



Submissions for Diagnostic Protocols

I. General information

Submission number	2023-011
Title of Proposal	Diagnostic Protocol for detection and identification of <i>Xylella</i> vectors
Submitted by (Country or Organization)	IPPC Contracting Party
IPPC Official Contact Point or RPPO	Australia
Supported by	Australia

2. Contact information

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3. Summary of proposal

Summary of justification for the proposal	Phytopathogenic bacteria in the genus <i>Xylella</i> can cause significant disease on a broad range of plant species world-wide. Insect species in the suborder Auchenorrhyncha that vector <i>Xylella</i> can spread the disease into new areas and be damaging pests even without the bacterium, so their movement is restricted by RPPOs around the world. Currently, there are numerous individual published diagnostic tools for identification of insect groups within the suborder Auchenorrhyncha. The most appropriate combination of validated tools/assays for identification and differentiation of <i>Xylella</i> vectors, potential <i>Xylella</i> vectors and closely related species, need to be identified and centralised in one protocol to enable trusted and comparable results for global trade.
Proposed priority	I (high)
Comments	<i>Xylella</i> is a high-risk pathogen with potential for global spread and impact with the help of vectors.

4. Literature review

Literature review

Phytopathogenic bacteria in the genus *Xylella* can cause significant disease on a broad range of plant species world-wide. Introduction into new geographic regions has historically occurred due to the movement of infected plant material, however vectors such as *Homalodisca vitripennis*, the glassy-winged sharpshooter, have also been recorded as hitch-hikers. Local movement and disease establishment is influenced by various factors including the vector species present, vector host range, vector host tissue preference, genetics of the bacterial lineage (e.g. subspecies and sequence type), host plant species, environmental factors, and crop management practices to name just a few. Each significant global *Xylella* epidemic has been characterised by its own unique combination of these factors. For example, Pierce's disease of grapevine in North America is caused by *Xylella fastidiosa* (Xf) subspecies *fastidiosa* and is predominantly vectored by sharpshooters, in particular the glassy-winged sharpshooter. Citrus Variegated Chlorosis of orange in Brazil is caused by Xf subspecies *pauca* and is transmitted by sharpshooter species. Olive Quick Decline Syndrome (OQDS) in Italy is caused by Xf subspecies *pauca*, and is primarily vectored by *Philaenus spumarius*, the meadow spittlebug. In every new *Xylella* detection, identifying not only the species/subspecies/ST of *Xylella* that is infecting the host, but also the vector/s that are disseminating the pathogen, is integral for informing response strategies. These diagnoses provide vital details (e.g. host range) which inform decision makers and guide surveillance strategies, risk assessment and eradication programs. All known vectors of *Xylella* are insects that belong to the Auchenorrhyncha suborder of Hemiptera and all xylem-feeding species belonging to this suborder are considered potential Xf vectors. This taxonomically diverse group includes species from the superfamily Ceropoidea (froghoppers and spittlebugs), the superfamily Cicadoidea (cicadas), and the subfamily Cicadellinae within the Cicadellidae family (sharpshooters). Species from these taxonomic groups are widespread, with representative species found in all zoogeographical regions except Antarctica. A diagnostic protocol for *Xylella* vectors is vital for accurate species identification, effective eradication responses, and to inform on-going management strategies where eradication is not technically feasible, all of which are essential to safeguard agriculture, ecosystems, social amenity and economies from the harmful impacts of these pests and the pathogens they carry. In a biosecurity setting, it is imperative that you can accurately identify known introduced *Xylella* vectors and differentiate them from look-alike or closely related native species. Diagnostics can be further complicated by the various life stages of these organisms that may be detected (e.g. adult insects vs eggs). Having accurate diagnostic tools to detect *Xylella* vectors will ensure rapid targeting of exotic vectors to delimitate the incursion area, to identify the plant hosts, and will also assist investigation of potential native vectors.

5. Criteria for prioritization of Diagnostic Protocols

Criteria	Information provided by Submitter
1. Need for international harmonization of the diagnostic techniques for the pest (e.g. due to difficulties in diagnosis or disputes on methodology)	<p>Within the suborder Auchenorrhyncha (Hemiptera), there are many genera that require specialised skills and/or molecular analysis for diagnosis to species level. This can be further confounded by the life stage of the insect being analysed. For example, there are 13 endemic species of Cicadellinae currently described in Australia, and there are likely further unrecorded and undescribed species that could confound diagnostics in an emergency response of known Xf vectors, for example, <i>Homalodisca vitripennis</i> (glassy-winged sharpshooter) and <i>Graphocephala atropunctata</i> (blue-green sharpshooter). Furthermore, these native species could also act as native vectors in an outbreak. In either case, an ability to accurately identify and differentiate these Cicadellinae from closely related species is imperative.</p> <p>It is proposed that a harmonised protocol will provide the following outcomes for trade, border protection, emergency responses and on-going management.</p> <ul style="list-style-type: none"> -Accurately identify known exotic <i>Xylella</i> vector species. -Identify potential native vector species. -Provide comparable results and reference material that can continue to aid other jurisdictions; for example, in relation to the following. <ul style="list-style-type: none"> ○ Harmonised standards for diagnostic results for use in domestic trade. ○ Use of the same molecular barcode region for identification and differentiation of key taxonomic groups to build a strong repository of sequence data from known vectors, closely related and/or look-alike species that would otherwise only be identified by specialists in their native countries. - Support high throughput surveillance and environmental scanning activities (e.g. consistent genetic target from broad taxa that could be used for eDNA, barcoding, metabarcoding and hybridisation capture).
2. The relevance of the diagnosis to the protection of plants including measures to limit the impact of the pest.	<p>In a quarantine situation (e.g. border protection) accurate detection and diagnosis of a regulated pest prevents the spread of an exotic organism into a new area protecting agriculture, the environment and social amenity. In an emergency response or outbreak situation, accurate and rapid detection and identification of the insect species provides key information such as life cycle, behaviour (e.g. feeding, dispersal) and host range. This information is integral in risk assessment and limiting the impact of the pest by informing surveillance, control, eradication, management and tracing activities. The speed and accuracy of the diagnosis directly impacts the success of these activities.</p>
3. Importance of the plants protected on the global level (e.g. relevant to many countries or of	<p><i>Xylella</i> spp. are pathogens of major importance worldwide. <i>X. fastidiosa</i> (Xf) has an extensive and growing host list, including 690 plant species belonging to 88 families (EFSA 2023). Xf can cause significant and devastating disease on economically</p>

major importance to a few countries).	important crops, in the native environment and on culturally significant amenity plants. Significant economic losses have been reported due to Xf disease. In Brazil approximately 200 million citrus trees and in Italy around one million olive trees are estimated to be infected by the disease. Xf disease causes over \$100 million in yearly losses to the grape industry in California. In Australia, where the pathogen is absent, Xf is listed as number one on the National Priority Plant Pest List. Diseases caused by Xylella have no cure. The bacterium is inoculated into xylem vessels by vector feeding, where it multiplies, and in combination with plant defence responses, blocks water and nutrients. Some species such as olives succumb quickly to the disease whereas others may die more slowly. Chemical control is not effective in managing the disease, thus robust and accurate diagnostic methods are required to prevent entry and establishment of the pest. Some countries effectively manage specific subspecies or sequence types of Xf and their associated vectors. However, due to the diverse nature of species within the genus and the potential consequences of the introduction of divergent strains into a naïve environment, even countries with endemic populations of Xylella remain vigilant about the movement of potentially infected plant material and vector species.																
4. Volume / importance of trade of the commodity that is subjected to the diagnostic procedures (e.g. relevant to many countries or of major importance to a few countries).	<p>Due to the extensive host range of Xylella spp., their known vectors and the potential of vector species to also spread as contaminating pests (hitchhikers) on plant material, there are numerous commodities that would be subject to biosecurity intervention including diagnostic procedures. These commodities include produce (fruit and vegetables), and live plants or planting material such as budwood, cuttings and rootstocks. The economic value of key Xf hosts (gross production value (constant 2014-2016) US\$) for the world listed at FAOSTAT in 2020 includes the following.</p> <table><tr><td>-Grapes</td><td>–</td><td>US\$68</td><td>billion.</td></tr><tr><td>-Oranges</td><td>–</td><td>US\$29</td><td>billion.</td></tr><tr><td>-Olives</td><td>–</td><td>US\$24</td><td>billion.</td></tr><tr><td>-Almonds</td><td>in shell –</td><td>US\$20</td><td>billion.</td></tr></table> <p>Due to the commodities impacted by Xylella spp. and its vectors, this diagnostic protocol would be relevant to many countries.</p>	-Grapes	–	US\$68	billion.	-Oranges	–	US\$29	billion.	-Olives	–	US\$24	billion.	-Almonds	in shell –	US\$20	billion.
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5. Other criteria for topics as determined by CPM that are relevant to determining priorities	<p>Contribution to the purpose of the IPPC as described in article I.1. This submission meets the core criteria outlined in CPM Appendix 8 The submission meets the following Supporting criteria points:</p> <p>Practical</p> <p>1) There is currently no overarching EPPO or IPPC protocol for Xylella vectors. There are a number of protocols that include relevant information:</p> <ul style="list-style-type: none">- PM 7/141 (1) Philaenus spumarius, Philaenus italosignus and Neophilaenus campestris (EPPO, 2019)- PM 7/129 DNA barcoding as an identification tool for a number of regulated pests (EPPO, 2016) <p>2) There is available expertise for development of the proposed standard.</p>																

	<p>Economic</p> <p>1) The economic value of key Xf hosts (gross production value (constant 2014-2016) US\$) for countries listed at FAOSTAT in 2020 includes:</p> <table><tr><td>-Grapes</td><td>–</td><td>US\$79</td><td>billion</td></tr><tr><td>-Oranges</td><td>–</td><td>US\$33</td><td>billion</td></tr><tr><td>-Olives</td><td>–</td><td>US\$24</td><td>billion</td></tr><tr><td>-Almonds</td><td>in shell</td><td>–</td><td>US\$12 billion</td></tr></table> <p>Environmental</p> <p>The submission meets points 3 & 4.</p> <p>Strategic</p> <p>The submission meets points 4 & 5</p>	-Grapes	–	US\$79	billion	-Oranges	–	US\$33	billion	-Olives	–	US\$24	billion	-Almonds	in shell	–	US\$12 billion
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<p>6. The balance between pests of importance in different climatic zones (temperate, tropics etc) and commodity classes.</p>	<p>Xylella spp. and their vectors are of importance to a significant proportion of countries worldwide and impact a wide range of agricultural commodity classes. Xylella spp. and their vectors are known to occur over a wide range of climatic zones in tropical and subtropical climates (e.g. Brazil, Costa Rica, Taiwan and southern California), in temperate and Mediterranean climates (e.g. Italy, Spain and France) and even continental climate regions (e.g. British Columbia, southern Ontario and Saskatchewan in Canada, the north-eastern regions of the USA and Argentina). Some of these countries are among the top countries exporting agricultural goods in the world (Top exporters of agricultural goods worldwide by country 2020 Statista). Research published in EFSA 2019 identified the prevalent Köppen–Geiger climate type climate subgroups in which Xylella has been recorded are: Csa (temperate with dry-hot summer), Cfa (temperate without dry season and hot summer) and Aw (tropical, savannah). At lower frequency, other subgroups with X. fastidiosa presence include BSk (arid, steppe, cold), Cwa (temperate with dry winter and hot summer) and BWWh (arid, desert, hot). These climate subgroups encompass regions of nearly all countries globally. The Auchenorrhyncha suborder of Hemiptera that includes the known and predicted xylem-feeding vectors of Xylella spp. occur in all zoogeographic regions except Antarctica.</p>																
<p>7. Number of labs undertaking the diagnosis.</p>	<p>In Australia, frontline federal diagnostics laboratories (at the border) and state-based laboratories all need to be equipped to diagnose Xylella vectors. This situation would be repeated across regional plant protection organizations (RPPOs) globally due to the climatic range of the pathogen and its vectors and the status of Xylella spp. as a regulated or high priority pest in many RPPOs (e.g. Australia, European Union, New Zealand, USA, Brazil, China, Japan and Egypt). It is likely that 100s of laboratories are undertaking diagnosis of Xylella vectors. The production of an annex to ISPM 27 protocol to harmonise diagnostic approaches for detection and identification of Xylella vectors would be a valuable tool for rapid adoption by laboratories in an incursion or emergency response.</p>																
<p>8. Feasibility of production of a protocol, including</p>	<p>The production of an annex to ISPM 27 to harmonise diagnostic approaches for detection and identification of Xylella vectors is highly feasible. Currently, there are numerous individual</p>																

availability of knowledge and expertise.	<p>published diagnostic tools for identification of insects in the suborder Auchenorrhyncha. These include morphological keys and molecular tools such as PCR, sequencing, barcoding and phylogenetic analysis, to identify and differentiate species. The most appropriate combination of validated tools/assays for identification and differentiation of <i>Xylella</i> vectors, potential <i>Xylella</i> vectors and closely related species, need to be identified and centralised in one protocol to enable trusted and comparable results. There is ample diagnostic expertise globally in the Auchenorrhyncha suborder with many recognised authorities. However, representation of specific regional expertise on closely related species that could confound diagnostics (e.g. cryptic species) from countries with and without <i>Xylella</i> would be a valuable inclusion in a protocol on <i>Xylella</i> vectors.</p>
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