TP	MLT	ET	McP	MLT	CH	YP	RB	RS	YP	PALz	RS	YP	PALz	GS
<i>Anastrepha fraterculus</i>															X	X										
<i>Anastrepha grandis</i>															X	X										
<i>Anastrepha ludens</i>													X		X	X										
<i>Anastrepha obliqua</i>													X		X	X										
<i>Anastrepha striata</i>															X	X										
<i>Anastrepha suspensa</i>													X		X	X										
<i>Bactrocera carambolae</i>															X	X										
<i>Bactrocera caryeae</i>															X	X										
<i>Bactrocera citri</i> (B. minax)															X	X										
<i>Bactrocera correcta</i>															X	X										
<i>Bactrocera cucumis</i>															X	X										
<i>Bactrocera cucurbitae</i>							X								X	X										
<i>Bactrocera dorsalis</i>															X	X										
<i>Bactrocera invadens</i>							X								X	X										
<i>Bactrocera kandiensis</i>															X	X										
<i>Bactrocera latifrons</i>															X	X										
<i>Bactrocera occipitalis</i>															X	X										
<i>Bactrocera oleae</i>														X	X	X	X	X			X	X				
<i>Bactrocera papayae</i>															X	X										
<i>Bactrocera philippinensis</i>															X	X										
<i>Bactrocera tau</i>															X	X										
<i>Bactrocera tryoni</i>															X	X										
<i>Bactrocera tsuneonis</i>															X	X										
<i>Bactrocera umbrosa</i>															X	X										
<i>Bactrocera zonata</i>							X								X	X										
<i>Ceratitis capitata</i>	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X										

Fruit fly species	Attractant and trap (see below for abbreviations)																									
	3C							2C-2					2C-1	PA			SK+AC		AS (AA, AC)				BuH			MVP
	ET	SE	MLT	OBDT	LT	MM	TP	ET	MLT	LT	MM	TP	MLT	ET	McP	MLT	CH	YP	RB	RS	YP	PALz	RS	YP	PALz	GS
<i>Ceratitis cosyra</i>			x						x						x	x										
<i>Ceratitis rosa</i>		x	x						x						x	x										
<i>Dacus ciliatus</i>			x												x	x										
<i>Myiopardalis pardalina</i>															x	x										
<i>Rhagoletis cerasi</i>																			x	x	x	x	x	x	x	
<i>Rhagoletis cingulata</i>																					x	x		x	x	
<i>Rhagoletis indifferens</i>																				x	x					
<i>Rhagoletis pomonella</i>																			x		x	x	x			
<i>Toxotrypana curvicauda</i>																										x

**Attractant abbreviations**

3C	(AA+Pt+TMA)	AS	ammonium salts
2C-2	(AA+TMA)	AA	ammonium acetate
2C-1	(AA+Pt)	BuH	butyl hexanoate
PA	protein attractant	MVP	papaya fruit fly pheromone (2-methyl vinylpyrazine)
SK	spiroketal	Pt	putrescine
AC	ammonium (bi)carbonate	TMA	trimethylamine

**Trap abbreviations**

CH	ChamP trap	McP	McPhail trap	RS	Red sphere trap
ET	Easy trap	MLT	Multilure trap	SE	Sensus trap
GS	Green sphere	OBDT	Open bottom dry trap	TP	Tephri trap
LT	Lynfield trap	PALz	Fluorescent yellow sticky "cloak" trap	YP	Yellow panel trap
MM	Maghreb-Med or Morocco trap	RB	Rebell trap		

**Table 3.** List of attractants and field longevity

Common name	Attractant abbreviations	Formulation	Field longevity <sup>1</sup> (weeks)
<b>Parapheromones</b>			
Trimedlure	TML	Polymeric plug	4–10
		Laminate	3–6
		Liquid	1–4
		PE bag	4–5
Methyl eugenol	ME	Polymeric plug	4–10
		Liquid	4–8
Cuelure	CUE	Polymeric plug	4–10
		Liquid	4–8
Capilure (TML plus extenders)	CE	Liquid	12–36
<b>Pheromones</b>			
Papaya fruit fly ( <i>T. curvicauda</i> ) (2-methyl-6-vinylpyrazine)	MVP	Patches	4–6
Olive Fly (spiroketal)	SK	Polymer	4–6
<b>Food-based attractants</b>			
Torula yeast/borax	PA	Pellet	1–2
Protein derivatives	PA	Liquid	1–2
Ammonium acetate	AA	Patches	4–6
		Liquid	1
		Polymer	2–4
		Patches	4–6
Ammonium (bi)carbonate	AC	Liquid	1
		Polymer	1–4
		Salt	1
		Patches	6–10
Ammonium salts	AS	Patches	6–10
Putrescine	Pt	Vial	2
Trimethylamine	TMA	Cone/patches	6–10
Butyl hexanoate	BuH		
Ammonium acetate + Putrescine + Trimethylamine	3C (AA+Pt+TMA)		
Ammonium acetate + Putrescine + Trimethylamine	3C (AA+Pt+TMA)	Long-lasting patches	18–26
Ammonium acetate + Trimethylamine	2C-2 (AA+TMA)	Patches	6–10
Ammonium acetate + Putrescine	2C-1 (AA+Pt)	Patches	6–10
Ammonium acetate / Ammonium carbonate	AA/AC	PE bag w. alufoil cover	3–4

<sup>1</sup> Based on half-life. Attractant longevity is indicative only. Actual timing should be supported by field testing and validation.

### 3.2 Killing and preserving agents

Traps retain attracted fruit flies through the use of killing and preserving agents. In some dry traps, killing agents are a sticky material or a toxicant. Some organophosphates may act as a repellent at higher doses. The use of insecticides in traps is subject to the registration and approval of the product in the respective national legislation.

In other traps, liquid is the killing agent. When liquid protein attractants are used, mix borax 3% concentration to preserve the captured fruit flies. There are protein attractants that are formulated with borax, and thus no additional borax is required. When water is used in hot climates, 10% propylene glycol is added to prevent evaporation of the attractant and to preserve captured flies.

### 3.3 Commonly used fruit fly traps

This section describes commonly used fruit fly traps. The list of traps is not comprehensive; other types of traps may achieve equivalent results and may be used for fruit fly trapping.

Based on the killing agent, there are three types of traps commonly used:

- **Dry traps.** The fly is caught on a sticky material board or killed by a chemical agent. Some of the most widely used dry traps are Cook and Cunningham (C&C), ChamP, Jackson/Delta, Lynfield, open bottom dry trap (OBDT) or Phase IV, red sphere, Steiner and yellow panel/Rebell traps.
- **Wet traps.** The fly is captured and drowns in the attractant solution or in water with surfactant. One of the most widely used wet traps is the McPhail trap. The Harris trap is also a wet trap with a more limited use.
- **Dry or wet traps.** These traps can be used either dry or wet. Some of the most widely used are Easy trap, Multilure trap and Tephri trap.

#### Cook and Cunningham (C&C) trap

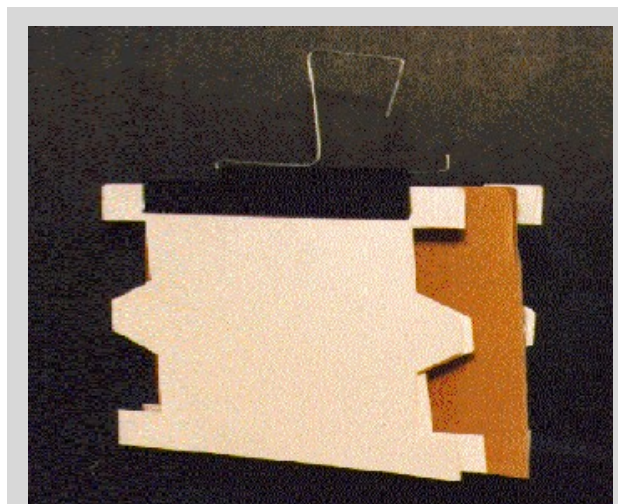
##### *General description*

The C&C trap consists of three removable creamy white panels, spaced approximately 2.5 cm apart. The two outer panels are made of rectangular paperboard measuring 22.8 cm × 14.0 cm. One or both panels are coated with sticky material (Figure 1). The adhesive panel has one or more holes which allow air to circulate through. The trap is used with a polymeric panel containing an olfactory attractant (usually trimedlure), which is placed between the two outer panels. The polymeric panels come in two sizes – standard and half panel. The standard panel (15.2 cm × 15.2 cm) contains 20 g of TML, while the half size (7.6 cm × 15.2 cm) contains 10 g. The entire unit is held together with clips, and suspended in the tree canopy with a wire hanger.

##### *Use*

As a result of the need for economic highly sensitive delimiting trapping of *C. capitata*, polymeric panels were developed for the controlled release of greater amounts of TML. This keeps the release rate constant for a longer period of time reducing hand labour and increasing sensitivity. The C&C trap with its multipanel construction has significant adhesive surface area for fly capture.

- For the species for which the trap and attractant is used, see Table 2a.



**Figure 1.** Cook and Cunningham (C&C) trap.

- For rebaiting (field longevity), see Table 3.
- For use under different scenarios and recommended densities, see Table 4d.

### ChamP trap (CH)

#### *General description*

The ChamP trap is a hollow, yellow panel-type trap with two perforated sticky side panels. When the two panels are folded, the trap is rectangular in shape (18 cm × 15 cm), and a central chamber is created to place the attractant (Figure 2). A wire hanger placed at the top of the trap is used to place it on branches.

#### *Use*

The ChamP trap can accommodate patches, polymeric panels, and plugs. It is equivalent to a Yellow panel/Rebell trap in sensitivity.

- For the species for which the trap and attractant is used, see Table 2 (a and b).
- For rebaiting (field longevity), see Table 3.
- For use under different scenarios and recommended densities, see Tables 4b and 4c.



Figure 2. ChamP trap.

### Easy trap (ET)

#### *General description*

The Easy trap is a two-part rectangular plastic container with an inbuilt hanger. It is 14.5 cm high, 9.5 cm wide, 5 cm deep and can hold 400 ml of liquid (Figure 3). The front part is transparent and the rear part is yellow. The transparent front of the trap contrasts with the yellow rear enhancing the trap's ability to catch fruit flies. It combines visual effects with parapheromone and food-based attractants.

#### *Use*

The trap is multipurpose. It can be used dry baited with parapheromones (e.g. TML, CUE, ME) or synthetic food attractants (e.g. 3C and both combinations of 2C attractants) and a retention system such as dichlorvos. It can also be used wet baited with liquid protein attractants holding up to 400 ml of mixture. When synthetic food attractants are used, one of the dispensers (the one containing putrescine) is attached inside to the yellow part of the trap and the other dispensers are left free.



Figure 3. Easy trap.

The Easy trap is one of the most economic traps commercially available. It is easy to carry, handle and service, providing the opportunity to service a greater number of traps per man-hour than some other traps.

- For the species for which the trap and attractant is used, see Table 2 (a and b).
- For rebaiting (field longevity), see Table 3.
- For use under different scenarios and recommended densities, see Table 4d.

### Fluorescent yellow sticky “cloak” trap (PALz)

#### *General description*

The PALz trap is prepared from fluorescent yellow plastic sheets (36 cm × 23 cm). One side is covered with sticky material. When setting up, the sticky sheet is placed around a vertical branch or a pole in a “cloaklike” manner (Figure 4), with the sticky side facing outward, and the back corners are fastened together with clips.

#### *Use*

The trap uses the optimal combination of visual (fluorescent yellow) and chemical (cherry fruit fly synthetic bait) attractant cues. The trap is kept in place by a piece of wire, attached to the branch or pole. The bait dispenser is fastened to the front top edge of the trap, with the bait hanging in front of the sticky surface. The sticky surface of the trap has a capture capacity of about 500 to 600 fruit flies. Insects attracted by the combined action of these two stimuli are caught on the sticky surface.

- For the species for which the trap and attractant is used, see Table 2b.
- For rebaiting (field longevity), see Table 3.
- For use under different scenarios and recommended densities, see Table 4e.



**Figure 4.** Fluorescent yellow sticky cloak trap.

### Jackson trap (JT) or Delta trap

#### *General description*

The Jackson trap is hollow, delta shaped and made of a white waxed cardboard. It is 8 cm high, 12.5 cm long and 9 cm wide (Figure 5). Additional parts include a white or yellow rectangular insert of waxed cardboard which is covered with a thin layer of adhesive used to trap fruit flies once they land inside the trap body; a polymeric plug or cotton wick in a plastic basket or wire holder; and a wire hanger placed at the top of the trap body.

#### *Use*

This trap is mainly used with parapheromone attractants to capture male fruit flies. The attractants used with JT/Delta traps are TML, ME and CUE. When ME and CUE are used a toxicant must be added.

For many years this trap has been used in exclusion, suppression or eradication programmes for multiple purposes, including population ecology studies (seasonal abundance, distribution, host sequence, etc.); detection and delimiting trapping; and surveying sterile fruit fly populations in areas subjected to sterile fly mass releases. JT/Delta traps may not be suitable for some environmental conditions (e.g. rain or dust).



**Figure 5.** Jackson trap or Delta trap.

The JT/Delta traps are some of the most economic traps commercially available. They are easy to carry, handle and service, providing the opportunity of servicing a greater number of traps per man-hour than some other traps.

- For the species for which the trap and attractant is used, see Table 2a.
- For rebaiting (field longevity), see Table 3.
- For use under different scenarios and recommended densities, see Tables 4b and 4d.

### Lynfield trap (LT)

#### General description

The conventional Lynfield trap consists of a disposable, clear plastic, cylindrical container measuring 11.5 cm high with a 10 cm diameter base and 9 cm diameter screw-top lid. There are four entry holes evenly spaced around the wall of the trap (Figure 6). Another version of the Lynfield trap is the Maghreb-Med trap also known as Morocco trap (Figure 7).

#### Use

The trap uses an attractant and insecticide system to attract and kill target fruit flies. The screw-top lid is usually colour-coded to the type of attractant being used (red, CE/TML; white, ME; yellow, CUE). To hold the attractant a 2.5 cm screw-tip cup hook (opening squeezed closed) screwed through the lid from above is used. The trap uses the male-specific parapheromone attractants CUE, Capilure (CE), TML and ME.



Figure 6. Lynfield trap.



Figure 7. Maghreb-Med trap or Morocco trap.

CUE and ME attractants, which are ingested by the male fruit fly, are mixed with malathion. However, because CE and TML are not ingested by either *C. capitata* or *C. rosa*, a dichlorvos-impregnated matrix is placed inside the trap to kill fruit flies that enter.

- For the species for which the trap and attractant is used, see Table 2 (a and b).
- For rebaiting (field longevity), see Table 3.
- For use under different scenarios and recommended densities, see Tables 4b and 4d.

### McPhail (McP) trap type

#### General description

The conventional McPhail (McP) trap is a transparent glass or plastic, pear-shaped invaginated container. The trap is 17.2 cm high and 16.5 cm wide at the base and holds up to 500 ml of solution (Figure 8). The trap parts include a rubber cork or plastic lid that seals the upper part of the trap and a wire hook to hang traps on tree branches. A plastic version of the McPhail trap is 18 cm high and 16 cm wide at the base and holds up to 500 ml of solution (Figure 9). The top part is transparent and the base is yellow.

#### Use

For this trap to function properly it is essential that the body stays clean. Some designs have two parts



Figure 8. McPhail trap.

in which the upper part and base of the trap can be separated allowing for easy service (rebaitying) and inspection of fruit fly captures.

This trap uses a liquid food attractant, based on hydrolysed protein or torula yeast/borax tablets. Torula tablets are more effective than hydrolysed proteins over time because the pH is stable at 9.2. The level of pH in the mixture plays an important role in attracting fruit flies. Fewer fruit flies are attracted to the mixture as the pH becomes more acidic.

To bait with yeast tablets, mix three to five torula tablets in 500 ml of water or follow the manufacturer's recommendation. Stir to dissolve tablets. To bait with protein hydrolysate, mix protein hydrolysate and borax (if not already added to the protein) in water to reach 5–9% hydrolysed protein concentration and 3% of borax.

The nature of its attractant means this trap is more effective at catching females. Food attractants are generic by nature, and so McP traps tend to also catch a wide range of other non-target tephritid and non-tephritid fruit flies in addition to the target species.

McP-type traps are used in fruit fly management programmes in combination with other traps. In areas subjected to suppression and eradication actions, these traps are used mainly to monitor female populations. Female catches are crucial in assessing the amount of sterility induced to a wild population in a sterile insect technique (SIT) programme. In programmes releasing only sterile males or in a male annihilation technique (MAT) programme, McP traps are used as a population detection tool by targeting feral females, whereas other traps (e.g. Jackson traps), used with male-specific attractants, catch the released sterile males, and their use should be limited to programmes with an SIT component. Furthermore, in fruit fly-free areas, McP traps are an important part of the non-indigenous fruit fly trapping network because of their capacity to capture fruit fly species of quarantine importance for which no specific attractants exist.

McP traps with liquid protein attractant are labour intensive. Servicing and rebaiting take time, and the number of traps that can be serviced in a normal working day is half that of some other traps described in this appendix.

- For the species for which the trap and attractant is used, see Table 2b.
- For rebaiting (field longevity), see Table 3.
- For use under different scenarios and recommended densities, see Tables 4a, 4b, 4d and 4e.



**Figure 9.** Plastic McPhail trap.

### Modified funnel trap (VARs+)

#### General description

The modified funnel trap consists of a plastic funnel and a lower catch container (Figure 10). The top roof has a large (5 cm diameter) hole, over which an upper catch container (transparent plastic) is placed.

#### Use

Since it is a non-sticky trap design, it has a virtually unlimited catch capacity and very long field life. The bait is attached to the roof, so that the bait dispenser is positioned into the middle of the large hole on the roof. A small piece of matrix impregnated with a killing agent is placed inside both the upper and lower catch containers to kill fruit flies that enter.

- For the species for which the trap and attractant is used, see Table 2a.
- For rebaiting (field longevity), see Table 3.
- For use under different scenarios and recommended densities, see Table 4d.



Figure 10. Modified funnel trap.

### Multilure trap (MLT)

#### General description

The Multilure trap (MLT) is a version of the McPhail trap described previously. The trap is 18 cm high and 15 cm wide at the base and can hold up to 750 ml of liquid (Figure 11). It consists of a two-piece plastic invaginated cylinder-shaped container. The top part is transparent and the base is yellow. The upper part and base of the trap separate, allowing the trap to be serviced and rebaited. The transparent upper part of the trap contrasts with the yellow base enhancing the trap's ability to catch fruit flies. A wire hanger, placed on top of the trap body, is used to hang the trap from tree branches.

#### Use

This trap follows the same principles as those of the McP trap. However, an MLT used with dry synthetic attractant is more efficient and selective than an MLT or McP trap used with liquid protein attractant. Another important difference is that an MLT with a dry synthetic attractant allows for a cleaner servicing and is much less labour intensive than a McP trap. When synthetic food attractants are used, dispensers are attached to the inside walls of the upper cylindrical part of the trap or hung from a clip at the top. For this trap to function properly it is essential that the upper part stays transparent.

When the MLT is used as a wet trap a surfactant should be added to the water. In hot climates 10% propylene glycol can be used to decrease water evaporation and decomposition of captured fruit flies.

When the MLT is used as a dry trap, a suitable (non-repellent at the concentration used) insecticide such as dichlorvos or a deltamethrin (DM) strip is placed inside the trap to kill the fruit flies. DM is applied to a polyethylene strip placed on the upper plastic platform inside the trap. Alternatively, DM may be used



Figure 11. Multilure trap.

in a circle of impregnated mosquito net and will retain its killing effect for at least six months under field conditions. The net must be fixed on the ceiling inside the trap using adhesive material.

- For the species for which the trap and attractant is used, see Table 2b.
- For rebaiting (field longevity), see Table 3.
- For use under different scenarios and recommended densities, see Tables 4a, 4b, 4c and 4d.

### Open bottom dry trap (OBDT) or (Phase IV) trap

#### *General description*

This trap is an open-bottom cylindrical dry trap that can be made from opaque green plastic or wax-coated green cardboard. The cylinder is 15.2 cm high and 9 cm in diameter at the top and 10 cm in diameter at the bottom (Figure 12). It has a transparent top, three holes (each of 2.5 cm diameter) equally spaced around the wall of the cylinder midway between the ends, and an open bottom, and is used with a sticky insert. A wire hanger, placed on top of the trap body, is used to hang the trap from tree branches.

#### *Use*

A food-based synthetic chemical female biased attractant can be used to capture *C. capitata*. However, it also serves to capture males. Synthetic attractants are attached to the inside walls of the cylinder. Servicing is easy because the sticky insert permits easy removal and replacement, similar to the inserts used in the JT. This trap is less expensive than the plastic or glass McP-type traps.



**Figure 12.** Open bottom dry trap (Phase IV).

- For the species for which the trap and attractant is used, see Table 2b.
- For attractants used and rebaiting (field longevity), see Table 3.
- For use under different scenarios and recommended densities, see Table 4d.

### Red sphere trap (RS)

#### *General description*

The trap is a red sphere 8 cm in diameter (Figure 13). The trap mimics the size and shape of a ripe apple. A green version of this trap is also used. The trap is covered with a sticky material and baited with the synthetic fruit odour butyl hexanoate, which has a fragrance like a ripe fruit. Attached to the top of the sphere is a wire hanger used to hang it from tree branches.

#### *Use*

The red or green traps can be used unbaited, but they are much more efficient in capturing fruit flies when baited. Fruit flies that are sexually mature and ready to lay eggs are attracted to this trap.

Many types of insects will be caught by these traps. It will be necessary to positively identify the target fruit fly from the non-target insects likely to be present on the traps.



**Figure 13.** Red sphere trap.

- For the species for which the trap and attractant is used, see Table 2b.
- For rebaiting (field longevity), see Table 3.
- For use under different scenarios and recommended densities, see Table 4e.

### Sensus trap (SE)

#### General description

The Sensus trap consists of a vertical plastic bucket 12.5 cm in high and 11.5 cm in diameter (Figure 14). It has a transparent body and a blue overhanging lid, which has a hole just underneath it. A wire hanger placed on top of the trap body is used to hang the trap from tree branches.

#### Use

The trap is dry and uses male-specific parapheromones or, for female-biased captures, dry synthetic food attractants. A dichlorvos block is placed in the comb on the lid to kill the flies.

- For the species for which the trap and attractant is used, see Table 2 (a and b).
- For rebaiting (field longevity), see Table 3.
- For use under different scenarios and recommended densities, see Table 4d.

### Steiner trap (ST)

#### General description

The Steiner trap is a horizontal, clear plastic cylinder with openings at each end. The conventional Steiner trap is 14.5 cm long and 11 cm in diameter (Figure 15). There are a number of versions of Steiner traps. These include the Steiner trap of 12 cm long and 10 cm in diameter (Figure 16) and 14 cm long and 8.5 cm in diameter (Figure 17). A wire hanger, placed on top of the trap body, is used to hang the trap from tree branches.

#### Use

This trap uses the male-specific parapheromone attractants TML, ME and CUE. The attractant is suspended from the centre of the inside of the trap. The attractant may be a cotton wick soaked in 2–3 ml of a mixture of parapheromone or a dispenser with the attractant and an insecticide (usually malathion, dibrom or deltamethrin) as a killing agent.

- For the species for which the trap and attractant is used, see Table 2a.
- For rebaiting (field longevity), see Table 3.
- For use under different scenarios and recommended densities, see Tables 4b and 4d.

### Tephri trap (TP)

#### General description

The Tephri trap is similar to a McP trap. It is a vertical cylinder 15 cm high and 12 cm in diameter at the base and can hold up to 450 ml of liquid (Figure 18). It has a yellow base and a clear top, which can be separated to facilitate servicing. There are entrance holes around the top of the



Figure 14. Sensus trap.



Figure 15. Conventional Steiner trap.



Figure 16. Steiner trap version.



Figure 17. Steiner trap version.

periphery of the yellow base, and an invaginated opening in the bottom. Inside the top is a platform to hold attractants. A wire hanger, placed on top of the trap body, is used to hang the trap from tree branches.

#### *Use*

The trap is baited with hydrolysed protein at 9% concentration; however, it can also be used with other liquid protein attractants as described for the conventional glass McP trap or with the female dry synthetic food attractant and with TML in a plug or liquid as described for the JT/Delta and Yellow panel traps. If the trap is used with liquid protein attractants or with dry synthetic attractants combined with a liquid retention system and without the side holes, the insecticide will not be necessary. However, when used as a dry trap and with side holes, an insecticide solution (e.g. malathion) soaked into a cotton wick or other killing agent is needed to avoid escape of captured insects. Other suitable insecticides are dichlorvos or deltamethrin (DM) strips placed inside the trap to kill the fruit flies. DM is applied in a polyethylene strip, placed on the plastic platform inside the top of the trap. Alternatively, DM may be used in a circle of impregnated mosquito net and will retain its killing effect for at least six months under field conditions. The net must be fixed on the ceiling of the inside of the trap using adhesive material.

- For the species for which the trap and attractant is used, see Table 2 (a and b).
- For rebaiting (field longevity), see Table 3.
- For use under different scenarios and recommended densities, see Tables 4b and 4d.

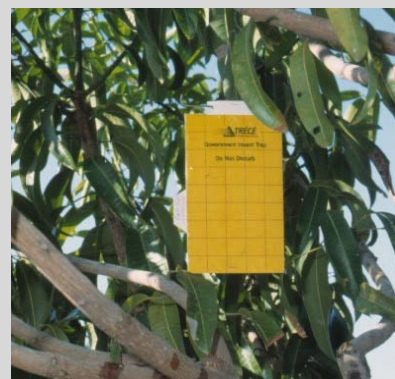
#### **Yellow panel trap (YP)/Rebell trap (RB)**

##### *General description*

The Yellow panel trap (YP) consists of a yellow rectangular cardboard plate (23 cm × 14 cm) coated with plastic (Figure 19). The rectangle is covered on both sides with a thin layer of sticky material. The Rebell trap is a three-dimensional YP-type trap with two crossed yellow rectangular plates (15 cm × 20 cm) made of plastic (polypropylene) making them extremely durable (Figure 20). The trap is also coated with a thin layer of sticky material on both sides of both plates. A wire hanger, placed on top of the trap body, is used to hang it from tree branches.



**Figure 18.** Tephri trap.



**Figure 19.** Yellow panel trap.

### Use

These traps can be used as visual traps alone and baited with TML, spiroketal or ammonium salts (ammonium acetate). The attractants may be contained in controlled-release dispensers such as a polymeric plug. The attractants are attached to the face of the trap. The attractants can also be mixed into the cardboard's coating. The two-dimensional design and greater contact surface make these traps more efficient, in terms of fly captures, than the JT and McPhail-type traps. It is important to consider that these traps require special procedures for transportation, submission and fruit fly screening methods because they are so sticky that specimens can be destroyed in handling. Although these traps can be used in most types of control programme applications, their use is recommended for the post-eradication phase and for fly-free areas, where highly sensitive traps are required. These traps should not be used in areas subjected to mass release of sterile fruit flies because of the large number of released fruit flies that would be caught. It is important to note that their yellow colour and open design allow them to catch other non-target insects including natural enemies of fruit flies and pollinators.



**Figure 20.** Rebell trap.

- For the species for which the trap and attractant is used, see Table 2 (a and b).
- For rebaiting (field longevity), see Table 3.
- For use under different scenarios and recommended densities, see Tables 4b, 4c, 4d and 4e.

## 4. Trapping procedures

### 4.1 Spatial distribution of traps

The spatial distribution of traps will be guided by the purpose of the survey, the intrinsic characteristics of the area, the biological characteristics of the fruit fly and its interactions with its hosts, as well as the efficacy of the attractant and trap. In areas where continuous compact blocks of commercial orchards are present and in urban and suburban areas where hosts exist, traps are usually deployed in a grid system, which may have a uniform distribution.

In areas with scattered commercial orchards, rural areas with hosts and in marginal areas where hosts exist, trap networks are normally distributed along roads that provide access to host material.

In suppression and eradication programmes, an extensive trapping network should be deployed over the entire area that is subject to surveillance and control actions.

Trapping networks are also placed as part of early detection programmes for target fruit fly species. In this case traps are placed in high-risk areas such as points of entry, fruit markets, urban areas garbage dumps, as appropriate. This can be further supplemented by traps placed along roadsides to form transects and at production areas close to or adjacent to land borders, port of entries and national roads.

### 4.2 Trap deployment (placement)

Trap deployment involves the actual placement of the traps in the field. One of the most important factors of trap deployment is selecting an appropriate trap site. It is important to have a list of the primary, secondary and occasional fruit fly hosts, their phenology, distribution and abundance. With this basic information, it is possible to properly place and distribute the traps in the field, and it also allows for effective planning of a programme of trap relocation.

When possible, pheromone traps should be placed in mating areas. Fruit flies normally mate in the crown of host plants or close by, selecting semi-shaded spots and usually on the upwind side of the crown. Other suitable trap sites are the eastern side of the tree which gets the sunlight in the early

hours of the day, resting and feeding areas in plants that provide shelter and protect fruit flies from strong winds and predators. In specific situations trap hangers may need to be coated with an appropriate insecticide to prevent ants from eating captured fruit flies.

Protein traps should be deployed in shaded areas in host plants. In this case traps should be deployed in primary host plants during their fruit maturation period. In the absence of primary host plants, secondary host plants should be used. In areas with no host plants identified, traps should be deployed in plants that can provide shelter, protection and food to adult fruit flies.

Traps should be deployed in the middle to the top part of the host plant canopy, depending on the height of the host plant, and oriented towards the upwind side. Traps should not be exposed to direct sunlight, strong winds or dust. It is of vital importance to have the trap entrance clear from twigs, leaves and other obstructions such as spider webs to allow proper airflow and easy access for the fruit flies.

Placement of traps in the same tree baited with different attractants should be avoided because it may cause interference among attractants and a reduction of trap efficiency. For example, placing a *C. capitata* male-specific TML trap and a protein attractant trap in the same tree will cause a reduction of female capture in the protein traps because TML acts as a female repellent.

Traps should be relocated following the maturation phenology of the fruit hosts present in the area and biology of the fruit fly species. By relocating the traps it is possible to follow the fruit fly population throughout the year and increase the number of sites being checked for fruit flies.

### 4.3 Trap mapping

Once traps are deployed at carefully selected sites at the correct density and distributed in an appropriate pattern, the location of the traps must be recorded. It is recommended that the location of traps should be geo-referenced with the use of global positioning system (GPS) equipment where available. A map or sketch of the trap location and the area around the traps should be prepared.

The application of GPS and geographic information systems (GIS) in the management of trapping network has proved to be a very powerful tool. GPS allows each trap to be geo-referenced through geographical coordinates, which are then used as input information in a GIS.

In addition to GPS location data or in the event that GPS data is not available for trap locations, reference for the trap location should include visible landmarks. In the case of traps placed in host plants located in suburban and urban areas, references should include the full address of the property where the trap was placed. Trap reference should be clear enough to allow control teams and supervisors who service the traps to find the trap easily.

A database or trapping book of all traps with their corresponding coordinates should be kept, together with the records of trap services, date of collection, collector, rebaiting, trap captures, and if possible notes on the collection site such as ecological characteristics. GIS provides high-resolution maps showing the exact location of each trap and other valuable information such as exact location of fruit fly detections, historical profiles of the geographical distribution patterns of the fruit flies, relative size of the populations in given areas and spread of the fruit fly population in case of an outbreak. This information is extremely useful in planning control activities, ensuring that bait sprays and sterile fruit fly releases are accurately placed and cost-effective in their application.

#### 4.4 Trap servicing and inspection

Trap servicing intervals are specific to each trapping system and are based on the half-life of the attractant noting that actual timings should be supported by field testing and validation (see Table 3). Capturing fruit flies will depend, in part, on how well the trap is serviced. Trap servicing includes rebaiting and maintaining the trap in a clean and appropriate operating condition. Traps should be in a condition to consistently kill and retain in good condition any target flies that have been captured.

Attractants have to be used in the appropriate volumes and concentrations and replaced at the recommended intervals, as indicated by the manufacturer. The release rate of attractants varies considerably with environmental conditions. The release rate is generally high in hot and dry areas, and low in cool and humid areas. Thus, in cool climates traps may have to be rebaited less often than in hot conditions.

Inspection intervals (i.e. checking for fruit fly captures) should be adjusted according to the prevailing environmental conditions, pest situations and biology of fruit flies, on a case-by-case basis. The interval can range from one day up to 30 days, e.g. seven days in areas where fruit fly populations are present and 14 days in fruit fly free areas. In the case of delimiting surveys inspection intervals may be more frequent, with two to three days being the most common interval.

Avoid handling more than one lure type at a time if more than one lure type is being used at a single locality. Cross-contamination between traps of different attractant types (e.g. Cue and ME) reduces trap efficacy and makes laboratory identification unduly difficult. When changing attractants, it is important to avoid spillage or contamination of the external surface of the trap body or the ground. Attractant spillage or trap contamination would reduce the chances of fruit flies entering the trap. For traps that use a sticky insert to capture fruit flies, it is important to avoid contaminating areas in the trap that are not meant for capturing fruit flies with the sticky material. This also applies to leaves and twigs that surround the trap. Attractants, by their nature, are highly volatile and care should be taken when storing, packaging, handling and disposing of lures to avoid compromising the attractant and operator safety.

The number of traps serviced per day per person will vary depending on type of trap, trap density, environmental and topographic conditions and experience of the operators. Where a large trap network is in place, it may need to be serviced over a number of days. In this case, the network may be serviced through a number of “routes” or “runs” which systematically ensure all traps within the network are inspected and serviced, and none are missed.

#### 4.5 Trapping records

The following information should be included in order to keep proper trapping records as they provide confidence in the survey results: trap location, plant where the trap is placed, trap and attractant type, servicing and inspection dates, and target fruit fly capture. Any other information considered necessary can be added to the trapping records. Retaining results over a number of seasons can provide useful information on spatial changes in fruit fly population.

#### 4.6 Flies per trap per day

Flies per trap per day (FTD) is a population index that indicates the average number of flies of the target species captured per trap per day during a specified period in which the trap was exposed in the field.

The function of this population index is to have a comparative measure of the size of the adult pest population in a given space and time.

It is used as baseline information to compare the size of the population before, during and after the application of a fruit fly control programme. The FTD should be used in all reports of trapping.

The FTD is comparable within a programme; however, for meaningful comparisons between programmes, it should be based on the same fruit fly species, trapping system and trap density.

In areas where sterile fruit fly release programmes are in operation FTD is used to measure the relative abundance of the sterile and wild fruit flies.

FTD is the result of dividing the total number of fruit flies captured (F) by the product obtained from multiplying the total number of inspected traps (T) by the average number of days between trap inspections (D). The formula is as follows:

$$\text{FTD} = \frac{F}{T \times D}$$

## 5. Trap densities

Establishing a trapping density appropriate to the purpose of the survey is critical and underpins confidence in the survey results. The trap densities need to be adjusted based on many factors including type of survey, trap efficiency, location (type and presence of host, climate and topography), pest situation and lure type. In terms of type and presence of hosts, as well as the risk involved, the following types of location may be of concern:

- production areas
- marginal areas
- urban areas
- points of entry (and other high-risk areas such as fruit markets).

Trap densities may also vary as a gradient from production areas to marginal areas, urban areas and points of entry. For example, in a pest free area, a higher density of traps is required at high-risk points of entry and a lower density in commercial orchards. Or, in an area where suppression is applied, such as in an area of low pest prevalence or an area under a systems approach where the target species is present, the reverse occurs, and trapping densities for that pest should be higher in the production field and decrease toward points of entry. Other situations such as high-risk urban areas should be taken into consideration when assessing trapping densities.

Tables 4a–4f show suggested trap densities for various fruit fly species based on common practice. These densities have been determined taking into consideration research results, feasibility and cost effectiveness. Trap densities are also dependent on associated surveillance activities, such as the type and intensity of fruit sampling to detect immature stages of fruit flies. In those cases where trapping surveillance programmes are complemented with fruit sampling activities, trap densities could be lower than the suggested densities shown in Tables 4a–4f.

The suggested densities presented in Tables 4a–4f have been made also taking into account the following technical factors:

- various survey objectives and pest status
- target fruit fly species (Table 1)
- pest risk associated with working areas (production and other areas).

Within the delimited area, the suggested trap density should be applied in areas with a significant likelihood of capturing fruit flies such as areas with primary hosts and possible pathways (e.g. production areas versus industrial areas).

**Table 4a.** Trap densities suggested for *Anastrepha* spp.

Trapping	Trap type <sup>1</sup>	Attractant	Trap density/km <sup>2</sup> <sup>(2)</sup> □			
			Production area	Marginal	Urban	Points of entry <sup>3</sup>
Monitoring survey, no control	MLT/McP	2C-1/PA	0.25–1	0.25–0.5	0.25–0.5	0.25–0.5
Monitoring survey for suppression	MLT/McP	2C-1/PA	2–4	1–2	0.25–0.5	0.25–0.5
Delimiting survey in an FF-ALPP after an unexpected increase in population	MLT/McP	2C-1/PA	3–5	3–5	3–5	3–5
Monitoring survey for eradication	MLT/McP	2C-1/PA	3–5	3–5	3–5	3–5
Detection survey in an FF-PFA to verify pest absence and for exclusion	MLT/McP	2C-1/PA	1–2	2–3	3–5	5–12
Delimitation survey in an FF-PFA after a detection in addition to detection survey <sup>4</sup>	MLT/McP	2C-1/PA	20–50	20–50	20–50	20–50

<sup>1</sup> Different traps can be combined to reach the total number.

<sup>(2)</sup> Refers to the total number of traps.

<sup>3</sup> Also other high-risk sites.

<sup>4</sup> This range includes high-density trapping in the immediate area of the detection (core area). However, it may decrease towards the surrounding trapping zones.

Trap type		Attractant	
McP	McPhail trap	2C-1	AA+Pt
		AA	Ammonium acetate
		Pt	Putrescine
MLT	Multilure trap	PA	Protein attractant

**Table 4b.** Trap densities suggested for *Bactrocera* spp. responding to methyl eugenol (ME), cuelure (CUE) and food attractants (PA = protein attractants)

Trapping	Trap type <sup>1</sup>	Attractant	Trap density/km <sup>2</sup> <sup>(2)</sup> □			
			Production area	Marginal	Urban	Points of entry <sup>3</sup>
Monitoring survey, no control	JT/ST/TP/LT/MM/MLT/McP/ET	ME/CUE/PA	0.25–1.0	0.2–0.5	0.2–0.5	0.2–0.5
Monitoring survey for suppression	JT/ST/TP/LT/MM/MLT/McP/ET	ME/CUE/PA	2–4	1–2	0.25–0.5	0.25–0.5
Delimiting survey in an FF-ALPP after an unexpected increase in population	JT/ST/TP/MLT/LT/MM/McP/YP/ET	ME/CUE/PA	3–5	3–5	3–5	3–5
Monitoring survey for eradication	JT/ST/TP/MLT/LT/MM/McP/ET	ME/CUE/PA	3–5	3–5	3–5	3–5
Detection survey in an FF-PFA to verify pest absence and for exclusion	CH/ST/LT/MM/MLT/McP/TP/YP/ET	ME/CUE/PA	1	1	1–5	3–12
Delimitation survey in a PFA after a detection in addition to detection survey <sup>4</sup>	JT/ST/TP/MLT/LT/MM/McP/YP/ET	ME/CUE/PA	20–50	20–50	20–50	20–50

<sup>1</sup> Different traps can be combined to reach the total number.

<sup>(2)</sup> Refers to the total number of traps.

<sup>3</sup> Also other high-risk sites.

<sup>4</sup> This range includes high-density trapping in the immediate area of the detection (core area). However, it may decrease towards the surrounding trapping zones.

Trap type		Attractant	
CH	ChamP trap	ME	Methyleugenol
ET	Easy trap	CUE	Cuelure
JT	Jackson trap	PA	Protein attractant
LT	Lynfield trap		
McP	McPhail trap		
MLT	Multilure trap		
MM	Maghreb-Med or Morocco		
ST	Steiner trap		
TP	Tephri trap		

YP Yellow panel trap

**Table 4c.** Trap densities suggested for *Bactrocera oleae*

Trapping	Trap type <sup>1</sup>	Attractant	Trap density/km <sup>2</sup> <sup>(2)</sup> □			
			Production area	Marginal	Urban	Points of entry <sup>3</sup>
Monitoring survey, no control	MLT/CH/YP/ET/McP	AC+SK/PA	0.5–1.0	0.25–0.5	0.25–0.5	0.25–0.5
Monitoring survey for suppression	MLT/CH/YP/ET/McP	AC+SK/PA	2–4	1–2	0.25–0.5	0.25–0.5
Delimiting survey in an FF-ALPP after an unexpected increase in population	MLT/CH/YP/ET/McP	AC+SK/PA	3–5	3–5	3–5	3–5
Monitoring survey for eradication	MLT/CH/YP/ET/McP	AC+SK/PA	3–5	3–5	3–5	3–5
Detection survey in an FF-PFA to verify pest absence and for exclusion	MLT/CH/YP/ET/McP	AC+SK/PA	1	1	2–5	3–12
Delimitation survey in a PFA after a detection in addition to detection survey <sup>4</sup>	MLT/CH/YP/ET/McP	AC+SK/PA	20–50	20–50	20–50	20–50

<sup>1</sup> Different traps can be combined to reach the total number.<sup>(2)</sup> Refers to the total number of traps.<sup>3</sup> Also other high-risk sites.<sup>4</sup> This range includes high-density trapping in the immediate area of the detection (core area). However, it may decrease towards the surrounding trapping zones.

Trap type		Attractant	
CH	ChamP trap □	AC	Ammonium bicarbonate
ET	Easy trap	PA	Protein attractant
McP	McPhail trap	SK	Spiroketal
MLT	Multilure trap		
YP	Yellow panel trap		

**Table 4d.** Trap densities suggested for *Ceratitidis* spp.

Trapping	Trap type <sup>1</sup>	Attractant	Trap density/km <sup>2</sup> <sup>(2)</sup> □			
			Production area	Marginal	Urban	Points of entry <sup>3</sup>
Monitoring survey, no control <sup>4</sup>	JT/MLT/McP/OBDT/ST/SE/ET/LT/TP/VARS+/CH	TML/CE/3C/2C-2/PA	0.5–1.0	0.25–0.5	0.25–0.5	0.25–0.5
Monitoring survey for suppression	JT/MLT/McP/OBDT/ST/SE/ET/LT/MMTP/VARS+/CH	TML/CE/3C/2C-2/PA	2–4	1–2	0.25–0.5	0.25–0.5
Delimiting survey in an FF-ALPP after an unexpected increase in population	JT/YP/MLT/McP/OBDT/ST/ET/LT/MM/TP/VARS+/CH	TML/CE/3C/PA	3–5	3–5	3–5	3–5
Monitoring survey for eradication <sup>5</sup>	JT/MLT/McP/OBDT/ST/ET/LT/MM/TP/VARS+/CH	TML/CE/3C/2C-2/PA	3–5	3–5	3–5	3–5
Detection survey in an FF-PFA to verify pest absence and for exclusion <sup>5</sup>	JT/MLT/McP/ST/ET/LT/MM/CC/VARS+/CH	TML/CE/3C/PA	1	1–2	1–5	3–12
Delimitation survey in a PFA after a detection in addition to detection survey <sup>5</sup>	JT/YP/MLT/McP/OBDT/ST/ET/LT/MM/TP/VARS+/CH	TML/CE/3C/PA	20–50	20–50	20–50	20–50

<sup>1</sup> Different traps can be combined to reach the total number.<sup>(2)</sup> Refers to the total number of traps.

- <sup>3</sup> Also other high-risk sites.
- <sup>4</sup> 1:1 ratio (1 female trap per male trap).
- <sup>5</sup> 3:1 ratio (3 female traps per male trap).
- <sup>6</sup> This range includes high-density trapping in the immediate area of the detection (core area). However, it may decrease towards the surrounding trapping zones (ratio 5:1, 5 female traps per male trap).

Trap type		Attractant	
CC	Cook and Cunningham (C&C) Trap (with TML for male capture)	2C-2	(AA+TMA)
CH	ChamP trap	3C	(AA+Pt+TMA)
ET	Easy trap (with 2C and 3C attractants for female-biased captures)	CE	Capilure
JT	Jackson trap (with TML for male capture)	AA	Ammonium acetate
LT	Lynfield trap (with TML for male capture)	PA	Protein attractant
McP	McPhail trap	Pt	Putrescine
MLT	Multilure trap (with 2C and 3C attractants for female-biased captures)	TMA	Trimethylamine
MM	Maghreb-Med or Morocco	TML	Trimedlure
OBDT	Open Bottom Dry Trap (with 2C and 3C attractants for female-biased captures)		
SE	Sensus trap (with CE for male captures and with 3C for female-biased captures)		
ST	Steiner trap (with TML for male capture)		
TP	Tephri trap (with 2C and 3C attractants for female-biased captures)		
VARs+	Modified funnel trap		
YP	Yellow panel trap		

**Table 4e.** Trap densities suggested for *Rhagoletis* spp.

Trapping	Trap type <sup>1</sup>	Attractant	Trap density/km <sup>2</sup> <sup>(2)</sup> □			
			Production area	Marginal	Urban	Points of entry <sup>3</sup>
Monitoring survey, no control	RB/RS/PALz/YP	BuH/AS	0.5–1.0	0.25–0.5	0.25–0.5	0.25–0.5
Monitoring survey for suppression	RB/RS/PALz/YP	BuH/AS	2–4	1–2	0.25–0.5	0.25–0.5
Delimiting survey in an FF-ALPP after an unexpected increase in population	RB/RS/PALz/YP	BuH/AS	3–5	3–5	3–5	3–5
Monitoring survey for eradication	RB/RS/PALz/YP	BuH/AS	3–5	3–5	3–5	3–5
Detection survey in an FF-PFA to verify pest absence and for exclusion	RB/RS/PALz/YP	BuH/AS	1	0.4–3	3–5	4–12
Delimitation survey in a PFA after a detection in addition to detection survey <sup>4</sup>	RB/RS/PALz/YP	BuH/AS	20–50	20–50	20–50	20–50

<sup>1</sup> Different traps can be combined to reach the total number.

<sup>(2)</sup> Refers to the total number of traps.

<sup>3</sup> Also other high-risk sites.

<sup>4</sup> This range includes high-density trapping in the immediate area of the detection (core area). However, it may decrease towards the surrounding trapping zones.

Trap type		Attractant	
RB	Rebell trap	AS	Ammonium salt
RS	Red sphere trap	BuH	Butyl hexanoate
PALz	Fluorescent yellow sticky trap		
YP	Yellow panel trap		

**Table 4f.** Trap densities suggested for *Toxotrypana curvicauda*

Trapping	Trap type <sup>1</sup>	Attractant	Trap density/km <sup>2</sup> <sup>(2)</sup> □			
			Production area	Marginal	Urban	Points of entry <sup>3</sup>
Monitoring survey, no control	GS	MVP	0.25–0.5	0.25–0.5	0.25–0.5	0.25–0.5

Monitoring survey for suppression	GS	MVP	2–4	1	0.25–0.5	0.25–0.5
Delimiting survey in an FF-ALPP after an unexpected increase in population	GS	MVP	3–5	3–5	3–5	3–5
Monitoring survey for eradication	GS	MVP	3–5	3–5	3–5	3–5
Detection survey in an FF-PFA to verify pest absence and for exclusion	GS	MVP	2	2–3	3–6	5–12
Delimitation survey in a PFA after a detection in addition to detection survey <sup>4</sup>	GS	MVP	20–50	20–50	20–50	20–50

<sup>1</sup> Different traps can be combined to reach the total number.

<sup>(2)</sup> Refers to the total number of traps.

<sup>3</sup> Also other high-risk sites.

<sup>4</sup> This range includes high-density trapping in the immediate area of the detection (core area). However, it may decrease towards the surrounding trapping zones.

**Trap type**

GS Green sphere

**Attractant**

MVP Papaya fruit fly pheromone (2-methyl-vinylpyrazine)

## 6. Supervision activities

Supervision of trapping activities includes assessing the quality of the materials used and reviewing the effectiveness of the use of these materials and trapping procedures.

The materials used should perform effectively and reliably at an acceptable level for a prescribed period of time. The traps themselves should maintain their integrity for the entire duration that they are anticipated to remain in the field. The attractants should be certified or bioassayed by the manufacturer for an acceptable level of performance based on their anticipated use.

The effectiveness of trapping should be officially reviewed periodically by individuals not directly involved in conducting trapping activities. The timing of review will vary by programme, but it is recommended to occur at least twice a year in programmes that run for six months or longer. The review should address all aspects related to the ability of trapping to detect targeted fruit flies within the timeframe required to meet programme outcomes e.g. Early detection of a fruit fly entry. Aspects of a review include quality of trapping materials, record-keeping, layout of the trapping network, trap mapping, trap placement, trap condition, trap servicing, trap inspection frequency and capability for fruit fly identification.

The trap deployment should be evaluated to ensure that the prescribed types and densities of traps are in place. Field confirmation is achieved through inspection of individual routes.

Trap placement should be evaluated for appropriate host selection, trap relocation schedule, height, light penetration, fruit fly access to trap, and proximity to other traps. Host selection, trap relocation and proximity to other traps can be evaluated from the records for each trap route. Host selection, placement and proximity can be further evaluated by field examination.

Traps should be evaluated for their overall condition, correct attractant, appropriate trap servicing and inspection intervals, correct identifying markings (such as trap identification and date placed), evidence of contamination and proper warning labels. This is performed in the field at each site where a trap is placed.

Evaluation of identification capability can occur via target fruit flies that have been marked in some manner in order to distinguish them from wild trapped fruit flies. These marked fruit flies are placed in traps in order to evaluate the operator's diligence in servicing the traps, competence in recognizing the targeted fruit fly species, and knowledge of the proper reporting procedures once a fruit fly is found. Commonly used marking systems are fluorescent dyes or wing clipping.

In some programmes that survey for eradication or to maintain FF-PFAs, the fruit flies may also be marked by using sterile irradiated fruit flies in order to further reduce the chances of the marked fruit

fly being falsely identified as a wild fruit fly and resulting in unnecessary actions by the programme. A slightly different method is necessary under a sterile fruit fly release programme in order to evaluate personnel on their ability to accurately distinguish target wild fruit flies from the released sterile fruit flies. The marked fruit flies used are sterile and lack the fluorescent dye, but are marked physically by wing clipping or some other method. These fruit flies are placed into the trap samples after they have been collected in the field but before they are inspected by the operators.

The review should be summarized in a report detailing how many inspected traps on each route were found to be in compliance with the accepted standards in categories such as trap mapping, placement, condition, and servicing and inspection interval. Aspects that were found to be deficient should be identified, and specific recommendations should be made to correct these deficiencies.

Proper record-keeping is crucial to the appropriate functioning of trapping. The records for each trap route should be inspected to ensure that they are complete and up to date. Field confirmation can then be used to validate the accuracy of the records. Maintenance of voucher specimens of collected species of regulated fruit fly species is recommended.

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This appendix is for reference purposes only and is not a prescriptive part of the standard.

## APPENDIX 2: Guidelines for fruit sampling

Information about sampling is available in the references listed below. The list is not exhaustive.

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**ISPM 5**



# **INTERNATIONAL STANDARDS FOR PHYTOSANITARY MEASURES**

**ISPM 5**

## **GLOSSARY OF PHYTOSANITARY TERMS**

Produced by the Secretariat of the International Plant Protection Convention  
*Adopted 2015; published 2015*



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## Publication history

*This is not an official part of the standard*

1986-05 RPPOs recommended creation of a *Core vocabulary of phytosanitary terms*

1988-02 RPPOs reviewed and approved for NAPPO and EPPO consultation

1989-09 RPPOs prepared draft *Core vocabulary of phytosanitary terms*

1990 FAO published *FAO Glossary of phytosanitary terms*; FAO Plant Protection Bulletin 38(1)

1991-05 TC-RPPOs endorsed topic *Glossary of phytosanitary terms* (1991-001)

1993-05 TC-RPPOs revised terms and recommended to establish WG for the *FAO Glossary* (GWG)

1994-02 1<sup>st</sup> meeting of the GWG

1994-03 CEPM-1 revised text and agreed to add new terms

1995-05 CEPM-2 decided publication of revised *Glossary of phytosanitary terms* as an ISPM

**ISPM 5**. 1995. *Glossary of phytosanitary terms*. Rome, IPPC, FAO

1996-05 CEPM-3 revised text of *Glossary of phytosanitary terms*

1997-10 CEPM-4 revised the text and 29<sup>th</sup> Session of the FAO Conference approved **ISPM 5**. 1997.

1999-02 GWG revised standard

1999-05 CEPM-6 revised standard for adoption

1999-10 ICPM-2 adopted revised **ISPM 5**. 1999

1999-09 GWG revised standard

2000-05 ISC-1 revised standard and approved for MC

2000-06 Sent for MC

2000-11 ISC-2 revised standard for adoption

2001-04 ICPM-3 adopted revised **ISPM 5**. 2001

2000-03 and 2001-03 GWG revised standard

2001-05 ISC-3 approved Specification 1 *Review and updating of the glossary of phytosanitary terms*

2001-05 ISC-3 revised standard and approved for MC

2001-06 Sent for MC

2001-11 ISC-4 revised standard for adoption

2002-03 ICPM-4 adopted revised **ISPM 5**. 2002

2002-02 GWG revised standard

2002-05 SC revised standard and approved MC

2002-06 Sent for MC

2002-11 SC revised standard for adoption

2003-04 ICPM-5 adopted revised **ISPM 5**. 2003

2003-02 GWG revised standard

2003-05 SC-7 agreed recommendations by TPG

2003-09 GWG revised standard

2003-11 SC revised standard and requested to add new terms on ISPMs

2004-02 GWG revised standard

2004-04 SC revised standard and approved MC

2004-06 Sent for MC

2004-11 SC revised standard for adoption

2005-04 ICPM-7 adopted revised **ISPM 5**. 2005

2004-10 & 2005-10 GWG revised standard

2006-05 SC revised standard and approved for MC

2006-06 Sent for MC

2006-11 SC revised standard for adoption

2007-03 CPM-2 adopted revised **ISPM 5**. 2007

2006-03 CPM-1 created the Technical panel for the glossary (TPG)

2006-10 1<sup>st</sup> meeting of the TPG. TPG revised standard

2007-05 SC revised standard and approved for MC

2007-06 Sent for MC

2007-11 revised standard for adoption

2008-04 CPM-3 adopted revised **ISPM 5**. 2008

2007-10 TPG revised standard

2008-05 SC-7 revised standard and approved for MC

2008-06 Sent for MC

2008-11 SC revised standard for adoption

2009-03 CPM-4 adopted revised **ISPM 5**. 2009

2008-10 TPG revised standard

2009-05 SC revised standard and approved for MC

2009-06 Sent for MC

2009-11 SC revised standard for adoption  
2010-03 CPM-5 adopted revised **ISPM 5**. 2010

2009-06 TPG started reviewing adopted standards for consistency in the use of terms  
2009-10 TPG proposed ink amendments to ISPMs 3, 10, 13, 14, 22 and Supplement 1 to ISPM 5  
2009-11 SC revised proposed ink amendments  
2010-03 CPM-5 noted ink amendments in the English version  
2010-10 TPG proposed ink amendments to ISPM 5  
2010-11 SC revised proposed ink amendments  
2011-03 CPM-6 noted ink amendments in the English version  
2011-05 IPPC Secretariat applied ink amendments as noted by CPM-6 (2011)

2010-10 TPG revised standard  
2011-05 SC revised standard and approved for MC  
2011-06 Sent for MC  
2011-11 SC revised standard for adoption  
2012-03 CPM-7 adopted revised **ISPM 5**. 2012

#### **Supplement 1**

1999-10 ICPM-2 added topic *Official control* (1999-002)  
2000-03 EWG developed draft text  
2000-05 ISC-1 revised draft text and approved for MC  
2000-06 Sent for MC  
2000-11 ISC-2 revised draft text for adoption  
2001-04 ICPM-3 adopted Supplement 1 to ISPM 5  
**ISPM 5. Supplement 1** *Guidelines on the interpretation and application of the concept of official control for regulated pests* (2001)  
2005-03 ICPM-7 added the topic not widely distributed (2005-008) (supplement to ISPM No. 5: Glossary of phytosanitary terms)  
2006-05 SC approved specification 33  
2008-05 SC-7 reviewed draft  
2010-03 revised to incorporate consistency ink amendments noted by CPM-5 (2010)  
2011-05 SC approved for member consultation  
2011-06 member consultation  
2011-11 TPG reviewed member comments  
2011-11 SC approved draft supplement to ISPM  
2012-03 CPM-7 adopted revised supplement 1 to ISPM 5

**ISPM 5. Supplement 1.** *Guidelines on the interpretation and application of the concepts of "official control" and "not widely distributed"* (2012)

#### **Supplement 2**

2001-04 ICPM-3 added topic *Defining economic importance* (2001-004)  
2002-02 GWG developed draft text  
2002-05 SC revised draft text and approved for MC  
2002-06 Sent for MC  
2002-11 SC revised draft text for adoption  
2003-04 ICPM-5 adopted Supplement 2 to ISPM 5  
**ISPM 5. Supplement 2** *Guidelines on the understanding of potential economic importance and related terms including reference to environmental considerations* (2003)

2013-08 IPPC Secretariat applied ink amendments as noted by CPM-8 (2013)

#### **Appendix 1**

2005-03 ICPM-7 IPPC and CBD (Convention on Biological Diversity) secretariats decided cooperation programme  
2006-04 CPM-1 agreed assess progress on the work programme (2006-033)  
2006-10 TPG developed draft text  
2007-05 SC requested TPG to develop draft text *CBD terms*  
2007-10 TPG developed draft text  
2008-05 SC revised draft text and approved for MC  
2008-06 Sent for MC  
2008-11 SC revised draft text for adoption  
2009-03 CPM-4 adopted Appendix 1 to ISPM 5  
**ISPM 5. Appendix 1** *Terminology of the Convention on Biological Diversity in relation to the Glossary of phytosanitary terms* (2009)

2013-08 IPPC Secretariat applied ink amendments as noted by CPM-8 (2013)

2015-03 CPM-10 adopted revised **ISPM 5**. 2015  
2015-03 IPPC Secretariat applied amendments and ink amendments as adopted and noted by CPM-10 (2015)  
2015-05 IPPC Secretariat corrected a mistake introduced in the definition of "pest free area" and "area of low pest prevalence"  
Publication history last modified: 2015-05-29

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## Adoption

This standard was first adopted by the Twenty-eighth Session of the FAO Conference in November 1995. It has undergone repeated modifications since that time. The current edition of ISPM 5 arises from an amendment adopted by the Tenth Session of the Commission on Phytosanitary Measures in March 2015.

Supplement 1 was first adopted by the Third Session of the Interim Commission on Phytosanitary Measures in April 2001. The first revision of Supplement 1 was adopted by the Seventh Session of the Commission on Phytosanitary Measures in March 2012. Supplement 2 was adopted by the Fifth Session of the Interim Commission on Phytosanitary Measures in April 2003. Appendix 1 was adopted by the Fourth Session of the Commission on Phytosanitary Measures in March–April 2009.

## INTRODUCTION

### Scope

This reference standard is a listing of terms and definitions with specific meaning for phytosanitary systems worldwide. It has been developed to provide a harmonized internationally agreed vocabulary associated with the implementation of the International Plant Protection Convention (IPPC) and International Standards for Phytosanitary Measures (ISPMs).

Within the context of the IPPC and its ISPMs, all references to plants should be understood to continue to include algae and fungi, consistent with the International Code of Nomenclature for algae, fungi, and plants.

### Purpose

The purpose of this reference standard is to increase clarity and consistency in the use and understanding of terms and definitions which are used by contracting parties for official phytosanitary purposes, in phytosanitary legislation and regulations, as well as for official information exchange.

### References

The references below correspond to the approval of terms and definitions, as indicated in the definitions. For ISPMs, they do not indicate the most recent version (which is available on the IPP at <https://www.ippc.int/core-activities/standards-setting/ispm>)

**CBD.** 2000. *Cartagena Protocol on Biosafety to the Convention on Biological Diversity*. Montreal, CBD.

**CEPM.** 1996. *Report of the Third Meeting of the FAO Committee of Experts on Phytosanitary Measures, Rome, 13–17 May 1996*. Rome, IPPC, FAO.

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**CPM.** 2007. *Report of the Second Session of the Commission on Phytosanitary Measures, Rome, 26–30 March 2007*. Rome, IPPC, FAO.

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- FAO**. 1995. *See ISPM 5, 1995*.
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- ISPM 3**. 2005. *Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms*. Rome, IPPC, FAO.
- ISPM 5**. 1995. *Glossary of phytosanitary terms*. Rome, IPPC, FAO. [published 1996]
- ISPM 8**. 1998. *Determination of pest status in an area*. Rome, IPPC, FAO.
- ISPM 10**. 1999. *Requirements for the establishment of pest free places of production and pest free production sites*. Rome, IPPC, FAO.
- ISPM 11**. 2001. *Pest risk analysis for quarantine pests*. Rome, IPPC, FAO.
- ISPM 11**. 2004. *Pest risk analysis for quarantine pests including analysis of environmental risks and living modified organisms*. Rome, IPPC, FAO.
- ISPM 14**. 2002. *The use of integrated measures in a systems approach for pest risk management*. Rome, IPPC, FAO.
- ISPM 15**. 2002. *Guidelines for regulating wood packaging material in international trade*. Rome, IPPC, FAO.
- ISPM 16**. 2002. *Regulated non-quarantine pests: concept and application*. Rome, IPPC, FAO.
- ISPM 17**. 2002. *Pest reporting*. Rome, IPPC, FAO.
- ISPM 18**. 2003. *Guidelines for the use of irradiation as a phytosanitary measure*. Rome, IPPC, FAO.
- ISPM 20**. 2004. *Guidelines for a phytosanitary import regulatory system*. Rome, IPPC, FAO.
- ISPM 22**. 2005. *Requirements for the establishment of areas of low pest prevalence*. Rome, IPPC, FAO.
- ISPM 23**. 2005. *Guidelines for inspection*. Rome, IPPC, FAO.
- ISPM 24**. 2005. *Guidelines for the determination and recognition of equivalence of phytosanitary measures*. Rome, IPPC, FAO.
- ISPM 25**. 2006. *Consignments in transit*. Rome, IPPC, FAO.

**ISPM 27.** 2006. *Diagnostic protocols for regulated pests*. Rome, IPPC, FAO.

**ISPM 28.** 2007. *Phytosanitary treatments for regulated pests*. Rome, IPPC, FAO.

**WTO.** 1994. *Agreement on the Application of Sanitary and Phytosanitary Measures*. Geneva, World Trade Organization.

### **Outline of Reference**

The purpose of this standard is to assist national plant protection organizations (NPPOs) and others in information exchange and the harmonization of vocabulary used in official communications and legislation pertaining to phytosanitary measures. The present version incorporates revisions agreed as a result of the approval of the International Plant Protection Convention (1997) and terms added through the adoption of additional International Standards for Phytosanitary Measures (ISPMs).

The Glossary contains all terms and definitions approved until the Seventh Session of the Commission on Phytosanitary Measures (CPM, 2012). References in square brackets refer to the approval of the term and definition, and not to subsequent adjustments in translation.

As in previous editions of the Glossary, terms in definitions are printed in bold to indicate their relation to other Glossary terms and to avoid unnecessary repetition of elements described elsewhere in the Glossary. Derived forms of words that appear in the Glossary, e.g. *inspected* from *inspection*, are also considered glossary terms.

## PHYTOSANITARY TERMS AND DEFINITIONS

*\* Indicates that the term, at the time of publishing, is on the work programme of the Technical Panel for the Glossary which means the terms or definitions may be revised or deleted in the future.*

<b>absorbed dose</b>	Quantity of radiating energy absorbed per unit of mass of a specified target [ISPM 18, 2003, revised CPM, 2012]
<b>additional declaration*</b>	A statement that is required by an importing country to be entered on a <b>phytosanitary certificate</b> and which provides specific additional information on a <b>consignment</b> in relation to <b>regulated pests</b> [FAO, 1990; revised ICPM, 2005]
<b>area</b>	An <b>officially</b> defined country, part of a country or all or parts of several countries [FAO, 1990; revised FAO, 1995; CEPM, 1999; based on the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (WTO, 1994)]
<b>area endangered</b>	See <b>endangered area</b>
<b>area of low pest prevalence</b>	An <b>area</b> , whether all of a country, part of a country, or all or parts of several countries, as identified by the competent authorities, in which a specific <b>pest</b> is present at low levels and which is subject to effective <b>surveillance</b> or <b>control</b> measures [IPPC, 1997; revised CPM, 2015]
<b>bark*</b>	The layer of a woody trunk, branch or root outside the cambium [CPM, 2008]
<b>bark-free wood</b>	<b>Wood</b> from which all <b>bark</b> , except ingrown bark around knots and bark pockets between rings of annual growth, has been removed [ISPM 15, 2002; revised CPM, 2008]
<b>biological control agent</b>	A <b>natural enemy</b> , <b>antagonist</b> or <b>competitor</b> , or other <b>organism</b> , used for <b>pest control</b> [ISPM 3, 1995; revised ISPM 3, 2005]
<b>buffer zone</b>	An <b>area</b> surrounding or adjacent to an <b>area officially</b> delimited for phytosanitary purposes in order to minimize the probability of <b>spread</b> of the target <b>pest</b> into or out of the delimited <b>area</b> , and subject to phytosanitary or other <b>control</b> measures, if appropriate [ISPM 10, 1999; revised ISPM 22, 2005; revised CPM, 2007]
<b>bulbs and tubers</b> (as a commodity class)	Dormant underground parts of <b>plants</b> intended for <b>planting</b> (includes corms and rhizomes) [FAO, 1990; revised ICPM, 2001; revised CPM, 2015]
<b>chemical pressure impregnation</b>	<b>Treatment</b> of <b>wood</b> with a chemical preservative through a process of pressure in accordance with an <b>official</b> technical specification [ISPM 15, 2002; revised ICPM, 2005]
<b>clearance</b> (of a consignment)	Verification of compliance with <b>phytosanitary regulations</b> [FAO, 1995]
<b>Commission</b>	The Commission on Phytosanitary Measures established under Article XI [IPPC, 1997]
<b>commodity</b>	A type of <b>plant</b> , <b>plant product</b> , or other article being moved for trade or other purpose [FAO, 1990; revised ICPM, 2001]

<b>commodity class</b>	A category of similar <b>commodities</b> that can be considered together in <b>phytosanitary regulations</b> [FAO, 1990]
<b>commodity pest list</b>	A list of <b>pests</b> present in an <b>area</b> which may be associated with a specific <b>commodity</b> [CEPM, 1996; revised CPM, 2015]
<b>compliance procedure</b> (for a <b>consignment</b> )	<b>Official</b> procedure used to verify that a <b>consignment</b> complies with <b>phytosanitary import requirements</b> or <b>phytosanitary measures</b> related to <b>transit</b> [CEPM, 1999; revised CPM, 2009]
<b>confinement</b> (of a <b>regulated article</b> )	Application of <b>phytosanitary measures</b> to a <b>regulated article</b> to prevent the escape of <b>pests</b> [CPM, 2012]
<b>consignment</b>	A quantity of <b>plants</b> , <b>plant products</b> or other articles being moved from one country to another and covered, when required, by a single <b>phytosanitary certificate</b> (a <b>consignment</b> may be composed of one or more <b>commodities</b> or <b>lots</b> ) [FAO, 1990; revised ICPM, 2001]
<b>consignment in transit</b>	A <b>consignment</b> which passes through a country without being imported, and that may be subject to <b>phytosanitary measures</b> [FAO, 1990; revised CEPM, 1996; revised CEPM 1999; revised ICPM, 2002; revised ISPM 25, 2006; formerly “country of transit”]
<b>containment*</b>	Application of <b>phytosanitary measures</b> in and around an infested <b>area</b> to prevent <b>spread</b> of a <b>pest</b> [FAO, 1995]
<b>contaminating pest*</b>	A <b>pest</b> that is carried by a <b>commodity</b> and, in the case of <b>plants</b> and <b>plant products</b> , does not infest those <b>plants</b> or <b>plant products</b> [CEPM, 1996; revised CEPM, 1999]
<b>contamination*</b>	Presence in a <b>commodity</b> , storage place, conveyance or container, of <b>pests</b> or other <b>regulated articles</b> , not constituting an <b>infestation</b> (see <b>infestation</b> ) [CEPM, 1997; revised CEPM, 1999]
<b>control</b> (of a <b>pest</b> )*	<b>Suppression</b> , <b>containment</b> or <b>eradication</b> of a <b>pest</b> population [FAO, 1995]
<b>corrective action plan</b> (in an <b>area</b> )	Documented plan of <b>phytosanitary actions</b> to be implemented in an <b>area</b> officially delimited for phytosanitary purposes if a <b>pest</b> is detected or a tolerance level is exceeded or in the case of faulty implementation of officially established procedures [CPM, 2009; revised CPM, 2013]
<b>country of origin</b> (of a <b>consignment</b> of <b>plant products</b> )*	Country where the <b>plants</b> from which the <b>plant products</b> are derived were grown [FAO, 1990; revised CEPM, 1996; revised CEPM, 1999]
<b>country of origin</b> (of a <b>consignment</b> of <b>plants</b> )*	Country where the <b>plants</b> were grown [FAO, 1990; revised CEPM, 1996; revised CEPM, 1999]
<b>country of origin</b> (of <b>regulated articles</b> other than <b>plants</b> and <b>plant products</b> )*	Country where the <b>regulated articles</b> were first exposed to <b>contamination</b> by <b>pests</b> [FAO, 1990; revised CEPM, 1996; revised CEPM, 1999]

<b>cut flowers and branches</b> (as a commodity class)*	<b>Fresh</b> parts of <b>plants</b> intended for decorative use and not for <b>planting</b> [FAO, 1990; revised ICPM, 2001; revised CPM, 2015]
<b>debarked wood</b>	<b>Wood</b> that has been subjected to any process that results in the removal of <b>bark</b> . (Debarked wood is not necessarily <b>bark-free wood</b> .) [CPM, 2008; replacing “debarking”]
<b>delimiting survey</b>	<b>Survey</b> conducted to establish the boundaries of an <b>area</b> considered to be infested by or <b>free from a pest</b> [FAO, 1990]
<b>detection survey</b>	<b>Survey</b> conducted in an <b>area</b> to determine if <b>pests</b> are present [FAO, 1990; revised FAO, 1995]
<b>detention</b>	Keeping a <b>consignment</b> in <b>official</b> custody or confinement, as a <b>phytosanitary measure</b> (see <b>quarantine</b> ) [FAO, 1990; revised FAO, 1995; CEPM, 1999; ICPM, 2005]
<b>devitalization</b>	A procedure rendering <b>plants</b> or <b>plant products</b> incapable of germination, growth or further reproduction [ICPM, 2001]
<b>dose mapping</b>	Measurement of the <b>absorbed dose</b> distribution within a <b>process load</b> through the use of <b>dosimeters</b> placed at specific locations within the <b>process load</b> [ISPM 18, 2003]
<b>dunnage</b>	<b>Wood packaging material</b> used to secure or support a <b>commodity</b> but which does not remain associated with the <b>commodity</b> [FAO, 1990; revised ISPM 15, 2002]
<b>ecosystem</b>	A dynamic complex of <b>plant</b> , animal and micro-organism communities and their abiotic environment interacting as a functional unit [ISPM 3, 1995; revised ICPM, 2005]
<b>efficacy (of a treatment)</b>	A defined, measurable, and reproducible effect by a prescribed <b>treatment</b> [ISPM 18, 2003]
<b>emergency action</b>	A prompt <b>phytosanitary action</b> undertaken in a new or unexpected phytosanitary situation [ICPM, 2001]
<b>emergency measure</b>	A <b>phytosanitary measure</b> established as a matter of urgency in a new or unexpected phytosanitary situation. An emergency measure may or may not be a <b>provisional measure</b> [ICPM, 2001; revised ICPM, 2005]
<b>endangered area*</b>	An <b>area</b> where ecological factors favour the <b>establishment</b> of a <b>pest</b> whose presence in the <b>area</b> will result in economically important loss [FAO, 1995; revised CPM, 2013]
<b>entry (of a consignment)</b>	Movement through a <b>point of entry</b> into an <b>area</b> [FAO, 1995]
<b>entry (of a pest)</b>	Movement of a <b>pest</b> into an <b>area</b> where it is not yet present, or present but not widely distributed and being <b>officially controlled</b> [FAO, 1995]
<b>equivalence (of phytosanitary measures)</b>	The situation where, for a specified pest risk, different <b>phytosanitary measures</b> achieve a contracting party’s appropriate level of protection [FAO, 1995; revised CEPM, 1999; based on the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (WTO, 1994); revised ISPM 24, 2005]

<b>eradication*</b>	Application of <b>phytosanitary measures</b> to eliminate a <b>pest</b> from an <b>area</b> [FAO, 1990; revised FAO, 1995; formerly eradicate]
<b>establishment</b> (of a <b>pest</b> )	Perpetuation, for the foreseeable future, of a <b>pest</b> within an <b>area</b> after <b>entry</b> [FAO, 1990; revised FAO, 1995; revised IPPC, 1997; formerly “established”]
<b>field</b>	A plot of land with defined boundaries within a <b>place of production</b> on which a <b>commodity</b> is grown [FAO, 1990]
<b>find free</b>	To <b>inspect</b> a <b>consignment</b> , <b>field</b> or <b>place of production</b> and consider it to be <b>free from</b> a specific <b>pest</b> [FAO, 1990]
<b>free from</b> (of a <b>consignment</b> , <b>field</b> or <b>place of production</b> )	Without <b>pests</b> (or a specific <b>pest</b> ) in numbers or quantities that can be detected by the application of <b>phytosanitary procedures</b> [FAO, 1990; revised FAO, 1995; revised CEPM, 1999]
<b>fresh</b>	Living; not dried, deep-frozen or otherwise conserved [FAO, 1990]
<b>fruits and vegetables</b> (as a <b>commodity class</b> )	<b>Fresh</b> parts of <b>plants</b> intended for consumption or processing and not for <b>planting</b> [FAO, 1990; revised ICPM, 2001; revised CPM, 2015]
<b>fumigation</b>	<b>Treatment</b> with a chemical agent that reaches the <b>commodity</b> wholly or primarily in a gaseous state [FAO, 1990; revised FAO, 1995]
<b>germplasm</b>	<b>Plants</b> intended for use in breeding or conservation programmes [FAO, 1990]
<b>grain</b> (as a <b>commodity class</b> )*	<b>Seeds</b> intended for processing or consumption and not for <b>planting</b> (see <b>seeds</b> ) [FAO, 1990; revised ICPM, 2001; revised CPM, 2015]
<b>growing medium</b>	Any material in which <b>plant</b> roots are growing or intended for that purpose [FAO, 1990]
<b>growing period</b> (of a <b>plant species</b> )	Time period of active growth during a <b>growing season</b> [ICPM, 2003]
<b>growing season</b>	Period or periods of the year when <b>plants</b> actively grow in an <b>area</b> , <b>place of production</b> or <b>production site</b> [FAO, 1990; revised ICPM, 2003]
<b>habitat</b>	Part of an <b>ecosystem</b> with conditions in which an <b>organism</b> is naturally present or can establish [ICPM, 2005; revised CPM, 2015]
<b>harmonization</b>	The establishment, recognition and application by different countries of <b>phytosanitary measures</b> based on common <b>standards</b> [FAO, 1995; revised CEPM, 1999; based on the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (WTO, 1994)]
<b>harmonized phytosanitary measures</b>	<b>Phytosanitary measures</b> established by contracting parties to the <b>IPPC</b> , based on <b>international standards</b> [IPPC, 1997]
<b>heat treatment</b>	The process in which a <b>commodity</b> is heated until it reaches a minimum temperature for a minimum period of time according to an <b>official</b> technical specification [ISPM 15, 2002; revised ICPM, 2005]

<b>host pest list</b>	A list of <b>pests</b> that infest a <b>plant</b> species, globally or in an <b>area</b> [CEPM, 1996; revised CEPM, 1999]
<b>host range</b>	Species capable, under natural conditions, of sustaining a specific <b>pest</b> or other <b>organism</b> [FAO, 1990; revised ISPM 3, 2005]
<b>import permit</b>	<b>Official</b> document authorizing importation of a <b>commodity</b> in accordance with specified <b>phytosanitary import requirements</b> [FAO, 1990; revised FAO, 1995; ICPM, 2005]
<b>inactivation</b>	Rendering micro-organisms incapable of development [ISPM 18, 2003]
<b>incidence (of a pest)</b>	Proportion or number of units in which a <b>pest</b> is present in a sample, <b>consignment</b> , <b>field</b> or other defined population [CPM, 2009]
<b>incursion</b>	An isolated population of a <b>pest</b> recently detected in an <b>area</b> , not known to be <b>established</b> , but expected to survive for the immediate future [ICPM, 2003]
<b>infestation (of a commodity)</b>	Presence in a <b>commodity</b> of a living <b>pest</b> of the <b>plant</b> or <b>plant product</b> concerned. <b>Infestation</b> includes infection [CEPM, 1997; revised CEPM, 1999]
<b>inspection</b>	<b>Official visual examination</b> of <b>plants</b> , <b>plant products</b> or other <b>regulated articles</b> to determine if <b>pests</b> are present or to determine compliance with <b>phytosanitary regulations</b> [FAO, 1990; revised FAO, 1995; formerly “inspect”]
<b>inspector</b>	Person authorized by a <b>national plant protection organization</b> to discharge its functions [FAO, 1990]
<b>integrity (of a consignment)*</b>	Composition of a <b>consignment</b> as described by its <b>phytosanitary certificate</b> or other <b>officially</b> acceptable document, maintained without loss, addition or substitution [CPM, 2007]
<b>intended use</b>	Declared purpose for which <b>plants</b> , <b>plant products</b> or other articles are imported, produced or used [ISPM 16, 2002; revised CPM, 2009]
<b>interception (of a consignment)</b>	The <b>refusal</b> or controlled <b>entry</b> of an imported <b>consignment</b> due to failure to comply with <b>phytosanitary regulations</b> [FAO, 1990; revised FAO, 1995]
<b>interception (of a pest)</b>	The detection of a <b>pest</b> during <b>inspection</b> or <b>testing</b> of an imported <b>consignment</b> [FAO, 1990; revised CEPM, 1996]
<b>intermediate quarantine</b>	<b>Quarantine</b> in a country other than the <b>country of origin</b> or destination [CEPM, 1996]
<b>International Plant Protection Convention</b>	International Plant Protection Convention, as deposited with FAO in Rome in 1951 and as subsequently amended [FAO, 1990]
<b>International Standard for Phytosanitary Measures</b>	An <b>international standard</b> adopted by the Conference of FAO, the Interim Commission on Phytosanitary Measures or the Commission on Phytosanitary Measures, established under the <b>IPPC</b> [CEPM, 1996; revised CEPM, 1999]

<b>international standards</b>	International <b>standards</b> established in accordance with Article X paragraphs 1 and 2 of the <b>IPPC</b> [IPPC, 1997]
<b>introduction</b> (of a <b>pest</b> )	The <b>entry</b> of a <b>pest</b> resulting in its <b>establishment</b> [FAO, 1990; revised FAO, 1995; IPPC, 1997]
<b>inundative release</b>	The release of large numbers of mass-produced <b>biological control agents</b> or <b>beneficial organisms</b> with the expectation of achieving a rapid effect [ISPM 3, 1995; revised ISPM 3, 2005]
<b>IPPC</b>	<b>International Plant Protection Convention</b> , as deposited in 1951 with FAO in Rome and as subsequently amended [FAO, 1990; revised ICPM, 2001]
<b>irradiation</b>	<b>Treatment</b> with any type of <b>ionizing radiation</b> [ISPM 18, 2003]
<b>ISPM</b>	<b>International Standard for Phytosanitary Measures</b> [CEPM, 1996; revised ICPM, 2001]
<b>kiln-drying*</b>	A process in which <b>wood</b> is dried in a closed chamber using heat and/or humidity control to achieve a required moisture content [ISPM 15, 2002]
<b>living modified organism</b>	Any living organism that possesses a novel combination of genetic material obtained through the use of <b>modern biotechnology</b> [Cartagena Protocol on Biosafety to the Convention on Biological Diversity (CBD, 2000)]
<b>LMO</b>	<b>living modified organism</b> [ISPM 11, 2004]
<b>lot</b>	A number of units of a single <b>commodity</b> , identifiable by its homogeneity of composition, origin etc., forming part of a <b>consignment</b> [FAO, 1990]
<b>mark*</b>	An <b>official</b> stamp or brand, internationally recognized, applied to a <b>regulated article</b> to attest its phytosanitary status [ISPM 15, 2002]
<b>minimum absorbed dose (Dmin)</b>	The localized minimum <b>absorbed dose</b> within the <b>process load</b> [ISPM 18, 2003]
<b>modern biotechnology</b>	The application of: <ol style="list-style-type: none"> <li>in vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles; or</li> <li>fusion of cells beyond the taxonomic family,</li> </ol> that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection. [Cartagena Protocol on Biosafety to the Convention on Biological Diversity (CBD, 2000)]
<b>monitoring</b>	An <b>official</b> ongoing process to verify phytosanitary situations [CEPM, 1996]
<b>monitoring survey</b>	Ongoing <b>survey</b> to verify the characteristics of a <b>pest</b> population [FAO, 1995]

<b>national plant protection organization</b>	<b>Official</b> service established by a government to discharge the functions specified by the <b>IPPC</b> [FAO, 1990; formerly “plant protection organization (national)”]
<b>natural enemy</b>	An <b>organism</b> which lives at the expense of another <b>organism</b> in its area of origin and which may help to limit the population of that <b>organism</b> . This includes <b>parasitoids</b> , <b>parasites</b> , <b>predators</b> , phytophagous organisms and <b>pathogens</b> [ISPM 3, 1995; revised ISPM 3, 2005]
<b>non-quarantine pest</b>	<b>Pest</b> that is not a <b>quarantine pest</b> for an <b>area</b> [FAO, 1995]
<b>NPPO</b>	<b>National plant protection organization</b> [FAO, 1990; ICPM, 2001]
<b>official</b>	Established, authorized or performed by a <b>national plant protection organization</b> [FAO, 1990]
<b>official control</b>	The active enforcement of mandatory <b>phytosanitary regulations</b> and the application of mandatory <b>phytosanitary procedures</b> with the objective of <b>eradication</b> or <b>containment</b> of <b>quarantine pests</b> or for the management of <b>regulated non-quarantine pests</b> [ICPM, 2001; revised CPM, 2013]
<b>outbreak</b>	A recently detected <b>pest</b> population, including an <b>incursion</b> , or a sudden significant increase of an established <b>pest</b> population in an <b>area</b> [FAO, 1995; revised ICPM, 2003]
<b>packaging</b>	Material used in supporting, protecting or carrying a <b>commodity</b> [ISPM 20, 2004]
<b>parasite</b>	An organism which lives on or in a larger organism, feeding upon it [ISPM 3, 1995]
<b>parasitoid</b>	An insect <b>parasitic</b> only in its immature stages, killing its host in the process of its development, and free living as an adult [ISPM 3, 1995]
<b>pathogen</b>	Micro-organism causing disease [ISPM 3, 1995]
<b>pathway</b>	Any means that allows the <b>entry</b> or <b>spread</b> of a <b>pest</b> [FAO, 1990; revised FAO, 1995]
<b>pest</b>	Any species, strain or biotype of plant, animal or <b>pathogenic</b> agent injurious to <b>plants</b> or <b>plant products</b> . Note: In the IPPC, plant pest is sometimes used for the term pest [FAO, 1990; revised FAO, 1995; IPPC, 1997; revised CPM, 2012]
<b>pest categorization</b>	The process for determining whether a <b>pest</b> has or has not the characteristics of a <b>quarantine pest</b> or those of a <b>regulated non-quarantine pest</b> [ISPM 11, 2001]
<b>pest diagnosis</b>	The process of detection and identification of a <b>pest</b> [ISPM 27, 2006]

<b>pest free area</b>	An <b>area</b> in which a specific <b>pest</b> is absent as demonstrated by scientific evidence and in which, where appropriate, this condition is being <b>officially</b> maintained [FAO, 1995; revised CPM, 2015]
<b>pest free place of production</b>	<b>Place of production</b> in which a specific <b>pest</b> is absent as demonstrated by scientific evidence and in which, where appropriate, this condition is being <b>officially</b> maintained for a defined period [ISPM 10, 1999; revised CPM, 2015]
<b>pest free production site</b>	A <b>production site</b> in which a specific <b>pest</b> is absent, as demonstrated by scientific evidence, and in which, where appropriate, this condition is being <b>officially</b> maintained for a defined period [ISPM 10, 1999; revised CPM, 2015]
<b>pest record</b>	A document providing information concerning the presence or absence of a specific <b>pest</b> at a particular location at a certain time, within an <b>area</b> (usually a country) under described circumstances [CEPM, 1997]
<b>pest risk (for quarantine pests)</b>	The probability of <b>introduction</b> and <b>spread</b> of a <b>pest</b> and the magnitude of the associated potential economic consequences [ISPM 2, 2007; revised CPM, 2013]
<b>pest risk (for regulated non-quarantine pests)</b>	The probability that a <b>pest</b> in <b>plants for planting</b> affects the <b>intended use</b> of those <b>plants</b> with an economically unacceptable impact [ISPM 2, 2007; revised CPM, 2013]
<b>pest risk analysis (agreed interpretation)</b>	The process of evaluating biological or other scientific and economic evidence to determine whether an <b>organism</b> is a <b>pest</b> , whether it should be regulated, and the strength of any <b>phytosanitary measures</b> to be taken against it [FAO, 1995; revised IPPC, 1997; ISPM 2, 2007]
<b>pest risk assessment (for quarantine pests)</b>	Evaluation of the probability of the <b>introduction</b> and <b>spread</b> of a <b>pest</b> and the magnitude of the associated potential economic consequences [FAO, 1995; revised ISPM 11, 2001; ISPM 2, 2007 ; revised CPM, 2013]
<b>pest risk assessment (for regulated non-quarantine pests)</b>	Evaluation of the probability that a <b>pest</b> in <b>plants for planting</b> affects the <b>intended use</b> of those <b>plants</b> with an economically unacceptable impact [ICPM, 2005; revised CPM, 2013]
<b>pest risk management (for quarantine pests)</b>	Evaluation and selection of options to reduce the risk of <b>introduction</b> and <b>spread</b> of a <b>pest</b> [FAO, 1995; revised ISPM 11, 2001]
<b>pest risk management (for regulated non-quarantine pests)</b>	Evaluation and selection of options to reduce the risk that a <b>pest</b> in <b>plants for planting</b> causes an economically unacceptable impact on the <b>intended use</b> of those <b>plants</b> [ICPM, 2005; revised CPM, 2013]
<b>pest status (in an area)</b>	Presence or absence, at the present time, of a <b>pest</b> in an <b>area</b> , including where appropriate its distribution, as <b>officially</b> determined using expert judgement on the basis of current and historical <b>pest records</b> and other information [CEPM, 1997; revised ICPM, 1998]
<b>PFA</b>	<b>Pest free area</b> [FAO, 1995; revised ICPM, 2001]
<b>phytosanitary action</b>	An <b>official</b> operation, such as <b>inspection</b> , <b>testing</b> , <b>surveillance</b> or <b>treatment</b> , undertaken to implement <b>phytosanitary measures</b> [ICPM, 2001; revised ICPM, 2005]

<b>phytosanitary certificate</b>	An <b>official</b> paper document or its <b>official</b> electronic equivalent, consistent with the model certificates of the <b>IPPC</b> , attesting that a <b>consignment</b> meets <b>phytosanitary import requirements</b> [FAO, 1990; revised CPM, 2012]
<b>phytosanitary certification</b>	Use of <b>phytosanitary procedures</b> leading to the issue of a <b>phytosanitary certificate</b> [FAO, 1990]
<b>phytosanitary import requirements</b>	Specific <b>phytosanitary measures</b> established by an importing country concerning <b>consignments</b> moving into that country [ICPM, 2005]
<b>phytosanitary legislation</b>	Basic laws granting legal authority to a <b>national plant protection organization</b> from which <b>phytosanitary regulations</b> may be drafted [FAO, 1990; revised FAO, 1995]
<b>phytosanitary measure</b> (agreed interpretation)	Any <b>legislation, regulation</b> or <b>official</b> procedure having the purpose to prevent the <b>introduction</b> or <b>spread</b> of <b>quarantine pests</b> , or to limit the economic impact of <b>regulated non-quarantine pests</b> [FAO, 1995; revised IPPC, 1997; ICPM, 2002; revised CPM, 2013]
<i>The agreed interpretation of the term phytosanitary measure accounts for the relationship of phytosanitary measures to regulated non-quarantine pests. This relationship is not adequately reflected in the definition found in Article II of the IPPC (1997).</i>	
<b>phytosanitary procedure</b>	Any <b>official</b> method for implementing <b>phytosanitary measures</b> including the performance of <b>inspections, tests, surveillance</b> or <b>treatments</b> in connection with <b>regulated pests</b> [FAO, 1990; revised FAO, 1995; revised CEPM, 1999; revised ICPM, 2001; revised ICPM, 2005]
<b>phytosanitary regulation</b>	<b>Official</b> rule to prevent the <b>introduction</b> or <b>spread</b> of <b>quarantine pests</b> , or to limit the economic impact of <b>regulated non-quarantine pests</b> , including establishment of procedures for <b>phytosanitary certification</b> [FAO, 1990; revised FAO, 1995; CEPM, 1999; ICPM, 2001; revised CPM, 2013]
<b>phytosanitary security</b> (of a <b>consignment</b> )*	Maintenance of the <b>integrity</b> of a <b>consignment</b> and prevention of its <b>infestation</b> and <b>contamination</b> by <b>regulated pests</b> , through the application of appropriate <b>phytosanitary measures</b> [CPM, 2009]
<b>place of production</b>	Any premises or collection of <b>fields</b> operated as a single production or farming unit. [FAO, 1990; revised CEPM, 1999; revised CPM, 2015]
<b>plant products</b>	Unmanufactured material of <b>plant</b> origin (including <b>grain</b> ) and those manufactured products that, by their nature or that of their processing, may create a risk for the <b>introduction</b> and <b>spread</b> of pests [FAO, 1990; revised IPPC, 1997; formerly “plant product”]
<b>plant protection organization (national)</b>	See <b>national plant protection organization</b>
<b>plant quarantine</b>	All activities designed to prevent the <b>introduction</b> or <b>spread</b> of <b>quarantine pests</b> or to ensure their <b>official control</b> [FAO, 1990; revised FAO, 1995; revised CPM, 2013]

<b>planting</b> (including <b>replanting</b> )	Any operation for the placing of <b>plants</b> in a <b>growing medium</b> , or by grafting or similar operations, to ensure their subsequent growth, reproduction or propagation [FAO, 1990; revised CEPM, 1999]
<b>plants</b>	Living plants and parts thereof, including <b>seeds</b> and <b>germplasm</b> [FAO, 1990; revised IPPC, 1997]
<b>plants for planting</b>	<b>Plants</b> intended to remain <b>planted</b> , to be <b>planted</b> or <b>replanted</b> [FAO, 1990]
<b>plants <i>in vitro</i></b> (as a <b>commodity class</b> )	<b>Plants</b> growing in an aseptic medium in a closed container [FAO, 1990; revised CEPM, 1999; ICPM, 2002; formerly “plants in tissue culture”; revised CPM, 2015]
<b>point of entry</b>	Airport, seaport, land border point or any other location <b>officially</b> designated for the importation of <b>consignments</b> , or the entrance of persons [FAO, 1995; revised CPM, 2015]
<b>post-entry quarantine</b>	<b>Quarantine</b> applied to a <b>consignment</b> after <b>entry</b> [FAO, 1995]
<b>PRA</b>	<b>Pest risk analysis</b> [FAO, 1995; revised ICPM, 2001]
<b>PRA area</b>	<b>Area</b> in relation to which a <b>pest risk analysis</b> is conducted [FAO, 1995]
<b>practically free*</b>	Of a <b>consignment</b> , <b>field</b> , or <b>place of production</b> , without <b>pests</b> (or a specific <b>pest</b> ) in numbers or quantities in excess of those that can be expected to result from, and be consistent with good cultural and handling practices employed in the production and marketing of the <b>commodity</b> [FAO, 1990; revised FAO, 1995]
<b>pre-clearance*</b>	<b>Phytosanitary certification</b> and/or <b>clearance</b> in the <b>country of origin</b> , performed by or under the regular supervision of the <b>national plant protection organization</b> of the country of destination [FAO, 1990; revised FAO, 1995]
<b>predator</b>	A <b>natural enemy</b> that preys and feeds on other animal <b>organisms</b> , more than one of which are killed during its lifetime [ISPM 3, 1995]
<b>process load</b>	A volume of material with a specified loading configuration and treated as a single entity [ISPM 18, 2003]
<b>processed wood material</b>	Products that are a composite of <b>wood</b> constructed using glue, heat and pressure, or any combination thereof [ISPM 15, 2002]
<b>production site</b>	A defined part of a <b>place of production</b> , that is managed as a separate unit for phytosanitary purposes [CPM, 2015]
<b>prohibition</b>	A <b>phytosanitary regulation</b> forbidding the importation or movement of specified <b>pests</b> or <b>commodities</b> [FAO, 1990; revised FAO, 1995]
<b>provisional measure</b>	A <b>phytosanitary regulation</b> or procedure established without full <b>technical justification</b> owing to current lack of adequate information. A <b>provisional measure</b> is subjected to periodic review and full technical justification as soon as possible [ICPM, 2001]

<b>quarantine*</b>	<b>Official</b> confinement of <b>regulated articles</b> for observation and research or for further <b>inspection, testing</b> or <b>treatment</b> [FAO, 1990; revised FAO, 1995; CEPM, 1999]
<b>quarantine area*</b>	An <b>area</b> within which a <b>quarantine pest</b> is present and is being <b>officially controlled</b> [FAO, 1990; revised FAO, 1995]
<b>quarantine pest</b>	A <b>pest</b> of potential economic importance to the <b>area endangered</b> thereby and not yet present there, or present but not widely distributed and being <b>officially controlled</b> [FAO, 1990; revised FAO, 1995; IPPC 1997]
<b>quarantine station</b>	<b>Official</b> station for holding <b>plants</b> or <b>plant products</b> or other <b>regulated articles</b> , including beneficial organisms, in <b>quarantine</b> [FAO, 1990; revised FAO, 1995; formerly quarantine station or facility; revised CPM, 2015]
<b>raw wood</b>	<b>Wood</b> which has not undergone processing or <b>treatment</b> [ISPM 15, 2002]
<b>re-exported consignment</b>	<b>Consignment</b> that has been imported into a country from which it is then exported. The <b>consignment</b> may be stored, split up, combined with other <b>consignments</b> or have its <b>packaging</b> changed [FAO, 1990; revised CEPM, 1996; CEPM, 1999; ICPM, 2001; ICPM, 2002; formerly country of re-export]
<b>reference specimen</b>	Specimen, from a population of a specific <b>organism</b> , conserved and accessible for the purpose of identification, verification or comparison. [ISPM 3, 2005; revised CPM, 2009]
<b>refusal</b>	Forbidding <b>entry</b> of a <b>consignment</b> or other <b>regulated article</b> when it fails to comply with <b>phytosanitary regulations</b> [FAO, 1990; revised FAO, 1995]
<b>regional plant protection organization</b>	An intergovernmental organization with the functions laid down by Article IX of the <b>IPPC</b> [FAO, 1990; revised FAO, 1995; CEPM, 1999; formerly “plant protection organization (regional)”]
<b>regional standards</b>	<b>Standards</b> established by a <b>regional plant protection organization</b> for the guidance of the members of that organization [IPPC, 1997]
<b>regulated area</b>	An <b>area</b> into which, within which or from which <b>plants, plant products</b> and other <b>regulated articles</b> are subjected to <b>phytosanitary measures</b> [CEPM, 1996; revised CEPM, 1999; ICPM, 2001; revised CPM, 2013]
<b>regulated article</b>	Any <b>plant, plant product</b> , storage place, <b>packaging</b> , conveyance, container, soil and any other <b>organism</b> , object or material capable of harbouring or spreading <b>pests</b> , deemed to require <b>phytosanitary measures</b> , particularly where international transportation is involved [FAO, 1990; revised FAO, 1995; IPPC, 1997]
<b>regulated non-quarantine pest</b>	A <b>non-quarantine pest</b> whose presence in <b>plants for planting</b> affects the <b>intended use</b> of those <b>plants</b> with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party [IPPC, 1997; revised CPM, 2013]

<b>regulated pest</b>	A <b>quarantine pest</b> or a <b>regulated non-quarantine pest</b> [IPPC, 1997]
<b>release</b> (into the environment)	Intentional liberation of an <b>organism</b> into the environment [ISPM 3, 1995; revised CPM, 2013]
<b>release</b> (of a consignment)	Authorization for <b>entry</b> after <b>clearance</b> [FAO, 1995]
<b>replanting</b>	See <b>planting</b>
<b>required response</b>	A specified level of effect for a <b>treatment</b> [ISPM 18, 2003]
<b>RNQP</b>	<b>Regulated non-quarantine pest</b> [ISPM 16, 2002]
<b>round wood</b>	<b>Wood</b> not sawn longitudinally, carrying its natural rounded surface, with or without <b>bark</b> [FAO, 1990]
<b>RPPO</b>	<b>Regional plant protection organization</b> [FAO, 1990; revised ICPM, 2001]
<b>sawn wood</b>	<b>Wood</b> sawn longitudinally, with or without its natural rounded surface with or without <b>bark</b> [FAO, 1990]
<b>Secretary</b>	<b>Secretary</b> of the <b>Commission</b> appointed pursuant to Article XII [IPPC, 1997]
<b>seeds</b> (as a <b>commodity class</b> )*	Seeds for <b>planting</b> or intended for planting and not for consumption or processing (see <b>grain</b> ) [FAO, 1990; revised ICPM, 2001; revised CPM, 2015]
<b>SIT</b>	<b>sterile insect technique</b> [ISPM 3, 2005]
<b>spread</b> (of a <b>pest</b> )	Expansion of the geographical distribution of a <b>pest</b> within an <b>area</b> [FAO, 1995]
<b>standard</b>	Document established by consensus and approved by a recognized body, that provides, for common and repeated use, rules, guidelines or characteristics for activities or their results, aimed at the achievement of the optimum degree of order in a given context [FAO, 1995; ISO/IEC Guide 2:1991 definition]
<b>sterile insect</b>	An insect that, as a result of a specific treatment, is unable to reproduce [ISPM 3, 2005]
<b>sterile insect technique</b>	Method of <b>pest control</b> using area-wide <b>inundative release</b> of <b>sterile insects</b> to reduce reproduction in a field population of the same species [ISPM 3, 2005]
<b>stored product</b>	Unmanufactured <b>plant product</b> intended for consumption or processing, stored in a dried form (this includes in particular <b>grain</b> and dried <b>fruits and vegetables</b> ) [FAO, 1990]
<b>suppression</b> *	The application of <b>phytosanitary measures</b> in an infested <b>area</b> to reduce <b>pest</b> populations [FAO, 1995; revised CEPM, 1999]

<b>surveillance</b>	An <b>official</b> process which collects and records data on <b>pest</b> presence or absence by <b>survey</b> , <b>monitoring</b> or other procedures [CEPM, 1996; revised CPM, 2015]
<b>survey*</b>	An <b>official</b> procedure conducted over a defined period of time to determine the characteristics of a <b>pest</b> population or to determine which species are present in an <b>area</b> [FAO, 1990; revised CEPM, 1996; revised CPM, 2015]
<b>systems approach</b>	A <b>pest risk management</b> option that integrates different measures, at least two of which act independently, with cumulative effect [ISPM 14, 2002; revised ICPM, 2005; revised CPM, 2015]
<b>technically justified</b>	Justified on the basis of conclusions reached by using an appropriate <b>pest risk analysis</b> or, where applicable, another comparable examination and evaluation of available scientific information [IPPC, 1997]
<b>test*</b>	<b>Official</b> examination, other than visual, to determine if <b>pests</b> are present or to identify <b>pests</b> [FAO, 1990]
<b>tolerance level (of a pest)</b>	<b>Incidence</b> of a <b>pest</b> specified as a threshold for action to <b>control</b> that <b>pest</b> or to prevent its <b>spread</b> or <b>introduction</b> [CPM, 2009]
<b>transience</b>	Presence of a <b>pest</b> that is not expected to lead to <b>establishment</b> [ISPM 8, 1998]
<b>transit</b>	See <b>consignment in transit</b>
<b>transparency</b>	The principle of making available, at the international level, <b>phytosanitary measures</b> and their rationale [FAO, 1995; revised CEPM, 1999; based on the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (WTO, 1994)]
<b>treatment</b>	<b>Official</b> procedure for the killing, <b>inactivation</b> or removal of <b>pests</b> , or for rendering <b>pests</b> infertile or for <b>devitalization</b> [FAO, 1990, revised FAO, 1995; ISPM 15, 2002; ISPM 18, 2003; ICPM, 2005]
<b>treatment schedule</b>	The critical parameters of a <b>treatment</b> which need to be met to achieve the intended outcome (i.e. the killing, <b>inactivation</b> or removal of <b>pests</b> , or rendering <b>pests</b> infertile, or <b>devitalization</b> ) at a stated <b>efficacy</b> [ISPM 28, 2007]
<b>visual examination*</b>	The physical examination of <b>plants</b> , <b>plant products</b> , or other <b>regulated articles</b> using the unaided eye, lens, stereoscope or microscope to detect <b>pests</b> or <b>contaminants</b> without <b>testing</b> or processing [ISPM 23, 2005]
<b>wood (as a commodity class)*</b>	<b>Round wood</b> , <b>sawn wood</b> , wood chips or <b>dunnage</b> , with or without <b>bark</b> [FAO, 1990; revised ICPM, 2001; revised CPM, 2015]
<b>wood packaging material</b>	<b>Wood</b> or wood products (excluding paper products) used in supporting, protecting or carrying a <b>commodity</b> (includes <b>dunnage</b> ) [ISPM 15, 2002]

This supplement was first adopted by the Third Session of the Interim Commission on Phytosanitary Measures in April 2001.

The first revision of this supplement was adopted by the Seventh Session of the

Commission on Phytosanitary Measures in March 2012.

The supplement is a prescriptive part of the standard.

## **SUPPLEMENT 1: Guidelines on the interpretation and application of the concepts of “official control” and “not widely distributed”**

### **INTRODUCTION**

#### **Scope**

This supplement provides guidance on:

- the official control of regulated pests, and
- determination of when a pest is considered to be present but not widely distributed, for the decision on whether a pest qualifies as a quarantine pest.

#### **References**

The present standard refers to ISPMs. ISPMs are available on the International Phytosanitary Portal (IPP – [www.IPPC.int](http://www.IPPC.int)).

#### **Definition**

Official control is defined as:

The active enforcement of mandatory phytosanitary regulations and the application of mandatory phytosanitary procedures with the objective of eradication or containment of quarantine pests or for the management of regulated non-quarantine pests.

### **BACKGROUND**

The words “present but not widely distributed and being officially controlled” express an essential concept in the definition of quarantine pest. According to that definition, a quarantine pest must always be of potential economic importance to an endangered area. In addition, it must either meet the criterion of not being present in that area or it must meet the combined criteria of being present but not widely distributed and subject to official control.

The *Glossary of phytosanitary terms* defines official as “established, authorized or performed by an NPPO” and control as “suppression, containment or eradication of a pest population”. However, for phytosanitary purposes, the concept of *official control* is not adequately expressed by the combination of these two definitions.

The purpose of this supplement is to describe more precisely the interpretation of:

- the concept of official control and its application in practice for quarantine pests that are present in an area as well as for regulated non-quarantine pests, and
- the concept of “present but not widely distributed and under official control” for quarantine pests.

“Not widely distributed” is not a term included in the description of pest status listed in ISPM 8.

## REQUIREMENTS

### 1. General Requirements

Official control is subject to ISPM 1, in particular the principles of non-discrimination, transparency, equivalence of phytosanitary measures and pest risk analysis.

#### 1.1 Official control

Official control includes:

- eradication and/or containment in the infested area(s)
- surveillance in the endangered area(s)
- restrictions related to the movement into and within the protected area(s) including phytosanitary measures applied at import.

All official control programmes have elements that are mandatory. At minimum, programme evaluation and pest surveillance are required in official control programmes to determine the need for and effect of control to justify phytosanitary measures applied at import for the same purpose. Phytosanitary measures applied at import should be consistent with the principle of non-discrimination (see section 2.2 below).

For quarantine pests, eradication and containment may have an element of suppression. For regulated non-quarantine pests, suppression may be used to avoid unacceptable economic impact as it applies to the intended use of plants for planting.

#### 1.2 Not widely distributed

“Not widely distributed” is a concept referring to a pest’s occurrence and distribution within an area. A pest may be categorized as present and widely distributed in an area or not widely distributed, or absent. In pest risk analysis (PRA), the determination of whether a pest is not widely distributed is carried out in the pest categorization step. Transience means that a pest is not expected to establish and therefore is not relevant to the concept of “not widely distributed”.

In the case of a quarantine pest that is present but not widely distributed, the importing country should define the infested area(s) and the endangered area(s). When a quarantine pest is considered not widely distributed, this means that the pest is limited to parts of its potential distribution and there are areas free from the pest that are at risk of economic loss from its introduction or spread. These endangered areas do not need to be contiguous but may consist of several distinct parts. In order to justify the statement of a pest being not widely distributed, a description and delimitation of the endangered areas should be made available if requested. There is a degree of uncertainty attached to any categorization of distribution. The categorization may also change over time.

The area in which the pest is not widely distributed should be the same as the area for which the economic impact applies (i.e. the endangered area) and where the pest is under or being considered for official control. The decision that a pest is a quarantine pest, including consideration of its distribution, and placing that pest under official control, is typically made with respect to an entire country. However, in some instances it may be more appropriate to regulate a pest as a quarantine pest in parts of a country rather than in the whole country. It is the potential economic importance of the pest for those parts that has to be considered in determining phytosanitary measures. Examples of when this may be appropriate are countries whose territories include one or more islands or other cases where there are natural or artificially created barriers to pest establishment and spread, such as large countries in which specified crops are restricted by climate to well-defined areas.

#### 1.3 Decision to apply official control

A national plant protection organization (NPPO) may choose whether or not to officially control a pest of potential economic importance that is present but not widely distributed, taking into account

relevant factors from PRA, for example the costs and benefits of regulating the specific pest, and the technical and logistical ability to control the pest within the defined area. If the pest is not subjected to official control, it does not then qualify as a quarantine pest.

## **2. Specific Requirements**

The specific requirements to be met relate to pest risk analysis, technical justification, non-discrimination, transparency, enforcement, mandatory nature of official control, area of application, and NPPO authority and involvement in official control.

### **2.1 Technical justification**

Domestic requirements and phytosanitary import requirements should be technically justified and result in non-discriminatory phytosanitary measures.

Application of the definition of a quarantine pest requires knowledge of potential economic importance, potential distribution and official control programmes (ISPM 2). The categorization of a pest as present and widely distributed or present but not widely distributed is determined in relation to its potential distribution. This potential distribution represents the areas where the pest could become established if given the opportunity, i.e. its hosts are present and environmental factors such as climate and soil are favourable. ISPM 11 provides guidance on the factors to be considered in assessing the probability of establishment and spread when conducting a pest risk analysis. In the case of a pest that is present but not widely distributed, the assessment of potential economic importance should relate to the areas where the pest is not established.

Surveillance should be used to determine the distribution of a pest in an area as a basis for the further consideration of whether the pest is not widely distributed. ISPM 6 provides guidance on surveillance, and includes provisions on transparency. Biological factors such as pest life cycle, means of dispersal and rate of reproduction may influence the design of surveillance programmes, the interpretation of survey data and the level of confidence in the categorization of a pest as not widely distributed. The distribution of a pest in an area is not a static condition. Changing conditions or new information may necessitate reconsideration of whether a pest is not widely distributed.

### **2.2 Non-discrimination**

The principle of non-discrimination between domestic requirements and phytosanitary import requirements is fundamental. In particular, requirements for imports should not be more stringent than the effect of official control in an importing country. There should therefore be consistency between domestic requirements and phytosanitary import requirements for a defined pest:

- Import requirements should not be more stringent than domestic requirements.
- Domestic and import requirements should be the same or have an equivalent effect.
- Mandatory elements of domestic and import requirements should be the same.
- The intensity of inspection of imported consignments should be the same as equivalent processes in domestic control programmes.
- In the case of non-compliance, the same or equivalent phytosanitary actions should be taken on imported consignments as are taken domestically.
- If a tolerance level is applied within a domestic official control programme, the same tolerance level should be applied to equivalent imported material. In particular, if no action is taken in the domestic official control programme because the pest incidence does not exceed the tolerance level concerned, then no action should be taken for an imported consignment if the pest incidence does not exceed that same tolerance level. Compliance with import tolerance levels is generally determined by inspection or testing at entry, whereas compliance with the tolerance level for domestic consignments should be determined at the last point where official control is applied.

- If downgrading or reclassifying is permitted within a domestic official control programme, similar options should be available for imported consignments.

### **2.3 Transparency**

Domestic requirements for official control and the phytosanitary import requirements should be documented and made available, on request.

### **2.4 Enforcement**

The domestic enforcement of official control programmes should be equivalent to the enforcement of phytosanitary import requirements. Enforcement should include:

- a legal basis
- operational implementation
- evaluation and review
- phytosanitary action in the case of non-compliance.

### **2.5 Mandatory nature of official control**

Official control is mandatory in the sense that all persons involved are legally bound to perform the actions required. The scope of official control programmes for quarantine pests is completely mandatory (e.g. procedures for eradication campaigns), whereas the scope for regulated non-quarantine pests is mandatory only in certain circumstances (e.g. official certification programmes).

### **2.6 Area of application**

An official control programme can be applied at national, subnational or local area level. The area of application of official control measures should be specified. Any phytosanitary import requirements should have the same effect as the domestic requirements for official control.

### **2.7 NPPO authority and involvement in official control**

Official control should:

- be established or recognized by the contracting party or the NPPO under appropriate legislative authority
- be performed, managed, supervised or, at minimum, audited/reviewed by the NPPO
- have enforcement assured by the contracting party or the NPPO
- be modified, terminated or lose official recognition by the contracting party or the NPPO.

Responsibility and accountability for official control programmes rests with the contracting party. Agencies other than the NPPO may be responsible for aspects of official control programmes, and certain aspects of official control programmes may be the responsibility of subnational authorities or the private sector. The NPPO should be fully aware of all aspects of official control programmes in its country.

This supplement was adopted by the Fifth Session of the Interim Commission on Phytosanitary Measures in April 2003.

The supplement is a prescriptive part of the standard.

## **SUPPLEMENT 2: Guidelines on the understanding of *potential economic importance* and related terms including reference to environmental considerations**

### **1. Purpose and Scope**

These guidelines provide the background and other relevant information to clarify *potential economic importance* and related terms, so that such terms are clearly understood and their application is consistent with the International Plant Protection Convention (IPPC) and the International Standards for Phytosanitary Measures (ISPMs). These guidelines also show the application of certain economic principles as they relate to the IPPC's objectives, in particular in protecting uncultivated/unmanaged plants, wild flora, habitats and ecosystems with respect to invasive alien species that are pests.

These guidelines clarify that the IPPC:

- can account for environmental concerns in economic terms using monetary or non-monetary values
- asserts that market impacts are not the sole indicator of pest impact
- maintains the right of contracting parties to adopt phytosanitary measures with respect to pests for which the economic damage caused to plants, plant products or ecosystems within an area cannot be easily quantified.

They also clarify, with respect to pests, that the scope of the IPPC covers the protection of cultivated plants in agriculture, horticulture and forestry, uncultivated/unmanaged plants, wild flora, habitats and ecosystems.

### **2. Background**

The IPPC has historically maintained that the adverse consequences of pests, including those concerning uncultivated/unmanaged plants, wild flora, habitats and ecosystems, are measured in economic terms. References to the terms *economic effects*, *economic impacts*, *potential economic importance* and *economically unacceptable impact* and the use of the word *economic* in the IPPC and in ISPMs has resulted in some misunderstanding of the application of such terms and of the focus of the IPPC.

The scope of the Convention applies to the protection of wild flora resulting in an important contribution to the conservation of biological diversity. However, it has been misinterpreted that the IPPC is only commercially focused and limited in scope. It has not been clearly understood that the IPPC can account for environmental concerns in economic terms. This has created issues of consistency with other agreements, including the Convention on Biological Diversity and the Montreal Protocol on Substances that Deplete the Ozone Layer.

### **3. Economic Terms and Environmental Scope of the IPPC and ISPMs**

The economic terms found in the IPPC and ISPMs may be categorized as follows.

Terms requiring judgement to support policy decisions:

- potential economic importance (in the definition for quarantine pest)
- economically unacceptable impact (in the definition for regulated non-quarantine pest)
- economically important loss (in the definition for endangered area).

Terms related to evidence that supports the above judgements:

- limit the economic impact (in the definition for phytosanitary regulation and the agreed interpretation of phytosanitary measure)
- economic evidence (in the definition for pest risk analysis)

- *cause economic damage* (in Article VII.3 of the IPPC, 1997)
- direct and indirect *economic impacts* (in ISPM 11 and ISPM 16)
- economic consequences and potential economic consequences (in ISPM 11)
- commercial consequences and non-commercial consequences (in ISPM 11).

ISPM 11 notes in section 2.1.1.5 with respect to pest categorization, that there should be a clear indication that the pest is likely to have an unacceptable economic impact, including environmental impact, in the PRA area. Section 2.3 of the standard describes the procedure for assessing potential economic consequences of a pest introduction. Pest effects may be considered to be direct or indirect. Section 2.3.2.2 addresses analysis of commercial consequences. Section 2.3.2.4 provides guidance on the assessment of the non-commercial and environmental consequences of pest introduction. It acknowledges that certain types of effects may not apply to an existing market that can be easily identified, but it goes on to state that the impacts could be approximated with an appropriate non-market valuation method. This section notes that if a quantitative measurement is not feasible, then this part of the assessment should at least include a qualitative analysis and an explanation of how the information is used in the PRA. Environmental or other undesirable effects of control measures are covered in section 2.3.1.2 (Indirect pest effects) as part of the analysis of potential economic consequences. Where a pest risk is found to be unacceptable, section 3.4 provides guidance on the selection of pest risk management options, including measurements of cost-effectiveness, feasibility and least trade restrictiveness.

In April 2001 the ICPM recognized that under the IPPC's existing mandate, to take account of environmental concerns, further clarification should include consideration of the following five proposed points relating to potential environmental risks of pests:

- reduction or elimination of endangered (or threatened) native plant species
- reduction or elimination of a keystone plant species (a species which plays a major role in the maintenance of an ecosystem)
- reduction or elimination of a plant species which is a major component of a native ecosystem
- causing a change to plant biological diversity in such a way as to result in ecosystem destabilization
- resulting in control, eradication or management programmes that would be needed if a quarantine pest was introduced, and impacts of such programmes (e.g. pesticides, non-indigenous predators or parasites) on biological diversity.

Thus it is clear, with respect to plant pests, that the scope of the IPPC covers the protection of cultivated plants in agriculture, horticulture and forestry, uncultivated/unmanaged plants, wild flora, habitats and ecosystems.

## **4. Economic Considerations in PRA**

### **4.1 Types of economic effect**

In PRA, economic effects should not be interpreted to be only market effects. Goods and services not sold in commercial markets can have economic value, and economic analysis encompasses much more than the study of market goods and services. The use of the term *economic effects* provides a framework in which a wide variety of effects (including environmental and social effects) may be analysed. Economic analysis uses a monetary value as a measure to allow policy makers to compare costs and benefits from different types of goods and services. This does not preclude the use of other tools such as qualitative and environmental analyses that may not use monetary terms.

### **4.2 Costs and benefits**

A general economic test for any policy is to pursue the policy if its benefit is at least as large as its cost. Costs and benefits are broadly understood to include both market and non-market aspects. Costs and benefits can be represented by both quantifiable measurements and qualitative measurements.

Non-market goods and services may be difficult to quantify or measure but nevertheless are essential to consider.

Economic analysis for phytosanitary purposes can only provide information with regard to costs and benefits, and does not judge if one distribution is necessarily better than another distribution of costs and benefits of a specific policy. In principle, costs and benefits should be measured regardless to whom they occur. Given that judgements about the preferred distribution of costs and benefits are policy choices, these should have a rational relationship to phytosanitary considerations.

Costs and benefits should be counted whether they occur as a direct or indirect result of a pest introduction or if a chain of causation is required before the costs are incurred or the benefits realized. Costs and benefits associated with indirect consequences of pest introductions may be less certain than costs and benefits associated with direct consequences. Often, there is no monetary information about the cost of any loss that may result from pests introduced into natural environments. Any analysis should identify and explain uncertainties involved in estimating costs and benefits and assumptions should be clearly stated.

## 5. Application

The following criteria<sup>1</sup> should be met before a pest is deemed to have *potential economic importance*:

- a potential for introduction in the PRA area
- the potential to spread after establishment
- a potential harmful impact on plants, for example:
  - crops (for example loss of yield or quality)
  - the environment, for example damage to ecosystems, habitats or species
  - some other specified value, for example recreation, tourism, aesthetics.

As stated in section 3, environmental damage, arising from the introduction of a pest, is one of the types of damage recognized by the IPPC. Thus, with respect to the third criterion above, contracting parties to the IPPC have the right to adopt phytosanitary measures even with respect to a pest that only has the potential for environmental damage. Such action should be based upon a pest risk analysis that includes the consideration of evidence of potential environmental damage. When indicating the direct and indirect impact of pests on the environment, the nature of the harm or losses arising from a pest introduction should be specified in pest risk analysis.

In the case of regulated non-quarantine pests, because such pest populations are already established, introduction in an area of concern and environmental effects are not relevant criteria in the consideration of *economically unacceptable impacts* (see ISPM 16 and ISPM 21).

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<sup>1</sup> With respect to the first and second criteria, IPPC (1997) Article VII.3 states that for pests that may not be capable of establishment, measures taken against these pests must be technically justified.

This appendix is for reference purposes only and is not a prescriptive part of the standard.

## APPENDIX TO SUPPLEMENT 2

This appendix provides additional clarification of some terms used in this supplement.

*Economic analysis:* It primarily uses monetary values as a measure to allow policy makers to compare costs and benefits from different types of goods and services. It encompasses more than the study of market goods and services. Economic analysis does not prevent the use of other measures that do not use a monetary value; for example, qualitative or environmental analysis.

*Economic effects:* This includes market effects as well as non-market effects, such as environmental and social considerations. Measurement of the economic value of environmental effects or social effects may be difficult to establish. For example, the survival and well-being of another species or the value of the aesthetics of a forest or a jungle. Both qualitative and quantitative worth may be considered in measuring economic effects.

*Economic impacts of plant pests:* This includes both market measures as well as those consequences that may not be easy to measure in direct economic terms, but which represent a loss or damage to cultivated plants, uncultivated plants or plant products.

*Economic value:* This is the basis for measuring the cost of the effect of changes (e.g. in biodiversity, ecosystems, managed resources or natural resources) on human welfare. Goods and services not sold in commercial markets can have economic value. Determining economic value does not prevent ethical or altruistic concerns for the survival and well-being of other species based on cooperative behaviour.

*Qualitative measurement:* This is the valuation of qualities or characteristics in other than monetary or numeric terms.

*Quantitative measurement:* This is the valuation of qualities or characteristics in monetary or other numeric terms.

This appendix was adopted by the Fourth Session of the Commission on Phytosanitary Measures in March–April 2009.

The appendix is for reference purposes only and is not a prescriptive part of the standard.

## **APPENDIX 1: Terminology of the Convention on Biological Diversity in relation to the Glossary of phytosanitary terms**

### **1. Introduction**

Since 2001, it has been made clear that the scope of the IPPC extends to risks arising from pests that primarily affect the environment and biological diversity, including harmful plants. The Technical Panel for the Glossary, which reviews ISPM 5 (*Glossary of phytosanitary terms*, hereinafter referred to as the Glossary), therefore examined the possibility of adding new terms and definitions to the standard to cover this area of concern. In particular, it considered the terms and definitions that are in use by the Convention on Biological Diversity (CBD)\*, with a view to adding them to the Glossary, as has previously been done in several cases for the terminology of other intergovernmental organizations.

However, study of the terms and definitions available from the CBD has shown that they are based on concepts different from those of the IPPC, so that similar terms are given distinctly different meanings. The CBD terms and definitions could not accordingly be used directly in the Glossary. It was decided instead to present these terms and definitions in the present Appendix to the Glossary, providing explanations of how they differ from IPPC terminology.

This Appendix is not intended to provide a clarification of the scope of the CBD, nor of the scope of the IPPC.

### **2. Presentation**

In relation to each term considered, the CBD definition is first provided. This is placed alongside an “Explanation in IPPC context”, in which, as usual, Glossary terms (or derived forms of Glossary terms) are shown in **bold**. These explanations may also include CBD terms, in which case these are also in **bold** and followed by “(CBD)”. The explanations constitute the main body of this Appendix. Each is followed by notes, providing further clarification of some of the difficulties.

### **3. Terminology**

#### **3.1 “Alien species”**

<i>CBD definition</i>	<i>Explanation in IPPC context</i>
A species, subspecies or lower taxon, introduced outside its natural past <sup>1</sup> or present distribution; includes any part, gametes, seeds, eggs, or propagules of such species that might survive and subsequently reproduce	An <b>alien<sup>2</sup> species (CBD)</b> is an individual <sup>3</sup> or population, at any life stage, or a viable part of an organism that is non-indigenous to an <b>area</b> and that has <b>entered<sup>4</sup></b> by human agency <sup>5</sup> into the <b>area</b>

*Notes:*

<sup>1</sup> The qualification concerning “past and present” distribution is not relevant for IPPC purposes, since the IPPC is concerned only with existing situations. It does not matter that the species was present in the past if it is present now. The word “past” in the CBD definition presumably allows for the re-introduction of a species into an area where it has recently become extinct and thus a reintroduced species would presumably not be considered an alien species.

\* The terms and definitions discussed in this document have resulted from discussion on invasive alien species by the Parties of the Convention on Biological Diversity (Secretariat of the Convention on Biological Diversity).

<sup>2</sup> “Alien” refers only to the location and distribution of an organism compared with its natural range. It does not imply that the organism is harmful.

<sup>3</sup> The CBD definition emphasizes the physical presence of individuals of a species at a certain time, whereas the IPPC concept of occurrence relates to the geographical distribution of the taxon in general.

<sup>4</sup> For CBD purposes, an alien species is already present in the **area** that is not within its native distribution (see **Introduction** below). The IPPC is more concerned with organisms that are not yet present in the area of concern (i.e. quarantine pests). The term “alien” is not appropriate for them, and terms such as “exotic”, “non-indigenous” or “non-native” have been used in ISPMs. To avoid confusion, it would be preferable to use only one of these terms, in which case “non-indigenous” would be suitable, especially as it can accompany its opposite “indigenous”. “Exotic” is not suitable because it presents translation problems.

<sup>5</sup> A species that is non-indigenous and has entered an **area** through natural means is not an **alien species (CBD)**. It is simply extending its natural range. For **IPPC** purposes, such a species could still be considered as a potential **quarantine pest**.

### 3.2 “Introduction”

<i>CBD definition</i>	<i>Explanation in IPPC context</i>
The movement by human agency, indirect or direct, of an alien species <sup>6</sup> outside of its natural range (past or present). This movement can be either within a country or between countries or areas beyond national jurisdiction <sup>7</sup>	The <b>entry</b> of a <b>species</b> into an <b>area where it is non-indigenous</b> , through movement by human agency, either directly from an area where the species is indigenous, or indirectly <sup>8</sup> (by successive movement from an area where the species is indigenous through one or several areas where it is not)

#### *Notes:*

<sup>6</sup> The CBD definition suggests that **introduction (CBD)** concerns an **alien species (CBD)**, and thus a species that has already entered the area. However, it may be supposed, on the basis of other documents made available by CBD, that this is not so, and that a non-indigenous species entering for the first time is being **introduced (CBD)**. For CBD, a species can be **introduced (CBD)** many times, but for IPPC a species, once established, cannot be **introduced** again.

<sup>7</sup> The issue of “areas beyond national jurisdiction” is not relevant for the IPPC.

<sup>8</sup> In the case of indirect movement, it is not specifically stated in the definition whether all the movements from one **area** to another must be **introductions (CBD)** (i.e. by human agency, intentional or unintentional), or whether some can be by natural movement. This question arises, for example, where a species is **introduced (CBD)** into one **area** and then moves naturally to an adjoining **area**. It seems that this may be considered as an indirect **introduction (CBD)**, so that the species concerned is an **alien species (CBD)** in the adjoining area, despite the fact that it **entered** it naturally. In the IPPC context, the intermediate country, from which the natural movement occurs, has no obligation to act to limit the natural movement, though it may have obligations to prevent intentional or unintentional **introduction (CBD)** if the importing country concerned establishes corresponding **phytosanitary measures**.

### 3.3 “Invasive alien species”

<i>CBD definition</i>	<i>Explanation in IPPC context</i>
An alien species whose introduction and/or spread threaten <sup>9</sup> biological diversity <sup>10, 11</sup>	An <b>invasive</b> <sup>12</sup> <b>alien species (CBD)</b> is an <b>alien species (CBD)</b> that by its <b>establishment</b> or <b>spread</b> has become injurious to <b>plants</b> <sup>13</sup> , or that by <b>risk analysis (CBD)</b> <sup>14</sup> is shown to be potentially injurious to <b>plants</b>

*Notes:*

<sup>9</sup> The word “threaten” does not have an immediate equivalent in IPPC language. The IPPC definition of a **pest** uses the term “injurious”, while the definition of a **quarantine pest** refers to “economic importance”. ISPM 11 makes it clear that **quarantine pests** may be “injurious” to **plants** directly, or indirectly (via other components of ecosystems), while Supplement 2 of the Glossary explains that “economic importance” depends on a harmful impact on crops, or on the environment, or on some other specific value (recreation, tourism, aesthetics).

<sup>10</sup> **Invasive alien species (CBD)** threaten “biological diversity”. This is not an IPPC term, and the question arises whether it has a scope corresponding to that of the IPPC. “Biological diversity” would then have to be given a wide meaning, extending to the integrity of cultivated plants in agro-ecosystems, non-indigenous **plants** that have been imported and **planted** for forestry, amenity or habitat management, and indigenous **plants** in any **habitat**, whether “man-made” or not. The **IPPC** does protect **plants** in any of these situations, but it is not clear whether the scope of the CBD is as wide; some definitions of “biological diversity” take a much narrower view.

<sup>11</sup> On the basis of other documents made available by CBD, **invasive alien species** may also threaten “ecosystems, habitats or species”.

<sup>12</sup> The CBD definition and its explanation concern the whole term **invasive alien species** and do not address the term “invasive” as such.

<sup>13</sup> The context of the IPPC is the protection of **plants**. It is clear that there are effects on biological diversity that do not concern **plants**, and so there are **invasive alien species (CBD)** that are not relevant to the **IPPC**. The IPPC is also concerned with **plant products**, but it is not clear to what extent the CBD considers **plant products** as a component of biological diversity.

<sup>14</sup> For the IPPC, organisms that have never entered the **endangered area** can also be considered as potentially injurious to **plants**, as a result of **pest risk analysis**.

### 3.4 “Establishment”

<i>CBD definition</i>	<i>Explanation in IPPC context</i>
The process <sup>15</sup> of an alien species in a new habitat successfully producing viable offspring <sup>16</sup> with a likelihood of continued survival	The <b>establishment</b> of an <b>alien species (CBD)</b> in a <b>habitat</b> in the <b>area</b> it has <b>entered</b> , by successful reproduction

*Notes:*

<sup>15</sup> **Establishment (CBD)** is a process, not a result. It seems that a single generation of reproduction can be **establishment (CBD)**, provided the offspring have a likelihood of continued survival (otherwise there would be a comma after “offspring”). The CBD definition does not express the **IPPC** concept of “perpetuation for the foreseeable future”.

<sup>16</sup> It is not clear how far “offspring” applies to organisms that propagate themselves vegetatively (many **plants**, most fungi, other micro-organisms). By using “perpetuation”, the **IPPC** avoids the

question of reproduction or replication of individuals altogether. It is the species as a whole that survives. Even the growth of long-lived individuals to maturity could be considered to be perpetuation for the foreseeable future (e.g. plantations of a non-indigenous **plant**).

### 3.5 “Intentional introduction”

<i>CBD definition</i>	<i>Explanation in IPPC context</i>
Deliberate movement and/or <sup>17</sup> release by humans of an alien species outside its natural range	Deliberate movement of a non-indigenous species into an <b>area</b> , including its <b>release</b> into the environment <sup>18</sup>

*Notes:*

<sup>17</sup> The “and/or” of the CBD definition is difficult to understand.

<sup>18</sup> Under most phytosanitary import regulatory systems the intentional introduction of regulated pests is prohibited.

### 3.6 “Unintentional introduction”

<i>CBD definition</i>	<i>Explanation in IPPC context</i>
All other introductions which are not intentional	<b>Entry</b> of a non-indigenous species with a traded <b>consignment</b> , which it <b>infests</b> or <b>contaminates</b> , or by some other human agency including <b>pathways</b> such as passengers’ baggage, vehicles, artificial waterways <sup>19</sup>

*Notes:*

<sup>19</sup> The prevention of unintentional introduction of regulated pests is an important focus of phytosanitary import regulatory systems.

### 3.7 “Risk analysis”

<i>CBD definition</i>	<i>Explanation in IPPC context</i>
1) the assessment of the consequences <sup>20</sup> of the introduction and of the likelihood of establishment of an alien species using science-based information (i.e., risk assessment), and 2) the identification of measures that can be implemented to reduce or manage these risks (i.e., risk management), taking into account socio-economic and cultural considerations <sup>21</sup>	<b>Risk analysis (CBD)</b> <sup>22</sup> is: 1) evaluation of the probability of <b>establishment</b> and <b>spread</b> , within an <b>area</b> <sup>23</sup> , of an <b>alien species (CBD)</b> that has entered that <b>area</b> , 2) evaluation of the associated potential undesirable consequences, and 3) evaluation and selection of measures to reduce the risk of such <b>establishment</b> and <b>spread</b>

*Notes:*

<sup>20</sup> It is not clear what kinds of consequences are considered.

<sup>21</sup> It is not clear at what stages in the process of **risk analysis (CBD)** socio-economic and cultural considerations are taken into account (during assessment, or during management, or both). No explanation can be offered in relation to ISPM 11 or Supplement 2 of ISPM 5.

<sup>22</sup> This explanation is based on the IPPC definitions of **pest risk assessment** and **pest risk management**, rather than on that of **pest risk analysis**.

<sup>23</sup> It is unclear whether **risk analysis (CBD)** may be conducted prior to **entry**, in which case the probability of **introduction** may also need to be assessed, and measures evaluated and selected to reduce the risk of **introduction**. It may be supposed (on the basis of other documents made available by CBD) that **risk analysis (CBD)** can identify measures restricting further introductions, in which case it relates more closely to **pest risk analysis**.

#### 4. Other concepts

The CBD does not propose definitions of other terms, but does use a number of concepts that do not seem to be considered in the same light by the IPPC and the CBD, or are not distinguished by the IPPC. These include:

- border controls
- quarantine measures
- burden of proof
- natural range or distribution
- precautionary approach
- provisional measures
- control
- statutory measures
- regulatory measures
- social impact
- economic impact.

#### 5. References

**CBD.** 1992. *Convention on Biological Diversity*. Montreal, CBD.

**CBD.** *Glossary of terms* (available at <http://www.cbd.int/invasive/terms.shtml>, accessed November 2008).



ISPM 28  
Annex 16

## INTERNATIONAL STANDARDS FOR PHYTOSANITARY MEASURES

### ISPM 28 PHYTOSANITARY TREATMENTS

#### PT 16

### Cold treatment for *Bactrocera tryoni* on *Citrus sinensis*

Adopted 2015; published 2015

#### Scope of the treatment

This treatment comprises the cold treatment of fruit of *Citrus sinensis* (orange) to result in the mortality of eggs and larvae of *Bactrocera tryoni* (Queensland fruit fly) at the stated efficacy<sup>1</sup>.

#### Treatment description

Name of treatment	Cold treatment for <i>Bactrocera tryoni</i> on <i>Citrus sinensis</i>
Active ingredient	N/A
Treatment type	Physical (cold)
Target pest	<i>Bactrocera tryoni</i> (Diptera: Tephritidae) (Queensland fruit fly)
Target regulated articles	Fruit of <i>Citrus sinensis</i> (orange)

#### Treatment schedule

##### 3 °C or below for 16 continuous days

For cultivar “Navel” the efficacy is effective dose (ED)<sub>99.9981</sub> at the 95% confidence level.

<sup>1</sup> The scope of phytosanitary treatments does not include issues related to pesticide registration or other domestic requirements for contracting parties' approval of treatments. IPPC adopted treatments may not provide information on specific effects on human health or food safety, which should be addressed using domestic procedures prior to contracting parties approving a treatment. In addition, potential effects of treatments on product quality are considered for some host commodities before their international adoption. However, evaluation of any effects of a treatment on the quality of commodities may require additional consideration. There is no obligation for a contracting party to approve, register or adopt the treatments for use in its territory.

For cultivar “Valencia” the efficacy is  $ED_{99,9973}$  at the 95% confidence level.

The fruit must reach the treatment temperature before treatment exposure time is started. The fruit temperature should be monitored and recorded, and the temperature should not exceed the stated level throughout the duration of the treatment.

### Other relevant information

In evaluating this treatment the Technical Panel on Phytosanitary Treatments (TPPT) considered issues associated with temperature regimes and thermal conditioning, taking into account the work of Hallman and Mangan (1997).

This schedule is based on the work of De Lima *et al.* (2007).

### References

- De Lima, C.P.F., Jessup, A.J., Cruickshank, L., Walsh, C.J. & Mansfield, E.R.** 2007. Cold disinfestation of citrus (*Citrus* spp.) for Mediterranean fruit fly (*Ceratitis capitata*) and Queensland fruit fly (*Bactrocera tryoni*) (Diptera: Tephritidae). *New Zealand Journal of Crop and Horticultural Science*, 35: 39–50.
- Hallman, G.J. & Mangan, R.L.** 1997. Concerns with temperature quarantine treatment research. In G.L. Obenauf, ed. *1997 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reduction*, San Diego, CA, USA, Nov. 3–5. pp. 79-1–79-4.

### Publication history

*This is not an official part of the standard*

2007-09 Treatment submitted in response to the Call for treatments

2007-12 TPPT meeting split Cold treatment of *Citrus sinensis* for *Bactrocera tryoni* from 2007-106 to create 2007-206E

2008-04 CPM-3 added subject under the topic Fruit fly treatments

2008-09 SC approved for member consultation via e-decision

2009-06 Sent for member consultation

2010-07 TPPT meeting revised the text and recommended to SC for CPM-7 (2012) adoption

2011-11 SC recommended to CPM for adoption

2012-03 Treatment received formal objection

2012-09 TPPT virtual meeting drafted response to formal objection (no revision recommended)

2012-12 TPPT meeting revised the text and recommended to SC for CPM adoption

2013-06 SC recommended to CPM-9 for adoption

2014-03 Treatment received formal objection

2014-06 TPPT meeting drafted response to formal objections and revised text

2014-11 SC reviewed TPPT response and approved draft for CPM adoption

2015-03 CPM-10 adopted the treatment

**ISPM 28. Annex 16** Cold treatment for *Bactrocera tryoni* on *Citrus sinensis* (2015). Rome, IPPC, FAO.

Publication history last modified: 2015-04



## INTERNATIONAL STANDARDS FOR PHYTOSANITARY MEASURES

### ISPM 28 PHYTOSANITARY TREATMENTS

#### PT 17

### Cold treatment for *Bactrocera tryoni* on *Citrus reticulata* x *C. sinensis*

Adopted 2015; published 2015

#### Scope of the treatment

This treatment comprises the cold treatment of fruit of *Citrus reticulata* × *Citrus sinensis*<sup>1</sup> (tangor) to result in the mortality of eggs and larvae of *Bactrocera tryoni* (Queensland fruit fly) at the stated efficacy<sup>2</sup>.

#### Treatment description

<b>Name of treatment</b>	Cold treatment for <i>Bactrocera tryoni</i> on <i>Citrus reticulata</i> × <i>Citrus sinensis</i>
<b>Active ingredient</b>	N/A
<b>Treatment type</b>	Physical (cold)
<b>Target pest</b>	<i>Bactrocera tryoni</i> (Diptera: Tephritidae) (Queensland fruit fly)
<b>Target regulated articles</b>	Fruit of <i>Citrus reticulata</i> × <i>Citrus sinensis</i> (tangor)

<sup>1</sup> *Citrus* species and hybrids are named according to the nomenclature in Cottin, R. 2002. *Citrus of the world: a citrus directory*. Montpellier, France, INRA-CIRAD.

<sup>2</sup> The scope of phytosanitary treatments does not include issues related to pesticide registration or other domestic requirements for contracting parties' approval of treatments. IPPC adopted treatments may not provide information on specific effects on human health or food safety, which should be addressed using domestic procedures prior to contracting parties approving a treatment. In addition, potential effects of treatments on product quality are considered for some host commodities before their international adoption. However, evaluation of any effects of a treatment on the quality of commodities may require additional consideration. There is no obligation for a contracting party to approve, register or adopt the treatments for use in its territory.

## Treatment schedule

### 3 °C or below for 16 continuous days

The efficacy is effective dose (ED)<sub>99,9986</sub> at the 95% confidence level.

The fruit must reach the treatment temperature before treatment exposure time is started. The fruit temperature should be monitored and recorded, and the temperature should not exceed the stated level throughout the duration of the treatment.

## Other relevant information

In evaluating this treatment the Technical Panel on Phytosanitary Treatments (TPPT) considered issues associated with temperature regimes and thermal conditioning, taking into account the work of Hallman and Mangan (1997).

This schedule is based on the work of De Lima *et al.* (2007) and developed using cultivars “Ellendale” and “Murcott”.

## References

- De Lima, C.P.F., Jessup, A.J., Cruickshank, L., Walsh, C.J. & Mansfield, E.R.** 2007. Cold disinfestation of citrus (*Citrus* spp.) for Mediterranean fruit fly (*Ceratitis capitata*) and Queensland fruit fly (*Bactrocera tryoni*) (Diptera: Tephritidae). *New Zealand Journal of Crop and Horticultural Science*, 35: 39–50.
- Hallman, G.J. & Mangan, R.L.** 1997. Concerns with temperature quarantine treatment research. In G.L. Obenauf, ed. *1997 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reduction*, San Diego, CA, USA, Nov. 3–5. pp. 79-1–79-4.

## Publication history

*This is not an official part of the standard*

2007-09 Treatment submitted in response to the Call for treatments

2007-12 TPPT meeting combined Cold treatment of *Citrus reticulata* x *C. sinensis* for *Bactrocera tryoni*. 2007-106 and 2007-206H to create 2007-206F

2008-04 CPM-3 added subject under the topic Fruit fly treatments

2008-09 SC approved for member consultation via e-decision

2009-06 Sent for member consultation

2010-07 TPPT meeting revised the text and recommended to SC for CPM-7 (2012) adoption

2011-11 SC recommended to CPM for adoption

2012-03 Treatment received formal objection

2012-09 TPPT virtual meeting drafted response to formal objections (no revision recommended)

2012-12 TPPT meeting revised the text and recommended to SC for CPM adoption

2013-06 SC recommended to CPM-9 for adoption

2014-03 Treatment received formal objection

2014-06 TPPT meeting drafted response to formal objections and revised text

2014-11 SC reviewed TPPT response and approved draft for CPM adoption

2015-03 CPM-10 adopted the treatment

**ISPM 28. Annex 17.** *Cold treatment for Bactrocera tryoni on Citrus reticulata* x *C. sinensis* (2015). Rome, IPPC, FAO.

Publication history last modified: 2015-04



**ISPM 28**  
**Annex 18**

## **INTERNATIONAL STANDARDS FOR PHYTOSANITARY MEASURES**

### **ISPM 28 PHYTOSANITARY TREATMENTS**

#### **PT 18**

### **Cold treatment for *Bactrocera tryoni* on *Citrus limon***

*Adopted 2015; published 2015*

#### **Scope of the treatment**

This treatment applies to the cold treatment of fruit of *Citrus limon* (lemon) to result in the mortality of eggs and larvae of *Bactrocera tryoni* (Queensland fruit fly) at the stated efficacy<sup>1</sup>.

#### **Treatment description**

<b>Name of treatment</b>	Cold treatment for <i>Bactrocera tryoni</i> on <i>Citrus limon</i>
<b>Active ingredient</b>	N/A
<b>Treatment type</b>	Physical (cold)
<b>Target pest</b>	<i>Bactrocera tryoni</i> (Diptera: Tephritidae) (Queensland fruit fly)
<b>Target regulated articles</b>	Fruit of <i>Citrus limon</i> (lemon)

#### **Treatment schedule**

##### **Schedule 1: 2 °C or below for 14 continuous days**

The efficacy is effective dose (ED)<sub>99,99</sub> at the 95% confidence level.

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<sup>1</sup> The scope of phytosanitary treatments does not include issues related to pesticide registration or other domestic requirements for contracting parties' approval of treatments. Treatments adopted by the CPM may not provide information on specific effects on human health or food safety, which should be addressed using domestic procedures prior to contracting parties approving a treatment. In addition, potential effects of treatments on product quality are considered for some host commodities before their international adoption. However, evaluation of any effects of a treatment on the quality of commodities may require additional consideration. There is no obligation for a contracting party to approve, register or adopt the treatments for use in its territory.

## Schedule 2: 3 °C or below for 14 continuous days

The efficacy is ED<sub>99,9872</sub> at the 95% confidence level.

The fruit must reach the treatment temperature before treatment commences. The fruit temperature should be monitored and recorded, and temperatures should not exceed the stated level throughout the duration of the treatment.

## Other relevant information

In evaluating this treatment the Technical Panel on Phytosanitary Treatments (TPPT) considered issues associated with temperature regimes and thermal conditioning, taking into account the work of Hallman and Mangan (1997).

Schedules 1 and 2 were based on the work of De Lima *et al.* (2007) and developed using cultivar “Lisbon”.

The TPPT also considered issues associated with chilling injury in lemons (TPPT, 2012).

## References

- De Lima, C.P.F., Jessup, A.J., Cruickshank, L., Walsh, C.J. & Mansfield, E.R.** 2007. Cold disinfestation of citrus (*Citrus* spp.) for Mediterranean fruit fly (*Ceratitis capitata*) and Queensland fruit fly (*Bactrocera tryoni*) (Diptera: Tephritidae). *New Zealand Journal of Crop and Horticultural Science*, 35: 39–50.
- Hallman, G.J. & Mangan, R.L.** 1997. Concerns with temperature quarantine treatment research. In G.L. Obenauf, ed. *1997 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reduction*, San Diego, CA, USA, Nov. 3–5. pp. 79-1–79-4.
- TPPT.** 2012. TPPT response to SC’s concerns about chilling injury in lemons during in-transit cold disinfestation. Appendix 9, TPPT meeting report, Dec. 2012, pp. 55–57.

## Publication history

*This is not an official part of the standard*

2007-09 Treatment submitted in response to the Call for treatments

2007-12 TPPT meeting split Cold treatment of *Citrus limon* for *Bactrocera tryoni* from 2007-106 to create 2007-206G

2008-04 CPM-3 added subject under the topic Fruit fly treatments

2008-09 SC approved for member consultation via e-decision

2009-06 Sent for member consultation

2010-07 TPPT meeting revised the text and recommended to SC for CPM-7 (2012) adoption

2011-11 SC commented by e-decision

2012-12 TPPT meeting finalized response to concern about chilling injury revised the text and recommended to SC for CPM adoption

2013-11 SC agreed to recommend the treatment for CPM for adoption

2014-03 Treatment received formal objection

2014-06 TPPT meeting drafted response to formal objection and revised text

2014-11 SC reviewed TPPT response and approved draft for CPM adoption

2015-03 CPM-10 adopted the treatment

**ISPM 28. Annex 18** Cold treatment for *Bactrocera tryoni* on *Citrus limon* (2015). Rome, IPPC, FAO.

Publication history last modified: 2015-04



## INTERNATIONAL STANDARDS FOR PHYTOSANITARY MEASURES

### ISPM 28 PHYTOSANITARY TREATMENTS

#### PT 19

### Irradiation treatment for *Dysmicoccus neobrevipes*, *Planococcus lilacinus* and *Planococcus minor*

Adopted 2015; published 2015

#### Scope of the treatment

This treatment describes the irradiation treatment of fruits and vegetables to prevent the reproduction of adult females of *Dysmicoccus neobrevipes*, *Planococcus lilacinus* and *Planococcus minor* at the stated efficacy level<sup>1</sup>.

#### Treatment description

<b>Name of treatment</b>	Irradiation treatment for <i>Dysmicoccus neobrevipes</i> , <i>Planococcus lilacinus</i> and <i>Planococcus minor</i>
<b>Active ingredient</b>	N/A
<b>Treatment type</b>	Irradiation
<b>Target pests</b>	<i>Dysmicoccus neobrevipes</i> Beardsley, <i>Planococcus lilacinus</i> (Cockerell) and <i>Planococcus minor</i> (Maskell) (Hemiptera: Pseudococcidae)
<b>Target regulated articles</b>	All fruits and vegetables that are hosts of the above mealybugs

#### Treatment schedule

Minimum absorbed dose of 231 Gy to prevent the reproduction of adult females of *Dysmicoccus neobrevipes*, *Planococcus lilacinus* and *Planococcus minor*.

Efficacy and confidence level of the treatment is ED<sub>99.99023</sub> at the 95% confidence level.

<sup>1</sup> The scope of phytosanitary treatments does not include issues related to pesticide registration or other domestic requirements for contracting parties' approval of treatments for use in their territory. Treatments adopted by the CPM may not provide information on specific effects on human health or food safety, which should be addressed using domestic procedures prior to contracting parties approving a treatment for use in its territory. In addition, potential effects of treatments on product quality are considered for some host commodities before their international adoption. However, evaluation of any effects of a treatment on the quality of commodities may require additional consideration. There is no obligation for a contracting party to approve, register or adopt the treatments for use in its territory.

This treatment should be applied in accordance with the requirements of ISPM 18 (*Guidelines for the use of irradiation as a phytosanitary measure*).

This irradiation treatment should not be applied to fruits and vegetables stored in modified atmospheres.

### Other relevant information

Because irradiation may not result in outright mortality, inspectors may encounter live but non-viable *Dysmicoccus neobrevipes* or *Planococcus lilacinus* or *Planococcus minor* (immatures or adults) during the inspection process. This does not imply a failure of the treatment.

This treatment schedule was based on the work of Doan *et al.* (2012). In this paper a minimum absorbed dose of 200 Gy prevented reproduction by adult females of *Dysmicoccus neobrevipes* and development to the next generation from all immature stages. A subsequent large scale confirmatory test showed that there was no reproduction at a maximum dose of 231 Gy. Further tests also showed that the other two species were more radio-susceptible than *Dysmicoccus neobrevipes*.

Very little data is available for other members of the Pseudococcidae and all papers are listed in the References. In each case a dose near to or less than 200 Gy was sufficient to ensure no reproduction providing additional confidence in the proposed dose.

### References

- Doan, T.T., Nguyen, T.K., Vo, T.K.L., Cao, V.C., Tran, T.T.A. & Nguyen, N.H.** 2012. Effects of gamma irradiation on different stages of mealybug *Dysmicoccus neobrevipes* (Hemiptera: Pseudococcidae). *Radiation Physics and Chemistry*, 81: 97–100 (with supplementary data provided by the submitter).
- Dohino, T. & Masaki, S.** 1995. Effects of electron beam irradiation on Comstock mealybug, *Pseudococcus comstocki* (Kuwana) (Homoptera: Pseudococcidae). *Research Bulletin of the Plant Protection Service Japan*, 31: 31–36.
- Dohino, T., Masaki, S., Takano, T., & Hayashi, T.** 1997. Effects of electron beam irradiation on sterility of Comstock mealybug, *Pseudococcus comstocki* (Kuwana) (Homoptera: Pseudococcidae). *Research Bulletin of the Plant Protection Service Japan*, 33: 31–34.
- Jacobsen, C.M. & Hara, A.H.** 2003. Irradiation of *Maconellicoccus hirsutus* (Homoptera: Pseudococcidae) for phytosanitation of agricultural commodities. *Journal of Economic Entomology*, 96(4): 1334–1339.
- Ravuiwasa, K.T., Lu, K.H., Shen, T.C., & Hwang, S.Y.** 2009. Effects of irradiation on *Planococcus minor* (Hemiptera: Pseudococcidae). *J. Econ. Entomol.* 102(5), 1774–1780.

### Publication history

*This is not an official part of the standard*

2012-11 SC added subject under topic: (2006-014) Irradiation treatments

2012-09 Submitted in response to 2012 call for treatments

2012-12 TPPT evaluated submission, drafted schedule and recommended to SC for member consultation

2013-02 Submitted for SC e-decision

2013-04 Approved for member consultation by SC e-decision

2014-04 Treatment lead addressed members and TPG comments

2014-06 TPPT finalized the response and recommended to the SC for adoption

2014-09 SC reviewed (no changes) and recommended for CPM adoption

2015-03 CPM-10 adopted the treatment

**ISPM 28. Annex 19** Irradiation treatment for *Dysmicoccus neobrevipes*, *Planococcus lilacinus* and *Planococcus minor* (2015). Rome, IPPC, FAO.

Publication history last modified: 2015-04



**ISPM 27  
Annex 5**

## **INTERNATIONAL STANDARDS FOR PHYTOSANITARY MEASURES**

### **ISPM 27 DIAGNOSTIC PROTOCOLS**

#### **DP 5:**

#### ***Phyllosticta citricarpa* (McAlpine) Aa on fruit**

**(2014)**

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## 1. Pest Information

*Phyllosticta citricarpa* (McAlpine) Aa, the causal agent of “citrus black spot” disease, is a leaf-spotting and fruit-blemishing fungus affecting *Citrus*, *Poncirus* and *Fortunella* and their hybrids. Except for *Citrus aurantium* and its hybrids and *Citrus latifolia*, all commercially grown *Citrus* species are susceptible (Aguilar-Vildoso *et al.*, 2002; Kotzé, 2000). *Citrus limon* is particularly susceptible and thus it is usually the first *Citrus* species to show symptoms of the disease once the pathogen is introduced into a new area (Kotzé, 2000).

Citrus black spot was first recorded in Australia in 1895 on *Citrus sinensis* (Benson, 1895). It is now present in some citrus-producing areas of Africa, Asia, Australia, and North and South America (CABI, 2011; NAPPO, 2010; Schubert *et al.*, 2012). The organism has not been reported from Europe, Central America or the Caribbean region (CABI, 2011; CABI/EPPO, 1998; EPPO/CABI, 1997; NAPPO, 2010).

*P. citricarpa* has economic impact mainly because of the external blemishes it causes, which makes citrus fruit unsuitable for the fresh market (Spósito, 2003). Severe infections may cause premature fruit drop (Kotzé, 2000). Some losses due to fruit drop occur in years favourable for pest development and when fruit is held on the trees past peak maturity (CABI, 2011). In addition, latently infected (asymptomatic) fruit at harvest may still develop symptoms during transport or storage (Kotzé, 1996).

The epidemiology of citrus black spot is influenced by the availability of inoculum, the occurrence of environmental conditions favourable for infection (i.e. warm, wet and humid conditions), the growth cycle of the citrus tree, and the age of the fruit and leaves in relation to their susceptibility to infection (Kotzé, 1981, 2000). In areas where rain is confined to a single season, pseudothecia with ascospores, produced exclusively on leaf litter, are the main source of inoculum. Where rain is not confined to a single season, where out-of-season fruit with lesions remains on the trees after flowering and fruit set, or where successive and irregular flowering occurs in the cultivated citrus species and varieties, pycnidia with conidia of *P. citricarpa* are also important as inoculum sources (Kotzé, 1981; Spósito *et al.*, 2008, 2011).

Pseudothecia develop 40–180 days after leaf drop, depending on the frequency of wetting and drying as well as on the prevailing temperatures (Kotzé, 1981). Citrus leaves drop all year round in some countries and seasonally in others, and this affects the availability of inoculum. The optimum temperature for pseudothecial formation is 21–28 °C; no pseudothecia are formed below 7 °C or above 35 °C (Lee and Huang, 1973). Ascospore release takes place during rainfall and occasionally during irrigation or when there is heavy dew (Kiely, 1949a; Kotzé, 2000). Ascospore discharges are closely influenced by the rainfall pattern (Kotzé, 1981). Ascospores are forcibly released up to a height of 1.2 cm above pseudothecia and are carried by air currents throughout the canopy and over long distances (Kiely, 1949a). The critical period for infection starts at fruit set and lasts 4–6 months, but the first symptoms on fruit do not appear until more than 6 months after fruit set (Baldassari *et al.*, 2006). In Brazil, fruit of *C. sinensis* “Valencia” and “Natal” varieties are susceptible until at least 24 weeks after the fall of 75% of the petals, when they are 5–6 cm in diameter (Baldassari *et al.*, 2006).

After infection, the fungus remains in a quiescent state until the fruit becomes fully grown or mature, with symptoms becoming apparent many months after infection has taken place (Kotzé, 2000). Leaves remain susceptible to infection from development up to 10 months of age (Truter *et al.*, 2007).

Pycnidia with conidia are produced on fruit, leaves, dead twigs, fruit pedicels and in abundance on leaf litter (Kotzé, 2000). They may be splash-dispersed onto the canopy or washed off infected late-hanging fruit onto younger fruit and leaves that are still at the susceptible stage (Agostini *et al.*, 2006; Spósito *et al.*, 2008). *P. citricarpa* also has a microconidial asexual state, described in the genus *Leptodothiorella* (Kiely, 1949a). This microconidial state, also referred to as the “spermogonial” state (Kiely, 1949a), usually appears on fallen leaves before pseudothecia develop. However, the role of microconidia in the biology of *P. citricarpa* is still unclear.

Symptom development on mature fruit is enhanced by rising temperature, high light intensity, drought and poor tree vigour. Older trees usually have more citrus black spot than younger trees (Kotzé, 2000). The spread of *P. citricarpa* to new areas is assumed to have taken place through infected nursery stock or other planting material rather than through citrus fruit (Kotzé, 2000; Timmer, 2004).

It should be noted that in symptomless citrus fruit or fruit with very small spots (<2 mm in diameter) without pycnidia, the non-pathogenic endophyte *Phyllosticta capitalensis* Henn (formerly incorrectly referred to as *Guignardia mangiferae* A.J. Roy) (Glienke *et al.*, 2011), recorded in many plant families, may be present. The cultural, morphological and molecular characteristics that differentiate *P. capitalensis* from *P. citricarpa* have been described by Baayen *et al.* (2002). Furthermore, symptoms of *P. citricarpa* may be confused with those caused by *Phyllosticta citriasiana* Wulandari, Crous & Gruyter, a newly described pathogen that has so far been found only on *Citrus maxima* (Wang *et al.*, 2012; Wulandari *et al.*, 2009). The pathogenicity of *P. citriasiana* to other *Citrus* species is unknown. The cultural, morphological and molecular characteristics that differentiate *P. citriasiana* from *P. citricarpa*, the species pathogenic to citrus, have been described by Wulandari *et al.* (2009). Two *Phyllosticta* species have recently been described associated with *Citrus* spp. *Phyllosticta citrichinaensis* causes small sunken grey–brown spots with a dark brown margin and olive green halos on pomelo leaves. The pathogen also induces small brown to black spots similar to melanose on mandarin and orange fruits (Wang *et al.*, 2012). *P. citribraziliensis* has been found as an endophyte in healthy leaves of *Citrus* spp. in Brazil (Glienke *et al.*, 2011).

## 2. Taxonomic Information

<b>Name:</b>	<i>Phyllosticta citricarpa</i> (McAlpine) Aa, 1973
<b>Synonyms:</b>	<i>Phoma citricarpa</i> McAlpine, 1899 <i>Guignardia citricarpa</i> Kiely, 1948 <i>Phyllostictina citricarpa</i> (McAlpine) Petr., 1953 <i>Leptodothiorella</i> sp. (spermatial state)
<b>Taxonomic position:</b>	Eukaryota, Fungi, Ascomycota, Pezizomycotina, Dothideomycetes, Botryosphaerales, Botryosphaeriaceae
<b>Common names:</b>	Citrus black spot (for common names in other languages, see CABI (2011))
<b>Reference:</b>	MycoBank 320327

## 3. Detection

Fruit, pedicels, leaves and twigs of *Citrus*, *Poncirus* and *Fortunella* and their hybrids may potentially harbour *P. citricarpa* (CABI, 2011).

### 3.1 Symptoms on fruit

Several symptoms (e.g. hard spot, freckle spot, false melanose, virulent spot) appear on fruit, depending on the temperature and on fruit maturity (Kotzé, 2000). The presence of *P. citricarpa* on fruit is unlikely to be accurately confirmed based on visual examination alone, as symptoms are

variable in appearance and can easily be confused with those caused by other citrus pathogens or by mechanical, cold or insect damage (Kotzé, 2000; Snowdon, 1990; L. Diaz, personal communication). The following four symptoms are widely recognized as described by Kiely (1949a, 1949b, 1960).

*Hard spot.* The most typical symptom of citrus black spot, consisting of shallow lesions, 3–10 mm in diameter, with a grey to tan centre and a dark brown to black margin (Figure 1A). At advanced stages of symptom development, the centre of the lesions becomes crater-like. Individual hard spot lesions may either remain small or coalesce to form larger lesions. A yellow halo, when the fruit is green, or a green halo, when the fruit is yellow or orange, may appear around these lesions. Quite often, pycnidia are produced in the centre of these spots (Figure 1a) and can be detected by using a hand lens or a dissecting microscope. Hard spot usually appears when fruit starts maturing, even before colour change, and on the side of the fruit most exposed to sunlight (Kotzé, 1981, 2000). In many cases, citrus black spot can be easily identified by hard spot lesions with pycnidia.

*Freckle spot.* Grey, tan, reddish or colourless spots, 1–3 mm in diameter, slightly depressed at the centre and with no halo around them (Figure 1B). The spots turn brown with age and are almost always devoid of pycnidia (Figure 1b). Freckle spots mostly develop after the fruit has changed colour and may also appear as satellite spots around hard spot lesions (Bonants *et al.*, 2003) (Figure 1C). Individual freckle spots may coalesce to form larger lesions that turn into virulent spots (Figure 2C), especially during fruit storage (Kotzé, 1981, 2000).

*False melanose or speckled blotch.* Usually appears on green fruit as small raised dark brown to black lesions, often surrounded by dark specks (FUNDECITRUS, 2005) (Figures 2A, 2a, 2B). The lesions are devoid of pycnidia and may coalesce as the season progresses (CABI, 2011). This symptom is observed in citrus-growing areas where *P. citricarpa* has been present for a long time (FUNDECITRUS, 2005).

*Virulent spot, spreading spot or galloping spot.* Sunken irregular red to brown or colourless lesions that appear on heavily infected mature fruit towards the end of the season (Figure 2C). Numerous pycnidia eventually develop in these lesions under conditions of high humidity (Kotzé, 2000). Virulent spots grow rapidly, covering two-thirds of the fruit surface within four to five days. It is the most damaging symptom, because, unlike the other symptoms, it extends deeply into the mesocarp (albedo), occasionally involving the entire thickness of the rind, causing premature fruit drop and serious post-harvest losses (Kotzé, 1981).

Two additional symptoms, as follows, have also been reported to occur on citrus fruit, though infrequently.

*Lacy spot.* Superficial yellow lesions with a dark yellow to brown centre, a smooth texture and no defined margins (Aguilar-Vildoso *et al.*, 2002) (Figure 2D). This symptom appears on green fruit and may cover a big part of its surface (Goes, 2001). The lesions are devoid of pycnidia and frequently appear as brown netting on a yellow background. Fruits showing lacy spot usually appear to be aggregated in the tree canopy (M. Spósito, personal communication).

*Cracked spot.* Superficial slightly raised dark brown to black lesions, variable in size, with a cracked surface and irregular margins (Goes *et al.*, 2000) (Figure 2E). The lesions are devoid of pycnidia and appear on fruit older than six months. This symptom has been associated with the presence of *Phyllocoptruta oleivora* Ashmead (FUNDECITRUS, 2005; Spósito, 2003).

It should be noted that more than one of the symptoms described above, or intermediate stages between symptoms, may be observed on the same fruit (Figure 1C, 1c).

In some areas with high inoculum pressure, symptoms may also appear on small fruit, calyxes and peduncles. The symptoms on calyxes are red to dark brown lesions similar to freckle spots. On small fruit and peduncles, symptoms appear as small black spots (Aguilar-Vildoso *et al.*, 2002). Such symptoms on small fruit, calyxes and peduncles have been reported from Brazil only.

### 3.2 Symptoms on leaves and twigs

Citrus black spot usually occurs on leaves as quiescent infection without visible symptoms (Sutton and Waterston, 1966). If symptoms do appear, they start as pinpoint spots visible on both leaf surfaces.

The spots, which may increase in size up to 3 mm in diameter, are circular, with their centres becoming grey or light brown in colour surrounded by a dark brown to black margin and a yellow halo (Kotzé, 2000) (Figure 3A). Pycnidia may occasionally be present in the centre of the lesions on the adaxial leaf surface.

Lesions similar to those on leaves may also occur on small twigs, more commonly on *C. limon* than on other citrus species (M. Truter, personal communication). Symptoms are small (0.5–2 mm in diameter) round slightly sunken lesions with a brown to black margin and a grey to light brown centre (Figure 3B). Pycnidia may occasionally be present in the centre of the lesions.

### 3.3 Comparison of citrus black spot symptoms with those caused by other organisms or abiotic factors

Symptoms on fruit are variable in appearance and often resemble those caused by other citrus pathogens (such as *P. citriasiana*, *P. citrichinaensis*, *Diaporthe citri*, *Mycosphaerella citri*, *Alternaria alternata* pv. *citri*, *Septoria* spp., *Colletotrichum* spp.) or by insect, mechanical or cold damage, particularly in the case of freckle spot (Bonants *et al.*, 2003; Snowdon, 1990; Wang *et al.*, 2012; Wulandari *et al.*, 2009; L. Diaz, personal communication).

As the symptoms caused by *P. citricarpa* on citrus fruit are similar to those caused by other pathogens, reliable diagnosis can be made only by using the methods described below.

## 4. Identification

This protocol describes the detection and identification of *P. citricarpa* on symptomatic citrus fruit. Citrus fruit should be inspected for any symptoms typical of citrus black spot (see section 3). If suspected symptoms are present in the form of spots or lesions, they are examined with a magnifying lens or a dissecting microscope for the presence of pycnidia. If pycnidia are present in hard spot lesions as described in section 3.1 and the morphological characteristics of the pycnidia and conidia are consistent with those in section 4.1.3, *P. citricarpa* may be present. However, as the pycnidia and conidia of *P. citricarpa* are very similar to those of *P. citriasiana*, the recently described pathogen on *C. maxima* (Wulandari *et al.*, 2009), the identity of *P. citricarpa* can only be confirmed with certainty by applying the diagnostic methods described below (Figure 4). Diagnostic Method A (isolation and culturing) is used for the identification of *P. citricarpa* on citrus fruit, but can also be used on leaves, twigs and pedicels, whereas Method B (molecular assay) applies to citrus fruit only.

If after applying Method A the cultural characteristics of the colonies grown on cherry decoction agar (CHA) and oatmeal agar (OA) media are not consistent with those of *P. citricarpa* (see section 4.1.4, requirements (i), (ii), (iii) and (iv)) then the plant material is considered free of *P. citricarpa*. On *P. citricarpa*-like cultures that do not produce mature pycnidia within 14 days, application of conventional polymerase chain reaction (PCR) and internal transcribed spacer (ITS) sequencing (see section 4.2.1) or real-time PCR (see section 4.2.2) is recommended. However, isolation and culturing of the organism on appropriate media followed by a direct molecular test of the cultures is a time-consuming procedure and thus undesirable in time-critical diagnosis of consignments.

There are two PCR methods (conventional and real-time) available for the detection and identification of *P. citricarpa* on citrus fruit (see sections 4.2.1, 4.2.2). However, it has been recently observed during routine testing of *C. maxima* fruit showing typical symptoms that the real-time PCR method developed by Gent-Pelzer *et al.* (2007) gives no amplification (J.P. Meffert, personal communication). The reason is that the citrus black spot-like symptoms on *C. maxima* are caused by *P. citriasiana*, a newly described species closely related to *P. citricarpa* (Wulandari *et al.*, 2009). As it is not clear whether *P. citricarpa* is able to cause typical symptoms on *C. maxima*, fruit of this *Citrus* species showing citrus black spot-like symptoms should also be tested for the presence of *P. citricarpa*.

The real-time PCR method developed by Gent-Pelzer *et al.* (2007) (see section 4.2.2) can be used for a positive diagnosis of *P. citricarpa*, as it will give a positive signal only when *P. citricarpa* is present, and not for *P. citriasiana* or *P. capitalensis*. The conventional PCR method (as described in section 4.2.1) will give amplification when either *P. citricarpa* or *P. citriasiana* is present. In this case, after a positive signal, isolation and culturing (see section 4.1), real-time PCR (see section 4.2.2) or ITS

sequencing (see section 4.2.1) should be performed to discriminate between the two species. There are no data available on reactions of the recently described *P. citrichinaensis* from China in these molecular assays.

It should be noted that occasionally acervuli of the common endophytic fungi *Colletotrichum* spp. may be present and may look similar to pycnidia of *P. citricarpa*. However, *Colletotrichum* spp. can be differentiated by the presence of setae in their acervuli, the production under humid conditions of pink or salmon-coloured masses of conidia on the surface of the lesions, and the morphology of their conidia (Kotzé, 2000).

In the present protocol, methods (including references to brand names) are described as published, as these define the original level of specificity achieved. Laboratory procedures presented may be adjusted to the standard of individual laboratories, provided that they are adequately validated.

#### 4.1 Method A: Isolation and culturing of *P. citricarpa*

Fruit lesions are excised with a cork borer or scalpel, dipped in 70% ethanol for 30 s, surface disinfested with 1% sodium hypochlorite (NaOCl) for 2 min, rinsed twice in sterile distilled water and blotted dry (Peres *et al.*, 2007). For increasing the isolation frequency, lesions must be excised carefully with any asymptomatic tissue being removed prior to plating (N.A. Peres, personal communication). Subsequently, the lesions are placed aseptically on Petri dishes (9 cm in diameter) with CHA or potato dextrose agar (PDA) (see section 4.1.1) or PDA with 50 µg/ml penicillin and 50 µg/ml streptomycin added (OEPP/EPPO, 2003). If PDA is used and slow-growing dark *P. citricarpa*-like cultures develop on it, they are subsequently transferred both to CHA dishes for testing the growth rate of the colonies and to OA (see section 4.1.1) dishes for evaluating the yellow pigment production. At the same time, the cultures grown on PDA medium should be placed under near-ultraviolet (NUV) light at 22 °C to facilitate the induction of pycnidia formation. Cultures that (i) grow slowly on CHA (see section 4.1.2); (ii) produce the characteristic pycnidia and conidia of *P. citricarpa* (see section 4.1.2); and (iii) produce a yellow pigment on OA – although not all *P. citricarpa* isolates produce such a pigment on OA (Baayen *et al.*, 2002) – are identified as belonging to *P. citricarpa*.

The method has the following shortcomings: (a) *P. citricarpa* is a rather slow-growing fungus and is often overgrown by other fungi in culture (e.g. *C. gloeosporioides*) (Peres *et al.*, 2007) as none of the culture media used is selective for *P. citricarpa*, and (b) it is a time-consuming method, as it requires 7–14 days for the production of pycnidia.

##### 4.1.1 Culture media

*Cherry decoction agar (CHA)*. Cherry juice is made by boiling 1 kg cherries, free of stones and petioles, in 1 litre tap water for approximately 2 h. The extract is filtered through cheesecloth, poured into bottles, sterilized for 30 min at 110 °C (pH 4.5) and stored until use. In a bottle containing 0.8 litres distilled water, 20 g technical agar no. 3 is added and the mixture is sterilized for 15 min at 121 °C. Immediately after sterilization, 0.2 litre sterilized cherry extract is added, mixed well and sterilized for 5 min at 102 °C (Gams *et al.*, 1998).

*Oatmeal agar (OA)*. OA is commercially available. Alternatively, it can be prepared by using the following method: 30 g oatmeal flakes is placed into cheesecloth and suspended in a pan containing tap water. After simmering for approximately 2 h, the flakes are squeezed, filtered through cheesecloth and the extract is sterilized for 15 min at 121 °C. In a bottle containing 1 litre oatmeal extract, 20 g of technical agar no. 3 is added and the mixture is sterilized for 15 min at 121 °C (Gams *et al.*, 1998).

*Potato dextrose agar (PDA)*. PDA is commercially available. Alternatively, it can be prepared according to the method described by Hawksworth *et al.* (1995).

##### 4.1.2 Cultural characteristics

*P. citricarpa* colonies grow slowly on CHA; they have an average diameter of 25–30 mm after 7 days at 22 °C in darkness (Baayen *et al.*, 2002). On PDA, the colonies have irregular margins lined by a much wider translucent zone of colourless submerged mycelium (Figure 5A). The centre of the colony is dark with grey to glaucous aerial mycelium, often with numerous small tufts. The reverse of the

colony is very dark in the centre and surrounded by areas of grey sepia and buff (Baayen *et al.*, 2002). Stromata start to develop after 7–8 days, whereas mature pycnidia with conidia are generally produced within 10–14 days (Figure 5B). On OA after 14 days at 25°C in the dark, colonies are flat, spreading, olivaceous-grey, becoming pale olivaceous-grey towards the margin, with sparse to moderate aerial mycelium (Glienke *et al.*, 2011). On OA a distinct yellow pigment is often produced that diffuses into the medium around the colony (Figure 6D, top row), although not all *P. citricarpa* isolates produce a yellow pigment (Baayen *et al.*, 2002). This yellow pigment is weakly produced on CHA and PDA.

### 4.1.3 Morphology

Published data on the morphology of *P. citricarpa* vary considerably, partly because of the confusion about the identity of the different *Phyllosticta* species associated with *Citrus* (Baayen *et al.*, 2002; Glienke *et al.*, 2011; Wang *et al.*, 2012; Wulandari *et al.*, 2009). The following morphological and morphometric characteristics refer to fructifications and spores of *P. citricarpa* produced mainly in culture; they are based on data from Sutton and Waterston (1966) and van der Aa (1973), as revised and amended by Baayen *et al.* (2002).

*Ascocarps*. Pseudothecia are formed on leaf litter and in culture (De Holanda Nozaki, 2007) but not on any other plant material (e.g. attached leaves, fruit). They are solitary or aggregated, globose to pyriform, immersed, dark brown to black, 125–360 µm, with a single papillate to rostrate ostiole, and their surface is often covered with irregular hyphal outgrowths. The outer wall layer is composed of angular cells with brown thickened walls, whereas the inner layer is composed of angular to globose cells with thinner colourless walls.

*Asci*. Fasciculate, bitunicate, clavate, eight-spored with a rounded apex. Their dimensions are 40–65 µm × 12–15 µm before the rupture of the outer wall, and they become cylindrical-clavate and extend in length to 120–150 µm prior to dehiscence.

*Ascospores*. Short, aseptate, hyaline, cylindrical, swollen in the middle, slightly curved, 12–16 µm × 4.5–6.5 µm, heteropolar with unequal obtuse ends. The smaller upper end has a truncate, non-cellular, mucoid cap-like appendage 1–2 µm long, and the lower end has an acute or ruffled appendage 3–6 µm long.

*Pycnidia*. Produced on fruit, attached leaves, dead twigs and leaf litter as well as in culture. They are solitary or occasionally aggregated, globose, immersed, mid- to dark brown, and 70–330 µm in diameter. The pycnidial wall is up to four cells thick, sclerotoid on the outside, pseudoparenchymatous within, with ostiole darker, slightly papillate, circular and 10–15 µm in diameter.

*Conidia*. Obovate to elliptical, hyaline, aseptate, multiguttulate, 9.4–12.7 µm × (5.0–8.5) µm, with a colourless subulate appendage and a barely visible, colourless, gelatinous sheath <1.5 µm thick (Figures 5C, 5D, 6A). They are formed as blastospores from hyaline, unicellular, cylindrical conidiophores up to 9 µm long.

*Spermatial state*. Described in the form genus *Leptodothiorella*, formed both on hosts and in pure culture. Spermatia dumbbell-shaped, rarely cylindrical, straight or slightly curved, 5–8 µm × 0.5–1 µm.

### 4.1.4 Comparison of *P. citricarpa* cultural and morphological characteristics with those of similar *Phyllosticta* species

Cultures of *P. citricarpa* are very similar to those of *P. citriasiana* (Wulandari *et al.*, 2009) and of the endophytic, non-pathogenic to citrus *P. capitalensis* (Baayen *et al.*, 2002; Glienke *et al.*, 2011).

Identification of *P. citricarpa* colonies is possible by combining:

- (1) the colony growth on CHA (although the ranges may overlap)
- (2) the thickness of the mucoid sheath surrounding the conidia (Figures 5C, 5D, 6A, 6B, 6C)
- (3) the length of the conidial appendage

- (4) the presence of yellow pigment on OA, although not all *P. citricarpa* isolates produce a yellow pigment (Baayen *et al.*, 2002; Wulandari *et al.*, 2009).

Detailed information of the distinctive characteristics of *P. citricarpa* and its related species are given in Table 1. In addition, *P. citrichinaensis* can be differentiated from *P. citricarpa* by its longer conidial appendage, 14–26 µm (Wang *et al.*, 2012).

**Table 1.** Main cultural and morphological characteristics of *Phyllosticta citricarpa*, *Phyllosticta citriasiana* and *Phyllosticta capitalensis* (Baayen *et al.*, 2002; Wulandari *et al.*, 2009)

Characteristic	<i>P. citricarpa</i>	<i>P. citriasiana</i>	<i>P. capitalensis</i>
Average conidia size (µm)	10–12 × 6–7.5	12–14 × 6–7	11–12 × 6.5–7.5
Mucoid sheath width (µm)	<1.5	1	1.5–2.5 (–3)
Apical appendage length (µm)	4–6 (–10)	7–10 (–14)	4–6 (–10)
Average ascospore size (µm)	12–16 × 4.5–6.5	Unknown	15–17.5 × 6.5–7.5
Average spermatia size (µm)	5–8 × 0.5–1	3–5 × 1–2	7–10 × 1.8–2.5
Average colony diameter (mm)*	25–30	18–20	>40
Maximum growth temperature (°C)	30–36	30–33	30–36
Production of yellow pigment on oatmeal agar (OA) medium	Yes <sup>†</sup>	No	No

\* On cherry decoction agar (CHA) medium after 7 days at 22 °C in darkness.

<sup>†</sup> It should be noted that not all *P. citricarpa* isolates produce a yellow pigment.

## 4.2 Method B: Molecular assays

Different molecular methods have been developed for the identification of *P. citricarpa* directly on pure cultures and fruit lesions (Bonants *et al.*, 2003; Gent-Pelzer *et al.*, 2007; Meyer *et al.*, 2006, 2012; Peres *et al.*, 2007; Stringari *et al.*, 2009). Two methods, a conventional PCR assay, developed by Peres *et al.* (2007), and a real-time PCR assay, developed by Gent-Pelzer *et al.* (2007), are described for the identification of *P. citricarpa*. It is noted that the real-time PCR method will generate a positive signal from a single citrus black spot lesion on fruit, whereas, in some cases, the conventional PCR may give inconclusive results. It is also noted that there are no data available on positive reactions in molecular assays of *P. citrichinaensis*, recently described on fruits in China.

### 4.2.1 Identification of *P. citricarpa* by conventional PCR

Specificity (analytical specificity) was assessed in a study with 36 isolates of *P. citricarpa*, 13 isolates of *P. capitalensis* and isolates of common citrus pests, including *Alternaria alternata*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, *Diaporthe citri*, *Mycosphaerella citri* and *Penicillium digitatum*. Only *P. citricarpa* gave a positive reaction. Sensitivity (analytical sensitivity; detection limit) is 1 pg DNA/µl (Peres *et al.*, 2007). The method will amplify either *P. citricarpa* or *P. citriasiana* DNA. There are three methods available to discriminate between the two species after conventional PCR: isolation and culturing (see section 4.1), real-time PCR assay (see section 4.2.2) and ITS sequencing (see section 4.2.3).

#### 4.2.1.1 General information

The protocol was developed by Peres *et al.* (2007). The nucleic acid source is mycelium or dissected fruit lesions. The assay is designed to amplify part of the ITS region producing an amplicon of 300 base pairs (bp). The oligonucleotide primers used are:

Forward primer: GCN (5'-CTG AAA GGT GAT GGA AGG GAG G -3')

Reverse primer: GCMR (5'-CAT TAC TTA TCG CAT TTC GCT GC -3').

2.5× Eppendorf®<sup>1</sup> MasterMix containing Taq DNA polymerase and reaction buffer containing Mg<sup>2+</sup> and nucleotides is used for PCR amplification. Molecular grade water (MGW) is used to make up the reaction mixes: the MGW should be purified (deionized or distilled), sterile (autoclaved or filtered through 0.45 µm) and nuclease-free. Amplification is performed in a Peltier-type thermocycler with heated lid.

#### 4.2.1.2 Methods

##### *Nucleic acid extraction and purification*

DNA is extracted either from fungal cultures grown for 7 days in potato-dextrose broth or from single fruit lesions. In the second case, the symptomatic tissue is dissected out, leaving behind as much mesocarp (albedo) and outer rind as possible.

DNA extraction from mycelium is done using commercially available DNA extraction kits (e.g. DNeasy Plant Mini Kit (Qiagen), QuickPick SML Plant DNA (Bio-Nobile), KingFisher® isolation robot (Thermo)) following the manufacturer's instructions. For the extraction of DNA from single fruit lesions, the following alkaline lysis DNA extraction protocol (Klimyuk *et al.*, 1993) followed by purification using a dipstick method can be used as it has proven to be the most effective (Peres *et al.*, 2007).

*Alkaline lysis DNA extraction method.* Symptomatic fruit tissue is placed into sterile 2 ml microtubes containing 40 µl 0.25 M NaOH and incubated in a boiling (100 °C) water bath for 30 s (critical period). The content of the tubes is neutralized by the addition of 40 µl 0.25 M HCl, 20 µl 0.5 M Tris-HCl, pH 8.0 and 0.25% (v/v) Nonidet P-40, and the tubes are placed again in the boiling water bath for 2 min. The material obtained can be either used directly for purification by applying the dipstick method (see below) or stored at 4 °C for several weeks. Prior to purification after storage, the samples are incubated in a boiling water bath for 2 min.

*Dipstick DNA purification method.* 150 µl 100% ethanol and a small piece of cellulose thin-layer chromatography plate (dipstick) are added to the 2 ml microtube after alkaline lysis (see above). Tubes are placed on their sides on ice and shaken for 30 min. The liquid is aspirated off and 500 µl wash buffer (10× (Tris, Na<sub>2</sub>ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite NaClO, pH 7.0) and 95% ethanol) diluted to 25% is added and the tubes are inverted to mix the contents. Washing is repeated twice. The dipsticks are placed in new tubes and dried under vacuum. The tubes are then placed on their sides and 50 µl Tris-EDTA buffer is added to each tube. After incubation for 5 min, the tubes are spun for 10 s, the dipsticks are removed and discarded, and the DNA is recovered. The purified DNA can be used immediately or stored at 4 °C overnight or at –20 °C for longer periods.

Alternatively, DNA can be extracted from fruit lesions using commercially available DNA extraction kits, according to the manufacturer's instructions.

##### *Polymerase chain reaction (PCR)*

The master mix (concentration per 20 µl single reaction) is composed of the following reagents:

Reagent	Working concentration	Volume per reaction (µl)	Final concentration
Molecular grade water	n/a	0.4	n/a
2.5× Eppendorf® <sup>1</sup> MasterMix (Taq DNA polymerase at 0.06 U/µl)	2.5×	8.0	1× (Taq 0.024 U/µl)
2.5× Taq reaction buffer (4 mM Mg <sup>2+</sup> , 500 µM of each dNTP)	2.5×	8.0	1× (1.6 mM Mg <sup>2+</sup> , 200 µM of each dNTP)

<sup>1</sup> The use of the brand Eppendorf® for PCR amplification in this diagnostic protocol implies no approval of it to the exclusion of others that may also be suitable. This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results.

Primer GCN	10 µM	0.8	0.4 µM
Primer GCMR	10 µM	0.8	0.4 µM
Subtotal	-	18.0	-
DNA	-	2.0	-
Total	-	20.0	-

The PCR cycling parameters are 94 °C denaturation for 2 min; 39 cycles of 94 °C for 30 s, 64 °C for 30 s and 72 °C for 1 min; and 72 °C extension for 10 min. A PCR product of 300 bp indicates the presence of *P. citricarpa* DNA.

#### 4.2.1.3 Essential procedural information

After amplification, 10 µl of the reaction mixture is mixed with 2 µl 6× DNA loading buffer (Promega) and loaded along with a molecular weight marker (100 bp DNA Ladder) onto a 1.5% agarose gel, separated by electrophoresis, stained with ethidium bromide or alternative reagents, and viewed and photographed under UV light (Sambrook *et al.*, 1989).

DNA from a reference strain of *P. citricarpa* (positive control) must be included as an additional sample to ensure that amplification has been successful. PCR amplification must also be performed on a sample in which the *P. citricarpa* DNA extract has been replaced with the DNA extract of other related species or on a sample of healthy exocarp (negative control). To monitor possible reagent contamination and false positives, a sample must be substituted by water (reaction control). It is advised to include an internal amplification control (IAC) to monitor inhibition.

#### 4.2.2 Identification of *P. citricarpa* by real-time PCR

Specificity (analytical specificity) was assessed with the *P. citricarpa* reference strain CBS 111.20 (representative for 10 *P. citricarpa* isolates ITS sequence group I; Baayen *et al.*, 2002), the *P. capitalensis* reference strain GC14 (representative for 22 *P. capitalensis* isolates ITS sequence group II; Baayen *et al.*, 2002), 12 other citrus pests (*Alternaria* spp., *Penicillium* spp., *Colletotrichum* spp.), *Phyllosticta artocarpina* and *Guignardia bidwellii*. Only *P. citricarpa* gave a positive reaction. The sensitivity (analytical sensitivity; detection limit) is 10 fg DNA per reaction and the diagnostic sensitivity is 100% (Gent-Pelzer *et al.*, 2007).

##### 4.2.2.1 General information

The protocol was developed by Gent-Pelzer *et al.* (2007). The nucleic acid source is mycelium or dissected fruit lesions. The assay is designed to amplify part of the ITS region producing an amplicon of 69 bp. The oligonucleotide primers used are:

Forward primer: GcF1 (5'-GGT GAT GGA AGG GAG GCC T-3')

Reverse primer: GcR1 (5'-GCA ACA TGG TAG ATA CAC AAG GGT-3').

Hydrolysis probe GcP1 (5'-AAA AAG CCG CCC GAC CTA CCT TCA-3') is labelled at the 5' end with the fluorescent reporter dye FAM (6-carboxy fluorescein) and modified at the 3' end with the dye TAMRA (6-carboxytetramethylrhod-amine) or Eclipse® Dark Quencher (Eurogentec).

2× Premix Ex Taq Master Mix (Takara)<sup>2</sup> containing Taq polymerase and reaction buffer containing MgCl<sub>2</sub> and nucleotides is used for PCR amplification. ROX Reference Dye (50× concentrated, Takara) is added to the Premix Ex Taq Master Mix. MGW is used to make up reaction mixes: the MGW

<sup>2</sup> The use of the brand Takara for the 2× Premix Ex Taq Master Mix in this diagnostic protocol implies no approval of it to the exclusion of others that may also be suitable. This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results.

should be purified (deionized or distilled), sterile (autoclaved or filtered through 0.45 µm) and nuclease-free. Amplification is performed using a real-time PCR thermal cycler.

#### 4.2.2.2 Methods

##### *Nucleic acid extraction and purification*

DNA is extracted either from plugs of mycelium (0.5 cm in diameter) taken from the edges of a colony grown on CHA (see section 4.1.1) at 22 °C in darkness or from fruit lesions. Lesions are dissected from the peel, removing as much as possible of the surrounding albedo and peel tissue. Mycelium plugs or lesions are cut into small pieces and placed in a 1.5 ml microcentrifuge tube with a secure-fitting flat-top cap containing a stainless steel bead (3.2 mm in diameter) and 125 µl extraction buffer (0.02 M phosphate-buffered saline (PBS), 0.5% Tween 20, 2% polyvinylpyrrolidone (PVP), 0.2% bovine serum albumin). The tube is shaken in a bead beater for 80 s at 5 000 r.p.m. The mixture is centrifuged for 5 s at maximum speed (16 100 g) in a microcentrifuge and 75 µl of the resulting supernatant is used for DNA extraction. DNA can be extracted using commercially available DNA extraction kits, according to the manufacturer's instructions. The final volume of the DNA solution is 50 µl. The DNA is further purified over spin columns filled with PVP. The columns are prepared by filling Axygen Multi-Spin separation columns (Dispolab) with 0.5 cm polyvinylpolypyrrolidone (PVPP), placing it on an empty reaction tube and washing twice with 250 µl MGW by centrifuging the column for 5 min at 4 000 g. The DNA suspension is applied to a PVP column and centrifuged for 5 min at 4 000 g. The flow-through fraction is used as input for the PCR assay. Purified DNA can be used immediately or stored at 4 °C overnight or at –20 °C for longer periods. PVP is used as soluble compound in the extraction buffer. PVPP is cross-linked PVP and is used as insoluble filtration material.

##### *Polymerase chain reaction*

The master mix (concentration per 30 µl single reaction) is composed of the following reagents:

Reagent	Working concentration	Volume per reaction (µl)	Final concentration
MGW	n/a	13.1	n/a
2x Premix Ex Taq Master Mix (Takara) <sup>2</sup>	2x	15.0	1x
Primer GcF1	50 µM	0.15	0.25 µM
Primer GcR1	50 µM	0.15	0.25 µM
Probe GcP1	5 µM	0.6	0.10 µM
Subtotal	-	29.0	-
DNA	-	1.0	-
Total	-	30.0	-

0.6 µl of 50x ROX Reference Dye can be added if applicable; in that case, 12.5 µl PCR grade water is used.

The PCR cycling parameters are 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. The cycle cutoff value of 40 was obtained using the ABI PRISM® 7700 or 7900 Sequence Detection System (Applied Biosystems) and materials and reagents used as described above. It should be noted that:

- The amplification curve should be exponential.
- A sample will be considered positive if it produces a Ct value of <40, provided the contamination controls are negative.

- A sample will be considered negative if it produces a Ct value of  $\geq 40$ , provided the assay and extraction inhibition controls are positive.

The cycle cutoff value needs to be verified in each laboratory when implementing the test for the first time.

#### 4.2.2.3 Essential procedural information

DNA from a reference strain of *P. citricarpa* (positive control) must be included as an additional sample to ensure that amplification has been successful. PCR amplification must also be performed on a sample in which the *P. citricarpa* DNA extract has been replaced with the DNA extract of other related species (e.g. *P. citriasiana*) or on a sample of healthy exocarp (negative control). To monitor possible reagent contamination and false positives, a sample must be substituted by water (reaction control).

To check for false negative reactions caused by inhibition of the amplification reaction, 12.5 fg of an IAC, 75 nM IAC forward primer FIAC (5'-TGG CCC TGT CCT TTT ACC AG-3'), 75 nM IAC reverse primer RIAC (5'-TTT TCG TTG GGA TCT TTC GAA-3'), and 50 nM IAC MGB hydrolysis probe (5'-ACA CAA TCT GCC-3') labelled with the fluorescent reporter dye VIC™ (Eurogentec) and the quencher dye Eclipse® Dark Quencher (Eurogentec) can be added to the reaction mixes.

#### 4.2.3 Identification of *P. citricarpa* by ITS sequencing

##### 4.2.3.1 General information

The identity of positive samples obtained by conventional PCR can be confirmed by sequencing (Baayen *et al.*, 2002). The method for sequencing of the ITS 1 and 2 regions of the fungal ribosomal RNA gene is described below.

The oligonucleotide primers used are:

Forward primer: ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3')

Reverse primer: ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') (White *et al.*, 1990).

##### 4.2.3.2 Methods

###### *Nucleic acid extraction and purification*

DNA should be extracted from a 1 cm<sup>2</sup> plug taken from a pure culture of the test isolate. A suitable DNA extraction kit is used or DNA is extracted following a more traditional method, such as that described in Hughes *et al.* (2000). Extracted DNA should be stored at 4 °C for immediate use or at –20 °C if testing is not to be performed on the same day.

###### *Polymerase chain reaction (PCR)*

The total reaction volume of a single PCR is 50 µl, and is composed of the following reagents:

Reagent	Working concentration	Volume per reaction (µl)	Final concentration
MGW	n/a	37.5	n/a
10x PCR reaction buffer (+15 mM MgCl <sub>2</sub> ) (Roche) <sup>3</sup>	2x	5.0	1x (Taq 0.024 U/µl)
dNTPs	10 mM (each)	4.0	0.8 mM (each)
Primer ITS1	10 µM	0.6	0.12 µM

<sup>3</sup> The use of the brand Roche for the PCR reaction buffer and the DNA Taq polymerase in this diagnostic protocol implies no approval of them to the exclusion of others that may also be suitable. This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results.

Primer ITS4	10 µM	0.6	0.12 µM
DNA Taq polymerase (Roche) <sup>3</sup>	5 U/µl	0.3	0.03 U/µl
Subtotal	-	48.0	-
DNA	-	2.0	-
Total	-	50.0	-

The PCR cycling parameters are 94 °C for 30 s; 40 cycles of 94 °C for 15 s, 55 °C for 60 s and 72 °C for 30 s; and 72 °C for 5 min. The amplicon size is 550 bp (Baayen *et al.*, 2002).

### ***Sequencing of amplicons***

The amplified mixture (5 µl of it) is run on a 1.5% agarose gel to check for positive test reactions. The remaining 45 µl from positive test reactions is purified using a suitable PCR purification kit, following the manufacturer's instructions. Sequencing is performed with forward primer ITS1 and reverse primer ITS4.

### **4.2.3.3 Essential procedural information**

#### ***Amplification and analysis***

Extracted DNA should be defrosted, if necessary. Enough reaction mix should be prepared for testing at least one sample of the unknown isolate, a positive control containing amplifiable DNA and a negative control loaded with water rather than DNA. Samples are resolved on a 1.5% agarose gel. Consensus sequences for test samples (excluding primer sequences) are compared with a confirmed strain for the ex-epitype of *P. citricarpa* CBS 127454 (GenBank accession number JF343583) on the National Center for Biotechnology Information (NCBI) database GenBank (<http://www.ncbi.nlm.nih.gov/>). The level of identity should be between 99% and 100%.

## **5. Records**

The records and evidence detailed in section 2.5 of ISPM 27:2006 should be kept.

In cases where other contracting parties may be adversely affected by the results of the diagnosis, records and evidence of the results (in particular cultures, slides, photos of fungal structures, photos of symptoms and signs, photos of DNA extracts and separation gels) should be retained for at least one year.

## **6. Contact Points for Further Information**

Further information on *P. citricarpa* and the methods for its detection and identification can be obtained from (in alphabetical order):

ARC-Plant Protection Research Institute, Biosystematics Division: Mycology, Private Bag x134, Queenswood 0121, South Africa (Dr Mariette Truter; tel.: +27 12 8088281; fax: +27 12 8088297; e-mail: truterm@arc.agric.za).

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University of Florida, Citrus Research and Education Center (CREC), 700 Experiment Station Rd, Lake Alfred, FL 33850, USA (Dr Lavern W. Timmer; tel.: +1 863 9561151; fax: +1 863 9564631; e-mail: lwtimmer@ufl.edu).

A request for a revision to a diagnostic protocol may be submitted by national plant protection organizations (NPPOs), regional plant protection organizations (RPPOs) or Commission on

Phytosanitary Measures (CPM) subsidiary bodies through the IPPC Secretariat ([ippc@fao.org](mailto:ippc@fao.org)), which will in turn forward it to the Technical Panel to develop Diagnostic Protocols (TPDP).

## 7. Acknowledgements

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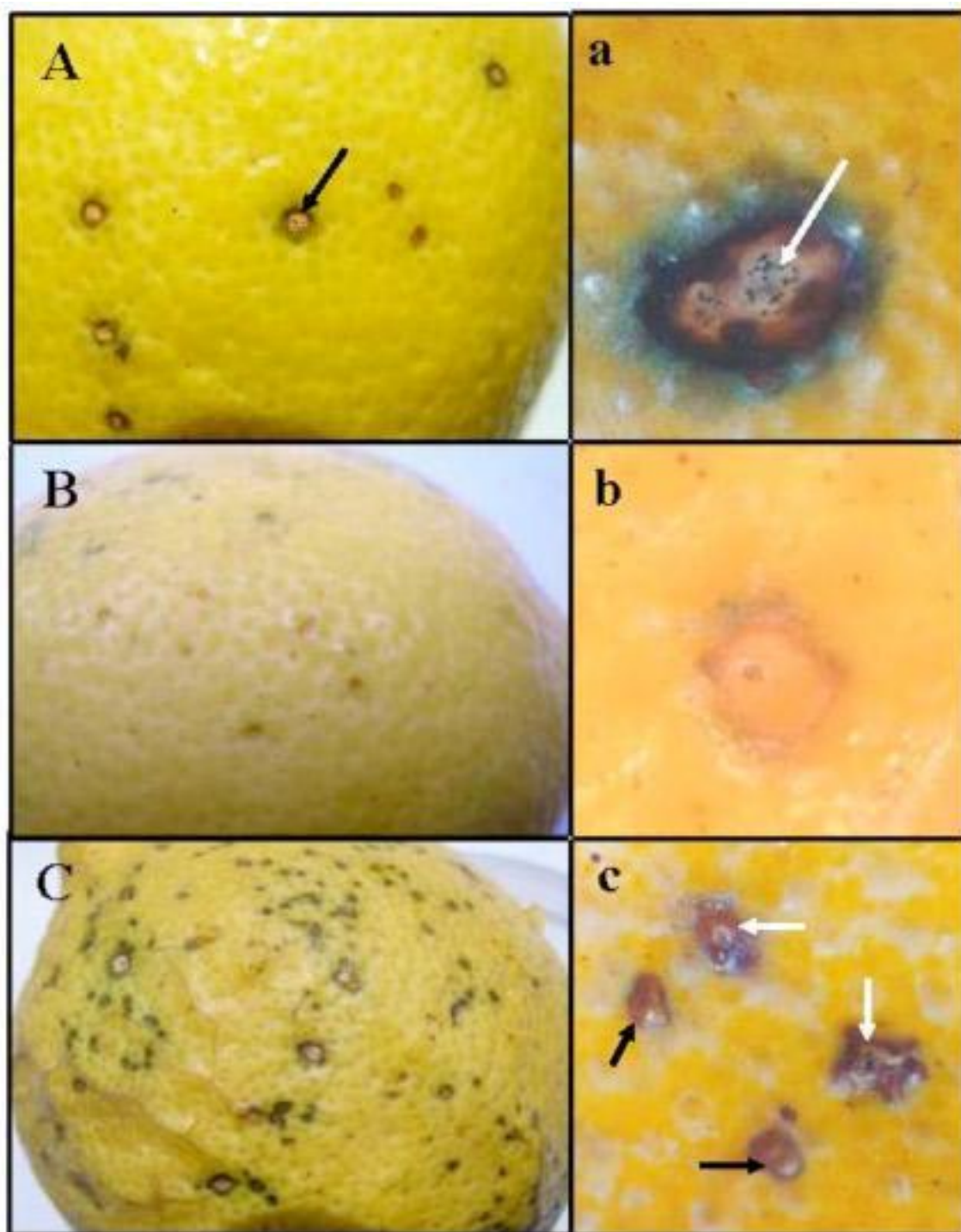
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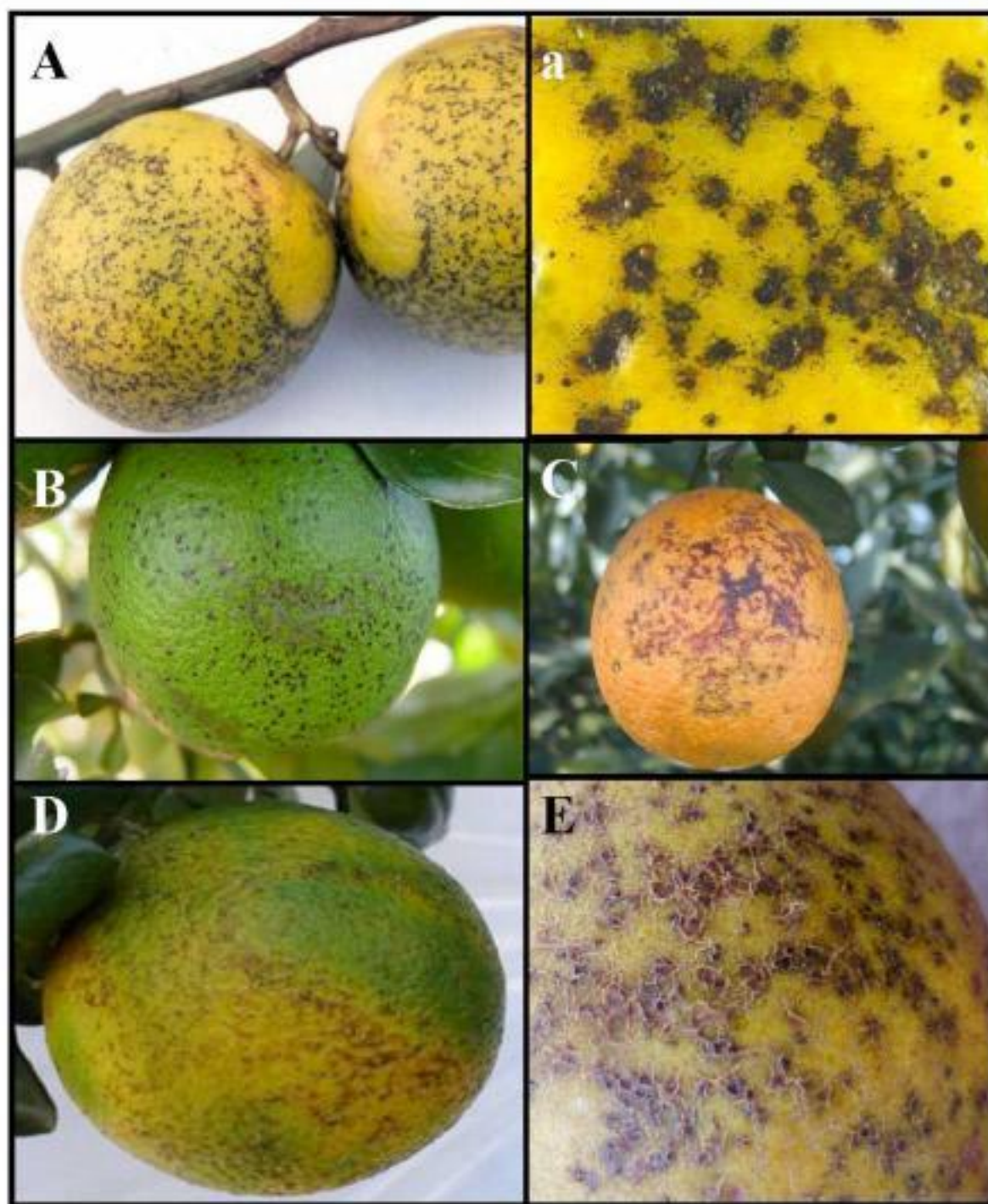
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## 9. Figures



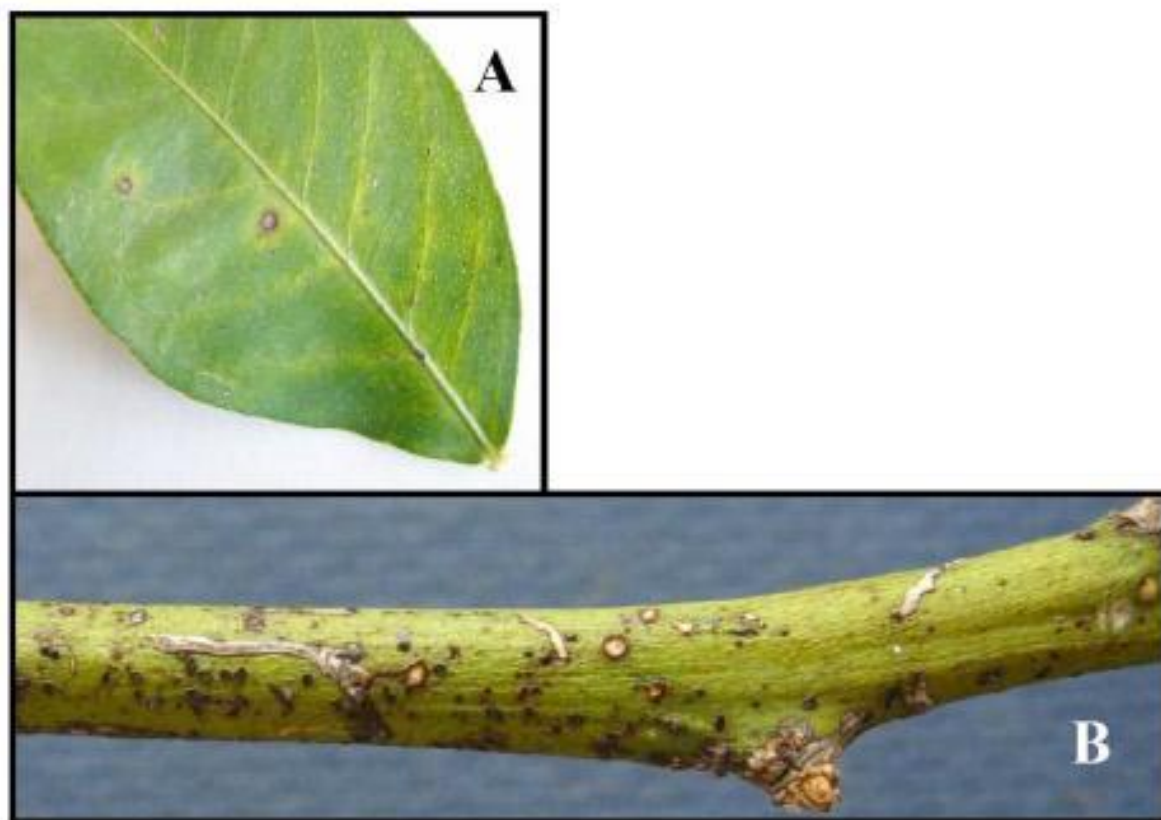
**Figure 1.** Hard spot and freckle spot symptoms caused by *Phyllosticta citricarpa* on sweet orange (*Citrus sinensis*) and lemon (*Citrus limon*) fruits: (A, a) hard spot lesions on sweet orange with the larger lesions containing pycnidia of the anamorph *Phyllosticta citricarpa* (arrows); (B) freckle spot lesions on lemon; (b) freckle spot lesions on sweet orange (the lesions are slightly depressed in the centre and devoid of pycnidia); (C) hard and freckle spot lesions on lemon; (c) freckle spot lesions (black arrows) and intermediate stage between freckle and hard spot lesions with pycnidia (white arrows) on sweet orange.

Photos courtesy E. Feichtenberger, Instituto Biológico, Sorocaba, Brazil.



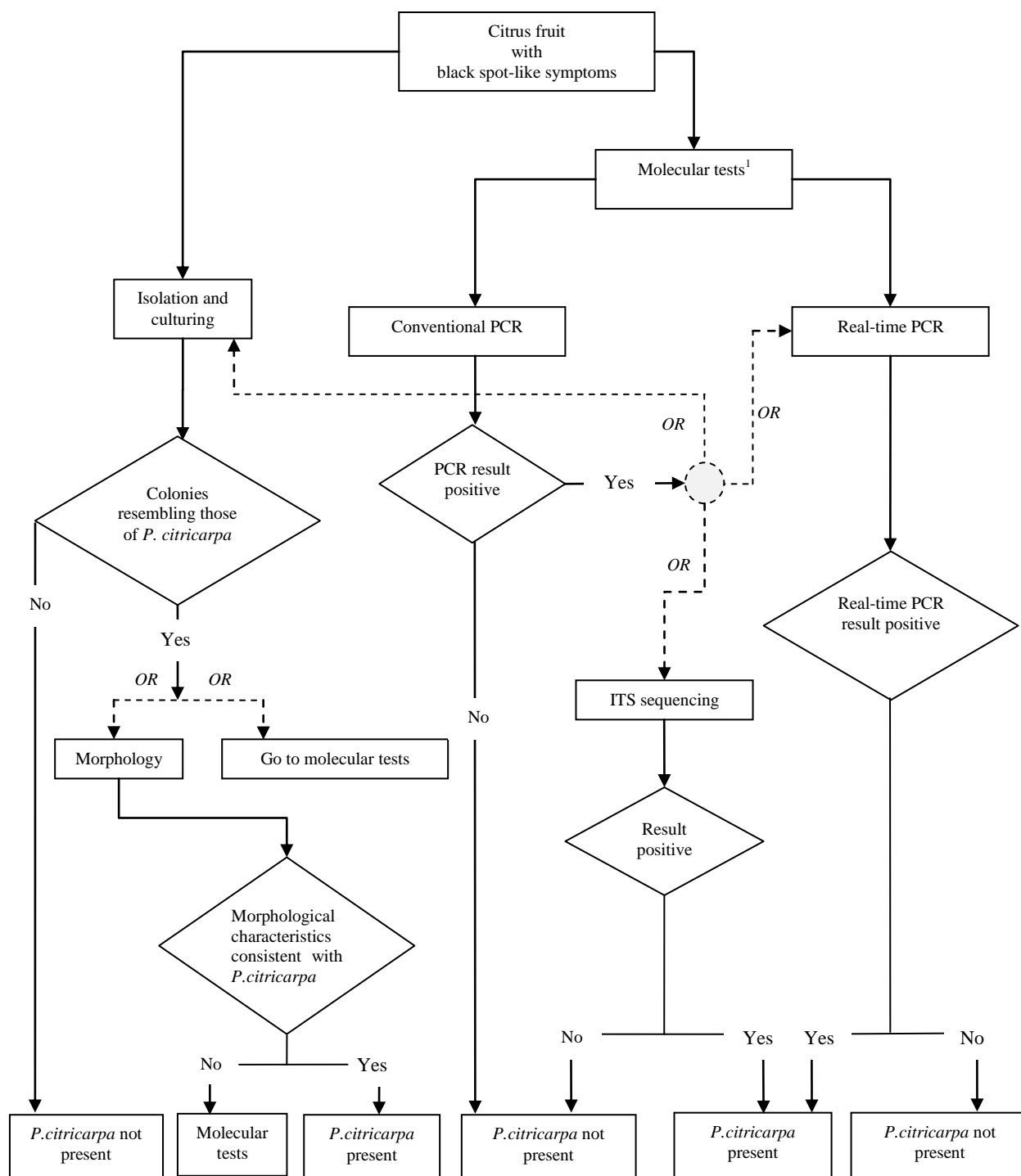
**Figure 2.** False melanose, virulent spot, lacy spot and cracked spot symptoms caused by *Phyllosticta citricarpa* on sweet orange (*Citrus sinensis*) and lemon (*Citrus limon*) fruits: (A) false melanose lesions on mature sweet orange; (a) false melanose lesions surrounded by dark specks on mature sweet orange; (B) false melanose lesions on a green sweet orange; (C) virulent spot lesions on sweet orange (the lesions are depressed and extend deeply into the albedo); (D) lacy spot symptoms on a green sweet orange; (E) cracked spot lesions on sweet orange (the lesions are slightly raised, cracked with irregular margins and devoid of pycnidia).

Photos courtesy FUNDECITRUS (A, B, C, D, E) and E. Feichtenberger, Instituto Biológico, Sorocaba, Brazil (a).



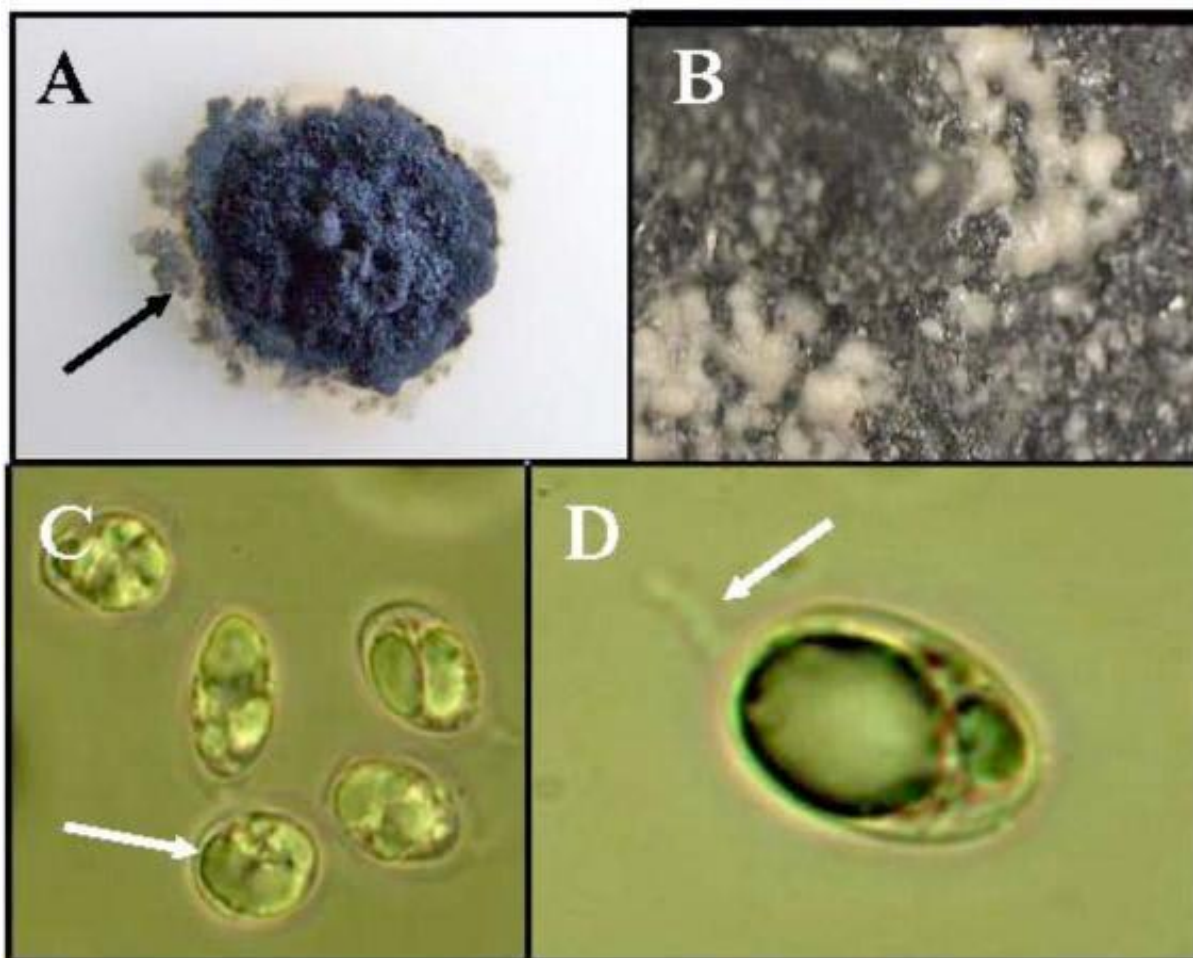
**Figure 3.** Symptoms of citrus black spot caused by *Phyllosticta citricarpa* on lemon (*Citrus limon*) leaves (A) and twigs (B).

Photos courtesy E. Feichtenberger, Instituto Biológico, Sorocaba, Brazil (A) and M. Truter, Plant Protection Research Institute, Agricultural Research Council, Pretoria, South Africa (B).



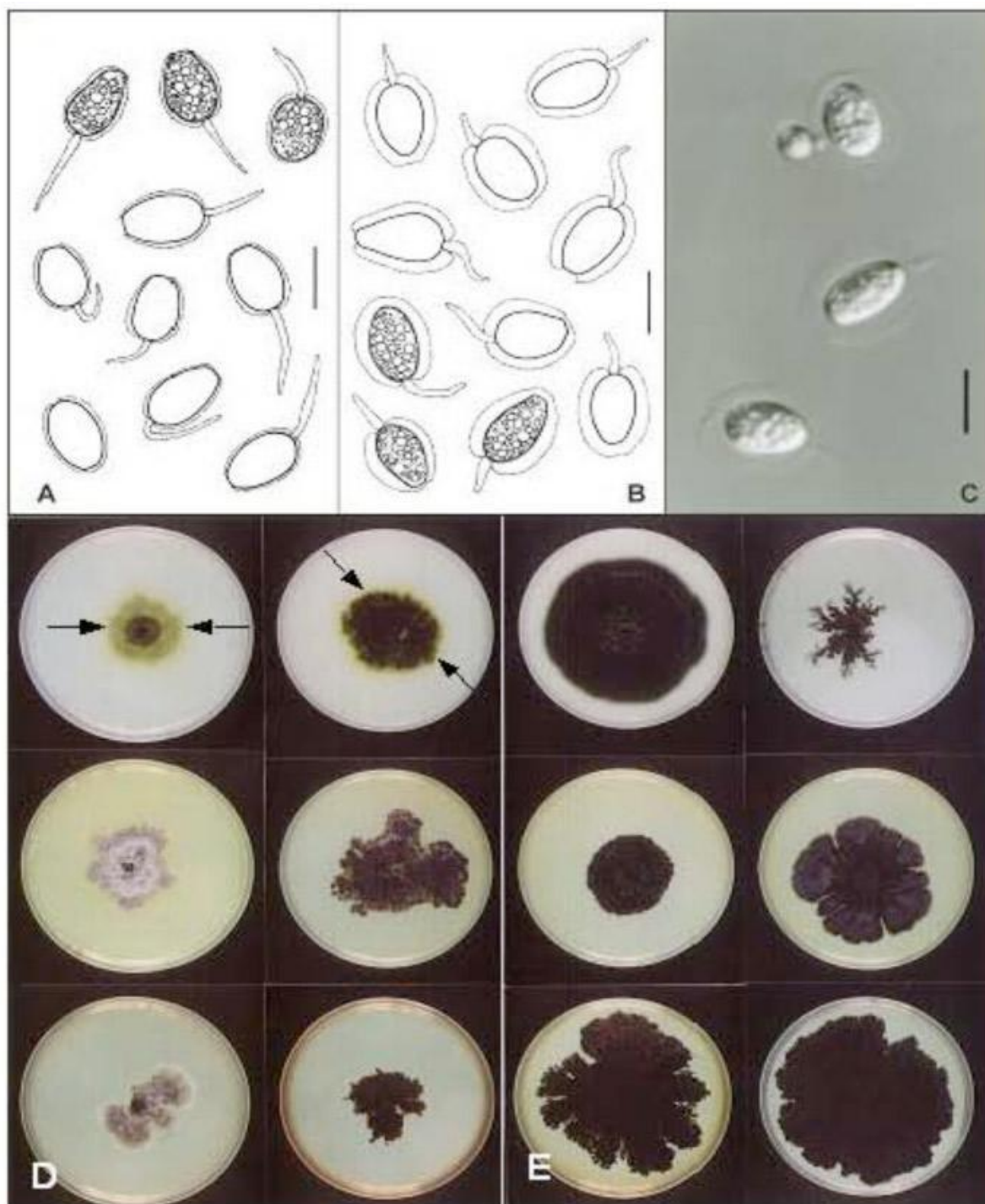
**Figure 4.** Flow diagram for the identification of *Phyllosticta citricarpa* on citrus fruit

<sup>1</sup>The molecular assays have been validated for the identification of the organism on pure cultures and fruit lesions and not on any other plant material (e.g. leaves, twigs). ITS, internal transcribed spacer; PCR, polymerase chain reaction.



**Figure 5.** Colony characteristics and conidial morphology of *Phyllosticta citricarpa*: (A) colony with irregular margin surrounded by a translucent zone of colourless submerged mycelium (arrow) after 30 days of growth on potato dextrose agar (pH 5.5) at 25 °C and a 12 h photoperiod; (B) conidial slime oozing from mature pycnidia; (C, D) conidia with a thin mucoid sheath (C, arrow) and a colourless subulate appendage (D, arrow, magnification 1 000× with immersion oil).

*Photos courtesy L.E. Diaz, Ministry of Husbandry, Agriculture and Fisheries, Montevideo, Uruguay.*



**Figure 6.** Conidial morphology and cultural characteristics of *Phyllosticta citricarpa* and *Phyllosticta capitalensis*: (A) conidia of *P. citricarpa* with thin (<1.5 µm) mucoid sheath; (B, C) conidia of *P. capitalensis* with thick (>1.5 µm) mucoid sheath (scale bar = 10 µm) (photo C was taken under a light microscope equipped with differential interference contrast); (D, E) colonies of *P. citricarpa* (D) and *P. capitalensis* (E) after 7 days of growth on oatmeal agar (top row), malt extract agar (middle row) and cherry decoction agar (bottom row) (note the production of a yellow pigment around the colony of *P. citricarpa* grown on oatmeal agar (D, arrows) and the absence of this pigment in cultures of *P. capitalensis* grown on the same medium (E)).

Photos courtesy G. Verkley, Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands (A, B, C) and W. van Lienden, Plant Protection Service, Wageningen, The Netherlands (D, E).

**Publication history**

*This is not an official part of the standard*

2006-03 CPM-1 added Work programme topic: Fungi and fungus-like organisms 2006-006

2004-11 SC added subject *Guignardia citricarpa* (2004-023)

2011-11 SC approved for member consultation via e-decision (2011\_eSC\_Nov\_06)

2012-07 Member consultation

2013-03 Title changed to *Phyllosticta citricarpa* (McAlpine) Aa on fruit (2004-023)

2013-07 TPDP revised and submitted to the SC for approval for adoption (2013\_eTPDP\_Jun\_01)

2013-10 SC approved for the 45 days notification period via e-decision (2013\_eSC\_Nov\_13)

2014-12/01 DP notification period - formal objection received

2014-02/03 TPDP revised in virtual meeting

2014 SC approved for the 45 days notification period via e-decision (2014\_eSC\_Nov\_01)

2014-07/08 DP notification period

2014-08 SC adopted DP on behalf of CPM

**ISPM 27. 2006: Annex 5. *Phyllosticta citricarpa* (McAlpine) Aa on fruit (2014). Rome, IPPC, FAO.**

Publication history last updated: 2014-08-29



**ISPM 27  
Annex 6**

## **INTERNATIONAL STANDARDS FOR PHYTOSANITARY MEASURES**

### **ISPM 27 DIAGNOSTIC PROTOCOLS**

#### **DP 6: *Xanthomonas citri* subsp. *citri* (2014)**

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## 1. Pest Information

*Xanthomonas citri* subsp. *citri* is the major causal agent of citrus bacterial canker. It causes damage to many cultivated species of Rutaceae (EPPO, 1979) – primarily *Citrus* spp., *Fortunella* spp. and *Poncirus* spp. – grown under the tropical and subtropical conditions that are prevalent in many countries in Asia, South America, Oceania and Africa as well as in Florida, United States (CABI, 2006; EPPO, 2006). Atypical strains of *X. citri* subsp. *citri* with a restricted host range have been identified and are designated as strains A\* and A<sup>w</sup> (Sun *et al.*, 2004; Vernière *et al.*, 1998). Strain A\* affects *Citrus aurantiifolia* (Mexican lime) under natural conditions in Asia. Strain A<sup>w</sup> causes canker in *Citrus aurantiifolia* (Mexican lime) and *Citrus macrophylla* (Alemow) in Florida, United States under natural conditions (Cubero and Graham, 2002, 2004). Both of these strains have been reported to cause atypical lesions in other citrus species experimentally (Escalon *et al.*, 2013).

Citrus bacterial canker typically occurs on seedlings and on young and adult trees of susceptible hosts in which there is a flush of actively growing shoots and leaves from late summer through to autumn in most citrus growing areas. Canker lesions are formed on the leaves, shoots, twigs and fruits of susceptible hosts. Wounds caused by wind, thorns, insects, and physical or mechanical damage facilitate infection of mature tissues. Attacks of *Phyllocnistis citrella*, the citrus leaf miner, can increase the susceptibility of leaves to citrus canker (Hall *et al.*, 2010).

*X. citri* subsp. *citri* can survive in diseased plant tissues, as an epiphyte on host and non-host plants, and as a saprophyte on straw mulch or in soil. However, overwintering lesions, particularly those formed on angular shoots, are the most important source of inoculum for the following season. The main mechanisms of short distance dispersal are wind-driven rain and splashing of water within and between plants: the bacteria are disseminated by rainwater running over the surface of lesions and then splashing onto healthy shoots (CABI, 2006). The movement of infected plant material, including budwood, rootstock seedlings and budded trees, has been implicated in long distance dispersal. There is no evidence that this pathogen is seed-borne (CABI, 2006).

## 2. Taxonomic Information

<b>Name:</b>	<i>Xanthomonas citri</i> subsp. <i>citri</i> (Gabriel <i>et al.</i> 1989) Schaad <i>et al.</i> 2007
<b>Synonyms:</b>	<i>Xanthomonas smithii</i> subsp. <i>citri</i> Gabriel <i>et al.</i> , 1989, Schaad <i>et al.</i> , 2007
	<i>Xanthomonas axonopodis</i> pv. <i>citri</i> (Hasse) Vauterin <i>et al.</i> , 1995
	<i>Xanthomonas citri</i> (ex Hasse, 1915) Gabriel <i>et al.</i> , 1989
	<i>Xanthomonas campestris</i> pv. <i>aurantifolii</i> Gabriel <i>et al.</i> , 1989
	<i>Xanthomonas campestris</i> pv. <i>citri</i> (Hasse) Dye, 1978
	<i>Xanthomonas citri</i> f.sp. <i>aurantifoliae</i> Namekata and Oliveira, 1972
	<i>Pseudomonas citri</i> Hasse, 1915

**Taxonomic position:** Bacteria, Proteobacteria, Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae

**Common names:** citrus canker, citrus bacterial canker, asiatic canker

**Note:** *X. citri* subsp. *citri* has been recently reclassified from *X. axonopodis* pv. *citri* (*X. campestris* pv. *citri* group A strains). The nomenclature of Gabriel *et al.* (1989) has been reinstated and the accepted name for the citrus bacterial canker pathogen is now *X. citri* subsp. *citri* (Bull *et al.*, 2010; Schaad *et al.*, 2006). The other group strains of *X. campestris* pv. *citri* have been reclassified as *Xanthomonas fuscans* subsp. *aurantifolii* (groups B, C and D) and *Xanthomonas alfalfae* subsp. *citrumelonis* (group E) (Schaad *et al.*, 2006).

### 3. Detection

#### 3.1 Detection in symptomatic plants

Diagnosis of citrus canker can be achieved by observing morphological characteristics of the colonies on nutrient media and by serological testing (by immunofluorescence (IF)), molecular testing (by polymerase chain reaction (PCR)) and bioassay of leaf discs or detached leaves. Positive and negative controls must be included for all tests (see section 4 for reference controls).

##### 3.1.1 Symptoms

The disease characteristically causes scabs or crater-like lesions on the rind of fruits and on leaves, stems and shoots. Symptoms of citrus canker can occur on seedlings in any season and on young trees from late summer through to autumn, when a flush of abundant growth of angular shoots occurs (CABI, 2006) (Figures 1–4). The disease becomes sporadic as trees reach full fruiting development, because fewer angular shoots are produced and older leaf tissue and mature fruit are more resistant to citrus canker infection under natural conditions. Disease severity also depends on the susceptibility of the host plant species and cultivars (Goto, 1992).

*Symptoms on fruits.* Crater-like lesions develop on the surface of the fruit; they may be scattered singly over the fruit or several lesions may occur together with an irregular pattern. Exudation of resinous substances may be observed on young infected fruits. The lesions never extend through the rind.

*Symptoms on branches.* In dry conditions, the canker spot is corky or spongy, is raised, and has a ruptured surface. In moist conditions, the lesion enlarges rapidly, and the surface remains unruptured and is oily at the margin. In the less susceptible cultivars, a callus layer may form between the diseased and healthy tissues. The scar of a canker may be identified by scraping the rough surface with a knife to remove the outer corky layer, revealing light to dark brown lesions in the healthy green bark tissues. The discoloured area can vary in shape and in size from 5 to 10 mm, depending on the susceptibility of the host plant.

*Symptoms on leaves.* Bright yellow spots are first apparent on the underside of leaves, followed by erumpent brownish lesions on both sides of the leaves, which become rough, cracked and corky. The canker may be surrounded by a water-soaked yellow or chlorotic halo margin.

Confusion may occur between symptoms on branches, leaves and fruit of citrus canker and scab or leaf spot-like symptoms caused by other bacteria or fungi that infect citrus or by physiological disorders. Other bacteria that can cause citrus canker-like symptoms are *X. alfalfae* subsp. *citrumelonis* and *X. fuscans* subsp. *aurantifolii*. Both of these bacteria have a limited host range, cause less aggressive symptoms and rarely produce lesions on fruit (Schaad *et al.*, 2005, 2006). Citrus scab caused by the fungus *Elsinoë fawcettii* has been reported to have symptoms similar to citrus canker, especially on host varieties that exhibit resistance to citrus scab (Taylor *et al.*, 2002), but in general its scab lesions are drier and more irregular than those of citrus canker and sometimes lack the characteristic yellow halo. Citrus scab can be differentiated from citrus canker by the lack of bacterial ooze.

### 3.1.2 Isolation

Freshly prepared sample extracts are essential for successful isolation of *X. citri* subsp. *citri* from symptomatic plant material. Plant material should be analysed as soon as possible after collection; it may be stored at 4–8 °C until processing. When symptoms are very advanced or when environmental conditions are not favourable, the number of *X. citri* subsp. *citri* culturable cells can be very low and isolation can result in plates being overcrowded with competing saprophytic or antagonistic bacteria. Particular care should be taken not to confuse *X. citri* subsp. *citri* colonies with *Pantoea agglomerans*, which is also commonly isolated from canker lesions, and produces morphologically similar colonies on standard bacteriological media. *P. agglomerans* is generally faster growing and the colonies are a brighter yellow than the pale yellow/lemon colonies of *X. citri* subsp. *citri*.

Isolation of the causal organism can be performed by streaking lesion extracts onto plates of suitable media, on which colonies of *X. citri* subsp. *citri* have a characteristic appearance. There are as yet no exclusively selective media available for *X. citri* subsp. *citri*.

Lesions are macerated in 0.5–1.0 ml saline (distilled sterile water with NaCl to 0.85%, pH 7.0), and when required they may be disinfected beforehand with 1% NaClO for 1 min, rinsed three times with sterile distilled water, and pulverized. An aliquot of the extract is streaked on nutrient media. Suitable general isolation media are nutrient agar supplemented with 0.1% glucose (NGA), yeast peptone glucose agar (YPGA) (yeast extract, 5 g; Bacto Peptone, 5 g; glucose, 10 g; agar, 20 g; distilled water, 1 litre; pH 7.0) and Wakimoto medium: (potato broth 250 ml; sucrose, 15 g; peptone, 5 g; Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, 0.8 g; Ca(NO<sub>3</sub>)<sub>2</sub>·7 H<sub>2</sub>O, 0.5 g; Bacto™ Agar, 20 g; distilled water, 1 litre; pH 7.2). Filter-sterilized cycloheximide (100 mg/litre) can be added when necessary as a fungicide after autoclaving the media.

The colony morphology on all three media is round, convex and smooth-edged, and the colony is mucoid and creamy yellow. Growth is evaluated after incubation at 25–28 °C for three to five days. In commercial fruit samples, the bacteria can be stressed and may not be easily cultured; therefore, longer incubations may be required or bioassays can be used to recover the bacteria from the samples, as described in section 3.1.6.2. Integration of kasugamycin and cephalixin in the medium (semi-selective KC or KCB medium) inhibits several saprophytic bacteria and facilitates isolation of the pathogen (Graham *et al.*, 1989; Pruvost *et al.*, 2005).

In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and reproducibility achieved. The use of names of chemicals (e.g. brand names) implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

### 3.1.3 Serological detection: Indirect immunofluorescence

For serological detection (IF and enzyme-linked immunosorbent assay (ELISA)), appropriate controls are essential to ensure that test results are reliable. A positive and negative control should be included in each test. Positive controls can consist of a reference *X. citri* subsp. *citri* strain resuspended in healthy host plant extract (for detection in plant material) or in phosphate-buffered saline (PBS) (for identification of bacterial cultures). Negative controls should consist of healthy host plant extract (for detection in plant material) or a suspension of a non-target bacterial species (for identification of bacterial cultures).

For serological detection of bacterial cells, a loopful of fresh culture is collected from the plate and resuspended in 1 ml PBS (NaCl, 8 g; KCl, 0.2 g; Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, 2.9 g; KH<sub>2</sub>PO<sub>4</sub>, 0.2 g; distilled water to 1 litre; pH 7.2) to make approximately 10<sup>8</sup> colony-forming units (cfu)/ml (EPPO, 2009).

For serological detection in plant tissue, samples with symptoms – shoots, twigs, leaves and fruits, all with necrotic lesions, or tissue from cankers on twigs, branches, the trunk or the collar – should be chosen. The samples should be processed following the general procedure recommended for the

specific serological test to be used. Generally, plant tissue is ground in freshly prepared antioxidant maceration buffer (polyvinylpyrrolidone (PVP)-10, 20 g; mannitol, 10 g; ascorbic acid, 1.76 g; reduced glutathione, 3 g; PBS, 10 mM, 1 litre; pH 7.2) or in PBS (NaCl, 8 g; KCl, 0.2 g; Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, 2.9 g; KH<sub>2</sub>PO<sub>4</sub>, 0.2 g; distilled water to 1 litre; pH 7.2) before use in serological tests. Both solutions are filter-sterilized using a sterile 0.22 µm membrane.

Aliquots of 25 µl of each bacterial preparation or plant sample to be tested are pipetted onto a plastic-coated multi-window microscope slide, allowed to air-dry and then gently heat-fixed over a flame. Separate slides are set up for each test bacterium or sample, and also for positive and negative controls as are used for ELISA. Commercially available antiserum or monoclonal antibodies are diluted with PBS (pH 7.2) and 25 µl of appropriate dilutions are added to the windows of each slide. Negative controls can consist of normal (pre-immune) serum at one dilution and PBS. Slides are incubated in a humid chamber at room temperature for 30 min. The droplets are shaken off the slides and they are rinsed with PBS and then washed three times for 5 min each in PBS. The slides are gently blotted dry before 25 µl of the appropriate anti-species gamma globulin-fluorescein isothiocyanate conjugate (FITC) at the appropriate dilution is pipetted into each window. The slides are incubated in the dark at room temperature for 30 min, rinsed, washed and blotted dry. Finally, 10 µl of 0.1 mmol/litre phosphate-buffered glycerine (pH 7.6) with an anti-fading agent is added to each window, which is then covered with a coverslip.

The slides are examined under immersion oil with a fluorescence microscope at 600× or 1 000× magnification. FITC fluoresces bright green under the ultraviolet light of the microscope. If the positive control with known bacterium shows fluorescent rod-shaped bacterial cells and the negative controls of normal serum and PBS do not show fluorescence, the sample windows are examined for fluorescent bacterial cells with the size and form of *X. citri* subsp. *citri*. This method permits detection of approximately 10<sup>3</sup> cfu./ml.

### 3.1.4 Molecular detection

#### 3.1.4.1 Controls for molecular testing

For the test result obtained to be considered reliable, appropriate controls – which will depend on the type of test used and the level of certainty required – are essential. For PCR, a positive nucleic acid control, an internal control and a negative amplification control (no template control) are the minimum controls that should be used. These and other controls that should be considered for each series of nucleic acid extractions from your test samples as described below.

**Positive nucleic acid control.** Pre-prepared (stored) nucleic acid, whole genome DNA or a synthetic control (e.g. a cloned PCR product) may be used as a control to monitor the efficiency of PCR amplification.

**Internal controls.** For conventional and real-time PCR, a plant housekeeping gene (HKG) such as COX (Weller *et al.*, 2000), 16S ribosomal (r)DNA (Weisberg *et al.*, 1991) or GADPH (Mafra *et al.*, 2012) should be incorporated into the PCR protocol as a control to eliminate the possibility of false negatives due to nucleic acid extraction failure or degradation or the presence of PCR inhibitors.

**Negative amplification control (no template control).** For conventional and real-time PCR, PCR-grade water that was used to prepare the reaction mixture is added at the amplification stage to rule out false positives due to contamination during preparation of the reaction mixture.

**Positive extraction control.** This control is used to ensure that nucleic acid from the target is of sufficient quality for PCR amplification. Nucleic acid is extracted from infected host tissue or healthy plant tissue that has been spiked with the target at the concentration considered the detection limit of the protocol.

The positive control should be approximately one-tenth of the amount of leaf tissue used per plant for the DNA extraction. For PCR, care needs to be taken to avoid cross-contamination due to aerosols

from the positive control or from positive samples. If required, the positive control used in the laboratory should be sequenced so that the sequence can be readily compared with sequences obtained from PCR amplicons of the correct size. Alternatively, synthetic positive controls can be made with a known sequence, which, again, can be compared to PCR amplicons of the correct size.

**Negative extraction control.** This control is used to monitor contamination during nucleic acid extraction and cross-reaction with the host tissue. The control comprises of nucleic acid that is extracted from uninfected host tissue and subsequently amplified. Multiple controls are recommended when large numbers of positive samples are tested.

### 3.1.4.2 DNA extraction from infected citrus tissue

DNA extraction from infected citrus tissue was originally performed by Hartung *et al.* (1993) with a hexadecyltrimethylammonium bromide (CTAB) protocol, but there are commercial methods and an isopropanol protocol (not requiring phenol) that have been extensively evaluated (Llop *et al.*, 1999). DNA has also been successfully extracted from citrus tissue using commercial DNA extraction kits (e.g. Promega Wizard Genomic DNA Purification Kit) (Coletta-Filho *et al.*, 2006).

In the isopropanol protocol, lesions or plant material suspected to be infected are cut into small pieces, covered with PBS and shaken in a rotary shaker for 20 min at room temperature. The supernatant is filtered (to remove plant material) and then centrifuged at 10 000 *g* for 20 min. The pellet is resuspended in 1 ml PBS: 500 µl is saved for further analysis or for direct isolation on agar plates, and 500 µl is centrifuged at 10 000 *g* for 10 min. The pellet is resuspended in 500 µl extraction buffer (200 mM Tris-HCl, pH 7.5; 250 mM NaCl; 25 mM ethylenediaminetetraacetic (EDTA); 0.5% sodium dodecyl sulphate (SDS); 2% PVP), vortexed and left for 1 h at room temperature with continuous shaking. The suspension is then centrifuged at 5 000 *g* for 5 min, after which 450 µl of the supernatant is transferred to a new tube and mixed with 450 µl isopropanol. The suspension is mixed gently and left for 1 h at room temperature. Precipitation can be improved by the use of Pellet Paint co-precipitant (Cubero *et al.*, 2001). The suspension is centrifuged at 13 000 *g* for 10 min, the supernatant is discarded, and the pellet is dried. The pellet is resuspended in 100 µl water. A 5 µl sample is used in a 50 µl PCR.

### 3.1.4.3 Conventional PCR

Several primer pairs are available for diagnosis of *X. citri* subsp. *citri*. Hartung *et al.* (1993) primers 2 and 3 target a *Bam*HI restriction fragment length polymorphic DNA fragment specific to *X. citri* subsp. *citri* and are the most frequently used in assays on plant material because of their good specificity and sensitivity (approximately 10<sup>2</sup> c.f.u./ml). Primers *J-pth1* and *J-pth2* target a 197 base pair (bp) fragment of the nuclear localization signal in the virulence gene *pthA* in *Xanthomonas* strains that cause citrus canker symptoms. These strains include *X. citri* subsp. *citri*, *X. fuscans* subsp. *aurantifolii* and the atypical *X. citri* subsp. *citri* strains A\* and A<sup>w</sup> detected in Florida (Cubero and Graham, 2002). The primers are universal, but they have lower sensitivity (10<sup>4</sup> cfu/ml in plant material) than the Hartung *et al.* (1993) primers. However, the Hartung primers do not detect the *X. citri* subsp. *citri* strain A<sup>w</sup> and all A\* strains or *X. fuscans* subsp. *aurantifolii*. In situations where the presence of atypical *X. citri* subsp. *citri* strains A\* and A<sup>w</sup> is suspected – for example, where citrus canker symptoms are observed on the hosts *C. aurantiifolia* (Mexican lime) and *C. macrophylla* (Alemow) – both primer sets should be used.

#### PCR protocol of Hartung *et al.* (1993)

The primers are:

2 (Reverse): 5'-CAC GGG TGC AAA AAA TCT-3'

3 (Forward): 5'-TGG TGT CGT CGC TTG TAT-3'.

The PCR mixture is prepared in a sterile tube and consists of PCR buffer (50 mM Tris-HCl, pH 9; 20 mM NaCl; 1% Triton X-100; 0.1% gelatin; 3 mM MgCl<sub>2</sub>), 1 µM each primer 2 and 3, 0.2 mM each deoxynucleotide triphosphate (dNTP) and 1.25 U Taq DNA polymerase. Extracted DNA sample

volume of 5 µl is added to 45 µl of the PCR mixture to give a total of 50 µl per reaction. The reaction conditions are an initial denaturation step of 95 °C for 2 min followed by 35 cycles of 95 °C for 60 s, 58 °C for 70 s and 72 °C for 75 s, and a final elongation step of 72 °C for 10 min. The amplicon size is 222 bp.

### PCR protocol of Cubero and Graham (2002)

The primers are:

*J-pth1* (Forward): 5'-CTT CAA CTC AAA CGCC GGA C-3'

*J-pth2* (Reverse): 5'-CAT CGC GCT GTT CGG GAG-3'.

The PCR mixture is prepared in a sterile tube and consists of 1× Taq buffer, 3 mM MgCl<sub>2</sub>, 1 µM each primer *J-pth1* and *J-pth2*, 0.2 mM each dNTP and 1 U Taq DNA polymerase. Extracted DNA sample volume of 2.5 µl is added to 22.5 µl of the PCR mixture to give a total of 25 µl per reaction. The reaction conditions are an initial denaturation step of 94 °C for 5 min followed by 40 cycles of 93 °C for 30 s, 58 °C for 30 s and 72 °C for 45 s, and a final elongation step of 72 °C for 10 min. The amplicon size is 198 bp.

Nested PCR, immunocapture and colorimetric detection of nested PCR products for direct and sensitive detection of *X. citri* subsp. *citri* in plants have also been developed (Hartung *et al.*, 1993). A review of the comparative sensitivity of the different protocols and primers in pure culture and fruit extracts has been reported (Golmohammadi *et al.*, 2007).

#### 3.1.4.4 Real-time PCR

After obtaining DNA from plant material by using the protocol previously described by Llop *et al.* (1999), the pellet is resuspended in 100 µl sterile ultrapure water and stored at –20 °C until use.

A set of primers, *J-pth3* (5'-ACC GTC CCC TAC TTC AAC TCA A-3') and *J-pth4* (5'-CGC ACC TCG AAC GAT TGC-3'), and the corresponding TaqMan probe (*J-Taqpht2*) (5'-ATG CGC CCA GCC CAA CGC-3') labelled at the 5' end with 6-carboxyfluorescein (FAM) and at the 3' end with tetramethylrhodamine were designed based on sequences of the *pth* gene, a major virulence gene used in other studies specifically to detect *X. citri* subsp. *citri* strains (Cubero and Graham, 2005). These strains include *X. citri* subsp. *citri*, *X. fuscans* subsp. *aurantifolii* and the atypical *X. citri* subsp. *citri* strains A<sup>\*</sup> and A<sup>w</sup> detected in Florida.

Real-time PCR is carried out by adding 2 µl template DNA to a reaction mixture containing 12.5 µl QuantiMix Easy Kit, which comprises QuantiMix Easy Master Mix and MgCl<sub>2</sub> (50 mM), 1 µl of 10 µM forward primer (*J-RTpth3*), 1 µl of 10 µM reverse primer (*J-RTpth4*) and 0.5 µl of 10 µM TaqMan probe (*J-Taqpht2*) and made up to a final reaction volume of 25 µl with sterile distilled water. The protocol for real-time PCR has been developed using an ABI PRISM 7000 Sequence Detection System. Other equipment has provided similar results (María Lopez, pers. comm., 2013). Amplification conditions for primers and probes are an initial activation step of 15 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. A complete real-time PCR kit based on this protocol and including master mix and enzyme is available from Plant Print Diagnostics (<http://www.plantprint.net>).

The real-time PCR provides similar specificity to the *pth* gene primers used in the conventional PCR method (Cubero and Graham, 2002, 2005) and enables reliable detection of approximately 10 cfu of *X. citri* subsp. *citri* from diseased leaf lesions and from a dilution of cultured cells (Mavrodieva *et al.*, 2004). This method has recently been compared with standard and nested PCR (Golmohammadi *et al.*, 2007) and the sensitivity of detection of *X. citri* subsp. *citri* in fruit lesions was reported to be 10 cfu/ml.

### 3.1.5 Interpretation of results from conventional and real-time PCR

#### Conventional PCR

The pathogen-specific PCR will be considered valid only if the below criteria are met:

- the positive control produces the correct size amplicon for the bacterium
- no amplicons of the correct size for the bacterium are produced in the negative extraction control and the negative amplification control.

If 16S rDNA internal control primers are also used, then the negative (healthy plant tissue) control (if used), positive control, and each of the test samples will produce an approximately 1.6 kilobase (kb) band (amplicon size will depend on which 16S rDNA primers are used (Weisberg *et al.*, 1991)). Note that synthetic and plasmid positive controls will not produce a 1.6 kb band. Failure of the samples to amplify with the internal control primers suggests, for example, that the DNA extraction has failed, the nucleic acid has not been included in the reaction mixture, compounds inhibitory to PCR are present in the DNA extract, or the DNA has degraded.

A sample will be considered positive if it produces an amplicon of the correct size.

#### Real-time PCR

The real-time PCR will be considered valid only if the below criteria are met:

- the positive control produces an amplification curve with the pathogen-specific primers
- no amplification curve is seen (i.e. cycle threshold (Ct) value is 40) with the negative extraction control and the negative amplification control.

If the COX internal control primers are also used, then the negative control (if used), positive control, and each of the test samples must produce an amplification curve. Failure of the samples to produce an amplification curve with the internal control primers suggests, for example, that the DNA extraction has failed, the DNA has not been included in the reaction mixture, compounds inhibitory to PCR are present in the DNA extract, or the DNA has degraded.

A sample will be considered positive if it produces a typical amplification curve. The cycle cut-off value needs to be verified in each laboratory when implementing the test for the first time.

### 3.1.6 Detection by bioassays

#### 3.1.6.1 Inoculation test in leaf discs

In this test, citrus leaf tissue susceptible to *X. citri* subsp. *citri* is inoculated with diseased sample extracts and incubated under appropriate conditions for bacterial multiplication and development of incipient pustules of the disease.

The procedure for this bioassay begins by sterilizing ELISA plates for 15 min in a microwave oven and adding to their wells 200 µl of 1.5% agar in sterile water in a laminar flow chamber at room temperature. Young citrus leaves from *Citrus paradisi* var. Duncan (grapefruit) or other susceptible hosts, for example, *Citrus aurantifolia* (Mexican lime) or *Poncirus trifoliata* (trifoliate orange), are surface-disinfected for 1 min with 1% NACIO. The leaves should be fully expanded but not mature and hard. The leaves are rinsed three times with sterile distilled water and then surface-dried in a laminar flow chamber at room temperature. The leaf discs, obtained with a hole punch (disinfected with 95% ethanol), are placed adaxial surface down on the water agar in each well. Fifty microlitres of macerated citrus canker lesions (four replicate wells for each plant sample) are added.

An *X. citri* subsp. *citri* suspension of 10<sup>5</sup> cfu/ml is used as a positive control and sterile saline as a negative control (four replicates each). Plates are sealed (e.g. Parafilm), achieving a relative humidity of almost 100%, and incubated at 28 °C for 12 days under constant light, with progress checked regularly. The formation of incipient whitish pustules in each of the leaf discs is evaluated from the third day using stereoscopic microscopy and isolation techniques for *X. citri* subsp. *citri* as described

in section 3.1.2. The symptomless discs can be further analysed for the presence of living bacteria by isolation onto semi-selective media (Verdier *et al.*, 2008). After 12 days, if *X. citri* subsp. *citri* is present, the bacterial cells have multiplied on the plant tissue and can be isolated onto media in higher numbers. This bioassay is a very specific and sensitive ( $10^2$  cfu/ml) diagnostic method (Verdier *et al.*, 2008).

### 3.1.6.2 Detached leaf enrichment

*X. citri* subsp. *citri* can also be selectively enriched in wounded detached leaves of *C. paradisi* var. Duncan (grapefruit) or other highly susceptible hosts, for example, *C. aurantifolia* (Mexican lime) or *P. trifoliata* (trifoliate orange). Young terminal leaves from glasshouse-grown plants are washed for 10 min in running tap water, surface-disinfected in 1% NACIO for 1 min, and aseptically rinsed thoroughly with sterile distilled water. The lower surface of each leaf is aseptically wounded by puncturing it with a needle or by making small cuts with a scalpel, and the whole leaves are placed onto 1% agar in sterile water in the wells of ELISA plates with their lower surface up. Droplets of 10–20 µl of macerated citrus canker lesions are added to the wounds. Positive and negative controls as for the leaf disc bioassay are used. After 4–12 days at 25 °C in a lighted incubator, pustule development is evaluated and *X. citri* subsp. *citri* can be isolated from either the pustules or the symptomless wounded leaf tissue as described above (EPPO, 1998).

## 3.2 Detection in asymptomatic plants

Detection of *X. citri* subsp. *citri* in asymptomatic plants can be achieved by isolation and enrichment on semi-selective media (see below), serological techniques (IF (section 3.1.3)) and molecular testing (section 3.1.4).

Isolation of *X. citri* subsp. *citri* from asymptomatic plants on semi-selective media can be achieved by washing the leaf or fruit samples in peptone buffer, concentrating the supernatant, and then plating onto the media (Verdier *et al.*, 2008). Ten leaves or one fruit constitute a sample.

Samples are shaken for 20 min at room temperature in 50 ml peptone buffer (NaCl, 8.5 g; peptone, 1 g; Tween 20, 250 µl; distilled water, 1 litre; pH 7.2). For bulked samples, 100 leaves in 200 ml peptone buffer can be used. Individual fruits are shaken for 20 min at room temperature in sterile bags containing 50 ml peptone buffer.

The suspension is then centrifuged at 6 000 g for 20 min. The supernatant is decanted and the pellet resuspended in 10 ml of 0.85% saline. Aliquots (100 µl) of 1:100 and 1:1000 dilutions of each suspension are streaked in triplicate onto XOS semi-selective medium (sucrose, 20 g; peptone, 2 g; monosodium glutamate, 5 g;  $\text{Ca}(\text{NO}_3)_2$ , 0.3 g;  $\text{K}_2\text{HPO}_4$ , 2 g; EDTA-Fe, 1 mg; cycloheximide, 100 mg; cephalaxine, 20 mg; kasugamycin, 20 mg; methyl violet 2B, 0.3 mg; Bacto Agar, 17 g; distilled water, 1 litre; pH 7.0) (Monier, 1992). After incubation at 28 °C for 5–6 days, growth as well as colony type and morphology are evaluated (section 3.1.2).

## 4. Identification

Identification of presumptive *X. citri* subsp. *citri* colonies should be verified by several techniques because other species of *Xanthomonas*, such as *X. fuscans* subsp. *aurantifolii* and *X. alfalfae* subsp. *citrumelonis*, can be isolated from citrus. Techniques in addition to observing morphological characteristics on nutrient media, include serological testing, molecular testing, bioassay of leaf discs or detached leaves, and pathogenicity testing.

The minimum requirements for identification of a pure culture are a positive result from each of the following three techniques: (1) PCR using two sets of primers (section 4.1); (2) a serological technique (IF, double antibody sandwich (DAS)-ELISA or indirect ELISA sections 4.2, and 4.2.1 and 4.2.2) using specific monoclonal antibodies; and (3) pathogenicity testing by inoculation of citrus hosts to fulfil the requirements of Koch's postulates (sections 4.3 and 3.1.6). Additional tests (sections 4.4 and 4.5) may be done to further characterize the strain present. In all tests, positive and

negative controls must be included. The recommended techniques are described in the following sections.

The following collections, among others, can provide *X. citri* subsp. *citri* reference strains (the *X. citri* subsp. *citri* isolates recommended for use as positive controls are given):

- NCPPB 3234 from National Collection of Plant Pathogenic Bacteria, Central Science Laboratory, York, United Kingdom
- CFPB 2911 from Collection Française de Bactéries Phytopathogènes, INRA Station Phytobactériologie, Angers, France (this is a *X. citri* subsp. *citri* A\* strain)
- ICMP 24 from International Collection of Microorganisms from Plants, Landcare Research (Manaaki Whenua) New Zealand Ltd, Auckland, New Zealand
- ATTC 49118 from American Type Culture Collection, Manassas, VA, United States
- IBSBF 1594 from Biological Institute Culture Collection of Phytopathogenic Bacteria, Centro Experimental Central do Instituto Biológico - Laboratório de Bacteriologia Vegetal, Campinas, Brazil.

The authenticity of the strains can be guaranteed only if obtained directly from the culture collections.

#### 4.1 PCR methods

It is recommended that in addition to the PCR protocol described in section 3.1.4.3, the identification of pure cultures of suspect strains is confirmed by using two different sets of primers. One set should be the *J-ptH1/J-ptH2* or *J-Rxg/J-Rxc2* primers (Cubero and Graham, 2002) and the other set the *Xac01/Xac02* (Coletto-Filho *et al.*, 2005) or *XACF/XACR* primers (Park *et al.*, 2006) (Table 1). This is because of the findings that most published primer pairs lack specificity (Delcourt *et al.*, 2013). Identification can be further confirmed by sequencing the resulting PCR amplicons and comparing their sequences with those of *X. citri* subsp. *citri* strains deposited in the National Center for Biotechnology Information (NCBI) GenBank database.

**PCR protocol of Cubero and Graham (2002)** developed PCR primers for the internal transcribed spacer (ITS) regions of 16S and 23S rDNAs specific to *X. citri* subsp. *citri*. Variation in the ITS sequences allowed the design of specific primers for *X. citri* subsp. *citri* and these primers detect the atypical strains A\* and A<sup>w</sup> (Cubero and Graham, 2002). The primers are:

*J-Rxg*: 5'-GCGTTGAGGCTGAGACATG-3'

*J-Rxc2*: 5'-CAAGTTGCCTCGGAGCTATC-3'.

PCR is carried out in 25 µl reaction mixtures containing 1× Taq buffer, 1.5 mM MgCl<sub>2</sub>, 0.04 µM primer *J-Rxg*, 0.04 µM primer *J-Rxc2*, 0.2 mM each dNTP and 1 U Taq DNA polymerase. The PCR amplification conditions are the same as those used with the *pthA* primers described in section 3.1.4.3.

**PCR protocol of Coletto-Filho *et al.* (2006)** developed primers based on the *rpf* gene cluster. The primers are:

*Xac01*: 5'-CGCCATCCCCACCACCACGAC-3'

*Xac02*: 5'-AACCGCTCAATGCCATCCACTTCA-3'.

PCR is carried out in 25 µl reaction mixtures containing 1× Taq buffer, 2.0 mM MgCl<sub>2</sub>, 0.36 µM each primer, 0.25 mM each dNTP and 1 U Taq DNA polymerase. The PCR amplification conditions are an initial denaturation step of 94 °C for 3 min followed by 36 cycles of 94 °C for 45 s, 60 °C for 45 s and 72 °C for 45 s, and a final elongation step of 72 °C for 5 min. The amplicon size is 582 bp.

**PCR protocol of Park *et al.* (2006)** developed primers based on the *hrpW* gene sequences. The primers are:

*XACF*: 5'-CGTCGCAATACGATTGGAAC-3'

*XACR*: 5'-CGGAGGCATTGTCTGAAGGAA-3'.

PCR is carried out in 25 µl reaction mixtures containing 1× Taq buffer, 1.5 mM MgCl<sub>2</sub>, 0.10 µM each primer, 0.25 mM each dNTP, 0.01% gelatin and 2 U Taq DNA polymerase. The PCR amplification conditions are an initial denaturation step of 94 °C for 5 min followed by 30 cycles of 94 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s, and a final elongation step of 72 °C for 7 min. The amplicon size is 561 bp.

**Table 1.** Summary of PCR methods described in this diagnostic protocol.

Specificity data are taken from Delcourt *et al.* (2013). \* Non-specific detection refers to the percentage of pathogenic xanthomonads and saprophytes that tested positive. \*\* Did not test positive with saprophytic strains.

Primer pair	Reference	Amplicon size (bp)	<i>X. citri</i> subsp. <i>citri</i> strain detection	Non-specific detection (%)*	Limits of detection in plant material
2/3	Hartung <i>et al.</i> (1993)	224	Does not detect A <sup>w</sup> and all A <sup>*</sup> strains	17	10 <sup>2</sup> cfu/ml
<i>J-pth1/J-pth2</i>	Cubero and Graham (2002)	198	All strains	51	10 <sup>4</sup> cfu/ml
<i>J-Rxg/J-Rxc2</i>	Cubero and Graham (2002)	179	All strains	30	10 <sup>4</sup> cfu/ml
<i>Xac01/Xac02</i>	Coletto-Filho <i>et al.</i> (2005)	582	All strains	16	10 <sup>4</sup> cfu/ml
<i>XACF/XACR</i>	Park <i>et al.</i> (2006)	561	All strains	6**	Not reported

## 4.2 Serological detection

It is recommended that in addition to the IF protocol described in section 3.1.3, different antibodies should be used for identification of pure cultures. DAS- ELISA or Indirect ELISA can also be used as alternative serological tests for the identification of pure cultures.

### 4.2.1 DAS-ELISA

For the DAS-ELISA, microtitre plates are coated with 100 µl/well carbonate coating buffer (Na<sub>2</sub>CO<sub>3</sub>, 1.59 g; NaHCO<sub>3</sub>, 2.93 g; NaN<sub>3</sub>, 0.2 g; distilled water, 1 litre; pH 9.6) containing appropriately diluted anti-*X. citri* subsp. *citri* immunoglobulins (IgG) and incubated overnight at 4 °C. After washing the plates three times with PBS-Tween (NaCl, 8 g; KH<sub>2</sub>PO<sub>4</sub>, 0.2 g; Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, 2.9 g; KCl, 0.2 g; NaN<sub>3</sub>, 0.2 g; Tween 20, 0.25 ml; distilled water, 1 litre; pH 7.4), test sample, negative control (healthy plant material) or positive control (reference strain of *X. citri* subsp. *citri*) is added (200 µl/well). The plates are incubated for 2 h at 37 °C. After washing, anti-*X. citri* subsp. *citri* IgG conjugated with alkaline phosphatase at the appropriate dilution in PBS-Tween is added (200 µl/well) and the plates are incubated for 2 h at 37 °C. After washing, p-nitrophenyl phosphate substrate buffer (1 mg/ml) is added (200 µl/well) and the plates are incubated for 30–60 min at room temperature. The absorbances are measured using a spectrophotometer equipped with a 405 nm filter. The criterion for determination of a sample as positive is two times the optical density (OD) value of the healthy plant material control. The detection limit of DAS-ELISA is 10<sup>4</sup>–10<sup>5</sup> cfu/ml (Civerolo and Fan, 1982). This method is not recommended for direct detection in plant tissue.

Monoclonal antibodies are available for ELISA, but are advised to be used only for identification of pure cultures because of their low sensitivity of detection in plant tissue. Commercial kits for detection of *X. citri* subsp. *citri* by ELISA are available (e.g. from Agdia, Inc.). For specificity data, refer to the technical information provided by the manufacturer. Some monoclonal antibodies have been reported to cross-react with *X. axonopodis* pv. *phaseoli*, *X. campestris* pv. *zinniae*, *X. alfalfae* subsp. *citrumelonis* and *Xanthomonas hortorum* pv. *pelargonii*; however, these pathovars are unlikely to be present on citrus.

### 4.2.2 Indirect ELISA

Indirect ELISA with monoclonal antibodies described by Alvarez *et al.* (1991) can be used for culture identification. ELISA kits containing all the necessary components for the identification of *X. citri* subsp. *citri* are available commercially (e.g. from Agdia, Inc.). In theory, all *X. citri* subsp. *citri* strains can be identified, but it has been reported that some phenotypically distinct strains isolated in South-West Asia do not react with the available monoclonal antibodies (Vernière *et al.*, 1998).

Pure culture suspensions are centrifuged at approximately 10 000 *g* for 2 min and the supernatant is discarded. One ml of 1× PBS is added and the cells are resuspended by vortexing. The operation is repeated twice more. After the third wash, the cells are resuspended in coating buffer. The bacterial concentration is adjusted spectrophotometrically to OD<sub>600</sub> 0.01 (approximately  $2.5 \times 10^7$  cfu/ml). Aliquots of the samples are loaded onto microtitre plates (two wells per sample, 100 µl/well). A positive control (a reference culture or sample provided by the manufacturer) and negative buffer control with another bacterium should be included. The plates are incubated overnight at 37 °C until they are dry. Blocking solution (5% non-fat dried milk powder in PBS) is added (200 µl/well). The plates are incubated for 30 min at room temperature and then washed twice with 1× PBS-Tween. Primary antibody at the appropriate dilution in 2.5% dried milk powder in PBS-Tween is added (100 µl/well). The plates are incubated for 1 h at room temperature and then washed five times with 1× PBS-Tween. Enzyme conjugate at the appropriate dilution in 2.5% dried milk powder in PBS-Tween is added (100 µl/well). The plates are incubated for 1 h at room temperature and then washed five times with 1× PBS-Tween. Freshly prepared substrate solution containing 1 mg/ml p-nitrophenyl phosphate in diethanolamine buffer (pH 9.8) is added (100 µl/well). The plates are incubated for 30–60 min at room temperature. The OD is measured using a spectrophotometer with a 405 nm filter. Positive samples are determined as for DAS-ELISA.

### 4.3 Pathogenicity testing

*X. citri* subsp. *citri* should be identified by pathogenicity on a panel of indicator hosts such as *C. paradisi* var. Duncan (grapefruit), *Citrus sinensis* (Valencia sweet orange) or *C. aurantiifolia* (Mexican lime) for confirmation of the diagnosis.

Leaf assays by infiltration with a syringe with or without needle on susceptible cultivars of *Citrus* hosts allow demonstration of pathogenicity of bacterial colonies. Immature leaves that are 50–70% to fully expanded are preferred due to their higher level of susceptibility. Lesions develop 7–14 days after inoculation of intact leaves or detached leaves (Francis *et al.*, 2010; Koizumi, 1971) after incubation at 25 °C in high humidity. With these assays, the eruptive callus-like reaction of *X. citri* subsp. *citri* can readily be distinguished. Bacteria grown in liquid media or colonies from a freshly streaked agar plate are resuspended in sterile distilled water and the concentration is adjusted to  $10^6$ – $10^8$  cfu/ml for inoculation into hosts. A negative and a positive control should always be included. Plants inoculated with the positive control strain should be kept separate from test plants.

### 4.4 Description and biochemical characteristics

*X. citri* subsp. *citri* is a Gram-negative, straight, rod-shaped bacterium measuring  $1.5\text{--}2.0 \times 0.5\text{--}0.75$  µm. It is motile by means of a single polar flagellum. It shares many physiological and biochemical properties with other members of the genus *Xanthomonas*. It is chemoorganotrophic and obligatorily aerobic with an oxidative metabolism of glucose. The yellow pigment is xanthomonadin. Some of the biochemical characteristics that identify *X. citri* subsp. *citri* are listed in Table 2.

**Table 2.** Key biochemical characteristics of *Xanthomonas citri* subsp. *citri*

Test	Result
Catalase	+
Oxidase	– or weak
Nitrate reduction	–
Hydrolysis of:	
starch	+
casein	+
Tween 80	+
aesculin	+
Gelatin liquefaction	+
Pectate gel liquefaction	+
Utilization of asparagine	–
Growth requires:	
methionine	+
cysteine	+
0.02% triphenyl tetrazolium chloride (TTC) (w/v)	–

#### 4.5 Molecular identification

Features of citrus-attacking xanthomonads including *X. citri* subsp. *citri* and the genus *Xanthomonas* as a whole have been characterized at the molecular level to develop quick and accurate methods for reclassification and identification. The procedures include DNA–DNA hybridization (Vauterin *et al.*, 1995), genomic fingerprinting (Hartung *et al.*, 1987; Lazo *et al.*, 1987), multilocus sequence analysis (Young *et al.*, 2008) and rep-PCR (Cubero and Graham, 2002, 2004).

##### 4.5.1 Multilocus sequence analysis

A multilocus sequence analysis (MLSA) approach has been used for the specific identification of *X. citri* subsp. *citri*. (Almeida *et al.*, 2010; Bui Thi Ngoc *et al.*, 2010; Young *et al.*, 2008). Housekeeping genes are amplified using primers and PCR conditions as described by Almeida *et al.* (2010), Bui Thi Ngoc *et al.* (2010) and Young *et al.*, (2008). MLSA consists of sequencing multiple loci (typically four to eight housekeeping genes) and comparing these sequences with reference sequences of *Xanthomonas* species deposited in nucleotide databases; for example, the Plant Associated Microbes Database (PAMDB) (<http://genome.ppws.vt.edu/cgi-bin/MLST/home.pl>) (Almeida *et al.*, 2010) and the MLVAbank for microbe genotyping ([https://bioinfo-prod.mpl.ird.fr/MLVA\\_bank/Genotyping/](https://bioinfo-prod.mpl.ird.fr/MLVA_bank/Genotyping/)).

##### 4.5.2 Rep-PCR fingerprinting

Rep-PCR fingerprinting using primers designed from repetitive extragenic palindromic (REP) elements – enterobacterial repetitive intergenic consensus (ERIC) sequences and the BOX element (Louws *et al.*, 1994) – can be used for strain identification and characterization under specific PCR conditions (Cubero and Graham, 2002).

DNA can be extracted from bacterial suspensions (absorbance at 600 nm from 0.2 to 0.5) in a single step with phenol-chloroform-isoamyl alcohol, precipitated in ethanol, and resuspended in ultrapure water. DNA is stored at –20 °C until use. The DNA extraction procedure described in section 3.1.4.2 can also be used.

BOX PCR is carried out in 25 µl reaction mixtures containing 1× Taq buffer, 6 mM MgCl<sub>2</sub>, 2.4 µM primer BOX1R (5'-CTACG-GCAAGGCGACGCTGCAG-3') (Louws *et al.*, 1994), 0.2 mM each dNTP, 2 U Taq DNA polymerase and 5 µl DNA extracted from xanthomonad strains. The reaction

conditions are an initial step of 94 °C for 5 min followed by 40 cycles of 94 °C for 30 s, 48 °C for 30 s and 72 °C for 1 min, and a final step of 72 °C for 10 min. PCR products are analysed in 3% agarose gels in 1× Tris-acetate-EDTA (TAE) buffer (40 mmol/litre Tris-acetate; 1 mmol/litre EDTA; pH 8.0) run for 2 h at 110 V and stained with ethidium bromide.

ERIC PCR is carried out in 25 µl reaction mixtures containing 1× Taq buffer, 3 mM MgCl<sub>2</sub>, 1.2 µM primer ERIC1R (5'-ATGTAAGCTCCT-GGGGATTCAC-3') and ERIC2 (5'-AAGTAAGTGACT-GGGGTGAGCG-3') (Louws *et al.*, 1994), 0.2 mM each dNTP, 2 U Taq DNA polymerase and 5 µl DNA extracted from xanthomonad strains. The reaction conditions are the same as for BOX PCR. Visualization of PCR products is as for BOX PCR.

Fingerprints (band patterns) can be compared and analysed for similarity by eye, but patterns can also be transformed into peak patterns and strains compared using a computer software program such as BioNumerics (Applied Maths). Identification should be based on similarity to patterns of control (reference) strains (section 4).

Schemes for detection and identification of *Xanthomonas citri* subsp. *citri* on symptomatic and asymptomatic plant material are shown in figures 5 and 6, respectively.

## 5. Records

Records and evidence should be retained as described in section 2.5 of ISPM 27:2006.

In instances where other contracting parties may be affected by the results of the diagnosis, retention of the original sample (labelled for traceability) culture(s) of the pest, preserved or mounted specimens, or test materials (e.g. photograph of gels, ELISA results printout, PCR amplicons) for at least for one year is recommended, especially in cases of non-compliance (ISPM 13:2001, *Guidelines for the notification of non-compliance and emergency action*) and where pests are found for the first time in a country or an area.

## 6. Contact Points for Further Information

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Instituto Nacional de Investigación Agraria y Tecnología Alimentaria, INIA, Ctra de La Coruña km 6, Madrid, Spain (Jaime Cubero; e-mail: [cubero@inia.es](mailto:cubero@inia.es); tel.: +34 913473900; fax: +34 913572293).

A request for a revision to a diagnostic protocol may be submitted by national plant protection organizations (NPPOs), regional plant protection organizations (RPPOs) or Commission on Phytosanitary Measures (CPM) subsidiary bodies through the IPPC Secretariat ([ippc@fao.org](mailto:ippc@fao.org)), which will forward it to the Technical Panel on Diagnostic Protocols (TPDP).

## 7. Acknowledgements

The first draft of this protocol was written by Mr E.F. Verdier, General Direction of Agricultural Services, Biological Laboratories Department, Uruguay (see section 6 for details), and revised by Ms R. Lanfranchi, Plant Pests and Disease Laboratory, National Service of Agrifood Health and Quality, SENASA, Av. Ing. Huergo 1001 CP 1107, Buenos Aires, Argentina (Rita Lanfranchi; e-mail: [ritalanfranchi@hotmail.com](mailto:ritalanfranchi@hotmail.com); tel.: +5411 43621177 int. 118); Mr Ed Civerolo, USDA, United States (e-mail: [emciv@comcast.net](mailto:emciv@comcast.net)) and Ms M.M. López, IVIA, Spain (see section 6 for details). In addition, Mr J. Cubero, INIA, Spain (see section 6 for details) was significantly involved in the development of this protocol.

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## 9. Figures



**Figure 1.** Typical citrus canker symptoms on leaves, stems and fruit of grapefruit (*Citrus paradisi*).



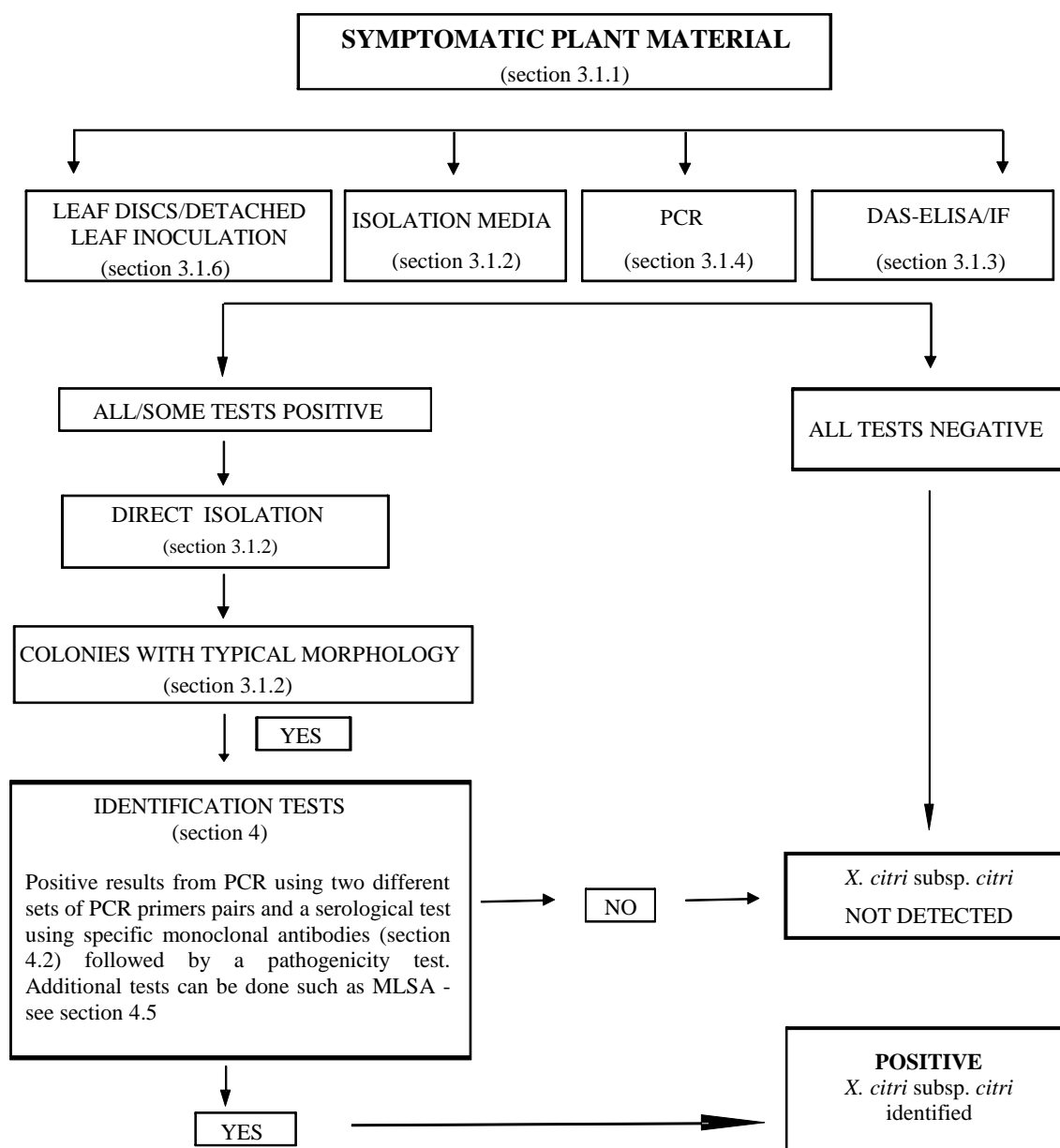
**Figure 2.** Twig symptoms of citrus canker: early lesions on grapefruit (*Citrus paradisi*).



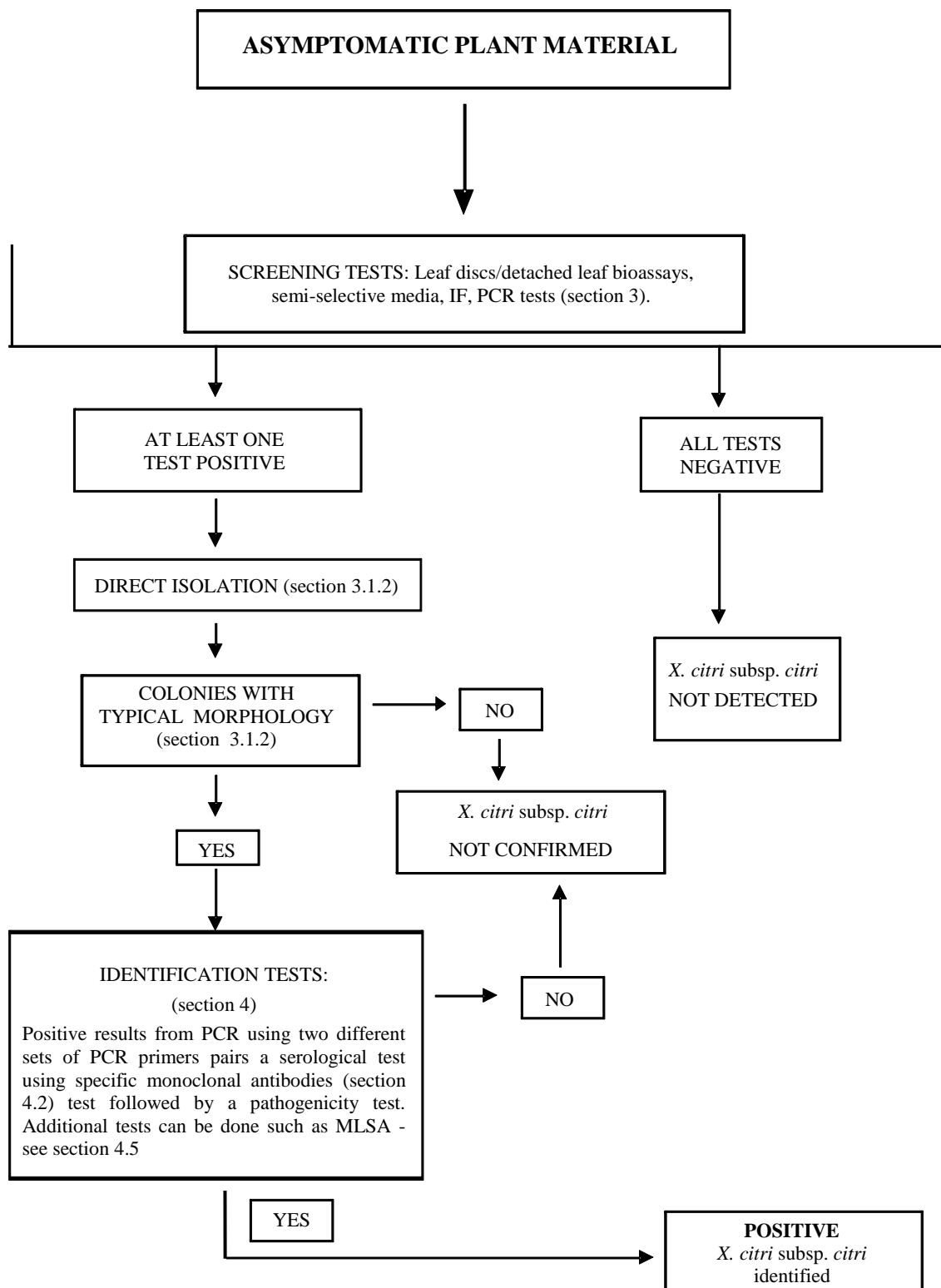
**Figure 3.** Fruit symptoms of citrus canker on sweet orange (*Citrus sinensis*) (left) and grapefruit (*Citrus paradisi*) (centre and right).



**Figure 4.** Leaf symptoms of citrus canker on lemon (*Citrus limon*) exacerbated by citrus leaf miner wounds.



**Figure 5.** Scheme for detection and identification of *Xanthomonas citri* subsp. *citri* on symptomatic plant material.



**Figure 6.** Scheme for detection and identification of *Xanthomonas citri* subsp. *citri* on asymptomatic plant material.

**Publication history**

2004-11 SC added subject *Xanthomonas axonopodis* pv. *citri* (2004-011) to the work programme

CPM-1 (2006) added subject *Xanthomonas axonopodis* pv. *citri* (2004-011) topic under the topic: Bacteria (2006-005)

2012-11 TPDP revised draft protocol

2013-04 SC approved draft for member consultation via e-decision (2013\_eSC\_May\_12)

2013-07 member consultation

2014-02 TPDP revised and submitted to the SC for approval for adoption (2014\_eTPDP\_Feb\_02)

2014-04 To SC for approval for adoption via e-decision (2014\_eSC\_May\_16)

2014-06 SC approved for the 45 days notification period via e-decision (2014\_eSC\_Nov\_03)

2014-07 SC adopted DP on behalf of CPM (no formal objections received)

2014-10 Secretariat corrected minor editorial mistakes

2014-11 Secretariat corrected minor editorial mistake

**ISPM 27. 2006: Annex 6** *Xanthomonas citri* subsp. *citri* (2014). Rome, IPPC, FAO.

Publication history last updated: 2014-11-11



## **INTERNATIONAL STANDARDS FOR PHYTOSANITARY MEASURES**

### **ISPM 27 DIAGNOSTIC PROTOCOLS**

#### **DP 7: *Potato spindle tuber viroid* (2015)**

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## 1. Pest Information

Viroids are unencapsidated, covalently closed circular single-stranded RNA molecules, 239–401 nucleotides in length that are replicated by host enzymes (Hammond & Owens, 2006). *Potato spindle tuber viroid* (PSTVd; genus *Pospiviroid*) is commonly 359 nucleotides in length but PSTVd isolates consisting of 341–364 nucleotides have been reported (Wassenegger *et al.*, 1994; Shamloul *et al.*, 1997; Jeffries, 1998). Mild and severe strains have been described based on symptoms produced in sensitive tomato cultivars; for example, *Solanum lycopersicum* L. (tomato) cv. *Rutgers* (Fernow, 1967).

The natural host range of PSTVd is relatively narrow. The primary natural hosts are stolon- and tuber-forming *Solanum* spp.; for example, *Solanum tuberosum* L. (potato) and *S. lycopersicum* (tomato). PSTVd has been found also in *Capsicum annuum*, *Persea americana* and *S. muricatum*. PSTVd has been detected in mainly vegetatively propagated ornamental plant species in the family Solanaceae – namely, *Brugmansia* spp., *Cestrum* spp., *Datura* sp., *Lycianthes rantonetti*, *Petunia* spp., *Physalis peruviana*, *Solanum* spp. and *Streptosolen jamesonii* – but also in *Chrysanthemum* sp. and *Dahlia* × *hybrida* in the family Asteraceae (for natural host details, see CABI (n.d.)). The experimental host range of PSTVd is wide and includes species in the family Solanaceae, but also some species in at least nine other families. Most hosts express few or no disease symptoms (Singh, 1973; Singh *et al.*, 2003).

PSTVd has been found infecting *S. tuberosum* in some countries or states in Africa, Asia, Eastern Europe, North America (EPPO/CABI, 1997), Central America (Badilla *et al.*, 1999), South America and the Middle East (Hadidi *et al.*, 2003). However, it has a wider geographical distribution in ornamental plant species and other hosts (see CABI (n.d.) for geographical distribution).

In *Solanum tuberosum* the main means of spread of PSTVd is vegetative propagation. It is also spread by contact, mainly by machinery in the field and by cutting seed potato tubers (Hammond & Owens, 2006). PSTVd is transmitted in true potato seed – up to 100% of the seed may be infected (Fernow *et al.*, 1970; Singh, 1970) – and also in pollen (Grasmick & Slack, 1985; Singh *et al.*, 1992). De Bokx and Pirone (1981) reported a low rate of transmission of PSTVd by the aphid *Macrosiphum euphorbiae* but not by the aphids *Myzus persicae* or *Aulacorthum solani*. However, experimental acquisition and transmission of PSTVd by *M. persicae* from plants co-infected with PSTVd and *Potato leafroll virus* (PLRV) have been reported (Salazar *et al.*, 1995; Singh & Kurz, 1997). PSTVd was subsequently shown to be heterologously encapsidated within particles of PLRV (Querci *et al.*, 1997), a phenomenon that may have important implications for the epidemiology and spread of PSTVd under field conditions.

In *Solanum lycopersicum*, PSTVd is easily spread by contact and has been shown to be transmitted by pollen and seed (Kryczynski *et al.*, 1988; Singh, 1970). Transmission via tomato seeds has been shown to contribute to the international spread of PSTVd (van Brunschot *et al.*, 2014). It is possible that PSTVd is also spread in infected capsicum seeds (Lebas *et al.*, 2005).

Infected ornamental plant species may act as an inoculum source if they are handled before touching other susceptible plants, and they have been shown to be a pathway for the international spread of PSTVd (Navarro *et al.*, 2009; Verhoeven *et al.*, 2010). No transmission of PSTVd was shown with *Apis mellifera*, *Bombus terrestris*, *Frankliniella occidentalis* or *Thrips tabaci* (Nielsen *et al.*, 2012).

PSTVd is the only viroid known to naturally infect cultivated species *Solanum*. However, *Mexican papita viroid* (MPVd) infects the wild species *S. cardiophyllum* (Martinez-Soriano *et al.*, 1996). Experimentally, other viroid species in the genus *Pospiviroid* infect *S. tuberosum* (Verhoeven *et al.*, 2004).

In addition to PSTVd, other pospiviroids have been found infecting *S. lycopersicum* naturally, including *Citrus exocortis viroid* (CEVd; Mishra *et al.*, 1991), *Columnnea latent viroid* (CLVd; Verhoeven *et al.*, 2004), *Mexican papita viroid* (MPVd; Ling & Bledsoe, 2009), *Pepper chat fruit viroid* (PCFVd; Reanwarakorn *et al.*, 2011) *Tomato apical stunt viroid* (TASVd; Walter, 1987), *Tomato*

*chlorotic dwarf viroid* (TCDVd; Singh *et al.*, 1999) and *Tomato planta macho viroid* (TPMVd; Galindo *et al.*, 1982).

## 2. Taxonomic Information

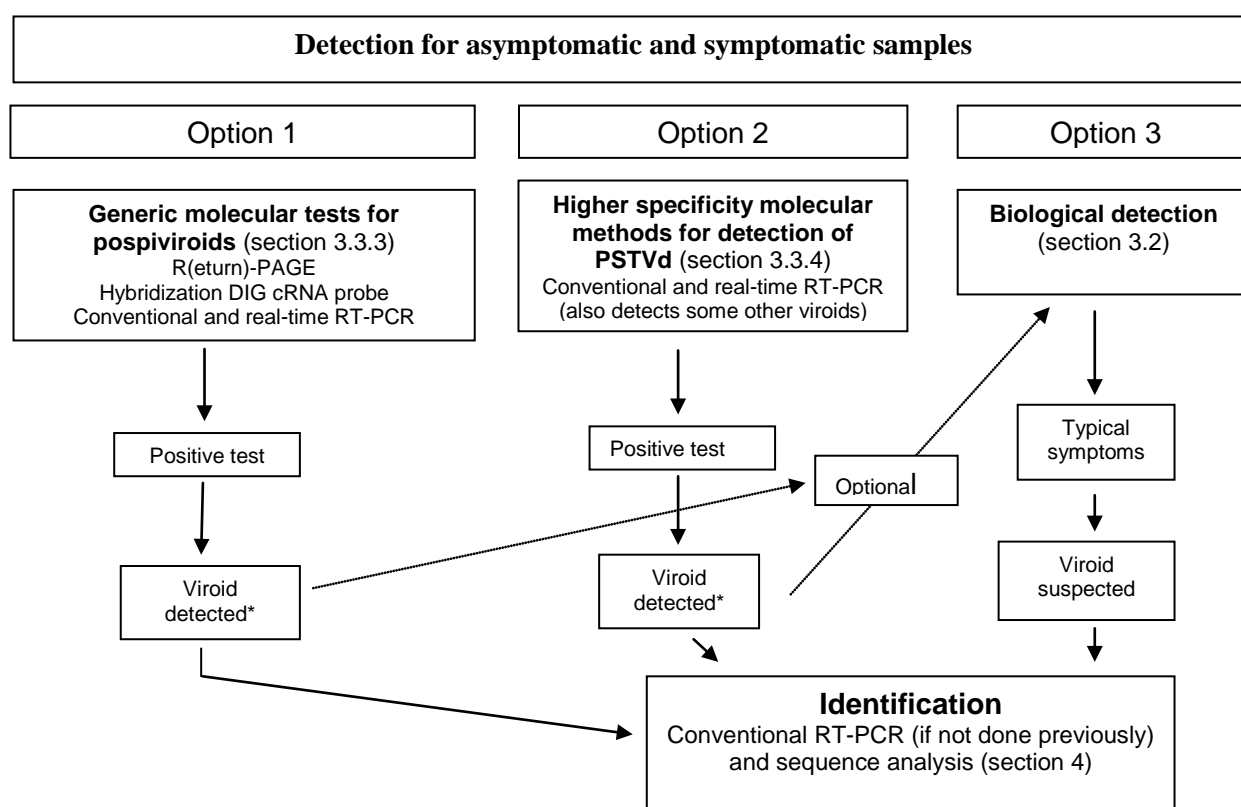
<b>Name:</b>	Potato spindle tuber viroid (acronym PSTVd)
<b>Synonyms:</b>	potato spindle tuber virus, potato gothic virus, tomato bunchy top virus
<b>Taxonomic position:</b>	Pospiviroidae, <i>Pospiviroid</i>
<b>Common names:</b>	potato spindle tuber

## 3. Detection

Symptom appearance and severity depend on PSTVd strain, cultivar and environment. In *S. tuberosum*, infection may be symptomless or produce symptoms ranging from mild to severe (reduction in plant size and uprightness and clockwise phyllotaxy of the foliage when the plants are viewed from above; dark green and rugose leaves). Tubers may be reduced in size, misshapen, spindle- or dumbbell-shaped, with conspicuous prominent eyes that are evenly distributed (EPPO, 2004). In *S. lycopersicum*, symptoms include stunting, epinasty, rugosity and lateral twisting of new leaflets, leaf chlorosis, reddening, brittleness, necrosis, reduction in fruit size, and fruit not fully ripening (Mackie *et al.*, 2002; Hailstones *et al.*, 2003; Lebas *et al.*, 2005). In *C. annuum*, symptoms are subtle, with leaves near the top of the plant showing a wavy-edged margin (Lebas *et al.*, 2005). All ornamental plant species investigated to date do not show symptoms (Verhoeven, 2010).

Because PSTVd infections may be asymptomatic, tests are required for detection and identification of the viroid. Detection of PSTVd can be achieved using the biological and molecular tests shown as options in Figure 1, but for identification, the polymerase chain reaction (PCR) product must be sequenced as the tests are not specific for PSTVd and will detect other viroids. Sequencing will also contribute to preventing the reporting of false positives. If pathogenicity is considered to be important, biological indexing may be done. If the identification of PSTVd represents the first finding for a country, the laboratory may have the diagnosis confirmed by another laboratory.

Appropriate controls should be included in all tests to minimize the risk of false positive or false negative results.



**Figure 1.** Minimum requirements for the detection and identification of *Potato spindle tuber viroid* (PSTVd)

\* Identification may not be needed for every viroid-positive sample in certain situations; for example, when dealing with a PSTVd outbreak.

Note: If a viroid is suspected in a sample (i.e. typical symptoms are present) but a test gives a negative result, another of the tests should be carried out for confirmation of the result.

This annex is for the detection of PSTVd; it has not been developed for the detection and identification of other pospiviroid species. However, the possible presence of other viroids needs to be considered when choosing a detection and an identification method. Therefore, this annex describes non-specific detection methods that will detect all known viroids; including pospiviroids such as PSTVd. For identification, the PCR product will need to be sequenced.

Protocols for the detection of PSTVd in leaf, tuber and botanical (true) seed tissue are described, however, reliable detection in seed tissue is particularly challenging.

In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. Use of names of reagents chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated. Recommendations on method validation in phytodiagnostics are provided by EPPO (2014).

The performance of a molecular test is determined by both the matrix to be tested and the choice of subsequent sample preparation, nucleic acid extraction, and detection and identification methods. Table 1 provides an overview of validation data that are available for different matrices and combinations of methods. Details of these methods are described in the corresponding paragraphs or indicated references.

### 3.1 Sampling

General guidance on sampling methodologies is described in ISPM 31 (*Methodologies for sampling of consignments*).

***S. tuberosum* microplants and glasshouse-grown *S. tuberosum* plants** For microplants the whole plant should be used as the sample or the top two-thirds of the plant should be sampled under aseptic conditions so as to enable the rest of the plant to continue growing. Microplants should be four to six weeks old with stems of about 5 cm in length and with well-formed leaves. For glasshouse-grown plants a fully expanded leaflet from each plant should be used. Viroid concentration is lower at low temperature and low light levels, so plants should be grown at a temperature of at least 18 °C and with a photoperiod of at least 14 h. Microplants or leaves may be bulked; the bulking rate will depend on the test method used and must be validated.

**Field-grown *S. tuberosum* plants** A fully expanded non-senescent terminal leaflet from the top of each plant should be used. Leaves may be bulked together for testing; the bulking rate will depend on the test method used and must be validated.

***S. tuberosum* tubers** PSTVd is systemically distributed in infected *S. tuberosum* tubers (Shamloul *et al.*, 1997). It also occurs in almost equal amounts in different parts of both primarily and secondarily infected tubers (Roenhorst *et al.*, 2006). The highest concentration is found immediately after harvest. In tubers stored at 4 °C the concentration does not decrease significantly for up to three months but after six months of storage, it may decrease by more than 10<sup>4</sup> times. A single core from any part of the tuber can be used as a sample and may be bulked; the bulking rate will depend on the test method used and must be validated.

**Leaves of other crops and ornamental plant species** Fully expanded young leaves are used. Leaves may be bulked together for testing; the bulking rate will depend on the test method used and must be validated. Note that the viroid concentration is influenced by the age/maturity of the plants, and there are often seasonal fluctuations. In addition, some species contain biochemicals that may inhibit transmission to test plants (e.g. *Brugmansia* spp.) or RT-PCR (e.g. *Calibrachoa* spp., *Solanum jasminoides* and *S. jamesonii*).

**Seed** Viroid concentration may vary greatly between seeds and the level of infection may vary from less than 1 to 100%. This makes it very difficult to recommend a sample size and bulking rate (EUPHRESO, 2010). For *S. lycopersicum*, bulking rates of 100–1 000 have been used for a single test. The bulking rate will depend on the test method used and must be validated.

Potato seeds may be sown in growing medium (e.g. compost) in trays and the seedlings/plants tested non-destructively using the same procedure described for glasshouse-grown plants (EPPO, 2006).

### 3.2 Biological detection

Inoculation of *S. lycopersicum* plants (cultivars Rutgers, Moneymaker or Sheyenne) will allow the detection of many but not all viroids (e.g. tomato is not a host of the pospiviroid *Iresine viroid 1* (IrVd-1; Spieker, 1996; Verhoeven *et al.*, 2010)) and will provide visual evidence of pathogenicity. However, some isolates may not be detected because of the absence of symptoms. Moreover, symptoms may not be diagnostic for PSTVd. Biological indexing may require a great deal of greenhouse space, it is labour intensive, and several weeks or more may be needed before the test is completed. No work has been done to compare the sensitivity of this method with other methods described in this protocol. If it is less sensitive than the molecular methods, it might be less suitable for testing seed. However, it is possible that the viroid may be amplified in biological indexing to a level that allows detection by other methods.

Approximately 200–500 mg leaf, root or tuber tissue is ground in a small quantity of 0.1 M phosphate inoculation buffer (a 1:1 dilution is adequate) containing carborundum (400 mesh). Phosphate buffer

(pH 7.4) is made by combining 80.2 ml of 1 M  $K_2HPO_4$  with 19.8 ml of 1 M  $KH_2PO_4$  and adjusting the volume to 1 litre with distilled water.

Young tomato plants with one or two fully expanded leaves are inoculated. Using a gloved finger, a cotton bud, or a cotton swab dipped into the inoculum, the leaf surface is gently rubbed with the inoculum and then the leaves are immediately rinsed with water until the carborundum has been removed. The plants are grown with a diurnal temperature fluctuation of 24–39 °C under a photoperiod of 14 h supplemented with sodium vapour illumination of approximately 650  $\mu E/m^2/s$  (Grassmick & Slack, 1985). Lower temperatures and less illumination may reduce the sensitivity of the assay. The plants are inspected weekly for symptoms for up to six weeks after inoculation. Symptoms of PSTVd infection include stunting, epinasty, rugosity and lateral twisting of new leaflets, leaf chlorosis, reddening, brittleness and necrosis.

A bioassay on tomato will allow detection of many pospiviroids (except IrVd-1, see above); therefore, RT-PCR should be carried out on the nucleic acid extracted from symptomatic indicator plants and the PCR product should be sequenced for identification.

### 3.3 Molecular detection

#### 3.3.1 Sample preparation

**Microplants, leaf material and roots** Mortars and pestles or homogenizers (e.g. Homex 6 (Bioreba)) with extraction bags (Bioreba) have been used successfully to grind material. Adding a small quantity of water or lysis buffer (the composition of which depends on the method used for nucleic acid extraction) or freezing the sample (e.g. in liquid nitrogen) may facilitate homogenization.

The following procedure has been validated (see Table 1) in combination with nucleic acid extraction using the magnetic bead extraction method 2 and the real-time RT-PCR GenPospi assay described in this annex. About 1 g tissue is homogenized in an extraction bag using a Homex 6 or handheld homogenizer (Bioreba) with 3.5 ml (range 1:2–1:5 (w/v)) GH plus lysis buffer (6 M guanidine hydrochloride; 0.2 M sodium acetate, pH 5.2; 25 mM ethylenediaminetetraacetic acid (EDTA); 2.5% polyvinylpyrrolidone (PVP)-10). Samples are then incubated for 10 min at 65 °C at 850 r.p.m. in a thermomixer (or by shaking (invert the tube 3 times) and additional centrifugation for 2 min at 16 000 g) before nucleic acid extraction.

***S. tuberosum* tubers** Tuber cores are thoroughly homogenized in water or lysis buffer (the composition of which depends on the method used for nucleic acid extraction; 1 ml per g tuber core). A grinder such as the Homex 6 with extraction bags has been used successfully. Freezing the cores (e.g. at –20°C) before adding the water or lysis buffer facilitates homogenization.

**Seeds** For small numbers of seeds (<100), a tissue lyser (e.g. Retsch TissueLyser (Qiagen)) may be used. For larger numbers of seeds, a paddle blender (e.g. MiniMix (Interscience)) or homogenizer (e.g. Homex 6) with a minimum quantity of lysis buffer (the composition of which depends on the method used for nucleic acid extraction) may be used. Seeds may also be crushed with a hammer (Bertolini *et al.*, 2014b) or by using a mortar and pestle. The latter may not be practical for routine use as cross-contamination may be difficult to control. Alternatively, liquid nitrogen may be used to freeze the sample, after which it is ground in a cell mill (this method can also be used for other tissue types).

The following procedure has been validated (see Table 1) in combination with nucleic acid extraction using the magnetic bead extraction method 2 and the real-time RT-PCR assay of Boonham *et al.* (2004) described in this annex. Each of three subsamples of 1 000 seeds are soaked in 20 ml GH plus lysis buffer in a 100 ml BagPage (Interscience) for 30–60 min at room temperature, homogenized for 90 s using a BagMixer (Interscience) and incubated (or shaken and centrifuged as described for microplants, leaf material and roots) before nucleic acid extraction

**Tissue print and/or squash** Leaf pedicels or detached shoots are pressed onto nylon membranes. Several partially overlapping imprints or squashes from different leaves and/or detached shoots may

be made on approximately 0.5 cm<sup>2</sup> nylon membrane according to Bertolini *et al.* (2008, 2014a). The membrane containing the immobilized sample is cut and inserted into a micro tube. The immobilized sample should be handled with clean tweezers. The tissue-printed or squashed samples can be stored at room temperature in a dark and dry environment for at least three months. For extraction of target RNA from the membranes, 100 µl glycine buffer is added to each micro tube containing an immobilized sample, which is then vortexed and placed on ice until PCR amplification.

### 3.3.2 Nucleic acid extraction

A wide range of nucleic acid extraction methods may be used, from commercial kits to methods published in scientific journals. The following nucleic acid extraction kits, buffers and procedures have been used successfully for the detection of PSTVd.

**Commercial kits** Commercial extraction kits such as RNeasy (Qiagen), MasterPure (Epicentre) and Sbeadex maxi plant kit (LGC Genomics) may be used according to the manufacturer's instructions. RNeasy was evaluated for the extraction of PSTVd RNA from different matrices as part of the EUPHRESKO Detection and Epidemiology of Pospiviroids (DEP) project (EUPHRESKO, 2010).

**Method described by Mackenzie *et al.* (1997)** Plant tissue is homogenized (1:10 (w/v)) in lysis buffer (4 M guanidine isothiocyanate, 0.2 M sodium acetate, 25 mM EDTA, 2.5% PVP-40 (w/v, and 1% 2-mercaptoethanol (v/v) added just before use). One millilitre of homogenate is then mixed with 100 µl of 20% sarkosyl (w/v) and incubated at 70 °C for 10 min in a thermomixer, with agitation at 1 200 r.p.m.. This method can be used to extract quality RNA from a wide range of plant species.

**Method using EDTA buffer** Plant tissue may be homogenized (1:4 (w/v)) in a simple lysis buffer (50 mM NaOH, 2.5 mM EDTA) and then incubated (at approximately 25° C for 15 min) or centrifuged (at 12 000 g at 4 °C for 15 min). The supernatant can then, depending on the level of sensitivity required, either be used directly for RT-PCR (less sensitive) or spotted onto a nitrocellulose membrane and eluted using sterile distilled water (more sensitive) (Singh *et al.*, 2006). Although the concentration of viroid is lower for the EDTA method than for the other extraction methods described, this should not be a limiting factor when the method is used with RT-PCR or the digoxigenin (DIG) probe. The method has been used with *S. lycopersicum* and *S. tuberosum* and a range of ornamental plant species.

**Phenol–chloroform and two-step PEG extraction** Plant tissue is homogenized and nucleic acid extracted as described by EPPO (2004). This method has been used in combination with return (R)-polyacrylamide gel electrophoresis (PAGE), DIG-RNA probe and the conventional RT-PCR methods described in this diagnostic protocol for a wide range of plant species and tissue types (e.g. leaves and potato tubers).

**CTAB extraction** Plant tissue is homogenized and nucleic acid extracted as described in EPPO (2004). The cetyl trimethylammonium bromide (CTAB) method has been used with real-time RT-PCR for a wide range of plant species and tissue types (e.g. leaves and tomato seeds; EUPHRESKO, 2010).

**Magnetic bead extraction method 1** The following automated procedure is based on use of the KingFisher mL Magnetic Particle Processor (Thermo Scientific). With appropriate adjustment of volumes, other KingFisher models may be used.

For each sample, at least 200 mg leaf or tuber tissue or up to 100 seeds are macerated, and then extraction buffer is added immediately at a ratio of 1g leaf or tuber tissue to 10 ml buffer and 1 g seed to 20 ml buffer. Maceration is continued until a clear cell lysate with minimal intact tissue debris is obtained. Extraction buffer consists of 200 µl of 8.39% (w/v) tetrasodium pyrophosphate (TNaPP) solution (pH 10.0–10.9) and 100 µl Antifoam B Emulsion (Sigma) added to 9.8 ml guanidine lysis buffer (GLB). GLB consists of: 764.2 g guanidine hydrochloride, 7.4 g disodium EDTA dehydrate, 30.0 g PVP-10, 5.25 g citric acid monohydrate, 0.3 g tri-sodium citrate, 5 ml Triton X-100, 250 ml absolute ethanol and 750 ml water.

Approximately 2 ml lysate is decanted into a fresh microcentrifuge tube, which is centrifuged at approximately 5 000 g for 1 min. One millilitre of supernatant is removed and placed in the first tube (A) of the KingFisher mL rack, to which 50 µl vortexed MAP Solution A magnetic beads (Invitex) are added. Tube B has 1 ml GLB added to it; tubes C and D, 1 ml of 70% ethanol; and tube E, 200 µl water or 1× Tris-EDTA buffer.

The tube strip is placed in the KingFisher mL and the programme (see Figure 2) is run. After 20 min, the machine will pause to allow a heating step. The tube strip is placed in an oven at 65–70 °C for 5 min and then returned to the KingFisher mL, and the programme is resumed. Other models may have a heating or holding evaporation step built in. On completion, the eluted nucleic acids are transferred to a new microcentrifuge tube.

This method has been used for a wide range of plant species as well as for potato tubers and tomato seeds. The method has been used with two of the real-time RT-PCR assays described in this annex (see sections 3.3.3.4 and 3.3.4.2). Cycle threshold (Ct) values several cycles higher than those for the other extraction methods described in this annex may be expected using the magnetic bead extraction method 1, but the increased throughput of samples that is achievable makes it a valuable extraction method (Roenhorst *et al.*, 2005).

*Plate layout* Default: Plate type = KingFisher tubestrip 1000 µl; Plate change message = Change Default  
**A:** volume = 1000, name = Cell lysate or tissue homogenate; volume = 50, name = Magnetic particles;  
**B:** volume = 1000, name = Washing buffer 1 (Various); **C:** volume = 1000, name = Washing buffer 2 (Various);  
**D:** volume = 1000, name = Washing buffer 3 (Various); **E:** volume = 200, name = Elution buffer (Various)  
**STEPS** COLLECT BEADS Step parameters: Name = Collect Beads; Well = A, Default; Beginning of step: Premix = No; Collect parameters: Collect count = 1. BIND Step parameters: Name = Lysing, Well = A, Default; Beginning of step: Release = Yes, time = 1min 0s, speed = Fast dual mix; Bind parameters: Bind time = 4min 0s, speed = Slow; End of step: Collect beads = No. BIND Step parameters: Name = Lysing, Well = A, Default; Beginning of step: Release = Yes, time = 1min 0s, speed = Fast dual mix Bind; Bind parameters: Bind time = 4min 0s, speed = Slow; End of step: Collect beads = No. BIND Step parameters: Name = Lysing, Well = A, Default; Beginning of step: Release = Yes, time = 1min 0s, speed = Fast dual mix; Bind parameters: Bind time = 4min 0s, speed = Slow; End of step: Collect beads = Yes, count = 4. WASH Step parameters: Name = Washing, Well = B, Default; Beginning of step: Release = Yes, time = 0s, speed = Fast; Wash parameters: Wash time = 3min 0s, speed = Fast dual mix; End of step: Collect beads = Yes, count = 3. WASH Step parameters: Name = Washing, Well = C, Default; Beginning of step: Release = Yes, time = 0s, speed = Fast; Wash parameters: Wash time = 3min 0s, speed = Fast dual mix; End of step: Collect beads = Yes, count = 3. WASH Step parameters: Name = Washing, Well = D, Default; Beginning of step: Release = Yes, time = 0s, speed = Fast; Wash parameters: Wash time = 3min 0s, speed = Fast dual mix; End of step: Collect beads = Yes, count = 3. ELUTION Step parameters: Name = Elution, Well = E, Default; Beginning of step: Release = Yes, time = 10s, speed = Fast; Elution parameters: Elution time = 20s, speed = Bottom very fast; Pause parameters: Pause for manual handling = Yes, message = Heating, Post mix time = 30s, speed = Bottom very fast; Remove beads: Remove beads = Yes, collect count = 4, disposal well = D

**Figure 2.** Programme for the KingFisher mL Magnetic Particle Processor (Thermo Scientific)

**Magnetic bead extraction method 2** This automated procedure uses the Sbeadex maxi plant kit (LGC Genomics) with the KingFisher 96 system (Thermo Scientific). The manufacturer's instructions should be followed except that GH plus lysis buffer is used instead of lysis buffer PN that is part of the kit.

### 3.3.3 Generic molecular methods for pospiviroid detection

#### 3.3.3.1 R-PAGE

R-PAGE has been recommended as a detection method for PSTVd infecting *S. tuberosum* leaves (EPPO, 2004), but it was less sensitive (limit of detection (LOD) 87 893 pg PSTVd) than the other molecular methods evaluated (LOD at least 17 pg PSTVd) in a ring test with DIG-labelled cRNA probe, two-step conventional RT-PCR using the primers of Shamloul *et al.* (1997) and the real-time method of Boonham *et al.* (2004) (Jeffries & James, 2005; see also Table 1).

This method has also been used successfully with other host plants; for example, *C. annuum*, *S. tuberosum* (tubers) and *S. lycopersicum*. Because of its low sensitivity, bulking of samples would need to be validated.

R-PAGE will detect all known pospiviroids; therefore, for identification of PSTVd, RT-PCR on the nucleic acid followed by sequencing of the PCR product must be carried out.

### 3.3.3.2 Hybridization with a DIG-labelled cRNA probe

This method has been recommended for detection of PSTVd infecting *S. tuberosum* leaves (EPPO, 2004). Sensitivity for the detection of PSTVd in *S. tuberosum* leaves was at least 17 pg PSTVd (Jeffries & James, 2005). Other hosts have been tested successfully, including *Petunia* spp., *S. jasminoides*, *S. lycopersicum* and *S. tuberosum* (tubers).

The probe used is based on a full-length monomer of PSTVd produced by Agdia, Inc.<sup>9</sup> (cat. no. DLP 08000/0001). This probe should be used according to the manufacturer's instructions, or refer to EPPO (2004) for details of the method. In addition to the Ames buffer (EPPO, 2004), polyethylene glycol (PEG) and other extraction buffers may be used for nucleic acid extraction.

This DIG-labelled cRNA probe method will detect all known pospiviroids, therefore, for identification of PSTVd, RT-PCR on the nucleic acid followed by sequencing of the PCR product must be carried out.

### 3.3.3.3 Conventional RT-PCR using the primers of Verhoeven *et al.* (2004)

The primers used in this assay are the Posp1 and Vid primers of Verhoeven *et al.* (2004). The Posp1 primers will detect CEVd, *Chrysanthemum stunt viroid* (CSVd), IrVd-1, MPVd, PCFVd, PSTVd, TASVd, TCDVd and TPMVd. The Vid primers will detect PSTVd, TCDVd and, additionally, CLVd. Using the Posp1 and Vid primers in two separate reactions will allow detection of all pospiviroids. However, sequence mismatch at critical positions of the primer target site may prevent the detection of some pospiviroid isolates (e.g. an isolate of CLVd was not detected using these primers; Steyer *et al.*, 2010) and additional primers to detect these isolates will be required. *In silico* studies have shown that the following PSTVd isolates may not be detected because of primer–sequence mismatch at critical positions: Posp1 primers: EU879925, EU273604, EF459697, AJ007489, AY372398, AY372394, FM998551, DQ308555, E00278; Vid primers: EU273604<sup>2</sup>. The Posp1 primers are much more sensitive than the Vid primers for the detection of PSTVd.

#### Primers

Posp1-FW: 5'-GGG ATC CCC GGG GAA AC-3' (nucleotide (nt) 86–102)

Posp1-RE: 5'-AGC TTC AGT TGT (T/A)TC CAC CGG GT-3' (nt 283–261)

Vid-FW: 5'-TTC CTC GGA ACT AAA CTC GTG-3' (nt 355–16)

Vid-RE: 5'-CCA ACT GCG GTT CCA AGG G-3' (nt 354–336)

#### Reaction conditions

The One-Step RT-PCR Kit (Qiagen) has been shown to be reliable when used for the detection of PSTVd, CEVd, CLVd, CSVd, TASVd and TCDVd in individual samples (EUPHRESCO, 2010) and for other pospiviroids listed at the start of this section. It is not necessary to use the Q-solution described by EUPHRESCO (2010). Although various RT-PCR kits and reaction conditions may be used, they should be validated to check that they are fit for the purpose intended, with all relevant pospiviroids detected.

Two microlitres of template is added to 23 µl master mix comprising 1.0 µl each of forward and reverse primer (10 µM), 5 µl of 5× One-Step RT-PCR buffer, 1.0 µl One-Step RT-PCR enzyme mix, 1.0 µl dNTPs (10 mM each dNTP) and 14 µl water. The thermocycling programme is as follows: 50 °C for 30 min; 95 °C for 15 min; 35 cycles of 94 °C for 30 s, 62 °C for 60 s and 72 °C for 60 s; and a final extension step of 72 °C for 7 min.

### ***Gel electrophoresis***

After RT-PCR, the PCR products (approximately 197 bp and 359 bp for the Posp1 and Vid primers, respectively) should be analysed by gel electrophoresis (2% agarose gel) and the PCR amplicons of the correct size sequenced to identify the viroid species. In practice, sequencing the 197 bp product has always resulted in the same identification as sequencing the complete viroid genome.

### **3.3.3.4 Real-time RT-PCR using the GenPosp1 assay (Botermans *et al.*, 2013)**

The GenPosp1 assay uses TaqMan real-time RT-PCR to detect all known species of the genus *Pospiviroid*. It consists of two reactions running in parallel: the first (reaction mix 1) targets all pospiviroids except CLVd (Botermans *et al.*, 2013); the second (reaction mix 2) specifically targets CLVd (Monger *et al.*, 2010). To monitor the RNA extraction a *nad5* internal control based on primers developed by Menzel *et al.* (2002) to amplify mRNA from plant mitochondria (the mitochondrial *NADH dehydrogenase* gene) is included. Method validation (see Table 1) on tomato leaves showed that the GenPosp1 assay detected isolates from all the known pospiviroid species up to a relative infection rate of 0.13% (which equals a 1:770 dilution). The assay was specific as no cross-reactivity was observed with other viroids, viruses or nucleic acid from host plants. Repeatability and reproducibility were 100% and the assay appeared robust in an inter-laboratory comparison. The GenPosp1 assay has been shown to be a suitable tool for large-scale screening for pospiviroid species. The assay will need to be validated for matrices other than tomato leaves.

#### ***Primers***

TCR-F 1-1: 5'-TTC CTG TGG TTC ACA CCT GAC C-3' (Botermans *et al.*, 2013)

TCR-F 1-3: 5'-CCT GTG GTG CTC ACC TGA CC-3' (Botermans *et al.*, 2013)

TCR-F 1-4: 5'-CCT GTG GTG CAC TCC TGA CC-3' (Botermans *et al.*, 2013)

TCR-F PCFVd: 5'-TGG TGC CTC CCC CGA A-3' (Botermans *et al.*, 2013)

TCR-F IrVd: 5'-AAT GGT TGC ACC CCT GAC C-3' (Botermans *et al.*, 2013)

TR-R1: 5'-GGA AGG GTG AAA ACC CTG TTT-3' (Botermans *et al.*, 2013)

TR-R CEVd: 5'-AGG AAG GAG ACG AGC TCC TGT T-3' (Botermans *et al.*, 2013)

TR-R6: 5'-GAA AGG AAG GAT GAA AAT CCT GTT TC-3' (Botermans *et al.*, 2013)

CLVd-F: 5'-GGT TCA CAC CTG ACC CTG CAG-3' (Monger *et al.*, 2010)

CLVd-F2: 5'-AAA CTC GTG GTT CCT GTG GTT-3' (Monger *et al.*, 2010)

CLVd-R: 5'-CGC TCG GTC TGA GTT GCC-3' (Monger *et al.*, 2010)

*nad5*-F: 5'-GAT GCT TCT TGG GGC TTC TTG TT-3' (Menzel *et al.*, 2002)

*nad5*-R: 5'-CTC CAG TCA CCA ACA TTG GCA TAA-3' (Menzel *et al.*, 2002)

#### ***Probes***

pUCCR: 6FAM-5'-CCG GGG AAA CCT GGA-3'-MGB (Botermans *et al.*, 2013)

CLVd-P: 6FAM-5'-AGC GGT CTC AGG AGC CCC GG-3'-BHQ1 (Monger *et al.*, 2010)

*nad5*-P: VICr-5'-AGG ATC CGC ATA GCC CTC GAT TTA TGT G-3'-BHQ1 (Botermans *et al.*, 2013)

The two reaction mixes are based on the TaqMan RNA to Ct 1-Step Kit (Applied Biosystems).

#### ***Reaction mix 1 (all pospiviroids except CLVd + nad5)***

The reaction mix consists of 12.5 µl of 2× TaqMan RT-PCR mix, 0.6 µl of 1× TaqMan RT enzyme mix, 0.75 µl (10 µM) forward primers (TCR-F 1-1, TCR-F 1-3, TCR-F 1-4, TCR-F IrVd, TCR-F PCFVd and *nad5*-F) and reverse primers (TR-R1, TR-R CEVd, TR-R6 and *nad5*-R) (final concentration 0.3 µM each), 0.25 µl (10 µM) TaqMan probe pUCCR (final concentration 0.1 µM) and 0.5 µl (10 µM) TaqMan probe *nad5*-P (final concentration 0.2 µM). Molecular grade water and 2 µl RNA template are added to make a final volume of 25 µl.

### **Reaction mix 2 (CLVd + nad5)**

The reaction mix consists of 12.5 µl of 2× TaqMan RT-PCR mix, 0.6 µl of 1× TaqMan RT enzyme mix, 0.75 µl (10 µM) forward primers (CLVd-F, CLVd-F2 and *nad5*-F) and reverse primers (CLVd-R and *nad5*-R) (final concentration 0.3 µM each), 0.25 µl (10 µM) TaqMan probe CLVd-P (final concentration 0.1 µM) and 0.5 µl (10 µM) TaqMan probe *nad5*-P (final concentration 0.2 µM). Molecular grade water and 2 µl RNA template are added to make a final volume of 25 µl.

Thermocycling conditions for both reaction mixes are 48 °C for 15 min, 95 °C for 10 min, followed by 40 cycles of (95 °C for 15 s and 60 °C for 1 min).

For this method, Botermans *et al.* (2013) interpreted Ct values <32 as positive; those between 32 and 37 as inconclusive, requiring confirmation; and those ≥37 as negative. However, these values may exclude low levels of infection in some tissues, and will need to be defined in each laboratory.

## **3.3.4 Higher specificity molecular methods for the detection of PSTVd**

### **3.3.4.1 Conventional RT-PCR using the primers of Shamloul *et al.* (1997)**

The RT-PCR primers used in this assay are those of Shamloul *et al.* (1997), which are also described by Weidemann and Buchta (1998). The primers will detect MPVd, PSTVd, TCDVd and TPMVd. *In silico* studies have shown that the following PSTVd isolates may not be detected because of primer–sequence mismatch at critical positions: AY372394, DQ308555, EF459698 for the reverse primer. If RNA was not amplified using these primers, the Vid primers may be used.

#### **Primers**

3H1-F: 5′-ATC CCC GGG GAA ACC TGG AGC GAA C-3′ (nt 89–113)

2H1-R: 5′-CCC TGA AGC GCT CCT CCG AG-3′ (nt 88–69)

#### **Method 1 (SuperScript One-Step RT-PCR with Platinum Taq (Invitrogen))**

For each reaction, 1 µl template RNA is added to 24 µl master mix consisting of 1.7 µl each of forward and reverse primer (15 µM), 12.5 µl of 2× Reaction Buffer, 0.5 µl RT/Platinum Taq and 7.6 µl water. The thermocycling programme is as follows: 43 °C for 30 min, 94 °C for 2 min, then 10 cycles of 94 °C for 30 s, 68 °C for 90 s and 72 °C for 45 s, followed by 20 cycles of 94 °C for 30 s, 64 °C for 90 s and 72 °C for 45 s, with a final extension of 72 °C for 10 min and 20 °C for 1 min.

#### **Method 2 (two-step RT-PCR)**

Using the two-step RT-PCR, the sensitivity for the detection of PSTVd in *S. tuberosum* is at least 17 pg PSTVd – the lowest concentration tested, but the sensitivity achieved varies between laboratories, with most laboratories detecting at least 89 pg PSTVd (Jeffries & James, 2005). See EPPO (2004) for a description of method 2.

After RT-PCR, the PCR products (approximately 360 bp) are analysed by gel electrophoresis as described and PCR amplicons of the correct size are sequenced to identify the viroid species.

An internal control assay using *nad5* primers (Menzel *et al.*, 2002) has been used with this method in a simplex (separate) reaction (Seigner *et al.*, 2008). Primers are used at a final concentration of 0.2 µM. The amplicon is 181 bp.

*nad5* sense: 5′-GATGCTTCTTGGGGCTTCTTGTT-3′ (nt 968–987 and 1836–1838)

*nad5* antisense: 5′-CTCCAGTCACCAACATTGGCATAA-3′ (nt 1973–1995)

### **3.3.4.2 Real-time RT-PCR using the primers of Boonham *et al.* (2004)**

The primers and probe used for this assay are those described by Boonham *et al.* (2004). However, neither this assay nor any of the published real-time assays will specifically identify PSTVd. If a positive is obtained by real-time RT-PCR, the identity of the viroid will need to be determined using conventional RT-PCR and sequencing.

The assay will detect PSTVd, MPVd, TCDVd and TPMVd. Sensitivity for the detection of PSTVd in *S. tuberosum* using the CTAB extraction method was at least 17 pg PSTVd, the lowest concentration tested (Jeffries & James, 2005). By testing variants of PSTVd and synthetic oligonucleotides it has been shown that this assay detects all known sequence variants. These were identified from *in silico* studies as primer–sequence mismatches with the potential for failure of detection (Boonham *et al.*, 2005). However, the divergent isolates VIR-06/7L and VIR-06/10L described recently by Owens *et al.* (2009) may not be detected because of the insertion of (an) additional base(s) at the probe binding site (W. Monger, personal communication, 2011)<sup>1</sup>.

#### Primers

PSTV-231-F: 5′-GCC CCC TTT GCGCTG T-3′ (nt 232–247)

PSTV-296-R: 5′-AAG CGG TTC TCG GGA GCT T-3′ (nt 297–279)

PSTV-251T: FAM-5′-CAG TTG TTT CCA CCG GGT AGTAGC CGA-3′ TAMRA (nt 278–252)

The internal control COX primers amplify the *cytochrome oxidase 1* gene found in plant mitochondria (Weller *et al.*, 2000).

COX-F: 5′-CGT GCG ATT CCA GAT TAT CCA-3′

COX-R: 5′-CAA CTA CGG ATA TAT AAG RRC CRR ACC TG-3′

COXsol-1511T: VIC-5′-AGG GCA TTC CAT CCA GCG TAA GCA-3′ TAMRA

The reaction mix is for a 96-well plate and is a modification of the EPPO method (EPPO, 2004) as it incorporates a duplex reaction for detection of PSTVd and COX and a simplex reaction for detection of PSTVD (Roehorst *et al.*, 2005).

The reaction mix consists of 13.75 µl water, 25 µl of 2× Master Mix (Applied Biosystems), 1.25 µl of 40× MultiScribe Reverse Transcriptase (Applied Biosystems), 1.5 µl of each primer PSTV-231-F and PSTV-296-R (10 µM) and 1.0 µl probe PSTV-251T (5 µM). This reaction mix is divided equally into two volumes of 22 µl, A and B. Two microlitres of water is added to A and to B is added 0.75 µl of each COX primer (10 µM) and 0.5 µl of the probe COXsol-1511T (5 µM). One microlitre of RNA target is added to each of A and B to make a final reaction mix of 25 µl for each well of the reaction plate. With reaction mix A, PSTVd will be detected and with reaction mix B, PSTVd and COX will be detected in a duplex reaction.

Thermocycling conditions are 48 °C for 30 min, 95 °C for 2 min and 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

#### 3.3.4.3 Real-time RT-PCR (Plant Print Diagnostics kit)

The primers and probe used in this assay are those described by Bertolini *et al.* (2010) and they are available as a kit from Plant Print Diagnostics (Ref. PSTVd/100). The assay will detect CLVd, PSTVd and TCDVd. All 327 PSTVd isolates present in GenBank should be detected because *in silico* studies showed that all primer–sequence mismatches were in non-critical positions (N. Duran-Vila, personal communication, 2014).

Validation data are provided in Table 1.

#### Primers

PSTVd-F: 5′-CCT TGG AAC CGC AGT TGG T-3′ (nt 339–357)

PSTVd-R: 5′-TTT CCC CGG GGA TCC C-3′ (nt 87–102)

PSTVdP: FAM-5′-TCCTGTGGTTCACACCTGACCTCCTGA-3′ TAMRA (nt 19–45)

The PCR cocktail contains lyophilized primers and probe (provided in the kit) to which any commercial RT-PCR master mix can be added. For each reaction, 3 µl template RNA is added to 9 µl

<sup>1</sup> As of 1 March 2010 (W. Monger, personal communication, 2011)

PCR cocktail consisting of 6 µl commercial 2× RT-PCR buffer, 0.6 µl of each of forward and reverse primer (10 µM), 0.36 µl TaqMan probe (5 µM), 0.5 µl of 25× RT-PCR enzyme mix and 0.94 µl water to make a final reaction volume of 12 µl.

Thermocycling conditions are 45 °C for 10 min, 95 °C for 10 min and 40 cycles of (95 °C for 15 s and 60 °C for 1 min).

For this method a sample is considered positive when it produces a Ct value of <40 and negative controls are negative (no amplification). A sample is considered negative when it produces a Ct value of ≥40 and the positive controls show amplification.

### 3.4 Controls for molecular tests

For the test result obtained to be considered reliable, appropriate controls – which will depend on the type of test used and the level of certainty required – should be considered for each series of nucleic acid isolation and amplification of the target pest or target nucleic acid. For RT-PCR, a positive nucleic acid control, an internal control and a negative amplification control (no template control) are the minimum controls that should be used.

**Positive nucleic acid control** This control is used to monitor the efficiency of the assay (apart from the extraction). Pre-prepared (stored) viroid nucleic acid, whole genome amplified DNA or a synthetic control (e.g. cloned PCR product) generated using the same primer pair as used for detection may be used. A limit of detection control (not mandatory) may also be used.

**Internal control** For conventional and real-time RT-PCR, a plant housekeeping gene (HKG) such as COX or NAD should be incorporated into the RT-PCR protocol to eliminate the possibility of false negatives due to nucleic acid extraction failure or degradation or the presence of PCR inhibitors. Preferably, the internal control primers should be used in a duplex reaction with the pospiviroid/PSTVd primers. However, as this may be difficult to achieve without reducing the sensitivity of the test for the viroid, it is recommended, where practical, to run a duplex reaction of the pospiviroid/PSTVd primers with the HKG primers and also a simplex reaction with only pospiviroid/PSTVd primers.

The *nad5* mitochondrial *NADH dehydrogenase 5* gene fragment has been shown to be a reliable indicator of the performance of the extraction procedure and RT step for conventional RT-PCR (Menzel *et al.*, 2002). It has been tested against many plant species, including *S. tuberosum* and other *Solanum* species (*S. bonariensis*, *S. dulcamara*, *S. jasminoides*, *S. nigrum*, *S. pseudocapsicum*, *S. rantonnetii* and *S. sisymbirifolium*), *Acnistus arborescens*, *Atropa belladonna*, *Brugmansia* spp., *Capsicum* spp., *Cestrum* spp., *Lochroma cyanea*, *Nicotiana* spp. and *Physalis* spp. (Seigner *et al.*, 2008). The *nad5* primers span an intron and will therefore not amplify from DNA. RNA is amplified after the intron is removed.

Although COX has been used as an internal control in this protocol, COX primers will amplify RNA and DNA. It therefore provides only an indication of the quality of amplifiable DNA rather than RNA alone and does not control the RT step.

When the internal control COX or *nad5* is not mentioned in the description of a PCR method, the laboratory should choose an internal control and validate it.

**Negative amplification control (no template control)** This control is necessary for conventional and real-time RT-PCR to rule out false positives due to contamination during preparation of the reaction mixture. PCR-grade water that was used to prepare the reaction mixture is added at the amplification stage.

**Positive extraction control** This control is used to ensure that target viroid nucleic acid extracted is of sufficient quantity and quality for RT-PCR and that the target viroid is detectable. Viroid nucleic acid is extracted from infected host tissue or healthy plant tissue that has been spiked with the viroid.

The positive control should be approximately one-tenth of the amount of leaf tissue used per plant for the RNA extraction. If bulking of samples is done then the quantity of positive control should be adjusted accordingly (e.g. 10 lots of 20 mg sample bulked for RNA extraction, 2 mg infected leaf + 198 mg healthy potato tissue). If this is not detected then the test should be repeated or the bulking rate reduced until reliable detection is achieved.

For RT-PCR, care needs to be taken to avoid cross-contamination due to aerosols from the positive control or from positive samples. The positive control used in the laboratory should be sequenced so that this sequence can be readily compared with the sequence obtained from PCR amplicons of the correct size. Alternatively, synthetic positive controls can be made with a known sequence that, again, can be compared with PCR amplicons of the correct size.

**Negative extraction control** This control is used to monitor contamination during nucleic acid extraction and/or cross-reaction with the host tissue. The control comprises nucleic acid that is extracted from uninfected host tissue and subsequently amplified. Multiple controls are recommended to be included when large numbers of positive samples are expected.

### 3.5 Interpretation of results from conventional and real-time RT-PCR

#### 3.5.1 Conventional RT-PCR

The viroid-specific PCR will be considered valid only if:

- the positive nucleic acid control produces the correct size product for the viroid; and
- no amplicons of the correct size for the viroid are produced in the negative extraction control and the negative amplification control.

If the COX and/or *nad5* internal control primers are also used, then the negative (healthy plant tissue) control (if used), positive nucleic acid control, and each of the test samples must produce a 181 bp band (*nad5*). Failure of the samples to amplify with the internal control primers suggests, for example, that the nucleic acid extraction has failed, the nucleic acid has not been included in the reaction mixture, the RT step has failed, compounds inhibitory to PCR are present in the nucleic acid extract, or the nucleic acid has degraded.

A sample will be considered positive if it produces an amplicon of the correct size. For identification of the viroid species the PCR product must be sequenced.

#### 3.5.2 Real-time RT-PCR

The real-time RT-PCR will be considered valid only if:

- the positive nucleic acid control produces an amplification curve with the viroid-specific primers; and
- no amplification curve is seen (i.e. Ct value is 40 or other Ct value defined by the laboratory after validation) with the negative extraction control and the negative amplification control.

If the COX and *nad5* internal control primers are also used, then the negative control (if used), positive nucleic acid control, and each of the test samples must produce an amplification curve. Failure of the samples to produce an amplification curve with the internal control primers suggests, for example, that the nucleic acid extraction has failed, the nucleic acid has not been included in the reaction mixture, compounds inhibitory to PCR are present in the nucleic acid extract, or the nucleic acid has degraded.

A sample will be considered positive if it produces a typical amplification curve. Specific information on the Ct cut-off value for two methods is provided in sections 3.3.3.4 and 3.3.4.3.

## 4. Identification

PSTVd should be identified by sequencing the product obtained from the conventional RT-PCR methods using the Shamloul or Vid primers described in sections 3.3.4.1 and 3.3.3.3, respectively, and by searching for a sequence match on the public genetic sequence databases. Sequence analysis specialists may be needed to assist in identification. If the PCR product is weakly amplified or if the sample is infected by more than one pospiviroid, cloning the PCR product may be effective in enabling a sequence to be obtained.

A positive sample detected by real-time RT-PCR, should, if required for confirmation, be retested using conventional RT-PCR to enable the product to be sequenced and identified. Sequencing the real-time PCR product directly will give sequence information that does not allow reliable identification. It will allow the PCR product to be identified as a viroid but will not allow species identification or discrimination from the positive control used. However, because of the increased sensitivity of the real-time RT-PCR, a product may not be obtained with conventional RT-PCR. In the case of bulked samples, retesting smaller subsamples might increase the reliability of amplification by conventional RT-PCR. Alternatively, samples may be inoculated in tomato plants to increase the concentration of the viroid to levels that may be detectable by conventional RT-PCR. However, this approach has not been evaluated and if results are inconclusive then resampling and testing may be required.

### 4.1 Sequencing and sequence analysis

Sequence analysis should only be done by an experienced person. If facilities are not available for sequencing to be done in-house, a commercial company should be used. The company will specify their requirements for the sequencing of PCR products. The purified product (and forward and reverse primers if requested) is sent to the company to carry out the sequencing. Some companies may also purify the product if required.

If sequencing is done in-house, the methods should be established and followed. Each strand of the PCR product should be sequenced, using the PCR primers as the sequencing primers. The two independently sequenced DNA strands (from using forward and reverse primers) should be assembled into a single contig, confirming the base call (identity) of each nucleotide site. It is preferable to use assemblers (e.g. Geneious, CLC Genomics Workbench or Lasergene software) that use electropherograms (trace files) for the analysis. Disagreements between the two strands should be coded as ambiguous bases in the edited sequence. The edited consensus sequence (determined by comparing the two strands) can then be compared with pospiviroid sequences in a relevant database. In the case of a mixed infection, the chromatogram may not be readable and the PCR product should be cloned and sequenced.

Careful alignment is required for pospiviroids where a few nucleotide differences may be critical in identifying the viroid as a regulated or a non-regulated pest. For initial identification of PSTVd, the primer sequences (Shamloul or Vid primers) in the consensus sequence may be kept because these primers are located in the most conserved regions of the viroid genome and are not likely to influence identification. A-overhangs built in by the polymerase during elongation have to be removed if observed. For identification, it is advisable to use an edited consensus sequence starting at position 1 of the viroid genome for comparison with one of the comprehensive nucleotide databases. The search should be done in the GenBank non-redundant nucleotide database at the website of the National Centre for Biotechnology Information (NCBI) or the European Nucleotide Archive at the website of the European Molecular Biology Laboratory (EMBL) by using the Basic Local Alignment Search Tool (BLAST). In addition, identification should be based on specific clustering of BLAST hit results in (neighbour joining) tree view.

According to the International Committee on Taxonomy of Viruses (ICTV) the main criterion for species identification is more than 90% sequence identity (Owens *et al.*, 2011). However, if the sequence obtained shows identity close to 90%, additional parameters should be included, such as biological properties. The ICTV Viroid Study Group is currently discussing the viroid classification and the criteria for species demarcation.

When 100% sequence accuracy is required, for example when a sequence is to be submitted to a database or when a new viroid species is suspected, it is necessary to perform a second PCR. This PCR will cover the region of the primer sequences used for the first PCR as well as any ambiguous bases from the first PCR. Design of a new set of primers from the initial sequence may be required for this purpose, but the use of the Shamloul and Vid primer-pairs may be sufficient.

## 5. Records

Records and evidence should be retained as described in ISPM 27 (*Diagnostic protocols for regulated pests*).

In instances where other contracting parties may be affected by the results of the diagnosis, in particular in cases of non-compliance and where PSTVd is found in an area for the first time, the following additional material should be kept in a manner that ensures complete traceability:

- the original sample (if still available) should be kept frozen at  $-80^{\circ}\text{C}$  or freeze-dried and kept at room temperature
- if relevant, RNA extractions should be kept at  $-80^{\circ}\text{C}$
- if relevant, RT-PCR amplification products should be kept at  $-20^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$
- the DNA sequence trace files used to generate the consensus sequence for identification of samples.

If the isolate is shown to have different molecular or biological characteristics to previously recorded isolates, it should be offered to a recognized plant pest collection/archive (e.g. Q-bank (Comprehensive Database on Quarantine Plant Pests and Diseases), DSMZ (Leibniz Institute-German Collection of Microorganisms and Cell Cultures)).

If there is evidence of any of the tests described failing to detect an isolate of PSTVd, isolate details (preferably the GenBank accession number) should be sent to the IPPC Secretariat.

## 6. Contact Points for Further Information

Further information on this protocol can be obtained from:

Science and Advice for Scottish Agriculture (SASA), Roddinglaw Road, Edinburgh EH12 9FJ, Scotland, UK (Dr C.J. Jeffries, e-mail: [colin.jeffries@sasa.gsi.gov.uk](mailto:colin.jeffries@sasa.gsi.gov.uk)).

National Plant Protection Organization, PO Box 9102, 6700 HC Wageningen, The Netherlands (Dr J.W. Roenhorst, e-mail: [j.w.roenhorst@nvwa.nl](mailto:j.w.roenhorst@nvwa.nl); Dr J.Th.J. Verhoeven, e-mail: [j.th.j.verhoeven@nvwa.nl](mailto:j.th.j.verhoeven@nvwa.nl)).

Department of Environment and Primary Industries, Biosciences Research Division, AgriBio, 5 Ring Road, La Trobe University, Bundoora, Victoria 3083, Australia (Dr B. Rodoni, e-mail: [brendan.rodoni@depi.vic.gov.au](mailto:brendan.rodoni@depi.vic.gov.au)).

Canadian Food Inspection Agency (CFIA), Charlottetown Laboratory, 93 Mt Edward Road, Charlottetown, PE, C1A 5T1, Canada (Dr H. Xu, e-mail: [huimin.xu@inspection.gc.ca](mailto:huimin.xu@inspection.gc.ca)).

Conselleria de Agricultura de la Generalitat Valenciana, Centro de Proteccion Vegetal y Biotecnologia (IVIA), 46113 Moncada (Valencia), Spain (Dr N. Duran-Vila, e-mail: [duran\\_nur@gva.es](mailto:duran_nur@gva.es)).

USDA-APHIS, Plant Germplasm Quarantine Program BARC-E, BLD 580, Powder Mill Road, Beltsville, MD 20705, USA (Dr J.A. Abad, e-mail: [jorge.a.abad@aphis.usda.gov](mailto:jorge.a.abad@aphis.usda.gov)).

Laboratorios Biológicos, Dirección General de Servicios Agrícolas, Ministerio de Ganadería, Agricultura y Pesca, Millán 4703, Montevideo, Uruguay (Dr A. Etchevers, e-mail: [anitaetchevers@hotmail.com](mailto:anitaetchevers@hotmail.com)).

A request for a revision to a diagnostic protocol may be submitted by national plant protection organizations (NPPOs), regional plant protection organizations (RPPOs) or Commission on Phytosanitary Measures (CPM) subsidiary bodies through the IPPC Secretariat ([ippc@fao.org](mailto:ippc@fao.org)), which will be forward it to the Technical Panel on Diagnostic Protocols (TPDP).

## 7. Acknowledgements

The first draft of this protocol was written by C.J. Jeffries (SASA, UK), J.W. Roenhorst (National Plant Protection Organization, the Netherlands), B. Rodoni (Department of Environment and Primary Industries, Australia), H. Xu (CFIA, Canada), N. Duran-Vila (IVIA, Spain), A. Etchevers (Laboratorios Biológicos, Uruguay) and J.A. Abad (USDA-APHIS, USA) (see section 6 for contact details). In addition, J.Th.J. Verhoeven (National Plant Protection Organization, the Netherlands) was significantly involved in the development of this protocol.

Thanks are due to S.L. Nielsen (Denmark); L. Seigner, S. Winter and M. Wassenegger (Germany); H. Koenraadt (the Netherlands); and A. Fox, T. James, W. Monger and V. Mulholland (UK) for helpful comments during development of this protocol.

## 8. References

The present standard also refers to other International Standards for Phytosanitary Measures (ISPMs). ISPMs are available on the IPP at <https://www.ippc.int/core-activities/standards-setting/ispms>.

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**Table 1.** Overview of and validation data for protocols used to detect *Potato spindle tuber viroid* in different types of host material

Matrix	Sample size	Sample preparation	Nucleic acid extraction	Detection method	Remarks on validation
Tomato leaves	1 g	3.5 ml (1:2–1:5 (w/v)) GH plus lysis buffer with Homex 6 (Bioreba)	RNeasy Plant Mini Kit (Qiagen) or Sbeadex maxi plant kit (LGC Genomics) on KingFisher 96 system (Thermo Scientific)	Real-time reverse transcription-polymerase chain reaction (RT-PCR): GenPospi assay, Botermans <i>et al.</i> (2013)	<b>Limit of detection:</b> detection of all pospiviroid species up to a relative infection rate <sup>1</sup> of 0.13% (equals 770 times dilution) with 99.7% certainty for dilution of infected tomato leaves in healthy tomato <b>Analytical specificity:</b> highly specific for pospiviroid species <b>Selectivity:</b> no influence of tomato leaves <b>Repeatability and reproducibility:</b> 100% (Naktuinbouw, 2012a; Botermans <i>et al.</i> , 2013; NPPO-NL, 2013d)
Tomato leaves	1 g	3.5 ml (1:2–1:5 (w/v)) GH plus lysis buffer with Homex 6	RNeasy Plant Mini Kit	Real-time RT-PCR: Boonham <i>et al.</i> (2004)	<b>Limit of detection:</b> detection up to 10 000 times dilution of infected tomato leaves in healthy tomato <b>Analytical specificity:</b> detection of <i>Mexican papita viroid</i> (MPVd), <i>Potato spindle tuber viroid</i> (PSTVd) <i>Tomato chlorotic dwarf viroid</i> (TCDVd), <i>Tomato planta macho viroid</i> (TPMVd) (some isolates) <b>Selectivity:</b> no influence of tomato leaves <b>Repeatability and reproducibility:</b> 100% (Naktuinbouw, 2012b)
Tomato leaves	1 g	3.5 ml (1:2–1:5 (w/v)) GH plus lysis buffer with Homex 6	RNeasy Plant Mini Kit	RT-PCR: Pospi1-FW Pospi1-RE primers, Verhoeven <i>et al.</i> (2004)	<b>Limit of detection:</b> detection of all pospiviroid species (except <i>Columnnea latent viroid</i> (CLVd)) up to at least a relative infection rate of 2.5% for dilution of infected tomato leaves in healthy tomato <b>Analytical specificity:</b> detection of <i>Hop latent viroid</i> (HpLVd, genus <i>Cocadviroid</i> ) and PSTVd <b>Selectivity:</b> no influence of tomato leaves <b>Repeatability and reproducibility:</b> 100% (NPPO-NL, 2013a)
Tomato leaves	1 g	3.5 ml (1:2–1:5 (w/v)) GH plus lysis buffer with Homex 6	RNeasy Plant Mini Kit	RT-PCR: Vid-FW/Vid-RE primers, Verhoeven <i>et al.</i> (2004)	<b>Limit of detection:</b> detection of CLVd, <i>Potato spindle tuber viroid</i> (PSTVd) and TCDVd up to at least a relative infection rate of 100% (10% for CLVd*) for dilution of infected tomato leaves in healthy tomato * Primers originally designed to detect CLVd complementary to the Pospi1-FW/Pospi1-RE RT-PCR (Verhoeven <i>et al.</i> , 2004) <b>Analytical specificity:</b> detection of CLVd, PSTVd and TCDVd <b>Selectivity:</b> no influence of tomato leaves <b>Repeatability and reproducibility:</b> 100% (NPPO-NL, 2013b)
Tomato leaves	1 g	3.5 ml (1:2–1:5 (w/v)) GH plus lysis buffer with Homex 6	RNeasy Plant Mini Kit	RT-PCR: Shamloul <i>et al.</i> (1997)	<b>Limit of detection:</b> detection up to at least a relative infection rate of 10% for dilution of infected tomato leaves in healthy tomato <b>Analytical specificity:</b> detection of MPVd, PSTVd, TCDVd, TPMVd (some isolates) <b>Selectivity:</b> no influence of tomato leaves <b>Repeatability and reproducibility:</b> 100% (NPPO-NL, 2013c)

Matrix	Sample size	Sample preparation	Nucleic acid extraction	Detection method	Remarks on validation
Tomato seeds	3 000 seeds (tested as three times 1 000)	20 ml (1:2–1:5 (w/v)) GH plus lysis buffer with BagMixer (Interscience)	Sbeadex maxi plant kit on KingFisher 96 system	Real-time RT-PCR: Boonham <i>et al.</i> (2004)	Performance characteristics assay as for tomato leaves Probability of detection of one infected seed in a sample of 1 000 is >95% when testing three subsamples each of 1 000 seeds. Owing to rapid cross-contamination of PSTVd from infected fruits to healthy seeds during processing (using fermentation and pectinase treatment) of the seeds there is a high probability that more contaminated seeds will be present in a sample (Naktuinbouw, 2012c).
Potato leaves (growth room grown) and <i>in vitro</i> potato plants	200 mg	20 µL of 10% sodium dodecyl sulphate (SDS), 180 µL LiCl extraction buffer, 400 µL phenol–chloroform with mortar and pestle	Phenol–chloroform and two-step polyethylene glycol (PEG) extraction	Return (R)-polyacrylamide gel electrophoresis (PAGE) <sup>2</sup>	<b>Limit of detection:</b> 2 465 pg PSTVd; this was the least sensitive of the molecular methods in an international ring test <b>Analytical specificity:</b> detection of all known pospiviroids <b>Selectivity:</b> no influence of potato variety, potato leaves or <i>in vitro</i> plants <b>Repeatability and reproducibility:</b> reproducibility 51% at 87 893 pg PSTVd (the highest concentration of PSTVd tested) and 42% at the limit of detection
Potato leaves (growth room grown) and <i>in vitro</i> potato plants	200 mg	1:1.5 (w/v) Ames buffer (EPPO, 2004) with mortar and pestle	Immobilization on membrane (Agdia, Inc.) phenol–chloroform and two-step PEG extraction	Digoxigenin (DIG) probe <sup>2</sup>	<b>Limit of detection:</b> at least 17 pg PSTVd (the lowest concentration tested) <b>Analytical specificity:</b> detection of all known pospiviroids <b>Selectivity:</b> no influence of potato variety, potato leaves or <i>in vitro</i> plants <b>Repeatability and reproducibility:</b> reproducibility 100% at 87 893 pg PSTVd and 23% at 17 pg PSTVd
Potato leaves (growth room grown) and <i>in vitro</i> potato plants	50–500 mg	1:9 (w/v) RH buffer (Qiagen) with microcentrifuge tube and micropestle or Homex 6	RNeasy Plant Mini Kit	Two-step <sup>2</sup> conventional RT-PCR using the primers of Shamloul <i>et al.</i> (1997)	<b>Limit of detection:</b> at least 17 pg PSTVd <b>Analytical specificity:</b> detection of MPVd, PSTVd, TCDVd and TPMVd <b>Selectivity:</b> no influence of potato variety, potato leaves or <i>in vitro</i> plants <b>Repeatability and reproducibility:</b> reproducibility 78% at 87 893 pg PSTVd (the highest concentration of PSTVd tested) and 44% at 17 pg PSTVd
Potato leaves (growth room grown) and <i>in vitro</i> potato plants	1 g	3.5 ml (1:2–1:5 (w/v)) GH plus lysis buffer with Homex 6	Sbeadex maxi plant kit on KingFisher 96 system	Real-time RT-PCR: GenPospi assay, Botermans <i>et al.</i> (2013)	Performance characteristics assay as for tomato leaves <b>Analytical specificity:</b> no cross-reaction with viruses commonly occurring in potato <b>Selectivity:</b> no influence of potato leaves and <i>in vitro</i> plants Validated for bulking rates up to 100 (100% detection in sample composed of 1 infected and 99 healthy leaves; NAK, 2011)
Potato leaves, (growth room grown) <i>in vitro</i> potato plants and tubers	1.5 g leaves or 5 g tubers	Approximately 600 µl buffer for leaves or approximately 3 ml buffer for tubers (buffer choice depending on method used for extraction)	RNeasy Plant Mini Kit, cetyl trimethylammonium bromide (CTAB) extraction or Purescript RNA isolation kit (Gentra Systems; note that this kit is not available anymore)	Real-time RT-PCR: Boonham <i>et al.</i> (2004)	<b>Limit of detection:</b> detection up to 10 000 times dilution of infected tissue in healthy tissue <b>Analytical specificity:</b> detection of MPVd, PSTVd, TCDVd, TPMVd (some isolates); no cross-reaction with viruses commonly occurring in potato <b>Selectivity:</b> no influence of potato leaves, <i>in vitro</i> plants or tubers <b>Repeatability and reproducibility:</b> 100% (ring test of four laboratories) Validated for bulking rates up to 100 (100% detection in sample composed of 1 infected and 99 healthy leaves; Roenhorst <i>et al.</i> , 2005, 2006)

Matrix	Sample size	Sample preparation	Nucleic acid extraction	Detection method	Remarks on validation
Ornamental plant species (leaves)	1 g	3.5 ml (1:2–1:5 (w/v)) GH plus lysis buffer with Homex 6	RNeasy Plant Mini Kit or Sbeadex maxi plant kit on KingFisher 96 system	Real-time RT-PCR: GenPospi assay, Botermans <i>et al.</i> (2013)	Performance characteristics assay as for tomato leaves <b>Analytical sensitivity:</b> concentration of pospiviroids and selectivity (inhibitory components) in leaf sap dependent on plant species Validated for bulking rates up to 25 for <i>Brugmansia</i> , <i>Calibrachoa</i> , <i>Cestrum</i> , <i>Dahlia</i> , <i>Nematanthus</i> , <i>Petunia</i> , <i>Solanum jasminoides</i> and <i>Streptosolen jamesonii</i> . Note that for <i>Calibrachoa</i> , <i>S. jasminoides</i> and <i>S. jamesonii</i> matrix effects have been observed at dilutions of more than 100. For some crops, such as <i>Dahlia</i> , only the summer period seems suitable for (reliable) testing (Naktuinbouw, 2012a).
Ornamental plant species (leaves)	1 g	3.5 ml (1:2–1:5 (w/v)) GH plus lysis buffer with Homex 6	RNeasy Plant Mini Kit or Sbeadex maxi plant kit on KingFisher 96 system	Real-time RT-PCR: Boonham <i>et al.</i> (2004)	Performance characteristics assay as for tomato leaves <b>Analytical sensitivity:</b> concentration of pospiviroids and selectivity (inhibitory components) in leaf sap dependent on plant species Validated for bulking rates up to 25 for <i>Brugmansia</i> , <i>Calibrachoa</i> , <i>Dahlia</i> , <i>Petunia</i> , <i>S. jasminoides</i> and <i>S. jamesonii</i> . Note that for <i>Calibrachoa</i> , <i>S. jasminoides</i> and <i>S. jamesonii</i> matrix effects have been observed at dilutions of more than 100. For some crops, such as <i>Dahlia</i> , only the summer period seems suitable for (reliable) testing (Naktuinbouw, 2012b).
Tomato leaves, potato leaves, tubers and seeds, and ornamental plant species (leaves)	1 g leaves or potato tubers or leaf prints on nylon membranes	10 ml (1:10 (w/v)) phosphate-buffered saline (PBS) with Homex 6	Direct methods (tissue print), RNeasy Plant Mini Kit or PowerPlant RNA Isolation Kit (Mo Bio)	Real-time RT-PCR: Bertolini <i>et al.</i> (2010)	<b>Limit of detection:</b> detection up to 10 000 times dilution of infected <i>S. jasminoides</i> leaves in healthy leaves of <i>S. jasminoides</i> and tomato <b>Analytical specificity:</b> detection of CLVd, PSTVd and TCDVd <b>Selectivity:</b> no influence of potato leaves, tubers or tomato seeds <b>Repeatability and reproducibility:</b> 100% (ring test of three laboratories) The diagnostic sensitivity was 100%, the diagnostic specificity was 100% and the relative accuracy compared with a molecular hybridization method (Murcia <i>et al.</i> , 2009) was 100%. Validation of the test was performed with 208 field samples of <i>S. jasminoides</i> , <i>Brugmansia</i> spp., <i>Datura</i> spp., <i>Petunia</i> spp., <i>Dendrathera</i> spp., potato and tomato. Of the 208 samples, 43 were true positive and 150 true negative by both techniques. Fifteen samples were false positive by hybridization in which <i>Tomato apical stunt viroid</i> (TASVd) and <i>Citrus exocortis viroid</i> (CEVd) were detected. No samples were false negative.

<sup>1</sup> Because viroid concentration in the original test material is not known, for some of the assays the limit of detection (sensitivity) is expressed as a relative value. Undiluted infected leaf sap is considered 100% infected (at a ratio of 1 g leaf material : 3 ml buffer). The relative limit of detection was determined by testing eight serial dilutions of infected leaf sap in healthy leaf sap. The relative limit of detection is defined as the average of the lowest relative infection rate of each isolate that could still be detected (cycle threshold (Ct) <32), and three standard deviations were added to give a conservative measure with 99.7% certainty (Botermans *et al.*, 2013).

<sup>2</sup> The three methods, R-PAGE, DIG probe and two-step conventional RT-PCR using the primers of Shamloul *et al.* (1997), were compared in an international ring test (Jeffries and James, 2005).

**Publication history**

*This is not an official part of the standard*

2007-03 CPM-2 added topic to work programme (2006-002)

2012-11 TPDP revised draft protocol

2013-03 SC approved by e-decision for member consultation  
(2013\_eSC\_May\_10)

2013-07 Member consultation

2014-07 TPDP reviewed draft protocol

2014-09 TPDP approved by e-decision to SC for approval for adoption  
(2014\_eTPDP\_September\_01)

2014-11 SC approved by e-decision for DP notification period  
(2014\_eSC\_Nov\_13)

2014-12 Notification period

2015-01 SC adopted DP on behalf of CPM (no formal objections received)

**ISPM 27. 2006: Annex 7 *Potato spindle tuber viroid* (2015).** Rome, IPPC, FAO.

Publication history last updated: 2015-02-09