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Protecting the world's plant resources from pests

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1. Opening of the Session

[1] The Secretary of the IPPC Secretariat, Jingyuan XIA, welcomed participants to the 14th Session of the Commission on Phytosanitary Measures. He informed CPM that it would be during this session that the Strategic Framework 2020-2030 would start taking the helm from the IPPC Strategic Objectives 2012-2019, marking a historical linkage between the two frameworks.

1.1 FAO Opening

[2] The FAO Assistant Director-General of the Agriculture and Consumer Protection Department, Mr Bukar TIJANI, opened the meeting welcoming all participants on behalf of the FAO Director-General, Mr Jose GRAZIANO Da Silva, and conveyed his appreciation to the 183 IPPC Contracting Parties (CPs) and ten Regional Plant Protection Organizations (RPPOs) for their commitment to the IPPC Work Programme. He further congratulated the IPPC for achieving the milestone of having 2020 proclaimed as the International Year of Plant Health (IYPH) by the United Nations General Assembly (UNGA), which would provide a significant platform to increase awareness of the importance of plant health for healthy living.

[3] Mr TIJANI acknowledged the important role the IPPC has played in protecting the world's plants from pests, thereby promoting food security, protection of the environment and facilitating trade. He further highlighted the heightened risks and devastating effects of migrating plant pests, associated with increased travel and trade. He also expressed appreciation for the work done by the IPPC Secretariat through its various activities, including, amongst others, the further development of an electronic phytosanitary certification system and the restructuring of its internal organizational structure.

1.2 Statement by the Minister for Agriculture and Rural Development of Mexico

[4] The Minister for Agriculture and Rural Development of Mexico, His Excellency Victor Manuel VILLALOBOS ARÁMBULA, delivered a statement to the CPM through a video address. The Minister highlighted the important role played by the CPM in bringing national plant health authorities together to discuss and establish phytosanitary norms that allow for the safe trade of plant products among members of the World Trade Organization (WTO). The Minister further highlighted some of the key themes on which CPM decisions have a direct impact, including the ending poverty, zero hunger, responsible production and consumption, safeguarding of the terrestrial ecosystems and climate action.

[5] The Minister expressed appreciation for the increasing importance given globally over the past years to phytosanitary issues, which culminated in the proclamation in December 2018 by the UNGA of 2020 as the International Year of Plant Health. The Minister noted that the challenge was for all governments who are party to the International Plant Protection Convention (IPPC) to give phytosanitary issues the same strategic value as they give to the Codex Alimentarius and to the World Organization for Animal Health (OIE).

2. Keynote Address on Plant Health and Capacity Development by the European Commission's Director-General for Health and Food Safety (DG SANTE)

[6] The keynote address on plant Health and Capacity Development was delivered by Ms Anne BUCHER, Director-General for Health and Food Safety of the European Commission. The address focused on the importance of healthy plants as a central element of the food chain, which provides over 80% of the food we eat, which help maintain the atmosphere and secure sustainable water supplies, and are the basis of many consumer products and habitats for animals. The address stressed the need to prevent and control the introduction and spread of pests through the required investment in resources and skills. Ms Bucher emphasized that institutional capacity and its development were the cornerstones for the proper functioning of plant health at national, regional and global levels, and that only with robust, institutional capacity could plant health policies have a positive impact on food security, trade facilitation and environment protection.

[7] Ms BUCHER detailed initiatives and programmes undertaken by the European Union (EU) within the framework of plant health, including new plant health laws which would become applicable at the end of 2019, support provided to capacity development projects, including co-funding of the Standards and Trade Development Facility (STDF) and FAO's "Framework for partnership for the sustainable management of the Fall Armyworm in Africa", as well as its financial contributions to the IPPC which have facilitated the participation in IPPC activities by plants health experts from developing countries. Ms BUCHER further called on FAO and its Governing Bodies to strengthen the funding of the IPPC from its Regular Budget.

3. Adoption of the Agenda

[8] The Chairperson informed the session of changes to the Provisional Agenda[1] with adding two items under the agenda item 20 "Any Other Business", i.e. IPPC Stakeholders Advisory Body and Presentation of IPP new structure. In addition, the title of agenda item 8.9 from "Antimicrobial Resistance (AMR)" to "Antimicrobial Resistance in relation to Plant Health aspects".

[9] The CPM:

(1) *Adopted* the Agenda with changes (Appendix 01) and *noted* the List of Documents. (Appendix 02)

3.1 European Union (EU) Statement of Competence

[10] The CPM:

(1) *Noted* the Declaration of Competences and Voting Rights submitted by the European Union (EU) and its 28 member states[2].

4. Election of the Rapporteur

[11] The CPM:

(1) *Elected* Ms Vlasta KNAPIC (Slovenia) and Ms Hellen MWAREY (Kenya) as Rapporteurs.

5. Establishment of the Credential Committee

[12] The CPM:

(1) *Appointed* a Credentials Committee composed of seven members, one per FAO region and one CPM Bureau member, in conformity with FAO rules.

(2) Credentials Committee *elected* Mr Khidir Gibril MUSA EDRES as its Chairperson (Sudan). The Credentials Committee endorsed a list of 121 valid credentials and noted the quorum for the Commission at 92 valid credentials.

6. Report by the Chairperson of the Commission on Phytosanitary Measures

[13] The Chairperson of the CPM, Mr Francisco Javier TRUJILLO-ARRIAGA, presented his report[3] on the key achievements and progress made by the CPM Bureau and IPPC Secretariat, in particular: highlighted the adoption by the United Nations General Assembly (UNGA) of a resolution endorsing the International Year of Plant Health (IYPH) in 2020; electronic phytosanitary certification (ePhyto) and acceptance by all FAO regions of the need for commodity and pathway standards.

[1] CPM 2019/01

[2] CPM 2019/CRP/05

[3] CPM 2019/25

[14] The Chairperson further called upon CPs to focus on the contributions that could be made by National Plant Protection Organizations (NPPO) to ensure that IYPH activities have the necessary visibility and support during its celebrations in 2020.

[15] The CPM:

(1) *Noted* the report.

7. Report by the IPPC Secretariat

[16] The IPPC Secretary pointed out the highest record number of participants at this CPM session, with 491 participants, which included 133 Contracting Parties and 30 and observer countries and organizations (List of Participants – Appendix 03).

[17] The IPPC Secretary presented the 2018 annual report of the IPPC Secretariat[4], highlighting ten important achievements, including:

- IPPC governance activities;
- standards setting;
- standards implementation;
- ePhyto;
- promotion of IYPH 2020;
- disseminating the 2018 annual theme;
- communications and advocacy;
- international cooperation;
- resource mobilization; and
- internal management.

[18] The IPPC Secretary further presented the key focus of the IPPC's activities for 2019, including:

- completion of activities for IYPH 2020;
- finalization of the SF 2020-2030;
- strengthening work on commodity and pathway standards;
- organization of activities for the IPPC annual theme; and
- expansion of external cooperation to mobilize resources for IYPH 2020.

[19] The CPM:

(1) *Noted* the report.

8. Governance and Strategy

8.1 Summary of the 2018 Strategic Planning Group Report (SPG)

[20] The Vice-Chairperson of the CPM Bureau, Mr Lucien KOUAME KONAN, presented the 2018 SPG report[5]. The report highlighted several areas of discussion by the SPG and issues raised during its October 2018 meeting, including:

[4] CPM 2019/36

[5] CPM 2019/34

- the IPPC Strategic Framework 2020-2030;
- IYPH 2020;
- the Focus Group on Commodity and Pathway Standards meeting held in October 2018;
- trade facilitation issues;
- the ePhyto project;
- plant health and emerging issues, including emerging pests;
- the increasing use of antimicrobial products for controlling plant pests;
- sustainable funding for the IPPC Secretariat; and
- the Five-year Investment Plan of the IPPC Secretariat.

[21] The CPM:

(1) *Noted* the report.

8.2 IPPC Strategic Framework for 2020-2030

[22] The draft IPPC Strategic Framework (SF) 2020-2030 was presented by its drafters.

[23] Some CPs welcomed the draft SF 2020-2030 and indicated that they are ready to endorse the draft presented which, once adopted at the Ministerial Session of CPM-15, would be a highlight in the IPPC's history.

[24] Some CPs raised concerns on some issues in the draft, which required consideration before their endorsement, in particular their comments proposed during the country consultation process which were not incorporated, as well as essential issues such as support for capacity development, clarity on the sustainability of actions and plans proposed in the SF, as well as editorial issues.

[25] One CP, while commending the work done on the document, indicated that there are further improvements to the draft SF 2020-2030 that needed to be addressed, including inconsistencies, editorial issues and the overall presentation of the SF.

[26] One CP indicated support for the SF 2020-2030, and, as a point of clarification, enquired whether CPM would still address new urgent issues regarding plant health not in the SF.

[27] One CP conveyed appreciation to the drafters of the SF 2020-2030 and indicated its endorsement of the SF and encouraged CPs to do the same.

[28] Several CPs raised concerns on the availability of resources for implementation of all the action plans and programmes contained in the SF, particularly for developing countries, who may not be in a position to implement the full extent of the SF. The CPs further noted that the strategic objective on capacity development had not been considered for inclusion in the SF and requested for it to be included.

[29] Considering the wide range of positions expressed by CPs regarding endorsement of the draft SF 2020-2030, one CP suggested that a working group be created to facilitate discussions in order to reach consensus.

[30] The CPM Chairperson agreed to have a Friends of the Chair (FoC) meeting. The FoC discussion took place on the margins of the CPM and resulted in a revised paper[6] (CRP) acceptable to all participants, which elaborated on, and took into account, the reservations and suggestions raised by CPs.

[6] CPM 2019/CRP/12

[31] The CPM:

(1) *Endorsed* the content of the IPPC Strategic Framework 2020-2030 in advance of the formal adoption during CPM-15 (2020), subject to adjusts as detailed in Appendix 04.

8.3 Five-year investment plan of the IPPC Secretariat

[32] The drafter presented the five-year investment plan[7] as a complement to the SF 2020-2030, noting that it should be read in conjunction with the Strategic Framework. The drafter also noted that the framework sets targets, and is not a roadmap, which should be reviewed annually. CPs were also invited to promote the investment plan to potential donors.

[33] Some contracting parties thought it would be useful to provide more detail on the various funding streams used by the IPPC so as to enhance the value of the document to potential donors.

[34] One CP welcomed the annual review of the plan, and suggested the inclusion of updates on funding availability.

[35] Other CPs observed that many of the regular activities undertaken under the strategic objectives will contribute to the implementation of activities under the Development Agenda and proposed that a detailed and costed operational plan be developed for the implementation of the entire Strategic Framework 2020-2030. This would consolidate all Action Plans and Operational Plans for the entire Strategic Framework 2020-2030 and allow the CPM to see the greater picture and would help avoid duplications, overlaps, contradictions etc.

[36] The CPM:

(1) Noted the investment plan.

8.4 Focus Group on Commodity and Pathway Standards

[37] A CPM Bureau member presented a report[8] on the work done by the Focus Group on Commodity and Pathway Standards, in particular the key challenges, decisions, and achievements.

[38] The conference room paper[9] related to this topic was introduced. Some CPs expressed their concerns regarding the principles related to the development and implementation of commodity and pathway standards. Others CPs expressed a concern that it was premature to develop specific draft standards as impacts first needed to be understood. Some were not in full agreement on the strategic values and purpose of commodity and pathway standards, as identified by the Focus Group. Moreover, some CPs felt that it had not been demonstrated that commodity or pathway standards, with harmonized measures, will facilitate trade. Some CPs further indicated disagreement with the proposal to establish a new Technical Panel for Phytosanitary Measures (TPPM) due to its potential burden on resources.

[39] One CP noted that there was still work to be done in developing cross-cutting standards. Regarding resources required for developing commodity standards, the CP indicated that it was necessary to prioritize available resources, and further that the same procedures and steps for developing ISPMs should be

[7] CPM 2019/30

[8] CPM 2019/27

[9] CPM 2019/CRP/07

followed. The CP further suggested that a revision of the Focus Group's Terms of Reference is required in order to extend its mandate for a second meeting.

[40] Some CPs conveyed appreciation for the work and progress made by the Focus Group, supporting several of the points in the CPM paper and reiterating that they were long-term promoters and supporters of commodity and pathway specific standards. In a show of support, the EU and its Member States indicated that they would support the activities related to the development of commodity and pathway standards with financial contributions of € 300,000 in the 2019-2021 period, in a co-funding arrangement, and invited other donors to also pledge support.

[41] One CP proposed that the decisions be revised to accommodate the concerns expressed by some CPs and further offered to host the second meeting of the Focus Group.

[42] One CP expressed its appreciation for the work of Focus Group and indicated that the promotion of the development of commodity and pathways standards could have a positive effect on the development of trade. They indicated their support for the basic principles, structure and content of commodity and pathways standards and indicated that the content of the commodity and pathways standards was complex as it related to trade. They further expressed support for the proposed work plan tabled by the Focus Group and recommended the establishment of the Technical Panel for Phytosanitary Measures (TPPM) as soon as possible, to which they were willing to send experts.

[43] One CP indicated that the development of this standard was an essential component of the IPPC's framework of standards and was a significant factor in the facilitation of safe trade of plant products. The CP further indicated that these standards would assist all CPs, including developing countries, by providing options for phytosanitary measures that could lead to new market access while protecting plant resources, and retaining existing sovereign rights.

[44] One CP considered that it was important that clear guidelines be provided for the implementation of the standards by the countries.

[45] Following a side session held during the CPM-14 meeting on "Commodity and pathways standards: Focus Group questions and answers", a revised set of decisions was proposed to CPM. It was further clarified that, due to existing logistical arrangements, this year's meeting of the Focus Group would need to be held at FAO headquarters.

[46] The CPM:

(1) *Noted* that work in this area had been identified as a development goal in the Strategic Framework and that the strategic value and purpose of commodity standards included:

- facilitation of safe trade;
- harmonization of measures;
- optimisation of efficiency of resource usage;
- support and assistance to developing countries, and;
- maintaining the relevance and influence of the IPPC.

(2) *Noted* that the development, adoption and implementation of commodity standards will not alter the sovereign rights and fundamental obligations under the IPPC and WTO-SPS Agreement, including that:

- the regulation of pests will remain firmly based on pest risk analysis and subject to technical justification;

- obligations will not be imposed on importing countries.

(3) *Agreed* that the proposed structure of these standards will apply equally to commodities and pathways.

(4) *Supported* the development of guidance on the process for the development of commodity standards and a template for commodity standards as a concept standard.

(5) *Supported* the development of specific governance processes by the Focus Group, which should consider options including:

- the establishment of a new Technical Panel;
- the Technical Panel will conduct its work under the mandate of the Standards Committee;
- the use of a permanent steward for the technical panel on commodity standards;
- a review of funding options to facilitate the development of commodity standards
- transition arrangements that might be assisted by the Focus Group as an advisory group.

(6) *Supported* the review of the IPPC Standards and Implementation Framework for inclusion of commodity standards.

(7) *Agreed* that commodity standards under development should remain "pending" until the new commodity standards approach has been adopted.

(8) *Noted* the conditions under which commodity standards would not be suitable.

(9) *Agreed* to a second meeting of the Focus Group on Commodity and Pathways Standards in 2019 to advance the aspects relating to governance and to draft an overarching concept standard, considering the discussions taken by the CPM, and prepare final proposals for adoption at CPM-15 in 2020.

(10) *Requested* the Bureau to review the Terms of Reference of the Focus Group, and amend as necessary.

8.5 Facilitating safe trade for plants and plant products – Action Plan

[47] A member of the CPM Bureau, Ms Lois RANSOM, presented the paper[10].

[48] Some CPs indicated that certain topics in the Action Plan still required adoption by CPM, such as commodity and pathways standards, while others were not mandatory, such as ePhyto, thereby creating concerns for the adoption of the Action Plan. The Secretariat was requested to provide further details on the agreement and joint work-plan between the IPPC and WCO in respect of their collaboration parameters and the implementation plans related to the Action Plan and CPs. It was further indicated that the Phytosanitary Capacity Evaluation (PCE) referred to in the Action Plan had not been undertaken by several of the Developing and Least Developed Countries (LDCs) due to the lack of financial capacity. It was suggested that further discussions and clarification was needed on the Action Plan framework.

[49] One CP suggested that the methods for monitoring of the Action Plan, and by who, should be clarified. The CP further suggested that the text referring to the PCE should be removed, as application of the PCE is discretionary and should therefore not be an element of the Action Plan.

[50] Some CPs welcomed the progress made in developing the Action Plan and suggested that it be incorporated and presented as a consolidated action plan within the SF 2020-2030.

[51] One CP enquired what mechanism would be put in place to monitor the Action Plan's progress.

[10] CPM 2019/33

[52] One CP indicated that there were some topics within the Action Plan that overlapped with other implementation plans and supported the suggestion that it be incorporated into the SF 2020-2030.

[53] One CP expressed its support for a closer working relationship between the WCO and the IPPC and suggested that the SF 2020-2030 be endorsed prior to the adoption of the Action Plan.

[54] The Secretary of the IPPC Secretariat informed the CPM that currently approximately 55% of standards are technical in nature while the rest are related to trade, prompting a need to give greater focus to trade facilitation matters within the IPPC community.

[55] Several CPs indicated that the lack of sufficient funding and capacity to implement the Action Plan needed to be addressed.

[56] One International Organization indicated it would support the IPPC's "Facilitating Safe Trade 2019-2021 Action Plan". It was further indicated that a number of the WCO-IPPC joint activities/initiatives were already listed in the Action Plan, such as the Cooperation Agreement, the Joint Work Plan, ongoing cooperation on E-commerce, ePhyto, Sea Containers. In regards to capacity building activities, the organization elaborated on the WCO Mercator Programme, which was a strategic initiative aimed at assisting governments worldwide in implementing the Trade Facilitation Agreement in a uniform manner.

[57] The CPM Bureau member noted the comments, in particular with regard to the importance of facilitating safe trade as a strategic outcome of the IPPC and welcomed the visibility of trade facilitating activities being progressed. Several CPs noted the need for collaboration with other border agencies, including the WCO, and ongoing capacity development to enable all countries to participate in safe trade. Having noted that several actions in the plan are included in the IPPC SF 2020-2030, it was proposed that the document form part of an integrated implementation plan and funding strategy that would direct the actions needed, and the funds required, to achieve the objectives set out in the Strategic Framework.

[58] The CPM:

(1) *Discussed* the action plan and *agreed* not to adopt it, noting that many of the elements of the plan are in the Strategic Framework 2020-2030 and implementation actions will be addressed through other CPM agenda items or in a consolidated implementation plan after the framework is adopted in 2020.

[59]

8.6 Five year Strategic Plan for ePhyto: Transitioning from project to "business-as-usual" operation

[60] The Secretariat presented the paper[11], reiterating that the ePhyto system was not a mandatory tool to be used by CPs, but rather an additional instrument for exchanging phytosanitary certificates.

[61] One CP supported the five year Strategic Plan, indicating that it was an important step towards assisting CPs with their trade facilitation efforts and would contribute directly and indirectly to cost savings for NPPOs. It was further emphasized that integration with the "single window platform" was critical and progress towards that goal should be intensified. It was also suggested that the GeNs platform be re-branded and promoted, not only as a tool developed for developing countries, but also designed to satisfy the needs of all NPPOs.

[62] Several CPs expressed their appreciation for the ePhyto certification system.

[11] CPM 2019/35

[63] One CP noted ePhyto as an alternative to the paper-based system currently employed and encouraged other developing countries to pursue establishing the system in their country to ensure they would not "be left behind".

[64] Some CPs stressed the importance of a clearly structured and robust governance for the ePhyto solution in a form of e.g. a governing board to ensure a reliable and uninterrupted operation of the system. It was suggested to involve donor's representative(s) in the governing board. They further suggested the future ePhyto unit and IFU complement their capacity building related activities focusing respectively on ePhyto IT aspects, and support to strengthening national phytosanitary certification systems. They further requested assurances that the system would be monitored around the clock to ensure assistance is always available to parties. The EU and its Member States further indicated that EUR 350,000 would be allocated to the project, in a co-funding arrangement, for the period 2019-2021, and EUR 200,000 annually for the 2022-2027 period. The CPs further suggested, that possible agreements between the Secretariat and Capacity Development actors be made available to the CPM.

[65] One CP indicated full support for the ePhyto project, having hosted two meetings in this regard and suggested that material be developed to guide countries in the preparation for, and implementation of the ePhyto Hub, and for there to be a sharing of experiences by countries in an effort to accelerate participation.

[66] One CP indicated that there was a need to ensure security of the system. It was further indicated that financial assistance and capacity development was required for the developing countries to implement this system and called on donors to assist where possible in this regard.

[67] One CP encouraged continued promotion and raising of awareness of the platform, to continue strengthening the cooperation of the IPPC Secretariat and WCO with the goal of realizing the "single window solution" and further requested the revision of ISPM 12 in this regard.

[68] One CP indicated that there was insufficient knowledge among decision-makers about the system and encouraged expanding awareness. It was further indicated that capacity building initiatives and financial resources to implement the system in developing countries was required.

[69] In response to a question raised during the discussions, one CP indicated that they were currently using ePhyto and paper certificates concurrently. It was also suggested that the time-line provided in the CPM paper for the ePhyto project to be self-sufficient could be shortened, and further suggested that the ePhyto Steering Group and the Finance Committee of the Bureau urgently propose a sustainable cost recovery model for adoption by CPM-15 (2020). These elements and others are further elaborated on in the paper provided to CPM[12].

[70] One CP expressed support for the international implementation of ePhyto. The CP further welcomed the political support by the G20 Ministers for Agriculture at their meeting in 2018 in Argentina, which concluded with a statement of support released in respect of the ePhyto project. The G20 members were further encouraged to implement this platform over the next two years, together with other global and regional groups.

[71] Some CPs expressed their appreciation to donors of the ePhyto project, including the EU, New Zealand, Republic of Korea and the United States of America, for their financial support towards the establishment of the ePhyto system and capacity development in this regard, and further called upon other donor members to participate in providing financial or other resources to assist the developing countries.

[72] One CP expressed concern about the sustainability of the system and proposed that contributions should be made according to each phytosanitary certificate. The CP further requested how the technological gap between the developed and least developed countries could be reduced, including in relation to

[12] CPM 2019/CRP/02

infrastructural capacity and IT systems. It was further proposed that an evaluation of the pilot ePhyto projects be made and for CPM to be provided with a report.

[73] One organization indicated that they were fully supportive of the "single window solution" with the goal of including all border agencies/authorities to be incorporated therein.

[74] In response to the question on the governance structure, the Secretariat indicated that a dedicated unit within the IPPC Secretariat would be created to manage ePhyto, and also confirmed that through the UNICC they had assurances that the system would be monitored around the clock.

[75] The Republic of Korea indicated that they would be contributing USD 50,000 to the ePhyto team.

[76] The CPM:

(1) *Approved* the implementation plan.

(2) *Agreed* to promote the use of the IPPC ePhyto Solution as an additional option for exchanging phytosanitary data between Contracting Parties to facilitate and enhance safe trade.

8.7 IPPC e-Commerce proposed project work plan and budget

[77] A member of the CPM Bureau presented the paper[13] jointly with the IPPC Secretariat outlining the history and collaborations related to e-Commerce, and in particular the Secretariat's continued joint activities with the WCO in relation to e-Commerce trade in plants and plant related products.

[78] Some CPs welcomed the proposal and suggested that the Secretariat should retain basic involvement in the e-Commerce related activities and meetings, such as those with the WCO. They further suggested that the two topics for ISPMs that are relevant for e-Commerce be added to the normal standard setting programme. They further requested that the IPPC Secretariat prepare a consolidated costed work programme and/or action plan for all activities related to the new Strategic Framework 2020-2030, so that the CPM can consider the work plan of the Secretariat in its entirety.

[79] An International Organization welcomed the support for the continued cooperation with the Secretariat on e-Commerce, and indicated that the WCO Framework of Standards on Cross-Border e-Commerce provided for close collaboration between Customs and relevant government agencies. They encouraged greater involvement of, and collaboration with, the IPPC community and NPPOs in the implementation of the Framework at national, regional and international levels, and that this would also include collaboration related to capacity building activities.

[80] One CP indicated that the increase of e-Commerce was raising significant phytosanitary concern and supported the IPPC's involvement in this topic. They acknowledged the challenge for sufficient resources to support action in this area and suggested a three pronged approach: continued interaction of the Secretariat with the WCO; dissemination by the Secretariat of relevant information, such as good practices, outreach material and other advisory information; and suggested that the SPG consider the funding requirements and its readiness to discuss the strategic merits to commit resources to support the Secretariat's work in this regard. The CP also pledged to sharing information on e-Commerce.

[81] One CP supported the Secretariat's involvement in e-Commerce and indicated that the threat of introduction of pests and invasive pest species including weeds was a real threat with the global increase in e-Commerce on plant and plant related products. The CP also recommended strengthening regulations and controls to prevent the introduction of such pests through internet trade.

[13] CPM 2019/16 and CPM 2019/INF/01

[82] One CP indicated that it was important to raise awareness with all IPPC stakeholders and considering the risks associated with increased internet trade in plant and plant products and requested partners to support e-Commerce related activities.

[83] Canada has committed support to the Secretariat in the form of an in-kind contribution of a staff member and related travel costs, and called on other CPs to also make resource contributions, reiterating that there was a significant benefit for all IPPC CPs to benefit from the IPPC's involvement on this topic.

[84] The CPM:

(1) *Reviewed* and discussed the draft project work plan and budget on e-Commerce, noting that the two related topics submitted in the 2018 Call for Topics will go through the normal Standard Setting process.

(2) *Noted* that activities to identify and address risks associated with e-Commerce are continuing through the WCO and in a number of countries with minimal involvement of the IPPC community and this is preventing the development of global solutions for managing phytosanitary risks.

(3) *Endorsed* the work plan and budget.

(4) *Co*nsidered to provide extra-budgetary resources to implement the draft project work plan.

(5) *Noted* that the IPPC Secretariat will not continue work on e-Commerce until it was fully resourced, with the exception of liaison with World Customs Organization.

(6) *Provided* the IPPC Secretariat updates on related e-Commerce actions in their region.

8.8 Concept of emerging pests and emergency issues

[85] A Member of the CPM Bureau, Mr Greg WOLFF, introduced the paper[14], clarifying that it was envisaged that additional information, comments and suggestions were expected to further develop the concept. It was further emphasized that there was awareness that for any decision taken to further develop this concept, a reallocation of resources would be required.

[86] One CP introduced the Summary Report on the International Conference on Brown Marmorated Stink Bug[15] and expressed willingness to share experience and expertise on managing emerging pests.

[87] Some CPs thanked FAO and donors for their support in managing Fall Armyworm (FAW). They called for a more synchronized response from FAO on emergency plans in the event of a pest outbreak, such as the FAW, and further called for a preventive approach.

[88] Several CPs expressed their appreciation and support for the topic and called for the creation of a dedicated trust fund that would support dealing with emerging pests and emergency issues. The CPs further encouraged FAO and the IPPC Secretariat to have a holistic rather than a country by country approach to deal with emerging pest issues.

[89] Some CPs encouraged the IPPC Secretariat to work closely with the relevant FAO departments. They further encouraged development of a framework, which would include researchers and policy makers, amongst others, to support CPs when dealing with emerging pest issues.

[90] One CP suggested that a mechanism be put in place to collect and disseminate information, which would enable the IPPC Secretariat to share information on emerging pests quickly, such as an alert system.

[14] CPM 2019/15

[15] CPM 2019/INF/20

[91] Some CPs called on FAO, and other partners, to strengthen CPs' phytosanitary systems, infrastructural facilities and technical support systems as this would assist CPs to reduce the cost of managing pest outbreaks.

[92] One RPPO indicated that effectively managing emerging risks is vital to maintain plant health, improve food security and preserve international plant trade and that without timely identification and understanding of emerging risks throughout the world, NPPOs would not be able to appropriately react, prepare and respond thereto. The experience of emergency intervention through commandos and preparation through simulations, in particular regarding Fusarium TR4 was reported as successful.

[93] A CP suggested that in order to establish IPPC systems and processes in relation to emerging pests, CPM should request the Bureau and/or SPG, with assistance from the IPPC Secretariat, to develop a draft action plan with time lines to achieve the Strategic Framework 2020-2030 objective of strengthening pest outbreak alert and response systems, for consideration at CPM-15 (2020). They further suggested that the CPM consider using workshops, with the participation of FAO, to facilitate global sharing of information on new and emerging pests with a standing agenda items on surveillance, diagnostics, field control, phytosanitary management, pest risk analysis, biological information, international cooperation and emergency response,

[94] Some CPs expressed concern regarding the time limitations for discussions in the CPM for a sufficiently broad and in-depth discussion on this important matter and suggested that the issue be discussed at a special meeting, such as the International Plant Health Conference, which is to be organized on the occasion of the celebration of IYPH 2020, taking into consideration the high-level participation expected at the conference. In support to the related activities of the IPPC Secretariat to deal with this issue, the EU and its Members States indicated they were prepared to provide financial contribution of EUR 300,000 in 2019-2021, in a co-funding arrangement, and called upon other CPs to contribute funds.

[95] In response to several interventions regarding the FAW, the FAO Plant Protection and Protection Division (AGP) was invited to share their experience. They indicated that emerging pests and emergency situation of plant health are increasingly important global issues and AGP, in collaboration with the IPPC Secretariat, other divisions in FAO, and RPPOs, are tackling some emerging pests and providing support to member countries on dealing with them - taking FAW as an example. Three teams in AGP are involved in pest management and activities consist of:

- Providing policy and guidance on sustainable management of emerging pests. For example, Sustainable Management of Emerging pests the FAW in Africa - FAO Programme for Action was implemented in October 2017.
- Facilitating development of projects to support urgent responses and emerging action in member countries. Forty-one (41) TCPs (technical co-operation program) have been developed and implemented.
- Providing various technical supports on monitoring and early warning at global, regional even national levels; developing agroecology-based IPM approaches; promoting biological control; transferring information and technology to extension service agencies and farmers through Farm Field School (FFS) approaches; and reducing risks of pesticides used for FAW etc.
- Monitoring overall outbreak and spread of the pests/FAW and provide updates including activities being done in regions and nations and sharing information through FAO website of FAW.

[96] The representatives from AGP expressed their willingness to collaborate with the IPPC Secretariat, FAO regional plant protection officers, CPs and other relevant partners to deal with this issue, as global approach is the best way.

[97] The IPPC Secretariat reminded CPs of their National Reporting Obligations, as contained in the IPPC, and encouraged CPs to report pest outbreaks in order to provide information for early warning and response.

The IPPC Secretariat also clarified that its mandate is to deal with prevention, and not with management actions of pests that are widespread.

[98] The CPM:

1) *Requested* the IPPC Secretariat to continue to engage with the division(s) of the FAO responsible for emergency situations and emerging pests to clarify what type and level of support is presently available for contracting parties.
2) *Confirmed* that updates on emerging pest situations be added to the CPM agenda as a standing item.
3) *Clarified*, however, that reports submitted and statements made as part of this standing CPM agenda item should:
   - be oriented towards pest outbreaks that are regional in nature or have the potential to have regional impacts;
   - identify the nature of the damage caused or expected, plant resources at risk, the endangered area(s) and other relevant potential plant health, environmental or economic consequences;
   - describe what measures if any, have been taken and what the results of these efforts have been;
   - indicate, if known, what role the FAO and any other international organizations are playing, or are planning, in relation to the outbreak;
   - clarify precisely what role the FAO, IPPC Secretariat or RPPOs could play in helping contracting parties respond to the outbreak.
4) *Noted* that contracting parties may donate targeted extra-budgetary funds through the multi-donor trust fund to support Secretariat activities identified through this standing agenda item.
5) *Called* on the IPPC Secretariat to establish an emergency trust fund to support addressing issues related to emerging pests and emergency issues.
6) *Requested* the CPM Bureau to draft an action plan on an IPPC emergency system to be submitted to the SPG for discussion and then presented to CPM-15 (2020)
7) *Requested* the Plant Health Conference to be held in 2020 in Finland to have an in-depth discussion on emerging pests and emergency situations.

8.9 Antimicrobial resistance (AMR) in relation to plant health aspects

[99] The CPM Chairperson highlighted key issues in the paper[16], and noted that it was a new topic for the IPPC. Representatives of FAO's Agriculture and Consumer Protection Department and Plant protection Division provided background on FAO's Antimicrobial programmes and the challenges faced with Antimicrobial Resistance (AMR) in human and animal health, highlighting that although Anti-Microbial products are not banned from use, they should be used prudently.

[100] CPs welcomed the discussions and applauded the Secretariat for initiating discussions in this regard, as the effects of the use of antimicrobial products for plants health, and its effects related to AMR had not been given the same focus as with its effects on human and animal health.

[101] Several CPs indicated that antimicrobial resistance has been a serious issue in human health and required careful attention in the area of animals and animal products, and noted that the use of antibiotics in plant protection is rare, partially due to cost but also because alternatives, including phytosanitary measures, are readily available.

[16] CPM 2019/INF/12

[102] Some CPs indicated that it was important to take decisions based on sound science and an assessment of risk and suggested that before the CPM or the Secretariat commit resources to this subject, credible and clearly assessed technical evidence should be produced to support the concern, which was consistent with the conclusions in the paper that the extent to which antimicrobial use on plants contributes to antimicrobial resistance was unclear.

[103] One CP advised that there was an understanding that AMR and pesticide resistance could lead to reduced plant yields and economic losses to the producer and that factors such as ineffective use of chemicals treatments, ineffective microbial treatment, coupled with other factors, such as climate change, were all contributing to the rise in AMR in plants. The CP suggested greater focus in the FAO task force on the impact study in plants.

[104] Several CPs indicated that in their countries, AMR was focused on its effects on animal and human health and that there was a challenge to include plant health in the discussion. They noted the One Health initiative and suggested that plant health should be integrated into it.

[105] Some CPs indicated that the Secretariat should collaborate with the leading role-players on this topic, including the World Organization for animal health (OIE) and the World Health Organization (WHO), and supported the call made by other CPs for the IPPC Community to monitor the effects of antimicrobial on plant health, and to collect more information in this regard.

[106] Some CPs agreed that prudent use of antimicrobials is essential to limiting the emergence and spread of antibiotic-resistant bacteria in humans, animals and plants. Since almost two decades, they carry out a regular monitoring of their efforts to limit the use of antimicrobial substances. They have noted that over these years there is a growing body of alternatives that can help further reduce the use of antimicrobials and phase out antibiotics. The CPs further suggested that a CPM Recommendation on AMR be developed in relation to plant health.

[107] In reply to a question from the FAO representatives, on use of antimicrobials in rice, one CP indicated that they had negative impacts, in the 1980s, not triggering resistance of bacteria causing bacterial blight, but favoring other harmful bacteria. Another negative impact was noticed by accumulation of streptomycin, which was absorbed by ginger roots and passed on to humans that consumed it. Nowadays these practices are not approved, as they are subject to careful assessment of these risks.

[108] One CP requested that FAO conduct a systematic review of the effects of antimicrobials used it plant health on AMR risks for possible reporting to the next meeting of the SPG in October 2019.

[109] One CP suggested that CPs encourage greater cooperation between Ministries dealing with public health, agriculture and livestock, as medicated animal feed, in their experience, had an effect on the animals and human health.

[110] The Secretariat pointed out the ongoing collaboration with the FAO Task Force on AMR on the plant health related AMR issues. It was also highlighted that IPPC involvement in AMR should be limited to the scope of the Convention that is supporting the prevention of the spread of the plant pests through developed International Standards for Phytosanitary Measures (ISPMs) and with regards to CPM priorities and Secretariat resources. IPPC should not be involved in the issues related the use of pesticide or antibiotics in pest management actions and their residues, as these issue fall under the scope of other international instruments, i.e. Codex Alimentarius Commission and the International Code of Conduct on Pesticide Management.

[111] The CPM:

1) *Noted* and conveyed appreciation for the discussion on the use of Antimicrobials and Antimicrobial Resistance in respect of plant health as an important topic to monitor.
2) *Supported* the IPPC Secretariat maintaining a watching brief on the contribution of plant health actions on Antimicrobial Resistance (AMR), through the FAO task force, subject to CPM and Secretariat priorities and resources.

8.10 CPM recommendations

CPM recommendation: High-throughput sequencing (HTS) technologies as a diagnostic tool for phytosanitary purposes

[112] The Secretariat introduced the papers regarding "High-throughput sequencing (HTS) technologies as a diagnostic tool for phytosanitary purposes"[17] as presented in CPM 2019/10_01, with the proposed adjustments presented in CPM 2019/CRP/03.

[113] The secretariat noted that in May 2018 the draft text was submitted to the consultation period via the Online Commenting System (OCS). The CPM Bureau then discussed the comments in its October meeting and among others noted that the title be adjusted to reflect a more accurate terminology from the original title "Next generation sequencing technologies as a diagnostic tool for phytosanitary purposes".

[114] The CPM:

1) *Adopted* the modified CPM Recommendation on "High-throughput sequencing (HTS) technologies as a diagnostic tool for phytosanitary purposes" (Appendix 10).

CPM recommendations - Safe provision of food and other aid to prevent the introduction of plant pests during an emergency situation (2018-026)

[115] One CP presented the paper[18] on a topic proposition for CPM recommendation "Safe provision of food and other aid to prevent the introduction of plant pests during an emergency situation (2018-026)".

[116] The Pacific Plant Protection Organization (PPPO) submitted a proposal for a concept standard on "safe import of food and other aid" in the 2018 Call for Topics: Standards and Implementation. The TFT however recommended that this guidance might appropriately take the form of a CPM Recommendation and that it be developed as a high priority for adoption at the CPM-15 Ministerial Conference in 2020.

[117] Some CPs supported the proposal for the issue to be brought for country consultations, and added some comments and suggestions for amendment in the text, including: adding food aid agencies to the list of addressees; it corresponds to the CP to distinguish between genuine emergencies that require urgent intervention and the less urgent emergencies which would allow for better planning and following of protocols; and lastly to include in the text "diversion from intended use", and that these modifications be considered prior to holding the envisaged country consultation.

[118] One CP indicated support in line with the normal procedure for adopting CPM Recommendations, adding that this was an important topic that could be highlighted during the CPM-15 (2020) Ministerial meeting.

[119] Some CPs indicated that phytosanitary safeguards, when either sending or receiving food aid, was critical.

[17] CPM 2019/10, CPM 2019/10_01, and CPM 2019/CRP/03

[18] CPM 2019/29

[120] One CP indicated that careful consideration be given to the measures and treatment options, which should be extended to the potential risk to human and animal health.

[121] The Secretariat indicated that it would assist the authors of the proposal before it goes for consultation and requested that comments in this regard be sent to the Secretariat before 30 April 2019.

[122] The CPM:

(1) *Noted* the background to this recommendation;

(2) *Agreed* to the inclusion of this topic - "Safe provision of food and other aid to prevent the introduction of plant pests during an emergency situation (2018-026)" - in the CPM Work Programme;

(3) *Considered* the need for a CPM recommendation to encourage contracting parties to prepare for managing the phytosanitary risks associated with the export and import of food and other aid during an emergency situation, in order to reduce the introduction of plant pests in these circumstances;

(4) *Agreed* that a draft CPM recommendation is circulated for country consultation between 1 July and 30 September 2019 using the OCS, with a view to presenting a final version for adoption at CPM-15 (2020);

(5) *Noted* that the CPM-15 (2020), Ministerial Conference, provides a potentially useful forum for raising awareness of the importance of managing pests on food and other aid including non-plant products such as packaging, equipment, machinery and vehicles;

(6) *Requested* that contracting parties provide additional information in their country comments that may assist others to manage phytosanitary risks associated with food and other aid, by:

- identifying frequently accessed goods and materials provided as humanitarian aid, from their experience as aid recipients in the last five years and adding these to the lists in Appendix 1 and Appendix 2;
- identifying, from their experience, phytosanitary risks associated with the import of these goods
- adding risk management options to manage these risks including widely available commercial manufacturing processes, which are recognized as effective in addressing phytosanitary risk;
- sharing information and experiences on the risks and their management with food and other aid at 2019 Regional Workshops, and providing this through their OCS response.

CPM recommendations - Facilitating safe trade by reducing the incidence of contaminating pests associated with traded goods

[123] One CP presented a paper[19] on another topic proposition for CPM recommendation "Facilitating safe trade by reducing the incidence of contaminating pests associated with traded goods (2019-002)".

[124] This recommendation was conceptualized after realization that the awareness of the scope of the Convention and the risks that pests associated with storage places, packaging, conveyances, containers, soil and any other organism, object or material capable of harboring or spreading plant pests pose to global plant health remains low. A CPM recommendation would therefore help to raise the profile of these risks and provide a stronger focus for addressing them.

[125] One CP indicated that they supported the proposals as contained in the papers but expressed reservations on the establishment of a small working group, as suggested in the papers, and further enquired about the availability of financial resources for this working group as well as its way forward.

[19] CPM 2019/37

[126] In response to the concern raised, one CP indicated that they would lead the small working group, which would elaborate on this issue and welcomed countries that wished to participate. Regarding the methodology of work, the CP indicated that should CPM support the project and the number of countries participating in the small working group was identified, the working methods would be developed.

[127] One CP indicated that the phytosanitary measures taken by an importing country should have a scientific basis and that sufficient time for consultations be permitted. In this regard should have a scientific basis and further indicated that they were interested in participating in the small working group.

[128] The CPM:

1) *Noted* the background to this recommendation.
2) *Agreed* to the inclusion of this topic - "Facilitating safe trade by reducing the incidence of contaminating pests associated with traded goods (2019-002)" - in the CPM Work Programme.
3) *Considered* the need for a CPM recommendation to encourage contracting parties to:
   a) raise awareness with governments and industries of the risks and impacts of significant pests moving internationally as contaminating pests on unregulated goods and conveyances.
   b) promote the benefits of preventing traded goods, and the conveyances, containers and others that carry them within and between countries, from being contaminated with phytosanitary risk materials such as soil, plant material and invertebrates, in terms of facilitating safer trade.
   c) collaborate with their exporting industries to develop commercial solutions that reduce the risk of contaminating pests moving in trade.
   d) act to gain the necessary legislative powers to regulate export pathways for the purpose of minimizing the spread of contaminating pests on traded goods, conveyances, containers and other non-plant regulated articles.
   e) negotiate agreed actions with importing countries that reduce exposure of plants and plant products to contaminating pests on trading pathways and through the movement of conveyances.
4) *Agreed* to establish a small working group of interested contracting parties to develop the recommendation further for consideration by the Bureau and Strategic Planning Group (SPG) in 2019, before it is presented to CPM-15 (2020) as a draft for country consultation.
5) *Requested* that the working group liaise with the Sea Container Task Force, International Year of Plant Health (IYPH) Steering Committee, Standards Committee and the Implementation and Capacity Development Committee to identify how the concept of managing phytosanitary risks in exports before they leave the exporting country can be integrated into their respective activities, and provide advice to the working group on existing standards and guidance that should be taken into account in further developing the recommendation.

9. Cooperation of Standards and Implementation

9.1 Task Force on Topics and 2018 Call for Topics: Standards and Implementation

[129] The Secretariat presented three papers, "TFT Recommendations to CPM for Submissions for 2018: Call for Topics: Standards and Implementation"[20], "Impacts and Benefits of the New Procedure for Call for Topics"[21], and List of Implementation and Capacity Development topics (paper 24).

[20] CPM 2019/22

[21] CPM 2019/23

A. TFT Recommendations to CPM for Submissions for 2018 Call for Topics: Standards and Implementation.

[130] Some CPs noted that recommended topics met the criteria for justification and prioritization and contributed to the objectives of the IPPC, and further indicated that two topics suggested for e-Commerce (2018-014 and 2018-021) were standard topics in their own right and should be added to the list of topics for Standards. They also indicated that the Standards Committee (SC) and Implementation and Capacity Development Committee (IC) had to reassess their respective lists of topics, review priorities and present the final lists for CPM endorsement.

[131] One CP noted that the reciprocal participation of SC and IC members in their respective committees had significantly improved the communication between the two committees.

[132] One CP indicated that topics not recommended should not be considered by the Bureau and CPM should not take any action on them.

[133] One CP indicated that risk-based inspection could be an effective and feasible tool beneficial to all NPPOs to use their resources efficiently without increasing phytosanitary risk, highlighting that it was not a 'national issue' as stated by the SC. The CP further emphasized that the risk-based approach was included in the SF 2020-2030 and certain Free Trade Agreements between countries. The CP requested CPM to put "pending status" on this topic and recommended that additional information be collected, including the North American Plant Protection Organization (NAPPO) manual currently under development, and requested the TFT to reconsider this topic.

[134] The CP also noted that, considering the special characteristic of diagnostic protocols (DPs), an analysis may be needed on the practical use and value of DPs before adding more to the list of topics. In this regard, the CP requested the SC and IC to conduct surveys of the utility of existing DPs using IRSS and to analyze means of developing DPs through the CPM Bureau and SPG to identify areas of improvement to have greater amounts of DPs quicker and more flexible.

[135] One CP indicated that given the risk of e-Commerce, the topic related to the subject should be prioritized in the list of topics.

[136] The Chairperson of the CPM noted the concerns raised and suggested that a Friends of the Chair (FoC) group review the decisions to be taken by CPM in consideration of the topics raised. The FoC meeting revised the set of decisions presented to CPM for adoption.

[137] The CPM:

1) *Noted* the TFT recommendation on SC subjects outlined in Table 1 of Appendix 09.
2) *Agreed* that the two topics suggested under the e-Commerce project be added to the *List of Topics for Standards.*
3) *Adopted* recommended topics and priorities in response to Call for topics: Standards and Implementation as presented in Table 2 of Appendix 09.
4) *Requested* the SC and IC to update their respective lists of topics, reviewing priorities as needed with consideration to the TFT recommendations and integrate the adopted topics into the Framework for Standards and Implementation.
5) *Encouraged* contracting parties, Regional Plant Protection Organizations and other interested parties to consider providing support and resources for the delivery of high priority topics on the lists of topics
6) *Requested* the Bureau to provide guidance to the TFT on the scope of the call for topics and the activities that should be solicited during the call for topics.
7) *Requested* the SC and IC to review the use and development of diagnostic protocols.

B. Impacts and Benefits of the New Procedure for Call for Topics

[138] Some CPs suggested that there be a postponement of the second Call for Topics until after the IYPH 2020 activities have been completed. One CP noted that the IYPH could serve as a platform to generate new ideas for topics for the IPPC work programme.

[139] Some CPs also recommended that the reviews of processes should be postponed until after a second call for topics has been completed.

[140] The Chairperson of the CPM also requested the FoC group to review the decisions to be taken by CPM considering the proposals raised. The FoC meeting reached a consensus and a revised set of decisions was drafted and presented to CPM for adoption.

[141] The CPM:

1) *Noted* that the joint Call for Topics process has operated effectively and will be refined and streamlined for the next Call in 2021.
2) *Acknowledged* the impacts and benefits analyzed by the IPPC Secretariat of the Call for Topics: Standards and Implementation.

C. List of Implementation and Capacity Development Topics

[142] One CP indicated that the work plan developed from the meetings of the IC included legacy projects from the previous and now dissolved Capacity Development Committee (CDC). They further indicated that with the additional topics allocated through the Task Force for Topics (TFT) process, the workload of the IC was not achievable. They suggested that the IC should focus on progressing topics added through the TFT process and that legacy work should be reviewed through the TFT criteria to assess their contemporary relevance and either cease these projects or adapt them to current priorities.

[143] Some CPs acknowledged the work on the Implementation and Capacity Development (ICD) topics and requested the Bureau to consider them high priority in the IPPC Secretariat work plan as standing elements of the IPPC Secretariat work plan, and prioritize the allocation of sufficient resources from the regular funds to support their implementation. In addition, they encouraged the Secretariat and the IC to disseminate and promote existing implementation resources that have been developed so that contracting parties and development partners can benefit from them.

[144] The CPM:

(1) *Noted* the List of Implementation and Capacity Development (ICD) topics.

9.2 Surveillance pilot project analysis

[145] The Secretariat presented the paper[22], which focused on the results of the evaluation undertaken on the project conducted in 2015-2018 and on a proposal to establish an *ad hoc* Surveillance Working Group to elaborate a clear management plan for new project on surveillance, provided extra budgetary resources are made available.

[146] Several CPs expressed disappointment with the implementation of the past project, pointing out the lack of clear and structured project planning, coordination, reporting and management accountability and

[22] CPM 2019/18

effectiveness. They stressed that the ability to deliver projects successfully is positively correlated to the willingness of potential donors to contribute resources, but recognized that the lack of extra-budgetary resource contributions may have had a negative impact on its success. They further indicated that for successful implementation of future projects, that clearly defined and achievable objectives and deliverables are required.

[147] Some CPs thanked the evaluators and agreed with the proposed recommendations, noting the importance of surveillance for NPPOs.

[148] One CP indicated that this project could have suffered a lack of commitment for collaboration between the Standards Setting and Implementation Facilitation Units of the IPPC Secretariat. The CP further questioned the rationale for providing financial resources to set up an ad-hoc working group, and requested clarity before agreeing to the recommendations sought.

[149] The Secretariat indicated that a lack of financial and human resources to implement the project was a crucial factor, preventing the good management of this project, as highlighted in the evaluation and felt this had been communicated to the CPM. It was further indicated that there were valuable lessons learnt. The Secretariat further indicated that the main objective of the ad hoc surveillance working group would be to develop a clear plan to identify governance, timelines, budget, procedures, activities and responsibilities to ensure a satisfactory implementation. The proposal made would be that the ad hoc surveillance working group be under the remit of the IC and be constituted of three experts from three different regions to develop this clear management plan as well as to identify clear strategies to mobilize resources.

[150] One CP suggested that this management plan be presented to SPG.

[151] It was decided to hold a FoC meeting with the relevant CPs from which a new set of decisions was agreed upon, as contained in the CRP[23] and indicated below.

[152] The CPM:

(1) *Reviewed* the evaluation.

(2) *Considered* and agreed to the following recommendations on the development and implementation of future programme initiatives:

a. CPM activities should be costed and extra budgetary resources should be identified prior to conducting any new activity.
b. CPM should investigate options for contracting parties to directly invest in specific components of a future programme initiatives through financial or in-kind contributions.
c. any future programme initiatives should be based around clear project management principles, with goals, objectives, outcomes, deliverables defined and an adequate allocation of resources.
d. significant effort should be invested by the IPPC Secretariat into coordination, management and planning components of any future programme initiatives, with this effort included in the relevant budgets and work plans.
e. future programme initiatives should include clearly defined and achievable requirements for programme: governance (resources (staffing and finance), engagement, etc.), deliverables or outputs (individual activity, workshop, meeting reports, etc.), and reporting (milestones reports: quarterly, annually, end of programme, etc.).
f. project management tools, such as Microsoft Project, should be utilized to manage scheduling, track resources and ensure milestones are met.

[23] CPM 2019/CRP/14

g. any future programme initiatives should be designed and structured around a clearly defined project monitoring and evaluation framework.

(3) *Requested* the CPM Bureau to consider what this Surveillance Implementation Programme should achieve and what should be the role of the IPPC Secretariat, how this programme supports implementation of ISPM 6 and how it contributes to the objectives and outcomes of the draft IPPC Strategic Framework (2020-2030). If necessary, the CPM Bureau would review the draft Terms of Reference for an *ad hoc* Surveillance Working Group. This guidance, along with the revised Terms of Reference, would then be submitted to the SPG.

(4) *Requested* the SPG to review the CPM Bureau guidance and draft Terms of Reference of the *ad hoc* Surveillance Working Group, and to identify very precisely the scope and objective of the Surveillance Implementation Programme.

(5) *Requested* the IC to review the SPG recommendation on the guidance and draft Terms of Reference with appropriate input from the SC and to present it to CPM-15 (2020) for approval and request funding for the *ad hoc* Surveillance Working Group.

(6) *Agreed* that no further work on surveillance should be done by the IPPC Secretariat until appropriate resources have been allocated.

9.3 Framework for standards and implementation

[153] The Secretariat presented the Framework for Standards and Implementation[24], which has been updated and maintained by the Secretariat after adoption at CPM-11 (2016) with responsibility for review and amendment resting jointly with the Standards Committee (SC) and Implementation and Capacity Development Committee (IC) and reviewed by the Strategic Planning Group (SPG).

[154] One CP indicated that they had been supporting the development of this framework, and encouraged continued improvement of design and readability of the framework.

[155] The CPM:

(1) *Endorsed* the updated Framework for Standards and Implementation as presented in Annex 1 of the paper.

10. Standard Setting

10.1 Report of the Standards Committee

[156] The Chairperson of the Standards Committee (SC) presented the report of the SC's activities in 2018[25], highlighting the key issues discussed during the SC meetings, as detailed in the report.

[157] Some CPs acknowledged and conveyed appreciation for the significant work done by the SC and was pleased with the emerging collaboration between the Implementation and Capacity Development Committee (IC) and SC.

[158] The CPM:

(1) *Noted* the report on the activities of the Standards Committee in 2018.

[24] CPM 2019/21

[25] CPM 2019/11

10.2 Adoption of International Standards for Phytosanitary Measures[26]

[159] The Secretariat introduced the papers[27] outlining the two draft ISPMs, as well as six Diagnostic Protocols (DPs) adopted by the SC on behalf of the CPM since last session of the CPM and activities related to language review groups (LRG) process on adopted standards. The SC requested the CPM to convey appreciation to the experts of the drafting groups for their active contribution in the development of the ISPMs (Appendix 07).

[160] The CPM:

1) *Adopted* the ISPM 43: Requirements for the use of Fumigation as a phytosanitary measure (2014-004) (Appendix 10).
2) *Adopted* the 2017 amendments to ISPM 5 (*Glossary of phytosanitary terms*) (1994-001) (Appendix 10).
3) *Noted* that the SC adopted on behalf of CPM the following six diagnostic protocols (DPs) as Annexes to ISPM 27 (*Diagnostic protocols for regulated pests*) (Appendix 10):
    - DP 2 revision: *Plum pox* virus (2016-007)
    - DP 25: *Xylella fastidiosa* (2004-024)
    - DP 26: *Austropuccinia psidii* (2006-018)
    - DP 27: *Ips* spp. (2006-020)
    - DP 28: *Conotrachelus nenuphar* (2013-002)
    - DP 29: *Bactrocera dorsalis* (2006-026)
4) *Noted* that the following five ISPMs have been reviewed by the Arabic, Chinese, Russian and Spanish LRGs and FAO Translation services and the IPPC Secretariat incorporated the modifications accordingly and *revoked* previously adopted versions:

    - ISPM 5 (*Glossary of phytosanitary terms*)
    - ISPM 6 (*Surveillance*)
    - Annex 1 and Annex 2 revisions to ISPM 15 (*Regulation of wood packaging material in international trade*), for inclusion of the phytosanitary treatment sulphuryl fluoride fumigation and revision of the dielectric heating section
    - ISPM 42 (Requirements for the use of temperature treatments as a phytosanitary measures)
    - Phytosanitary treatment (PT) 32 (Vapour heat treatment for *Bactrocera dorsalis* on *Carica papaya*), as annex to ISPM 28 (*Phytosanitary treatments for regulated pests*).

[161] These reviewed ISPMs are posted on the Adopted Standards page of the IPP, replacing previous versions.

5) *Thanked* Contracting Parties and Regional Plant Protection Organizations involved in the Language Review Groups (LRGs), as well as FAO Translation Services, for their efforts and hard work to improve the language versions of ISPMs.
6) *Acknowledged* the contributions of Contracting Parties, Regional Plant Protection Organizations and organizations who hosted or helped organize standard setting meetings in 2018:

[26] IPPC adopted ISPMs page: https://www.ippc.int/en/core-activities/standards-setting/ispms/

[27] CPM 2019/03, CPM 2019/03_01 and CPM 2019/03_02

- Malta: Expert Working Group (EWG) on *Guidance on Pest Risk Management* (2014-001)
- European and Mediterranean Plant Protection Organization (EPPO): Technical Panel on Diagnostic Protocols (TPDP)
- China: Technical Panel on Phytosanitary Treatments (TPPT)
- Italy: Technical Panel for the Glossary (TPG)

7) *Acknowledged* the contributions of the members of the Standards Committee (SC) who have left the SC in 2018:
   - Egypt, Ms Shaza OMAR
   - Indonesia, Mr HERMAWAN
   - Lebanon, Mr Youssef Al MASRI
   - Mexico, Ms Ana Lilia MONTEALEGRE LARA
   - Vietnam, Ms Thanh Huong HA
   - Yemen, Mr Gamil RAMADHAN
8) *Acknowledged* the contributions of the Technical Panel on Diagnostic Protocols (TPDP) Steward who left in 2018:
   - United Kingdom, Ms Jane CHARD
9) *Acknowledged* the contributions of the Technical Panel for the Glossary (TPG) member who left in 2018:
   - NAPPO, Ms Stephanie BLOEM
10) *Acknowledged* the contributions of the Technical Panel on Phytosanitary Treatments (TPPT) member who left in 2018
   - Australia, Mr Glenn BOWMAN (*in memoriam*)

[162] The Secretariat also presented Ink Amendments to CPM as contained in the relevant paper[28].

[163] The CPM:

1) *Noted* the ink amendments to the use of "contamination" to ensure a consistent use across adopted ISPMs (Appendix 08).

2) *Noted* that the ink amendments will be translated into FAO official languages and implemented into the language versions of the concerned standards as resources permit.

3) *Agreed* that, once the Secretariat has applied the ink amendments, the previous versions of the standards are revoked and replaced by the newly noted versions.

10.3 Standards Committee recommendations to the Commission on Phytosanitary Measures

[164] The Secretariat presented the SC's recommendations[29] to the CPM outlining that some points are related to agenda item 9.1.

[165] The CPM:

(1) *Added* the following topics, with the indicated priorities, to the *List of topics for IPPC standards* (see also agenda item 9.1):

[28] CPM 2019/07

[29] CPM 2019/05

i. ISPM 38 *International movement of seeds*: Annex 1 - *Design and use of systems approaches for phytosanitary certification of seeds* (2018-009), with priority 1; and

ii. *Criteria for the determination of host status for fruit flies based on available information* (Annex to ISPM 37) (2018-011), with priority 3.

(2) *Adopted* the List of topics for IPPC standards, with the above adjustments.

(3) *Requested* the Secretariat to incorporate these changes into the *List of topics for IPPC standards* database on the IPP[30].

(4) *Disestablished* the Technical Panel on Pest Free Areas and Systems Approaches for Fruit Flies (TPFF) in light of the topics submitted during the 2018 call for topics.

(5) *Agreed* to issue a *call for* an EWG for the drafting of new fruit fly standards, if needed.

(6) *Thanked* the members of the TPFF for their contributions over the years.

10.4 Conceptual challenges in standards development in terms of implementation

[166] The Secretariat presented the paper[31] on the Conceptual challenges in standards development in terms of implementation in particular with regards to the draft ISPM on the Authorization of entities to perform phytosanitary actions (2014-002) that completed the first round of consultation in 2018.

[167] Some CPs did not support the development of the draft standard as they had concerns that phytosanitary security would be compromised if commercial entities discharged functions that were the responsibility of NPPOs, while several CPs expressed support for its development and proposed improvements to the text (see also document CRP/04).

[168] Some CPs indicated that considering the need for further work on the draft standard, it was deemed necessary to specify cases when such delegation of authority was possible and to provide an exhaustive description of the audit system implemented by the NPPO, functions of the authorized persons, their responsibility, the control mechanism and the procedure for passing a no-confidence motion against the authorized persons as at present these aspects were only outlined in the standard but not elaborated on in full.

[169] Several CPs expressed their support for the development of the ISPM and international harmonization was required, as phytosanitary actions are often already authorized under national legislation for example with regards to phytosanitary activities specified in ISPM 15 (*Regulation of wood packaging material in international trade*). They considered the standard necessary as it provided guidance to NPPO's, particularly in light of the increased volume of trade and the often-limited resources of the NPPO.

[170] One CP indicated that it had utilized authorization programmes, which resulted in the optimization of its operations, while maintaining phytosanitary integrity, and suggested that the standard would bring clarity for CPs.

[171] One CP observed that although authorization is already applied in many countries, the operational structures of NPPOs used limited approaches and that the standard would benefit NPPOs in harmonizing the way they authorize entities.

[30] *List of topics for IPPC standards* database: https://www.ippc.int/en/core-activities/standards-setting/list-topics-ippc-standards/list

[31] CPM 2019/40

[172] Although several CPs opposed the standard, it was highlighted that there was no obligation to use third party entities, but rather that NPPOs had the authority to decide whether to delegate and to determine the functions to be delegated.

[173] The FAO Legal Division confirmed that Article V.2 (a) of the IPPC provided for the possibility to NPPOs to authorize entities to perform phytosanitary actions except phytosanitary certification, and indicated that the responsibility for the phytosanitary functions remained with the NPPO.

[174] One CP felt that the question is not whether to use of third parties should be allowed, but whether there should be harmonised guidance for NPPOs to manage the use of third parties in a consistent way. Such a standard will increase confidence in the use of third parties by those countries who choose to authorise third parties.

[175] In conclusion the CPM agreed that the SC analyze the comments of CPs submitted during the consultation period and at the CPM-14 session and would take them into account when revising the draft.

[176] The CPM

(1) *Recommended* that the Standards Committee consider the points raised by the CPM and revise the draft accordingly.

11. Implementation and Capacity Development

11.1 Report of the Implementation and Capacity Development Committee (IC)

[177] The Chairperson of the IC presented the IC's report[32] for 2018.

[178] One CP requested the Secretariat to urgently prioritise the development of the Implementation and Capacity Development portions of the IPP (www.ippc.int) to ensure access by CPs to the implementation materials.

[179] Some CPs welcomed the progress in the development of governance and procedures of the IC, as well as the transparent way in which it communicated which topics it is working on. They requested that for future CPMs, separate papers for the IC Sub-group activities be prepared and further requested that the IC reconsider their topics they are working on and focus on activities with higher priorities and also to prepare implementation and communication plans related to those activities.

[180] Some CPs indicated that it was concerned that the IPPC Secretariat may not be providing travel assistance for developing country members to participate in the IC meetings in 2019 due to lack of available funds. They highlighted the need for developing countries to be assisted financially to ensure their participation.

[181] The CPM:

(1) *Noted* the report of Implementation and Capacity Development Committee as presented by the IC Chairperson.

[32] CPM 2019/20

11.2 Proposed independent status for the Sea Containers Task Force

[182] The CPM Chairperson presented the proposal contained in CPM documents[33] [34] and referred CPM to the CPM Bureau's final deliberations to retain the oversight role of the IC over the SCTF to the advance the Complementary Action Plan.

[183] The CPM:

1) *Agreed* to maintain the IC oversight role over the SCTF *Requested* the IC to modify its rule 7 on decision-making of the IC Sub-group Rules of Procedure to make it clear that the IC Sub-groups develop recommendations to the IC.

11.3 Status of ISPM 15 Symbol Registration

[184] The Secretariat provided a report on the status of registration of ISPM 15 symbol worldwide.

[185] One CP encouraged CPs to continue to reimburse of the ISPM 15 registration renewal costs.

[186] One CP informed CPM that their country was in the process of renewing the ISPM 15 symbol registration and requested the IPPC Secretariat to update the attachment to indicate that the renewal is in process.

[187] One CP reminded the Secretariat that in 2023 there will be a large number of registration renewals required and proper planning should take place in advance.

[188] One CP enquired about the reason its request to FAO's Legal Office and the IPPC Secretariat in April 2018 for the registration of the ISPM 15 symbol in their country had not yet been processed.

[189] The CPM:

1) *Noted* the progress made in 2018 and the work plan for 2019 with regard to the registration of the ISPM 15 symbol.
2) *Encouraged* contracting parties to continuously support the process of registration of the ISPM 15 symbol, including renewals of registrations that are due to expire, and
3) *Encouraged* contracting parties to reimburse the IPPC Secretariat for registration and registration renewal costs as soon as practically possible.

12. International Year of Plant Health 2020 (IYPH 2020)

12.1 Report of the IYPH Steering Committee

[190] The Chairperson of the IPPC IYPH Steering Committee, Mr Ralf Lopian presented the report[35] of the IPPC IYPH Steering Committee (IPPC IYPH StC) highlighting three major areas, including the future role of the IPPC IYPH StC, programme of activities, and IYPH related resources.

[191] Several CPs agreed that the IPPC IYPH StC should be retained.

[192] Some CPs suggested that the efficient implementation of the IYPH in 2020 should have absolute priority for the IPPC's work in 2020. The CPs also observed that the Ministerial CPM session and the Global Plant Health Conference, to be held in Rome and Finland respectively in 2020, would constitute important events

[33] CPM 2019/32

[34] CPM 2019/CRP/09

[35] CPM 2019/39 Rev_01

for the IYPH in respect of political, scientific and technical importance. The CPs called on the IPPC Secretariat and CPs to take advantage of these meetings for advocacy and to raise awareness.

[193] One CP suggested that a special mention of the work done by the IPPC IYPH StC to date should be recorded in the CPM decision on this item. Several CPs agreed.

[194] One CP expressed hope that the budget would take into consideration decision point 9, and support the African Union and African, Caribbean and Pacific Group of States (ACP) NPPOs.

[195] Several CPs highlighted the need to re-align priorities for the IPPC Secretariat, NPPOs and RPPOs in 2020 to effectively promote the activities in the context of IYPH. In order to benefit from the exchange of information during the IYPH, the CPM decided to postpone the call for topics, originally planned for 2020, by one year.

[196] Several CPs reported on their plans to create national steering committees, in charge of developing national programmes in view of IYPH.

[197] The IPPC Secretary noted that total estimated costs for IYPH in 2019 and 2020 are 1.3 million USD, however only 285,000 USD are currently available. He informed CPM on the development of a needs analysis of the IPPC Secretariat towards IYPH, which had been reviewed by the CPM Bureau prior to CPM-14. In particular, he briefed CPM on the need for two dedicated project post staff (P-3 and P-2 level), and two experts (even as in-kind contributions), for a total of four P-level staff, in addition to resources for the organization of events. The IPPC Secretary also reported that the CPM Bureau agreed that if sufficient funds were not allocated by June 2019, there would be a need to re-organize the IPPC work plan and budget for 2020 and that this would be discussed by the CPM Bureau at its June 2019 meeting.

[198] The CPM:

(1) *Acknowledged* and *thanked* the IPPC IYPH StC for all of their efforts and contributions, in particular their efforts to help ensure UN proclaimed 2020 as the IYPH;

(2) *Agreed* that the work of the current IPPC IYPH Steering Committee should continue in form of a technical advisory body to CPM and the new IYPH International Steering Committee with the following main tasks:

- *Provide* technical support and advice as required by the IYPH ISC established by FAO
- *Serve* as programme and editorial committee for the International Plant Health Conference
- *Serve* as editorial committee for major IYPH publications to *Serve* as IPPC coordinating body for the IYPH 2020
- *Support* the evaluation of the IYPH in 2021;

(3) *Took note* of the preparations for the International Plant Health Conference;

(4) *Thanked* the Government of Finland for sponsoring the International Plant Health Conference;

(5) *noted* the removal of the video competition from the skeleton programme;

(6) *invited* the IPPC Secretariat to coordinate with other organizations the production of a flagship publication "*The global burden of plant pests*";

(7) *decided* to conduct a scientific/technical review of "*Plant Health and climate change*" to be published in 2020;

(8) *urgently encouraged* contracting parties and donors to provide financial or in-kind resources to support the IYPH 2020 at all levels (national, regional, global);

(9) *recommended* to NPPOs and RPPOs to establish national and regional IYPH 2020 coordination committees, respectively, to coordinate the IYPH 2020 planning and implementation;

(10) *noted* that the IPPC Secretariat had conducted an analysis of staff needs and commitments for IYPH, and had presented it to the CPM Bureau for their detailed consideration;

(11) *requested* the CPM Bureau to discuss possible rearrange the IPPC work plan in 2020, if necessary, in order to allow the IPPC Secretariat to deal with IYPH activities effectively and efficiently;

(12) *decided* that the IPPC call for topics planned in 2020 would be delayed by one year;

(13) *instructed* the SC and IC to create a small advisory group of experts each to help the IPPC Secretariat with scientific issues arising from IYPH.

12.2 IYPH action plan and budget

[199] The Chairperson of the IPPC IYPH StC Mr Ralf Lopian presented the IYPH work plan and budget[36], inclusive of global events and communication initiatives as approved by CPM-13.

[200] He reiterated the need for additional funding to cover the expected activities as detailed in the paper and elaborated by the IPPC Secretary.

[201] The following CPs pledged contributions to the IPPC multi-donor trust fund to cover costs for IYPH: Australia (25,000 Australian Dollars), European Union (300,000 EUR), Kenya (10,000 USD), Republic of Korea (60,000 USD), Sudan (10,000 USD), United Kingdom of Great Britain and Northern Ireland (77,586 USD), in addition to the already pledged contributions by Ireland (56,883 USD) and USA/NAPPO (30,000 USD).

[202] One CP commented on the use of the word "celebration" in association with IYPH, noting that while it was deemed appropriate celebrating the IYPH proclamation, the current messaging should focus on raising awareness, specifically in view or resource mobilization. The CP also suggested to rather use the expressions: "promoting IYPH" and "raising awareness of the importance health and protecting plant resources".

[203] Several CPs indicated that they are initiating national preparations for IYPH, and may further contribute to the IYPH budget at later stages.

[204] The CPM:

1) *Noted* the responsibilities, budget and actions plan for IYPH 2020.
2) *Acknowledged* the key contributions made by Ralf Lopian and Finland for the proclamation of the International Year of Plant Health, in addition to the other 93 UN Member States co-sponsoring the IYPH resolution, the IYPH Steering Committee members, and the IPPC Secretariat.
3) *Agreed* to establish national / regional committees or mechanisms in charge of coordinating activities within their country / region.
4) *Ensured* that IYPH-related initiatives for which they are responsible adhere to the FAO guidance provided in the IYPH communications handbook.
5) *Committed* to support the programme of IYPH at national and regional level.
6) *Thanked* several CPs for pledging contributions and noted contributions pledged by several CPs and *noted* the need to continue contributing financially and in-kind to the IYPH global events.

[36] https://www.ippc.int/en/publications/86904/

13. IPPC Network Activities

13.1 The IPPC Regional Workshops 2018 report

[205] The Secretariat presented the report[37] of the IPPC Regional Workshops held in 2018.

[206] One CP recognized and agreed with the importance of flexibility and ownership of the regional workshops which may have their own funding mechanisms, participation and regional interests. The Republic of Korea indicated that they would be hosting, and support the participation of the IPPC Regional Workshop in Asia from 2 to 6 September 2019.

[207] One CP indicated that the agenda for the workshops should be left to the discretion of the relevant region. It was further indicated that the online comment system (OCS) for regional workshop was used during regional workshop this year but that comments were not processed due to technical issues on the system and suggested that the Secretariat improve the OCS to avoid duplication of work. The IPPC Secretariat advised that this was in progress.

[208] One RPPO reported on the financial effort made to contribute to the participation of 10 participants for the IPPC Regional Workshop held in Africa, and expressed the organization will to proceed in 2019 with such financial contribution

[209] Some CPs indicated that they were disappointed that the Secretariat announced that there were no funds available to support the proposed regional workshop in Africa, and called on the Secretariat and donors to assist with mobilizing resources in this regard.

[210] Some CPs requested the Secretariat and donor countries to assist participants at the forthcoming Regional Workshop in Africa.

[211] One CP wished that CPM note their appreciation to the financial support provided by Australia for supporting the South West Pacific Regional Workshop in 2018.

[212] One CP expressed its appreciation for the IPPC Regional Workshop and indicated the stringent will to have a representative from the IPPC Secretariat attend such workshop for the Latin America region.

[213] Some CPs conveyed appreciation to the IPPC Secretariat and FAO-China South-South Cooperation Programme for participating in the African Regional Workshop in 2018.

[214] The CPM:

1) *Noted* the Guidelines for IPPC Regional Workshops (Appendix 06).
2) *Noted* the IPPC Regional Workshops 2018 report.
3) *Consider* that 2019 IPPC Regional Workshops should be held prior to the end of August 2019.

13.2 The 30th Technical Consultation (TC) among Regional Plant Protection Organizations (RPPOs)

[215] The TC-RPPO Chair, representative of the Secretary General of the Andean Community (CAN), Mr Camilo Beltrán presented the report[38] of the 30th TC-RPPO held in Lima, Peru, from 29 October to 2 November in 2018.

[37] CPM 2019/04

[38] CPM 2019/20

[216] One RPPO expressed appreciation for the report and welcomed the decision for the next TC-RPPO meeting to be held in Abuja, Nigeria.

[217] The CPM:

1) *Noted* the report.

14. Communication and International Cooperation

14.1 Report on Communication and Advocacy of the IPPC Secretariat

[218] The IPPC Secretariat presented its report[39] on its communication and advocacy activities in 2018 and plans for 2019.

[219] The CPM:

(1) *Noted* the report of communication and advocacy activities carried out by the IPPC Secretariat in 2018 and the action plan for 2019.

(2) *Agreed to* continue reporting on national level activities, which may be advertised via the IPPC communication channels.

(3) *Encouraged* national communication professionals to engage with the IPPC Secretariat in view of enhancing the impact of IPPC communications, with a focus on the International Year of Plant Health in 2020.

14.2 Report on international cooperation of the IPPC Secretariat

[220] The Secretariat provided its report on the Secretariats international cooperation activities in 2018.

[221] The CPM:

(1) *Noted* the Report on the 2018 international cooperation activities.

14.3 Written reports from relevant international organizations

[222] Several international organizations provided written presentations and written reports[40], including:

- The Inter-American Institute for Cooperation on Agriculture (IICA) – *Summary of Plant Health Activities*;
- Ozone Secretariat for the *Montreal Protocol on Substances that Deplete the Ozone Layer*;
- The World Customs Organization (WCO) – *Overview of the WCO;*
- International Seed Federation (ISF) – *Report*;
- The Convention on Biological Diversity (CBD) – *Report of the Secretariat*;
- International Forestry Quarantine Research Group (IFQRG)– *Report;*

[39] CPM 2019/08

[40] CPM 2019/INF/03, CPM 2019/INF/04, CPM 2019/INF/05, CPM 2019/INF/06, CPM 2019/INF/07, CPM 2019/INF/08, CPM 2019/INF/09, CPM 2019/INF/10, CPM 2019/INF/11, CPM 2019/INF/13, CPM 2019/INF/14, CPM 2019/INF/15, CPM 2019/INF/17, CPM 2019/INF/23,

- Centre International de Hautes études Agronomiques Méditerranéennes (CIHEAM) – *HighPrecise-IPM: A New Plant Health Paradigm*;
- International Advisory Group for Pest Risk Analysis (IAGPRA)– *Report*;
- The Europe-Africa-Caribbean-Pacific Liaison Committee (COLEACP) – *Report*;
- The Standards and Trade Facility (STDF) – *Overview*;
- World Trade Organization (WTO) – *Activities of the SPS Committee and other relevant WTO activities in 2018*;
- Joint FAO/IAEA Programme – *Report of Nuclear Techniques in Food and Agriculture*;
- Phytosanitary Measures Research Group (PMRG) – *Report*;
- World Bank Group – *Support to Capacity Development in Plant Health*.

[223] The CPM:

(1) *Thanked* the speakers for their oral presentation and noted their written reports.

14.4 Panel discussion on capacity development and plant health from selected international organizations (CABI, CBD, IAEA, STDF, WTO)

[224] A panel discussion was held with speakers and presentations focused on capacity development and plant health delivered by a number of international organization. The panel members and presenters included:

- Ms Ozelm SOSANLI, World Customs Organization;
- Mr Washington OTIENO, Centre for Agriculture and Bioscience International (CABI);
- Ms Junko SHIMURA, Convention on Biodiversity;
- Mr Rui Cardoso PERIERA, International Atomic Energy Agency;
- Mr Shane SELA, Trade Facilitation Support Programme, World Bank;
- Mr Melvin SPREIJ, Standards and Trade Development Fund;
- Ms Anneke HAMILTON, World Trade Organization.

15. Financial Report and Budget

15.1 Financial report of the IPPC Secretariat for 2018

[225] The IPPC Secretariat presented its financial report, detailing the resources available in 2018 from FAO's Regular Programme (RP) budget, the Extra-Budgetary (EB) and In-kind (non-financial) sources.

[226] Several CPs congratulated the IPPC Secretariat on improved financial transparency and clarity of report.

[227] One RPPO enquired about interpretation of Standards committee meetings. Secretariat replied that five languages per session were foreseen for 2019.

[228] Canada indicated that it had provided USD 288,000 to support the Multi-donor Trust Fund and encouraged other CPs to provide their support.

[229] Brazil pledged In-kind (staff) contribution to the IPPC Secretariat.

[230] One CP requested that a separate report on IYPH spending be included in the financial report for 2019.

[231] France pledged EUR 250,000 contribution to the IPPC Multi-donor trust fund dedicated to the TPG, drafting of commodity standards, and activities and IYPH. They further indicated that it provided an in-kind staff contribution valued at € 200,000 per year.

[232] The Republic of Korea pledged USD 160,000 contribution to the IPPC Multi-donor trust fund to support IYPH and the ePhyto project.

[233] The CPM:

(1) *Noted* the Financial Report for 2018 of the IPPC Secretariat;

(2) *Adopted* the Financial Report for 2018 of the IPPC Multi-Donor Trust Fund (Special Trust Fund of the IPPC) (Table 4);

(3) *Encouraged* contracting parties to contribute to the IPPC Multi-Donor Trust Fund (Special Trust Fund of the IPPC) and IPPC Projects, preferably on an ongoing basis;

(4) *Thanked* Contracting Parties that contributed to the IPPC Secretariat's programme of work in 2018.

15.2 Resource mobilization report of the IPPC Secretariat for 2018

[234] The IPPC Secretariat presented a report on its resource mobilization activities and achievements for 2018, led by the IPPC Secretariat Task Force for Resource Mobilization.

[235] Several CPs expressed appreciation for the clear and transparent report. One CP requested that the presentation by the IPPC Secretariat be posted with the other CPM documents on the IPP.

[236] One CP encouraged developing countries to consider contributing even small amounts towards the Multi-donor Trust Fund

[237] Sudan pledged USD 10,000 contribution to the IPPC Multi-donor trust fund in support of IYPH.

[238] Kenya pledged USD 10,000 contribution to the IPPC Multi-donor trust fund in support of IYPH.

[239] EU pledged financial support for the 2019-2021 period to the IPPC Secretariat, as stated previously.

[240] The CPM:

(1) *Noted* the resource mobilization activities and outcomes of the IPPC Secretariat in 2018.

(2) *Encouraged* CPs to make continuous financial support to the IPPC Work Programme.

15.3 Work plan and budget of the IPPC Secretariat for 2020

[241] The IPPC Secretariat presented the work plan and budget of the IPPC Secretariat for 2020.

[242] Some CPs conveyed appreciation to the IPPC Secretariat, with the support of the CPM Bureau and Finance Committee, for the "high-quality" document.

[243] One CP requested the IPPC Secretariat to consider cost-savings as a measure to fund IYPH activities.

[244] The CPM:

(1) *approved* "The Work Plan and Budget of the IPPC Secretariat for 2020"

15.4 Sustainable funding initiative - Support to IPPC work programme through increase FAO regular programme

[245] The Chairperson of the IPPC Financial Committee (FC) introduced the paper[41] and CRP[42]. He reiterated that the need for sustainable funding for the IPPC Secretariat has been on the CPM agenda for many years.

[246] The paper referred to the reports of the Committee on Agriculture (COAG-26), Committee on Forestry (COFO-24), Committee on Commodity Problems (CCP-72) and FAO Programme Committee (126th Session), where support for the increased FAO regular programme funding to the IPPC Secretariat was adopted.

The FC Chairperson stressed that the 161st Session of the FAO Council from 8-12 April 2019, and that there is an opportunity to discuss funding of the IPPC Secretariat and urged CPs to support the IPPC in the FAO Council meeting. He called on CPM to unanimously adopt the following statement contained in the CRP/10:

> *Contracting Parties to the IPPC participating at the 14th Session of the Commission of Phytosanitary Measures are unanimous in recognising the imperative need for sufficient and stable funding for the IPPC Secretariat to implement priority programmes, including enhanced support for implementation and capacity development, and the facilitation of safe trade. We call strongly upon the FAO to increase the funding basis of the IPPC Secretariat on an ongoing basis through reallocation of funds from the FAO's Regular Programme Budget. The sustainable funding that would be realised through such reallocation is urgently needed and would significantly support advancement of IPPC work programmes to the benefit of all 183 contracting parties of the IPPC and global plant protection. This allocation would also support the core mandate of the FAO's fundamental work, contributing directly to achieving the FAO's Strategic Objective 2 (Make agriculture, forestry and fisheries more productive and sustainable) and Strategic Objective 4 (Enable inclusive and efficient agricultural and food systems).*

[247] Some CPs reminded the CPM that plant health was the vanguard to prevent the introduction of dangerous pests and that investments in prevention would ultimately result in huge cost savings for control and eradication activities. They further suggested that the Fall Armyworm spread to Africa could have been prevented, or delayed, with efficient plant health policies and structures in place, and that for this reason it would be politically sound and opportune for FAO to strengthen the IPPC Secretariat financially.

[248] One CP requested how they could support the efforts if they were not Members of the FAO Council. The IPPC Secretariat indicated that they could work through their Permanent Representatives to ensure their support was advocated during the relevant Sessions of the FAO Council and Conference.

[249] The CPM:

(1) Unanimously *adopted* the statement above, also contained in the CRP/10.

(2) *Repeated* its call upon the FAO to increase the funding basis of the IPPC Secretariat through reallocation of funds from its Regular Programme Budget

(3) *Encouraged* CPs to request that their FAO Permanent Representatives strongly engage FAO management and members of the FAO Finance and Programme Committees, using the information in Attachment 1 as relevant, to increase the IPPC annual budget to USD 6 million

(4) *Noted* that the requested increase is very small relative to the total FAO biennial budget, but the activities that will be progressed by the IPPC community contribute significantly to progressing FAO priorities in food security, poverty alleviation and facilitating safe trade.

[41] CPM 2019/28

[42] CPM 2019/CRP/10

(5) *Noted* that the information contained in the paper[43] submitted to the COAG in 2018 contained information that can be used by CPs to inform FAO Council and Conference delegates on the imperative for sufficient stable and sustainable funding of the IPPC Secretariat to implement priority programs for CPs including enhanced support for implementation and capacity development, and innovation to facilitate safe trade.

(6) *Encouraged* CPs and Permanent Representatives to FAO to increase awareness of the benefit of increasing financial support to the IPPC to achieving FAO goals in candidates seeking election to the position of FAO Director-General.

(7) *Noted* that the FAO Council meets in Rome from 8 – 12 April 2019, and the FAO Conference meets from 22 – 29 June 2019.

16. Successes and Challenges of Implementation of the Convention

[250] CPs, observer countries and organizations, and Regional Plant Protection Organizations were invited to share their successes and challenges in implementing the IPPC:

- The North American Plant Protection Organization (NAPPO) – *"America's focused": ISPM 38 (international Movement of Seeds) Implementation Workshop[44];*
- New Zealand – *Successes and Challenges in Managing Brown Marmorated Stink Bug (BMSB)*[45]; and
- International Organization for Health in Agriculture (OIRSA)[46] – *Experience in phytosanitary emergencies*[47];
- Madagascar - *Conducting the Phytosanitary Capacity Evaluation in Madagascar: less rage than courage*;
- Nicaraguan Institute of Agricultural Protection and Health - *The use of Mobile apps technologies for surveillance in Nicaragua*[48].
- Kenya - *Is a systems approach the way to beat quarantine restrictions in the developing world? A case of Kenya Fresh avocado exports to South Africa*[49].
- Palestine - *Palestine takes the first step towards better Plant Health – The experience of Palestine*[50].
- Economic Community of West African States (ECOWAS) – ECOWAS Regional pre-preparation meetings on harmonization of matters of interest to be discussed at the CPM sessions of the IPPC[51].

17. Special Topics Session on Plant Health and Capacity Development

[251] The Special Topics session aimed at promoting the IPPC 2019 annual theme "Plant Health and Capacity Development ". Four speakers gave the CPM an overview on plant health capacity development tools and

[43] COAG/2018/INF/8
[44] CPM 2019/INF/18
[45] CPM 2019/INF/19
[46] CPM 2019/CRP/06
[47] CPM 2019/CRP/06
[48] CPM 2019/CRP/06
[49] CPM 2019/CRP/06
[50] CPM 2019/CRP/06
[51] CPM 2019/CRP/11

some examples of effective capacity development programmes that countries can benefit from to improve their phytosanitary systems.

17.1 Overview on support of the capacity development to the plant health[52]

[252] *Speaker: Brent Larson, Lead of the Implementation-Facilitation Unit, IPPC Secretariat.*

[253] The speaker provided an overview on the fundamental role that IPPC Secretariat plays in helping to build the capacity of contracting parties to implement the Convention, International Standards for Phytosanitary Measures and CPM Recommendations. The presentation included the IPPC capacity development tools, training materials, guidelines and manuals, and projects being implemented by the Secretariat.

17.2 Contribution of FAO South-South Cooperation to Capacity Development in Agriculture

[254] *Speaker: Jingyuan Xia, Secretary, IPPC Secretariat*

[255] The speaker familiarized the CPM-14 participants with the core objective of FAO South-South Cooperation (SSC) to help developing countries build sustainable food systems and enhance their capacities to improve their own livelihoods at institutional, national and regional levels. The speaker shaded a light on the IPPC global project on phytosanitary capacity development under the framework of the FAO-China SSC Programme. The overall objective of this global project is to bring more innovative ways and means for strengthening capacity of IPPC developing Contracting Parties (CPs) to better implement the IPPC and its international standards on phytosanitary measures (ISPMs). The project is the first project for FAO SSC Programme to specially support the IPPC activities with four-year (2017-2020) timeframe and four work components with total investment of USD 2 million.

17.3 Role of the Phytosanitary Capacity Evaluation (PCE) in strengthening the National Plant Protection Organizations (NPPOs)

[256] *Moderator: Sarah Brunel, Implementation-Facilitation Officer, IPPC Secretariat.*

[257] The speaker gave an overview on the Phytosanitary Capacity Evaluation (PCE) that has been conducted in more than 60 countries since 2000 with support of the IPPC Secretariat staff. It was noted that a PCE strategy is currently being developed to promote and improve PCEs and their implementation as well as their resulting impacts. The benefits of PCE for improvements of the phytosanitary systems within countries and funding opportunities for the NPPO were highlighted. The success of Guinea was presented that based on applied PCE, the country received funding from the Standards and Trade Development Facility (STDF) to improve the national phytosanitary system.

17.4 Phytosanitary Risk-Based Sampling: Next Steps

[258] Spea*ker: Robert L. Griffin, North American Plant Protection Organization (NAPPO).*

[259] The speaker pointed out the importance of the inspection as most used phytosanitary measure to make safe trade ensured. The guidance provided by the IPPC standards points to inspection as a procedure that is technically justified and fairly applied for risk management, however, it is important to understand the relevant statistical concepts and use them to advantage for inspection designs that are both fair to trade and informative to regulatory officials. The presentation shared some experience of the United States and Australia in particular that have started shifting their inspection designs toward statistically-based sampling that is consistent with the ISPMs. Other countries have similar plans or are interested in strategies that move

[52] CPM 2019/INF/22

in the same direction. It was emphasized that sharing views and experiences in this regard will contribute to a better understanding of the conceptual foundation, the operational and regulatory challenges, and responding to stakeholder perceptions that are needed to facilitate international harmonization.

18. IYPH: Promoting and celebrating the IYPH 2020 – Sharing Ideas

[260] An overview[53] was provided to the CPM on the status of preparations for IYPH 2020, including outreach, communications, roadmap and timeline for implementation in the lead-up to 2020. Presentation were delivered by the FAO Office of Corporate Communications as well as the FAO Partnerships Division, under the leadership of the IYPH Steering Committee Chairperson.

[261] The IPPC Secretariat reported on the results of the online survey on IYPH plans and ideas by the IPPC community. Several CPs expressed appreciation for the report[54] and agreed to continue sharing their plans for IYPH with the IPPC Secretariat.

[262] The CPM was also presented with the new logo for IYPH and slogan - "Protecting Plants, Protecting Life".

19. Confirmation of Membership and Potential Replacement Members for CPM Subsidiary Bodies

[263] Although the selection of IC members is not under this agenda point, the CPM was informed on a few adjustments.

[264] It was noted that the Bureau has approved Mr Ringolds ARNITIS (Latvia) as the alternative IC member for the European region. The Africa region also informed the CPM that they have submitted their nomination to the Bureau for an alternative IC member, Ms Raymonda JOHNSON (Sierra Leone).

19.1 CPM Bureau members and potential replacement members

[265] The IPPC Secretariat provided the CPM with the list of nominated Bureau members and potential replacement members[55] as revised during CPM.

[266] The CPM:

(1) *Confirmed* the current membership of Bureau members and potential replacement members (Appendix 05).

19.2 SC members and potential replacement members

[267] The IPPC Secretariat provided the CPM with the list of SC members and potential replacement members[56], with the revised document presented[57].

[268] The CPM:

(1) *Confirmed* the current membership of the Standards Committee and the potential replacements for the Standards Committee (Appendix 05);

(2) *Confirmed* new members and potential replacements;

(3) *Confirmed* the order in which potential replacements would be called upon for each region.

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[53] CPM 2019/CRP/15

[54] Ref. https://www.ippc.int/static/media/files/publication/en/2019/04/CPM-14_IYPH_BrainstormingSession.pdf

[55] CPM 2018/CRP/16

[56] CPM 2018/CRP/16

[57] CPM 2018/CRP/16

20. Any Other Business

20.1 IPPC Stakeholder Advisory Group

[269] The CPM discussed a proposal to develop and establish an IPPC Stakeholder Advisory Group as contained in the paper presented[58]. The CPM noted that CPM-12 (2017) had *encouraged* globally and regionally relevant stakeholders to explore the formation of an IPPC Stakeholder Advisory Group (SAG); and *requested* that the CPM Bureau and SPG, in consultation with relevant stakeholders, prepare draft Terms of Reference (ToR) and Rules of Procedure (RoP) for such an IPPC Stakeholder Advisory Group.

[270] One CP informed participants that CPM-12 (2017) intended for the Terms of Reference (ToR) and Rules of Procedure (RoP) to be developed and agreed at the IPPC/Stakeholder Workshop taking place under the auspices of IYPH 2020.

[271] One RPPO shared its experience of involving stakeholders noting that they bring practical and real life experience with phytosanitary issues.

[272] CP, though recognizing the important input of stakeholders, cautioned that they may advance their agenda therefore the principle of transparency was key to managing the engagement.

[273] The CPM:

(1) *Agreed* to establish an electronic working group to develop a stakeholder engagement framework for engaging with stakeholders for presentation and acceptance during the June 2019 Bureau meeting, taking into account relevant models for engagement that would inform this work,

(2) *Agreed* to building upon the CPM 12 decisions, encouraged the CPM Bureau to continue with implementation of the stakeholder engagement framework per the CPM-12 (2017) decisions for integration into IYPH 2020 plans as well as for a long-term relationship, and

(3) *Encouraged* the International Year of Plant Health International Steering Committee to include and prepare as part of the IYPH 2020 events an IPPC/Stakeholder Workshop, which would determine ToRs and RoPs for a long-term IPPC Stakeholders Advisory Group.

20.2 IPPC Webpage

[274] The Secretariat delivered a presentation to the CPM on the new structure and design of the IPPC Portal (IPP) that will be implemented soon after CPM-14 (2019). It was pointed out the new design has been developed based on the IPP users survey that has been carried out by the Secretariat. The new design aims improve the usability and ease the access to the information.

[275] The CPM:

(1) *Welcomed* the proposed improvement to the IPP.

21. Date and Venue of the Next Session[59]

[276] The Fifteenth Session of the Commission on Phytosanitary Measures (CPM-15) will take place from 30 March to 3 April 2020 at FAO Headquarters in Rome.

[277] The Ministerial segment[1] of CPM-15 (2020) is planned as a key event in the International Year of Plant Health (IYPH) in 2020, and is scheduled to take place on 2 April 2020, also at FAO Headquarters in Rome.

[58] CPM 2019/38

[59] CPM 2019/CRP/08

[278] CPM:

1) *Noted* the date and concept for the CPM-15 (2020) Ministerial segment.
2) *Urged* contracting parties to encourage their respective ministers to participate in the Ministerial segment organized during CPM-15 (30 March to 3 April 2020).

22. Adoption of the Report

[279] The report was adopted.

23. Closing of the Session

[280] The session was closed.

Appendix 01 – Agenda

1. Opening of the Session
1.1 FAO Opening
1.2 Statement of the Minister of Agriculture of Mexico
2. Keynote Address on Plant Health and Capacity Development by the European Commission's Director-General for Health and Food Safety (DG SANTE)
3. Adoption of the Agenda
3.1 EU Statement of Competence
4. Election of the Rapporteur
5. Establishment of the Credential Committee
6. Report from the CPM Chairperson
7. Report from the IPPC Secretariat
8. Governance and Strategy
8.1 Summary of the 2018 Strategic Planning Group report
8.2 IPPC Strategic Framework for 2020-2030
8.3 Five year investment plan of the IPPC Secretariat
8.4 Focus Group on Commodity and Pathways Standards
8.5 Facilitating safe trade for plants and plant products - Action Plan
8.6 Five year strategic plan for ePhyto: Transitioning from project to business as usual operation
8.7 IPPC e-Commerce proposed project work plan and budget
8.8 Concept of emerging pests and emergency issues
8.9 Antimicrobial resistance (AMR) in relation to Plant Health aspects
8.10 CPM recommendations
9. Cooperation of Standards and Implementation
9.1 Task Force on Topics and 2018 Call for Topics: Standards and Implementation
9.2 Surveillance pilot project analysis
9.3 Framework for standards and implementation
10. Standards Setting
10.1 Report of the Standards Committee (SC)
10.2 Adoption of International Standards for Phytosanitary Measures
10.3 Standards Committee recommendations to the Commission on Phytosanitary Measures
10.4 Conceptual challenges in standards development in terms of implementation
11. Implementation and Capacity Development
11.1 Report of the Implementation and Capacity Development Committee (IC)
11.2 Proposed independent status for the Sea Containers Task Force
11.3 Status of ISPM 15 Symbol Registration
12. International Year of Plant Health 2020 (IYPH 2020)
12.1 Report of the IYPH Steering Committee
12.2 IYPH action plan and budget
13. IPPC Network Activities
13.1 The IPPC Regional Workshops 2018 report
13.2 The 30th Technical Consultation (TC) among Regional Plant Protection Organizations (RPPOs)
14. Communication and International Cooperation
14.1 Report on Communication and Advocacy of the IPPC Secretariat
14.2 Report on international cooperation of the IPPC Secretariat
14.3 Written reports from relevant international organizations
14.4 Panel discussion on capacity development and plant health from selected international organizations (CABI, CBD, IAEA, STDF, WTO)
15. Financial Report and Budget
15.1 Financial report of the IPPC Secretariat for 2018

15.2 Resource mobilization report of the IPPC Secretariat for 2018
15.3 Work plan and budget of the IPPC Secretariat for 2020
15.4 Sustainable funding initiative - Support to IPPC work programme through increase FAO regular programme

16. Successes and Challenges in Implementation of the Convention

17. Special Topics Session on Plant Health and Capacity Development

17.1 Overview on support of the capacity development to the plant health
17.2 Contribution of FAO South-South Cooperation to Capacity Development in Agriculture
17.3 Role of the Phytosanitary Capacity Evaluation (PCE) in strengthening the National Plant Protection Organizations (NPPOs)
17.4 Phytosanitary Risk-Based Sampling: Next Steps

18. IYPH: Promoting and celebrating the IYPH - *Sharing ideas*

19. Confirmation of Membership and Potential Replacements for CPM Subsidiary Bodies

19.1 CPM Bureau members and potential replacement members
19.2 SC members and potential replacement members

20. Any other business

20.1 IPPC Stakeholders Advisory Body
20.2 Presentation of IPP new structure

21. Date and Venue of the Next Session

22. Adoption of the Report

23. Closing of the Session

Appendix 02 – List of Documents

Doc number	Title	Agenda	Available languages
01	Provisional Agenda	03	EN/FR/ES/AR/RU/ZH
02_Rev_01	Detailed Agenda	03	EN/FR/ES/AR/RU/ZH
03	Adoption of International Standards for Phytosanitary Measures	10.2	EN/FR/ES/AR/RU/ZH
03_01	2014-004_Fumigation	10.2	EN/FR/ES/AR/RU/ZH
03_02	1994_001_2017_Amendments Glossary	10.2	EN/FR/ES/AR/RU/ZH
04	The IPPC Regional Workshops 2018 report	13.1	EN/FR/ES/AR/RU/ZH
05	Recommendations of the Standards Committee to the Commission of Phytosanitary Measures	10.3	EN/FR/ES/AR/RU/ZH
06	SC members and potential replacement members	19.2	EN/FR/ES/AR/RU/ZH
07	Adoption of International Standards for Phytosanitary Measures - Ink Amendments to adopted ISPMs: “contamination” and its derivatives	10.2	EN/FR/ES/AR/RU/ZH
08	Report on Communication and Advocacy of the IPPC Secretariat - Report and Action Plan on Communication and Advocacy of the IPPC Secretariat	14.1	EN/FR/ES/AR/RU/ZH
09	IYPH action plan and budget	12.2	EN/FR/ES/AR/RU/ZH
10	CPM recommendations	08.10	EN/FR/ES/AR/RU/ZH
10_01	Attachment 01 – Draft CPM Recommendation: High-throughput sequencing (HTS) technologies as a diagnostic tool for phytosanitary purposes	08.10	EN/FR/ES/AR/RU/ZH
11	Report of the SC	10.1	EN/FR/ES/AR/RU/ZH
12	Resource mobilization report of the IPPC Secretariat for 2018	15.2	EN/FR/ES/AR/RU/ZH
13_Rev_01	Financial report of the IPPC Secretariat for 2018	15.1	
14	Work plan and budget of the IPPC Secretariat for 2020	15.3	EN/FR/ES/AR/RU/ZH
15	Concept of emerging pests and emergency issues - (Draft) role of the IPPC in relation to Plant Health emergencies and emerging pests	08.8	EN/FR/ES/AR/RU/ZH
16	IPPC e-Commerce proposed project work plan and budget	08.7	EN/FR/ES/AR/RU/ZH
17_Rev_01	Report on international cooperation of the IPPC Secretariat - Report from the IPPC Secretariat	14.2	EN/FR/ES/AR/RU/ZH
18	Surveillance pilot project analysis - Implementation Pilot Programme on Surveillance - Programme Review and Evaluation including recommendations	09.2	EN/FR/ES/AR/RU/ZH
19	The 30th Technical Consultation (TC) among Regional Plant Protection Organizations (RPPOs) - Summary Report	13.2	EN/FR/ES/AR/RU/ZH
20	Report of the Implementation and Capacity Development Committee (IC) - Activities of the IC	11.1	EN/FR/ES/AR/RU/ZH
21	Framework for standards and implementation	09.3	EN/FR/ES/AR/RU/ZH
22	Task Force on Topics and 2018 Call for Topics: Standards and Implementation - TFT Recommendations to CPM for Submissions for 2018 Call for Topics: Standards and Implementation	09.1	EN/FR/ES/AR/RU/ZH
23	Task Force on Topics and 2018 Call for Topics: Standards and Implementation - Impacts and Benefits of The New Procedure for Call for Topics	09.1	EN/FR/ES/AR/RU/ZH
24	Task Force on Topics and 2018 Call for Topics: Standards and Implementation - List of Implementation and Capacity Development Topics	09.1	EN/FR/ES/AR/RU/ZH
25	Report from the CPM Chairperson	06	EN/FR/ES/AR/RU/ZH
26	IPPC Strategic Framework for 2020-2030	08.2	EN/FR/ES/AR/RU/ZH
27	Focus Group on Commodity and Pathways Standards	08.4	EN/FR/ES/AR/RU/ZH

Doc number	Title	Agenda	Available languages
28	Sustainable funding initiative - Support to IPPC work programme through increase FAO regular programme	15.4	EN/FR/ES/AR/RU/ZH
29	CPM recommendations - Safe provision of food and other aid to prevent the introduction of plant pests during an emergency situation (2018-026)	08.10	EN/FR/ES/AR/RU/ZH
30	Five year investment plan of the IPPC Secretariat - in relation to the IPPC Strategic Framework 2020-2030	08.3	EN/FR/ES/AR/RU/ZH
31	Status of ISPM 15 Symbol Registration	11.3	EN/FR/ES/AR/RU/ZH
32	Proposed independent status for the Sea Containers Task Force	11.2	EN/FR/ES/AR/RU/ZH
33	Facilitating safe trade for plants and plant products - Action Plan	08.5	EN/FR/ES/AR/RU/ZH
34	Summary of the 2018 Strategic Planning Group report	08.1	EN/FR/ES/AR/RU/ZH
35	Five year strategic plan for ePhyto: Transitioning from project to business as usual operation	08.6	EN/FR/ES/AR/RU/ZH
36	Report from the IPPC Secretariat	07.	EN/FR/ES/AR/RU/ZH
37	CPM recommendations - Facilitating safe trade by reducing the incidence of contaminating pests associated with traded goods	08.10	EN/FR/ES/AR/RU/ZH
38	Any other business - IPPC Stakeholder Advisory Group	20	EN/FR/ES/AR/RU/ZH
39	Report of the IYPH Steering Committee - Report and recommendations of the IPPC IYPH Steering Committee	12.1	EN/FR/ES/AR/RU/ZH
40	Conceptual challenges in standards development in terms of implementation - Authorization of entities to perform phytosanitary actions	10.4	EN/FR/ES/AR/RU/ZH
INF 01	Template for proposals going to CPM that have resource implications to the budget of the IPPC Secretariat - Supplementary document to CPM 2019/16 (eCommerce)	08.7	EN only
INF 02	Template for proposals going to CPM that have resource implications to the budget of the IPPC Secretariat - Supplementary document to CPM 2019/18 (Surveillance)	09.2	EN only
INF 03	Written reports from relevant international organizations - The Inter-American Institute for Cooperation on Agriculture (IICA) Summary of 2018 Plant Health Activities	14.23	EN only
INF 04	Written reports from relevant international organizations - Report by the Ozone Secretariat for the Montreal Protocol on Substances that Deplete the Ozone Layer	14.3	EN only
INF 05	Written reports from relevant international organizations - The World Customs Organization (WCO) Overview	14.3	EN only
INF 06	Written reports from relevant international organizations - Written Report of the International Seed Federation (ISF)	14.3	EN only
INF 07	Written reports from relevant international organizations - Report of The Secretariat of The Convention on Biological Diversity (CBD)	14.3	EN only
INF 08	Written reports from relevant international organizations - Report from the International Forestry Quarantine Research Group (IFQRG)	14.3	EN only

Doc number	Title	Agenda	Available languages
INF 09	Written reports from relevant international organizations - HighPrecise-IPM: A New Plant Health Paradigm of CIHEAM	14.3	EN only
INF 10	Written reports from relevant international organizations - Report from The International Advisory Group for Pest Risk Analysis (IAGPRA)	14.3	EN only
INF 11	Written reports from relevant international organizations - Report from The Europe-Africa-Caribbean-Pacific Liaison Committee (COLEACP)	14.3	EN only
INF 12	Antimicrobial resistance (AMR) - Antimicrobial Resistance (AMR) in relation to plant health aspects	08.9	EN only
INF 13	Written reports from relevant international organizations - The Standards and Trade Development Facility (STDF) Overview	14.3	EN, FR, ES
INF 14	Written reports from relevant international organizations - Activities of the SPS Committee and other relevant WTO activities in 2018	14.3	EN, FR, ES
INF 15	Written reports from relevant international organizations - Report from the Joint FAO/IAEA Programme of Nuclear Techniques	14.3	EN only
INF 16	Adoption of the Agenda – Schedule of the CPM-14 main sessions and the Plenary time-table	03	EN only
INF 17	Written reports from relevant international organizations - Phytosanitary Measures Research Group (PMRG)	14.3	EN only
INF 18	Successes and Challenges in Implementation of the Convention - “Americas focused” ISPM 38 (International movement of seeds) Implementation Workshop	16	EN, ES
INF 19	Successes and Challenges in Implementation of the Convention - Successes and Challenges in Managing BMSB across Inanimate Pathways	16	EN only
INF 20	Concept of emerging pests and emergency issues - Summary Report on: International Conference Brown Marmorated Stink Bug (BMSB) – Phytosanitary Regulatory Framework	08.8	EN only
INF 21	Any other business - Programme and Background Information of CPM-14 Side Sessions	20	EN only
INF 22	Special Topics Session on Plant Health and Capacity Development - Information Note	17	EN only
INF 23	Written reports from relevant international organizations - The World Bank Group’s support to capacity development in plant health	14.3	EN only
INF 24	Work plan and budget of the IPPC Secretariat for 2020 - Standard Operating Procedure (SOP) Governing Programme Planning and Budgeting, Monitoring and Implementation and Reporting and Evaluation	15.3	EN only

Appendix 03 – List of Participants

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Appendix 04 – Agreed adjustments to IPPC Strategic Framework 2020-2030

[281] The Friends of the Chair meeting held during the CPM-14 to discuss concerns some countries raised with parts of the draft Strategic Framework 2020-2030 agreed on the below adjustments to be carried out to the Strategic Framework 2020-2030:

- Implementation and Capacity Building are core activities of the IPPC so should be more prominent in the document. To achieve this, the document should be reordered to place core activities ahead of strategic objectives (as per the diagram and in the body of the document).
- Additional comment should be inserted into the document making it clear that while the Development Agenda items present great opportunities to advance the Mission of the IPPC, progressing them is dependent on securing adequate resources.
- Text would be added to the document to make it clear that CPM can review and adapt the development agenda or other parts of the Strategic Framework as often as may be needed. This could potentially be a task assigned to SPG with recommendations provided to the CPM via the Bureau.
- Each pest case study would include photographs and more information on the impact of the pests.
- When final formatting is done by communication specialists, they should ensure the pest case studies are presented in a way that does not create confusion, and that they could achieve this by grouping them all together or by laying them out in an improved way.
- The Secretariat would use communication specialists to simplify the wording and ensure consistent use of terms that would be easily understood by readers.
- The glossary of terms would be expanded to provide more explanation for example, that an NPPO is the competent authority for a country and is responsible for providing and receiving government to government phytosanitary assurances, and should be resourced to fulfil their functions competently.

Appendix 05 – Current membership and potential replacements of the CPM Bureau and the Standards Committee

Table 1. Current membership of the Bureau of the CPM (following CPM-14 decisions)

Region	Country	Name	Nominated/ Re-nominated	Current term/duration	Term expires
Africa (Vice Chairperson)	Cote D'Ivoire	Mr Lucien KOUAME KONAN	CPM-7 (2012) CPM-9 (2014) CPM-11 (2016) CPM-13 (2018)	4th Term/2 years	2020
Asia	China	Mr Fuxiang WANG	CPM -13 (2018)	1st term/ 2 years	2020
Europe	Malta	Ms Marica GATT	CPM-13 (2018)	1st term/2 years	2020
Latin America and Caribbean (Chairperson)	Mexico	Mr Francisco Javier TRUJILLO ARRIAGA	CPM-11 (2016) CPM-13 (2018)	2nd term/ 2 years	2020
Near East	Yemen	Mr Gamil Anwar Mohammed RAMADHAN	CPM-13 (2018)	1st term/2 years	2020
North America	Canada	Mr Greg WOLFF	CPM-13 (2018)	1st term/ 2 years	2020
Southwest Pacific	New Zealand	Mr Stephen BUTCHER	CPM-14 (2019)	1st term/2 years	2021

Table 2. Current replacements of the Bureau of the CPM (following CPM-14 decision)

Region	Country	Name	Nominated/ Renominated	Current term/duration	Term expires
Africa	1 Democratic Republic of Congo	Mr Mamba Mamba DAMAS	CPM-14 (2019)	1st term/ 2 years	2021
	2 South Africa	Mr Kgabo MATLALA	CPM-13 (2018)	1st term/ 2 years	2020
Asia	1 Indonesia	Mr Antarjo DIKIN	CPM-11 (2016) CPM-13 (2018)	2nd term/ 2 years	2020
	2	VACANT			
Europe	1 United Kingdom	Mr Samuel BISHOP	CPM-12 (2017) CPM-13 (2018)	1st term/ 2 years	2020
	2	VACANT			
Latin America and Caribbean	1 Argentina	Mr Diego QUIROGA	CPM-11 (2016) CPM-13 (2018)	2nd term/ 2 years	2020
	2 Belize	Mr Francisco GUTIÉRREZ	CPM-13 (2018)	1st term/ 2 years	2020
Near East	1 Libya	Mr Salem Abdulkader HAROUN	CPM-13 (2018)	1st term/ 2 years	2020
	2 Egypt	Ahmed EL-ATTAR	CPM-13 (2018)	1st term/ 2 years	2020
North America	1 United States	Mr John K. GREIFER	CPM-11 (2016) CPM-13 (2018)	2nd term/ 2 years	2020
	2	VACANT			
Southwest Pacific	1 New Zealand	Mr Peter THOMSON	CPM-13 (2018)	1st term/ 2 years	2020
	2	VACANT			

Table 3. Standards Committee Membership and Potential Replacements

Standards Committee Membership

Region	Country	Name	Nominated/ Re-nominated	Current term/duration	Term expires
Africa	Kenya	Ms Esther KIMANI	CPM-9 (2014) CPM-12 (2017)	2nd term / 3 years	2020
	Congo	Ms Alphonsine LOUHOUARI TOKOZABA	CPM-13 (2018)	1st term / 3 years	2021
	Malawi	Mr David KAMANGIRA	CPM-11 (2016) CPM-14 (2019)	2nd term / 3 years	2022
	Nigeria	Mr Moses Adegboyega ADEWUMI	CPM-13 (2018)	1st term / 3 years	2021
Asia	Indonesia	Ms Chonticha RAKKRAI	CPM-14 (2019)	1st term / 3 years	2022
	Japan	Mr Masahiro SAI	CPM-13 (2018)	1st term / 3 years	2021
	Sri Lanka	Ms Jayani Nimanthika WATHUKARAGE	CPM-13 (2018)	1st term / 3 years	2021
	China	Mr Xiaodong FENG	CPM-13 (2018)	1st term / 3 years	2021
Europe	France	Ms Laurence BOUHOT-DELDUC	CPM-10 (2015) CPM-13 (2018)	2nd term / 3 years	2021
	Israel	Mr David OPATOWSKI	CPM-1 (2006) CPM-4 (2009) CPM-12 (2017)	3rd term / 3 years	2020
	Italy	Ms Mariangela CIAMPITTi	CPM-14 (2019)	1st term /3 years	2022
	United Kingdom	Mr Samuel BISHOP	CPM-13 (2018)	1st term /3 years	2021

Region	Country	Name	Nominated/ Re-nominated	Current term/duration	Term expires
Latin America and Caribbean	Argentina	Mr Ezequiel FERRO	CPM-8 (2013) CPM-11 (2016) CPM-14 (2019)	3rd term / 3 years	2022
	Brazil	Mr. Andre Felipe Carrapatoso Peralta DA SILVA	CPM-14 (2019)	1st term / 3 years	2022
	Costa Rica	Mr Hernando Morera GONZÁLEZ	CPM-13 (2018)	1st term / 3 years	2021
	Chile	Mr Álvaro SEPÚLVEDA LUQUE	CPM-10 (2015) CPM-13 (2018)	2nd term / 3 years	2021
Near East	Lebanon	Mr Nicholas EID	CPM-14 (2019)	1st term / 3 years	2022
	Syria	Mr Ouroba Alzitani ABOALBORGHOL	CPM-13 (2018)	1st term / 3 years	2021
	Sudan	Abdelmoneim Ismail ADRA ABDETAM	CPM-13 (2018)	1st term / 3 years	2021
	Iraq	Mr Abdulqader Khudhair ABBAS	CPM -13 (2018)	1st term / 3 years	2021
North America	Canada	Mr Rajesh RAMARATHNAM	CPM-11 (2016) CPM-14 (2019)	2nd term / 3 years	2022
	USA	Ms Marina ZLOTINA	CPM-10 (2015) CPM-13 (2018)	2nd term / 3 years	2021
Southwest Pacific	Australia	Ms Sophie Alexia PETERSON	Replacement member for Mr Bruce HANCOCKS CPM-12 (2017)	1st term / 3 years	2020
	New Zealand	Ms Joanne WILSON	CPM-14 (2019)	1st term / 3 years	2022
	Samoa	Mr Lupeomanu Pelenato FONOTI	CPM-12 (2017)	1st term / 3 years	2020

TABLE. 4 Standards Committee Potential Replacements

Region	Country	Name	Nominated / Re-nominated	Current term/duration	Term expires
Africa	1 Guinea Bissau	Mr Lois Antonio TAVARES	CPM-12 (2017)	1st term / 3 years	2020
	2 Burundi	Mr Eliakim SAKAYOYA	CPM-11 (2016) CPM-14 (2019)	2nd term / 3 years	2022
Asia	1 Philippines	Mr. Gerald Glenn F. PANGANIBAN	CPM-19 (2014)	1st term / 3 years	2022
	2	VACANT			
Europe	1 Estonia	Ms Olga LAVRENTJEVA	CPM-12 (2017)	1st term / 3 years	2020
	2 Russia	Mr Kostantin KORNEV	CPM-19 (2014)	1st term / 3 years	2022
Latin America and Caribbean	1 Panama	Ms Judith Ivette VARGAS AZCÁRRAGA	CPM-9 (2014) CPM-12 (2017)	1st term / 3 years	2020
	2 Dominica	Mr Nelson LAVILLE	CPM-11 (2016)	1st term / 3 years	2019
Near East	1Egypt	Mr Nader ELBADRY	CPM-14 (2019)	1st term / 3 years	2022
	2 United Arab Emirates	Ms Fatima Sad AL KALABANI	CPM-13 (2018)	1st term / 3 years	2021
North America	1 Canada	Mr Steve CÔTÉ	CPM-13 (2018)	1st term / 3 years	2021
	2 USA	Ms Stephanie DUBON	CPM-11 (2016) CPM-14 (2019)	2nd term / 3 years	2022

Region	Country	Name	Nominated / Re-nominated	Current term/duration	Term expires
Southwest Pacific	1 To replace New Zealand or Australia	Ms Sophie Alexia PETERSON	CPM-12 (2017)	1st term / 3 years	2020
	2 Fiji	Mr Nitesh DATT	CPM-13 (2018)	1st term / 3 years	2021

Appendix 06 – Guidelines for IPPC Regional Workshops

(Noted by CPM-14 (2019), agreed by the CPM Bureau in June 2018)

[1] The objectives of IPPC Regional Workshops are:

1) To analyse and prepare comments on draft ISPMs;
2) To build phytosanitary capacity and raise awareness on various activities of the IPPC Community; and
3) To provide a forum to exchanging experiences and ideas at the regional level.

[2] The workshop is normally for three days and the agenda includes the following:

1) IPPC Secretariat updates;
2) Discussion and formulation on draft International Standards for Phytosanitary Measures (ISPMs) for first and second consultation;
3) Phytosanitary capacity and raising awareness on all activities related to the IPPC community and exchanging regional experiences.

[3] Some regions may include additional day(s) for a field visit and/or to discuss issues of regional importance.

General

[4] Each IPPC Regional Workshop has organizational, logistical and funding peculiarities and efforts should be made to find a balance between addressing global and regional issues.

[5] The organization of the workshops include the following:

1) A regional workshop organizing committee should be established for each workshop and should be composed of the IPPC Secretariat, a representative from the Standards Committee (SC) and the Implementation and Capacity Development Committee (IC) and co-organizers which are representatives of RPPO(s), FAO regional and sub-regional offices, hosting country and any other relevant organizations supporting the workshop;
2) Each organizing committee and participant are encouraged to make efforts to help secure funding for their workshop;
3) These workshops will be named "IPPC Regional Workshop" for consistency and to help ensure the globally visibility of the IPPC. When other governments or institutions provide substantial financial support, their name may be inserted after IPPC, e.g. IPPC-[Institution's Name] Joint Regional Workshop;
4) Efforts should be made to ensure that at least one SC and one IC member are present;
5) Workshops comments on draft ISPMs should be submitted through the Online Comment System (OCS).

Roles and responsibilities of the different parties involved

IPPC Contact Point:

1) Nominates an individual(s) with the appropriate expertise to attend the workshop;
2) Mobilizes resources (full or at least partial) for the attendance of the designated participant;
3) Ensures that the participant selected to represent the NPPO in the workshop has analysed, before attending the workshop, the draft ISPMs and other documents and completed pre workshop exercises;

4) Ensures the NPPO's comments on draft ISPMs are entered into the Online Comment System (OCS) before the workshop;
5) If applicable, after the workshop, provides additional NPPO's comments on draft ISPMs to the IPPC Secretariat, by 30 September of each year or at least submits one general comment for each draft ISPM.

Workshop participant:

1) Mobilizes resources (full or at least partial) for his/her participation in the workshop;
2) Analyses draft ISPMs and other documents available and works towards agreed country comments, before attending the workshop;
3) Attends all sessions planned in the program of the workshop and participates actively in the discussions;
4) Provides comments on the draft ISPMs, and shares them within their region using the OCS before attending the workshop;
5) Practices using the OCS before attending the workshop. Guidelines on how to use the OCS are available at https://www.ippc.int/en/online-comment-system;
6) Conducts all pre-training activities and prepares all the requested information to be shared and discussed during the workshop;
7) Provides feedback to the workshop evaluation;
8) Shares information and results on the workshop within their NPPO after the workshop.

The Standards Committee Steward for draft ISPMs prepares a concise presentation on the draft ISPM explaining the key issues discussed during the development of the draft. This presentation should be provided to the IPPC Secretariat by the 15th of June.

[6] ***A Standards Committee representative*** is designated to attend the workshop, as agreed by the SC. They deliver the presentations related to the draft ISPMs and participate in discussions related to the standard setting procedures.

[7] ***An Implementation and Capacity Development Committee representative*** is designated to attend the workshop, as agreed by the IC. They deliver the presentations related to implementation and capacity development activities and exercises, and participate in discussions related to implementation and capacity development.

[8] ***A Chair and a rapporteur*** are to be elected by the participants. The role of the Chair is to facilitate discussions. The role of the rapporteur is to prepare the workshop report jointly with the Chair and the IPPC Secretariat. The report should be approved by the participants during or shortly after the meeting.

[9] ***The Online Comment System (OCS) expert*** is selected by the organizing committee. They are responsible to ensure that contracting parties provide comments through the OCS prior to the workshop, present and/or demonstrate how to best utilize the OCS, gather comments during the workshop and provide support to countries to submit comments after the workshop.

Co-organizers

1) liaise with contracting parties to comment on the draft agenda;
2) provide the facilities needed for the workshop;
3) provide additional logistical arrangements, as agreed with the IPPC Secretariat;
4) provide funds or help mobilize resources;

[10] ***Resource persons*** may be invited by the organizing committees. These includes Bureau members, stewards or experts from their regions or other regions and they may participate in discussions. A resource person should not influence discussions on regional issues, particularly comments on draft ISPMs.

[11] The organizing committee may agree to invite ***observers*** from relevant international organizations and NPPOs outside the region. Observers should not influence discussions on regional issues, particularly comments on draft ISPMs.

The IPPC Secretariat:

1) Develops a draft agenda through a consultation process with the SC, the IC and the Technical Consultation of Regional Plant Protection Organizations (TC-RPPOs). Subsequently, a draft agenda is circulated within the IPPC Secretariat and to all regional workshop co-organizers for further consultation;
2) Establishes an organizing committee for each workshop;
3) Establishes strong collaboration with co-organizers in the regions and discusses all logistical and financial arrangements well in advance;
4) Provides templates and prepares relevant presentations, training material and videos;
5) Coordinates the overall organization of IPPC Regional Workshops. This requires a consistent coordination at the IPPC Secretariat level including joint work between all units of the Secretariat, and between administrative and professional staff;
6) Organizes internal meetings for all IPPC Secretariat staff to become familiar with the regional workshops presentations, as well as training on the use of the OCS;
7) Drafts invitation letters; regions may wish to send their own invitation letter, if so, a copy of their regional letter should be sent to the Secretariat. In addition, a list of intended recipients should be sent to the IPPC Secretariat to help ensure that all contracting parties from the region are invited (regardless of whether they are funded or not);
8) Templates and publishes the report on the IPP up to two months after the workshop;
9) Develops and publishes a news item about the workshop on the IPP no later than 2 weeks after the workshop;
10) Develops and delivers a survey to collect feedback from participants to be used for improving the content and organization of the workshops;
11) Provides a summary of the workshops and information from the evaluation to the Commission on Phytosanitary Measures.

Appendix 07 – Recognition related to Standard Setting activities

[1] We would like to express gratitude to the experts of the drafting groups for their active contribution in the development of the following ISPMs, or Annexes to ISPMs, adopted in 2018/2019:

Table 1: ISPM on Requirements for the use of Fumigation as a phytosanitary measure (2014-004)

Country	Expert Name	Role
Israel	Mr David OPATOWSKI	Steward (2016-11), TPPT Steward
China	Mr Yuejin WANG	Steward (2014-05), Assistant Steward, (2016-11) TPPT member
New Zealand	Mr Michael ORMSBY	Assistant Steward (2014-05), TPPT member
USA	Mr Guy HALLMAN	TPPT member
Argentina	Mr Eduardo WILLINK	TPPT member
USA	Mr Scott MYERS	TPPT member
Australia	Mr Matthew SMYTH	TPPT member
Australia	Mr Glenn BOWMAN	TPPT member
China	Mr Daojian YU	TPPT member
Japan	Mr Toshiyuki DOHINO	TPPT member
USA	Mr Patrick GOMES	TPPT member
IAEA	Mr Andrew PARKER	TPPT member

Table 2: ISPM on 2017 amendments to ISPM 5 (*Glossary of phytosanitary terms*) (1994-001)

Country	Expert Name	Role
France	Ms Laurence BOUHOT-DELDUC	TPG Steward
USA	Ms Stephanie BLOEM	TPG English
New Zealand	Mr John HEDLEY	TPG English
Uruguay	Ms Beatriz MELCHO	TPG Spanish
China	Ms Hong NING	TPG Chinese
Denmark	Mr Ebbe NORDBO	TPG English, Assistant Steward
Egypt	Ms Shaza Roushdy OMAR	TPG Arabic
France	Mr Andrei ORLINSKI	TPG Russian

Table 3: ISPMs developed by the Technical Panel on Diagnostic Protocols as annexes to ISPM 27 (*Diagnostic protocols for regulated pests*)

Table 3-A: TPDP Steward:

Country	Steward Name
UK	Ms Jane Chard
Sri Lanka	Ms Jayani Nimanthika WATHAKURAGE

***Table 5-B:* Revision of DP2: *Plum pox virus* (2016-007)**

Country	Expert	Role
Canada	Mr Delano JAMES	Discipline lead, Lead author
Australia	Mr Brendan RODONI	Referee
Spain	Mr Mariano CAMBRA	Co-author
Spain	Mr Antonio OLMOS	Co-author

Table 5-C: DP 25: *Xylella fastidiosa* (2004-024)

Country	Expert	Role
France	Ms Géraldine ANTHOINE	Discipline lead
New Zealand	Mr Robert TAYLOR	Discipline lead, Lead author
Australia	Mr Brendan RODONI	Referee
USA	Mr Wenbin LI	Co-author
Austria	Ms Helga REISENZEIN	Co-author
USA	Mr John HARTUNG	Co-author

Table 5-D: DP 26: *Austropuccinia psidii* (2006-018)

Country	Expert	Role
New Zealand	Mr Robert TALOR	Discipline lead, Referee
Netherlands	Mr Hans DE GRUYTER	Referee, Discipline lead
Australia	Ms Jacqueline EDWARDS	Lead author
USA	Mr José HERNANDEZ	Co-author
Australia	Mr Morag GLEN	Co-author
France	Ms Jacqueline HUBERT	Co-author
Nigeria	Mr Kazeem SHAKIRU ADEWALE	Co-author

Table 5-E: DP 27: *Ips* spp. (2006-020)

Country	Expert	Role
USA	Mr Norman BARR	Discipline lead
China	Ms Liping YIN	Referee
Canada	Mr Hume DOUGLAS	Lead author
USA	Mr Anthony COGNATO	Co-author
Netherlands	Ms Brigitta WESSELS-BERK	Co-author
Jamaica	Ms Juliet GOLDSMITH	TPDP expert

Table 5-F: DP 28: *Conotrachelus nenuphar* (2013-002)

Country	Expert	Role
USA	Mr Norman BARR	Discipline lead
France	Ms Géraldine ANTHOINE	Referee
USA	Mr Samuel N. CRANE	Lead author
USA	Mr Charles W. O'BRIEN	Co-author
Jamaica	Ms Juliet GOLDSMITH	Co-author

Appendix 08 – Ink amendments to ensure a consistent use of "contamination" and its derivatives in adopted ISPMs (English only)

Table 1: Ink amendments to avoid using "contamination" or its derivatives where the intended meaning does not correspond to the Glossary definition of "contamination"

ISPM	ISPM SECTION	CURRENT TEXT	PROPOSED INK AMENDMENT	EXPLANATION
ISPM 11 (*Pest risk analysis for quarantine pests*)	1.1 Initiation points	*S2* The types of LMOs that an NPPO may be asked to assess for phytosanitary risk include: - plants for use (a) as agricultural crops, for food and feed, ornamental plants or managed forests; (b) in bioremediation (as an organism that cleans up contamination) […]	*S2* The types of LMOs that an NPPO may be asked to assess for phytosanitary risk include: - plants for use (a) as agricultural crops, for food and feed, ornamental plants or managed forests; (b) in bioremediation (as an organism that cleans up ~~contamination~~ pollution) […]	"Contamination" is not used according to its Glossary definition. Bioremediation is a waste management technique that involves the use of organisms to neutralize pollutants from a contaminated site. According to EPA (USA), bioremediation is a "treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non-toxic substances". Therefore, the suggested ink amendment is to replace the term "contamination" by the word "pollution".
ISPM 18 (*Guidelines for the use of irradiation as a phytosanitary measure*)	ANNEX 2 Criteria *3. Product handling, storage and segregation*	Commodities are handled in an environment that does not increase the risk of contamination from physical, chemical or biological hazards	Commodities are handled in an environment that does not increase the risk ~~of contamination~~ from physical, chemical or biological hazards	"Contamination" is not used according to its Glossary definition. The suggested ink amendment is to avoid using "contamination".
ISPM 21 (*Pest risk analysis for regulated non-quarantine pests*)	3.3.1 Pest effects	In some cases, economic consequences may only become apparent after a long period of time (e.g. a degenerative disease in a perennial crop, a pest with a long-lived resting stage). Furthermore, the infestation in the plants may result in contamination of places of production with a consequential impact on future crops. […]	In some cases, economic consequences may only become apparent after a long period of time (e.g. a degenerative disease in a perennial crop, a pest with a long-lived resting stage). Furthermore, the infestation in the plants may result in the pest remaining in the ~~contamination of~~ places of production with a consequential impact on future crops. […]	"Contamination" is not considered to be used strictly according to its Glossary definition, in which "places of production" are not listed. Rewording is suggested to avoid using "contamination".
ISPM 33 (*Pest free potato (*Solanum *spp.) micropropagative*	3.1 Establishment of pest free potato micropropagative material	[…] In addition to the laboratory testing procedure for regulated pests described below, potato micropropagative material should be inspected and found free from other	[…] In addition to the laboratory testing procedure for regulated pests described below, potato micropropagative material should be inspected and found free from other pests or their symptoms and from microbes in general ~~microbial contamination~~.	"Contamination" is not used according to its Glossary definition. Rewording is suggested to avoid using "contamination".

ISPM	ISPM SECTION	CURRENT TEXT	PROPOSED INK AMENDMENT	EXPLANATION
material and minitubers for international trade)		pests or their symptoms and general microbial contamination.		
ISPM 36 (*Integrated measures for plants for planting*)	APPENDIX 1 Table 1	6 Pests spread by water - Use of uncontaminated water sources, free of pests	6 Pests spread by water - Use of ~~uncontaminated~~ water sources~~,~~ free of pests	"Uncontaminated" is not considered to be used strictly according to the Glossary definition of "contamination", in which "water sources" are not listed. Furthermore, the word is redundant. Rewording is suggested to avoid using "uncontaminated".

Table 2: Ink amendments to ISPM 2 (*Framework for pest risk analysis*) where the term "contamination" is used in relation to biological control agents or beneficial organisms, but the use of "contaminants" is more appropriate

ISPM	ISPM SECTION	CURRENT TEXT	PROPOSED INK AMENDMENT	EXPLANATION
ISPM 2 (*Framework for pest risk analysis*)	1.2.2 Biological control agents and other beneficial organisms	Biological control agents and other beneficial organisms are intended to be beneficial to plants. Thus, when performing a PRA, the main concern is to look for potential injury to non-target organisms. Other concerns may include: - contamination of cultures of beneficial organisms with other species, the culture thereby acting as a pathway for pests	Biological control agents and other beneficial organisms are intended to be beneficial to plants. Thus, when performing a PRA, the main concern is to look for potential injury to non-target organisms. Other concerns may include: - ~~contamination~~ presence of other species as contaminants of cultures of beneficial organisms ~~with other species~~, the culture thereby acting as a pathway for pests.	"Contamination" is not used according its Glossary definition. The wording proposed instead uses "contaminants" because it is a word commonly used in this context and well understood. This is also in line with the note on "contaminant" in the *General recommendation in the use of terms in ISPMs* (as proposed by the TPG in their December 2017 meeting).

Table 3: Ink amendments to adopted ISPMs where "contaminant" is used but the Glossary terms "contamination" or "contaminating pest" should be used instead

ISPM	ISPM SECTION	CURRENT TEXT	PROPOSED INK AMENDMENT	EXPLANATION
ISPM 2 (*Framework for pest risk analysis*)	1.2.1 Plants as pests	Plants as pests may also be introduced unintentionally into a country, for example as contaminants of seeds for sowing, grain for consumption or fodder, wool, soil, machinery, equipment, vehicles, containers or ballast water.	Plants as pests may also be introduced unintentionally into a country, for example as ~~contaminants of~~ contaminating pests with seeds for sowing, grain for consumption or fodder, wool, soil, machinery, equipment, vehicles, containers or ballast water.	The meaning corresponds to that of the Glossary term "contaminating pest".
ISPM 11 *(Pest risk analysis for quarantine pests*)	ANNEX 4 Introduction	This annex provides specific guidance on conducting PRA to determine if a plant is a pest of ….. It focuses primarily on plants proposed for import, whether as plants for planting or for other intended uses. It does not cover the unintentional introduction of plants as contaminants in commodities or conveyances.	This annex provides specific guidance on conducting PRA to determine if a plant is a pest of… It focuses primarily on plants proposed for import, whether as plants for planting or for other intended uses. It does not cover the unintentional introduction of plants as ~~contaminants~~ contaminating pests in commodities or conveyances.	The meaning corresponds to that of the Glossary term "contaminating pest".
ISPM 11	ANNEX 4 *Probability of spread (refer to section 2.2.3)*	The likelihood and extent of spread depends on natural and human-mediated factors. […] Human-mediated factors, whether intentional or unintentional, may include: - intended use, consumer demand, economic value and ease of transport - the movement of propagules as a contaminant of soil or other materials (e.g. clothing, conveyances, machinery, tools, equipment)	The likelihood and extent of spread depends on natural and human-mediated factors. […] Human-mediated factors, whether intentional or unintentional, may include: - intended use, consumer demand, economic value and ease of transport - the movement of propagules of contaminating pests ~~as a contaminant of~~ with soil or other materials (e.g. clothing, conveyances, machinery, tools, equipment)	The meaning corresponds to that of the Glossary term "contaminating pest".
ISPM 14 (*The use of integrated measures in a systems approach for pest risk management*)	3. Relationship with PRA and Available Risk Management Options	**Harvest** - sanitation (e.g. removal of contaminants, "trash")	**Harvest** - sanitation (e.g. removal of contamination ~~contaminants~~, "trash")	The meaning corresponds to that of the Glossary term "contamination".

ISPM	ISPM SECTION	CURRENT TEXT	PROPOSED INK AMENDMENT	EXPLANATION
ISPM 20 (*Guidelines for a phytosanitary import regulatory system*)	5.1.6.2 Emergency action	Emergency action may be required in a new or unexpected phytosanitary situation, such as the detection of quarantine pests or potential quarantine pests: - as contaminants of conveyances, storage places or other places involved with imported commodities.	Emergency action may be required in a new or unexpected phytosanitary situation, such as the detection of quarantine pests or potential quarantine pests: - as ~~contaminants~~ contaminating pests of conveyances, storage places or other places involved with imported commodities.	The meaning corresponds to that of the Glossary term “contaminating pest”.
ISPM 23 (*Guidelines for inspection*)	2.3.2 Compliance of phytosanitary requirements	Inspection can be used to verify the compliance with some phytosanitary requirements. Examples include: freedom from contaminants (e.g. leaves, soil)	Inspection can be used to verify the compliance with some phytosanitary requirements. Examples include: - freedom from ~~contaminants~~contamination (e.g. leaves, soil)	The meaning corresponds to that of the Glossary term “contamination”.
ISPM 33 (*Pest free potato (*Solanum *spp.) micropropagative material and minitubers for international trade*)	Annex 2 Operating procedures	- a monitoring programme to check the level of air-borne contaminants in the subculture room, cabinets and growth room	- a monitoring programme to check the level of air-borne ~~contaminants~~contamination in the subculture room, cabinets and growth room	The meaning corresponds to that of the Glossary term “contamination”.
ISPM 41 (*International movement of used vehicles, machinery and equipment*)	Appendix 2 **Category:** Agricultural, forestry and horticultural used VME, such as:	**Contamination notes:** Contaminants: soil, pests.	**Contamination notes:** ~~Contaminants~~ Contamination by: soil, pests	The meaning corresponds to that of the Glossary term “contamination”.
ISPM 41	Appendix 2 **Category:** Earth moving used VME, such as: - bulldozers - graders	**Contamination notes:** Soil is the main contaminant; pests, plant debris and seeds can also be contaminants	**Contamination notes:** ~~Soil is the main contaminant;~~ Contamination mainly by soil; but also by pests, plant debris and seeds ~~can also be contaminants~~	The meaning corresponds to that of the Glossary term “contamination”.

ISPM	ISPM SECTION	CURRENT TEXT	PROPOSED INK AMENDMENT	EXPLANATION
	- surface mining equipment. Reconditioned or field-tested used VME are included. Pest risk is variable, but high levels of contamination may occur in this category			
ISPM 41	Appendix 2 **Category:** Used military VME, such as:	**Contamination notes:** Contaminants: soil, pests […]	**Contamination notes:** ~~Contaminants~~ Contamination by: soil, pests […]	The meaning corresponds to that of the Glossary term “contamination”.
ISPM 41	Appendix 2 **Category:** Waste management used VME, such as:	**Contamination notes:** Organic waste debris is the main contaminant, including: soil, pests […]	**Contamination notes:** Contamination mainly by o~~O~~rganic waste debris ~~is the main contaminant~~, including: soil, pests […]	The meaning corresponds to that of the Glossary term “contamination”.
ISPM 41	Appendix 2 **Category:** Deep mining used VME.	The most likely contaminants are soil and to a lesser extent pests. Pest risk is generally low unless used VME are contaminated with surface soil […]	~~The~~ Contamination is most likely ~~contaminants are~~ by soil and to a lesser extent by pests. Pest risk is generally low unless used VME are contaminated with surface soil […]	The meaning corresponds to that of the Glossary term “contamination”.
ISPM 41	Appendix 2 **Category:** Used vehicles, such as: - cars, vans, trucks, buses	**Contamination notes:** Contaminants: soil, pests […]	**Contamination notes:** ~~Contaminants~~ Contamination by: soil, pests […]	The meaning corresponds to that of the Glossary term “contamination”.

Appendix 09 - TFT recommendation to the CPM on proposed topics from 2018 Call for Topics: Standards and Implementation

[1] For detailed information on TFT discussions please refer to TFT meeting reports available at: https://www.ippc.int/en/core-activities/standards-and-implementation/call-for-topics-standards-and-implementation/task-force-on-topics/. Abbreviations used: N/A, not applicable; TBD, to be determined

Table1: Topics that were recommended by TFT, discussed and included by the SC in *List of Topics for IPPC Standards*[60]:

Row No.	Topic No.	Title	Submitter (Support)	TFT recommended material	TFT recommended priority
1	2018-010	Amendment to ISPM 5. Revision on the term "Incidence".	USA (Australia, New Zealand, Canada)	Glossary term (deletion)	N/A
2	2018-006	*Mononychelus tanajoa*	CAHFSA	Diagnostic Protocol	1
3	2018-025	*Citrus leprosis viruses*	CAHFSA	Diagnostic Protocol	1
4	2018-030	Psyllid vectors of *Candidatus* Liberibacter solanacearum	Japan	Diagnostic Protocol	1
5	2018-031	Pospiviroid species (except *Potato spindle tuber viroid* (DP 7))	Japan	Diagnostic Protocol	2
6	2018-032	*Acidovorax avenae* subsp. *Citrulli*	Japan	Diagnostic Protocol	2
7	2018-019	*Meloidogyne mali*	China	Diagnostic Protocol	3
8	2018-015	*Cronartium comandrae* Peck	China	Diagnostic Protocol	4

Table 2: Topics for Standards and Implementation recommended by TFT to CPM for adoption and to be added to the IC and SC lists of topics

Row No.	Topic No.	Title	Submitter (Support)	TFT recommended material	TFT recommended priority
9	2018-008	Development and implementation of regulations and legislation to manage phytosanitary risks on regulated articles for NPPOs	Australia	Implementation (Guide)	1
10	2018-009	ISPM 38- International movement of seeds: Annex 1 - Design and use of systems approaches for phytosanitary certification of seeds	NAPPO (Australia, Chile, USA, CAHFSA, OIRSA, ASTA, ISF, CSTA, SAA)	Standard (Annex)	1
11	2018-026	Safe Import of Food and Other Aid	PPPO	CPM Recommendation (for adoption during ministerial CPM in 2020)	1
12	2018-028	Developing Phytosanitary Security Procedures	New Zealand	Implementation (Guide)	1
13	2018-036	Guidance on assessing the risk of introduction of pests with seeds	EPPO	Implementation guide for ISPM 11	1
14	2018-037	Guidelines for surveillance of *Xylella fastidiosa*	EPPO	Implementation (Guide)	1
15	2018-038	Guidelines for inspection of consignments for *Xylella fastidiosa* at points of entry	EPPO	Implementation (Guide)	1

[60] https://www.ippc.int/en/core-activities/standards-setting/list-topics-ippc-standards/

Row No.	Topic No.	Title	Submitter (Support)	TFT recommended material	TFT recommended priority
16	2018-012	ISPM 15 implementation guidelines for non-compliance	USA	Implementation (Guide)	2
17	2018-017	Guidelines for the management of plants and plant products carried by entry passengers	China	Implementation materials (Awareness materials)	2
18	2018-027	Managing non-compliant treated consignments	New Zealand	Implementation (Guide)	2
19	2018-040	IPPC Guide on the development and implementation of programmes for the authorization of entities to perform phytosanitary actions	Canada (USA)	Implementation (Guide)	2
20	2018-007	Implementation of official control (ISPM 5; Supplement 1) and pest free areas (ISPM 4).	Australia	Implementation (Guide)	3
21	2018-011	Criteria for the determination of host status for fruit flies based on available information (Annex to ISPM 37).	USA	Standard (Annex)	3
22	2018-022	Risk based inspection of imported consignments	Republic of Korea (NAPPO)	TBD	pending
23	2018-013	Guidelines for designing of plant quarantine laboratories	China	Implementation (Reference material)	4
24	2018-014	Guidelines for Phytosanitary of International Mail Items	China	Standard	4
25	2018-021	Requirement for phytosanitary certificate on cross-border online-shopping plants, plant products and other regulated articles.	China	Standard	4
26	2018-023	Smart phone application to monitor *Xylella fastidiosa* for all relevant stakeholders and a mapping system to follow up on its global distribution	Libya	Tool available on the IPP[61]	4

Table 3: Topics not recommended by TFT

Row No.	Topic No.	Title	Submitter
27	2018-005	ISPM 5 definition of term "Harmful Organism"	Sri Lanka
28	2018-016	ISPM 27 Annex DP: *Cernuella virgata* (Da Costa, 1778)	China
29	2018-018	ISPM 27 Annex DP: *Hylotrupes bajulus*	China
30	2018-020	ISPM 27 Annex DP: Pathogenic fungus of Bull's-eye rot on apple（*Neofabraea* spp.）	China
31	2018-024	Pest free olive plants (*Olea europaea*) for international trade	Libya
32	2018-029	Guidelines for field inspection	Japan
33	2018-033	Symposium on implementation of the Convention and ISPMs	Japan
34	2018-034	Advocacy materials on ePhyto	Japan
35	2018-035	Revision of ISPM26 : Establishment of Pest Free Areas for Fruit Flies (Tephritidae)	Japan
36	2018-039	Amendment to ISPM 39 to address wooden logs with bark	India

[61] IPPC Implementation and Capacity Development Guides and training materials: https://www.ippc.int/en/core-activities/capacity-development/guides-and-training-materials/

Appendix 10 – Adoption of International Standards for Phytosanitary Measures (ISPMs) and the CPM Recommendation

[1] The CPM adopted the following ISPMs (attached to this report):

- ISPM 43 (*Requirements for the use Fumigation as a phytosanitary measure* (2014-004)
- 2017 amendments to ISPM 5 (*Glossary of phytosanitary terms*) (1994-001).

[2] The CPM noted that the SC adopted on behalf of CPM the following two diagnostic protocols (DPs) as Annexes to ISPM 27 (*Diagnostic protocols for regulated pests*) (attached to this report, and in English only):

- DP 2 revision: Plum pox virus (2016-007)
- DP 25: *Xylella fastidiosa* (2004-024)
- DP 26: *Austropuccinia psidii* (2006-018)
- DP 27: *Ips* spp. (2006-020)
- DP 28: *Conotrachelus nenuphar* (2013-002)
- DP 29: *Bactrocera dorsalis* (2006-026)

[3] The CPM adopted the following modified CPM Recommendation (attached to this report):

- High-throughput sequencing (HTS) technologies as a diagnostic tool for phytosanitary purposes.

DRAFT ISPM: *REQUIREMENTS FOR THE USE OF FUMIGATION AS A PHYTOSANITARY MEASURE* (2014-004)

Status box

This is not an official part of the standard and it will be modified by the IPPC Secretariat after adoption.	
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Adoption

[Text to this paragraph will be added following adoption.]

INTRODUCTION

Scope

[1] This standard provides technical guidance for national plant protection organizations (NPPOs) on the application of fumigation as a phytosanitary measure, encompassing treatments with chemicals that reach the commodity in a gaseous state. This standard also provides guidance for NPPOs on the authorization of treatment providers to conduct fumigation.

[2] This standard does not provide details on specific treatments with specific fumigants. Application of modified atmosphere when not in combination with fumigation is not part of this standard.

References

[3] The present standard refers to ISPMs. ISPMs are available on the International Phytosanitary Portal (IPP) at https://www.ippc.int/core-activities/standards-setting/ispms.

CPM R-03. 2017. Replacement or reduction of the use of methyl bromide as a phytosanitary measure. CPM Recommendation. Rome, IPPC, FAO. Available at https://www.ippc.int/en/publications/84230/ (last accessed 27 November 2018).

Definitions

[4] Definitions of phytosanitary terms used in this standard can be found in ISPM 5 (*Glossary of phytosanitary terms*).

Outline of Requirements

[5] NPPOs should ensure that the fumigation application is carried out effectively so that critical parameters are at the required level throughout the commodity to achieve the stated efficacy.

[6] The requirements for the application of fumigation, the use of fumigation equipment and the fumigation procedures should be met. Systems should be implemented to prevent the infestation or contamination of the fumigated commodity. Record keeping and documentation requirements should be met to enable auditing, verification or trace-back.

[7] The roles and responsibilities of parties involved in fumigation are described. Guidance is provided to NPPOs on responsibilities for authorizing, monitoring and auditing treatment providers.

BACKGROUND

[8] The purpose of this standard is to provide generic requirements for the application of fumigation as a phytosanitary measure, specifically for those treatments adopted under ISPM 28 (*Phytosanitary treatments for regulated pests*).

[9] ISPM 28 was adopted to harmonize effective phytosanitary treatments over a wide range of circumstances and to enhance the mutual recognition of treatment efficacy by NPPOs, which may facilitate trade. ISPM 28 provides requirements for submission and evaluation of efficacy data and other relevant information on phytosanitary treatments, and annexes with specific fumigations that have been evaluated and adopted by the Commission on Phytosanitary Measures.

[10] Fumigation is considered to be effective when the specific concentration of fumigant, at the minimum temperature and duration required for the stated efficacy, is achieved in the area of lowest concentration

of the fumigant within a fumigation enclosure. The effectiveness of the treatment process as a whole also includes measures applied to prevent infestation or contamination after fumigation.

IMPACTS ON BIODIVERSITY AND THE ENVIRONMENT

[11] Historically, fumigation has been widely applied to prevent the introduction and spread of regulated pests and has, therefore, been beneficial to biodiversity. However, fumigant gases, such as methyl bromide and sulphuryl fluoride, may have negative impacts on the environment. For example, the emission of methyl bromide into the atmosphere is known to deplete the ozone layer and sulphuryl fluoride is a recognized greenhouse gas. The CPM Recommendation on the replacement or reduction of the use of methyl bromide as a phytosanitary measure (CPM R-03, 2017) encourages contracting parties to use alternatives, where possible. Environmental impacts of fumigants can be mitigated through the use of destruction (chemical breakdown) or recapture technology to reduce gas emissions.

REQUIREMENTS

1. Fumigation Objective

[12] The objective of using fumigation as a phytosanitary measure is to achieve pest mortality at a specified efficacy.

2. Fumigation Application

[13] Fumigation is undertaken by treatment providers or the NPPO either in a treatment facility or at other suitable locations (e.g. cargo ship holds, shipping containers, warehouses and under tarpaulin).

[14] Fumigation may be applied at any point along the supply chain, for example:

- as an integral part of production or packaging operations
- after packaging (e.g. once the commodity is packaged for dispatch)
- during storage
- just before dispatch (e.g. at centralized locations at a port)
- during transport
- upon arrival in the importing country (before or after unloading).

[15] The fumigation procedure should ensure that the critical parameters (e.g. concentration or dose, temperature, duration) are at the required level throughout the commodity, allowing the stated efficacy to be achieved.

[16] Fumigation efficacy may be affected by factors such as the moisture content of the commodity and, within the enclosure used for the fumigation, the humidity, pressure, and changes in the atmospheric gas composition created by the packaging or by the commodity. Other factors to consider during fumigation are the penetration of the fumigant, sorption of the fumigant by the packaging or the commodity, fumigant specific gravity, circulation of the fumigant and leakage from the fumigation enclosure. For circulation of fumigants, the size of the enclosure and differences in the loading configuration between commodities loaded in boxes with spacing and commodities loaded in bulk should be taken into account.

[17] Some fumigants react with certain commodities or materials and this needs to be taken into consideration before fumigation (e.g. phosphine reacts strongly with copper and other metals, and may affect electronics used in verification equipment or in fans).

[18] The procedures approved by the NPPO for the application of a treatment should be clearly documented. These procedures should be designed to ensure that the critical parameters stated in the treatment schedule are achieved. The procedures should include the process of pre- and post-conditioning to reach the required dose, where these processes are critical to the treatment in achieving the required efficacy against the target pests while preserving commodity quality. They should also include contingency

procedures and guidance on corrective actions for treatment failures or problems with critical treatment parameters.

2.1 Single fumigant treatments

[19] The most common fumigations used are those that apply a single fumigant. General-use fumigants rely on a mode of action that is generally effective against all pest groups or against one particular group (e.g. arthropods, fungi, nematodes) and all or most life stages. Treatment schedules for single fumigants are generally simple, requiring a single application to achieve a required minimum concentration over a required duration to achieve the specified efficacy. A list of commonly used fumigants and their chemical properties is provided in Appendix 1.

2.2 Combination treatments

[20] Where a single fumigant may not achieve the required efficacy without rendering the commodity unmarketable, or for reasons of economy or logistics, another fumigant or treatment may be included in the treatment schedule.

[21] Another treatment may be applied sequentially immediately before or after fumigation to increase the effectiveness of the combination treatment. For example, fumigant and temperature treatments applied sequentially may be necessary where the commodity is vulnerable to damage from the increased severity required of either treatment alone, or where the most tolerant life stage of the target pest is different for the different treatments.

[22] Concurrent combinations of a fumigant with other fumigants or other type of treatments may also be beneficial in terms of effectiveness, commodity tolerance, economics, environmental impact or logistics, compared to treatment with a single fumigant alone.

2.3 Fumigation under special conditions

[23] Fumigation may also be conducted under the following special conditions.

2.3.1 Fumigation under modified atmosphere

[24] Increasing the atmospheric carbon dioxide concentration in the enclosure used for fumigation, either alone or in combination with increasing the nitrogen and decreasing or increasing the oxygen concentration, may be used to increase the efficacy of the fumigation. Changing the atmospheric gas concentrations in this way may directly enhance target pest mortality or may increase target pest respiration thereby increasing the efficacy of fumigants such as phosphine. Reducing the concentration of oxygen in the enclosure (e.g. by replacement with non-flammable gases such as carbon dioxide or nitrogen) may also be necessary where the fumigant is flammable, such as is the case with ethyl formate.

2.3.2 Fumigation under vacuum

[25] Applying a fumigant under lower atmospheric pressure can significantly increase the rate of fumigant penetration into a commodity, resulting in increased efficacy or the ability to reduce fumigant quantity or duration of treatment. Such treatments should be carried out in purpose-built vacuum chambers that can withstand the changes in pressure and ensure minimal vacuum loss during the fumigation, and using a vacuum pump capable of attaining the atmospheric pressure required within the time frame required.

3. Enclosures and Equipment used for Fumigation

[26] There are many types and designs for equipment and enclosures used in fumigation. These vary depending on the type of fumigant used, the nature of the commodity, and the conditions of the surrounding environment. The following enclosures and equipment may be necessary to ensure that a fumigation achieves the required efficacy.

3.1 Enclosures

[27] The enclosure should be a space that can be enclosed in a manner that ensures that appropriate fumigation conditions are maintained throughout the duration of the fumigation. Examples of enclosures include purpose-built fumigation chambers, silos, freight containers, warehouses, ship's holds or tarpaulin "tents". The enclosure should be constructed from materials that maintain adequate fumigant concentrations over the fumigation period and prevent fumigant escape (e.g. materials that are not porous or absorbent to the fumigant). Openings should be sealed effectively. Porous surfaces such as sand, base rock, wood and paving (stones or blocks) are not a suitable floor for a tent enclosure.

[28] All enclosures should allow adequate access for the equipment that is required to verify that the fumigation is conducted appropriately.

3.2 Fumigation equipment

[29] All equipment used for measuring fumigation parameters should be calibrated according to the manufacturer's instructions and, where applicable, NPPO specifications.

3.2.1 Dosing equipment

[30] Dosing equipment should enable the quantitative introduction of fumigant gas into an enclosure. Dosing equipment includes an appropriately safe and secure storage vessel for the fumigant, and lines that allow the fumigant to be delivered to the enclosure, and should include a device that can either measure the rate or volume of gas flow into the enclosure (e.g. a gas mass flow-meter) or measure the volume or weight loss from the gas containers supplying the enclosure (e.g. a scale or balance). In some cases, fumigant gas can be introduced into an enclosure as a solid (e.g. magnesium phosphide tablets), or from canisters of defined volume, that release a known volume of fumigant to achieve the required dose.

3.2.2 Gas vaporizer

[31] Some fumigants are stored as a compressed liquid in a metal cylinder. Release and vaporization of a significant quantity of the liquid as required for fumigation absorbs a significant amount of energy. A vaporizer may be used to provide energy (as heat) during the vaporization of the liquid to a gas to ensure that the required amount of gas is provided to the enclosure. Depending on the fumigant, an appropriate pressure-resistant vaporizer should be used.

3.2.3 Heating equipment

[32] When it is necessary to raise the temperature of the commodity and the air within the enclosure, exposed heating sources should not be used with flammable fumigants or fumigants that decompose at high temperatures (see Appendix 1 for fumigant chemical properties).

3.2.4 Gas circulation equipment

[33] Even and quick distribution of fumigant gas introduced into the enclosure may be important for successful fumigation of a large quantity of commodity, especially with gases that diffuse relatively slowly. Rapid circulation of gas is required for the fumigation of perishable commodities or commodities that sustain damage on extended exposure to the fumigant. For such commodities, one or more fans suitable for use with a fumigant and capable of providing adequate gas circulation should be used. For bulk commodities (e.g. grain), however, it is not always possible to use fans.

3.2.5 Instruments to measure moisture content

[34] For commodities where the moisture content affects the efficacy of the treatment, the moisture content should be measured. A moisture meter gives a reading of the approximate moisture content of the commodity. As moisture content usually varies within and between the commodities within the same lot, moisture meters need only measure within 5% of the actual moisture content. There are various instruments available for measuring moisture content. Their use should be consistent with the manufacturer's instructions.

[35] To ensure that the fumigation achieves the required efficacy, it may also be necessary to use instruments that measure the environmental humidity.

3.2.6 Instruments to measure reduced pressure

[36] When fumigation is performed under vacuum, a suitable vacuum gauge, of appropriate accuracy and sensitivity, should be used to measure and record the air pressure or vacuum drawn and maintained during the exposure or testing period. Suitable vacuum gauges may include a simple U-tube manometer or a Bourdon gauge, although specialized electronic measuring instruments are also available, and should measure within 1 kPa of the actual pressure.

3.2.7 Instruments to measure temperature

[37] Calibrated thermometers should be used to measure at suitable intervals the temperature in the enclosure space and, as appropriate, the external surfaces and inside the commodity before and during fumigation. The number of temperature sensors required depends on the size of the enclosure.

3.2.8 Instruments to measure gas concentration

[38] The equipment required to measure the fumigant concentration within the enclosure depends on the type of gas used. The equipment used should have an adequate accuracy (e.g. ±5% of the fumigant concentration to be achieved throughout the fumigation). The measuring equipment (e.g. sampling lines) exposed to the fumigant should be constructed from materials that do not absorb the fumigant. Fumigant sampling lines should be placed as far as possible from fumigant supply lines or dispensers, and in the area or areas of the enclosure likely to have the lowest concentration of fumigant.

4. Fumigation Procedures

[39] Many factors may affect fumigation efficacy. These include fumigant concentration, exposure time, commodity characteristics that relate to penetration or sorption of the fumigant, commodity temperature and atmospheric temperature. Gas tightness of the enclosure, load configuration and load ratio (ratio of occupied space to the entire space) directly influence gas distribution and gas concentration during fumigation. The fumigant supply and circulation equipment (where required) should be arranged within the enclosure in a way that ensures that the fumigant concentrations required by the treatment schedule are achieved and maintained within the enclosure during fumigation.

4.1 Commodity loading

[40] Before fumigation, the commodity should be loaded into the enclosure in a manner that ensures sufficient space for adequate circulation of the fumigant. In some cases, to ensure fumigant penetration into the commodity, separators should be used. For bulk loading, adequate circulation should be ensured, for instance by means of a recirculation system.

4.2 Packaging

[41] When used, packaging should be of a composition and construction that does not preclude fumigant gas penetration to the commodity and prevent fumigant concentrations achieving required levels. If this is not the case, fumigant-impenetrable packing material or coatings should be removed or punctured to ensure adequate penetration of the fumigant. Perforated packaging should not be overlapped, as holes may become blocked.

4.3 Sorption

[42] Sorption is the process of chemically or physically binding free fumigant on or within the fumigated commodity, packaging or enclosure. Sorption by packaging or enclosure may make the fumigant unavailable to kill pests but sorption by the commodity may be necessary to kill internal feeders such as fruit flies. The sorption rate is high at the start of the fumigation, then gradually reduces as fumigation progresses. Sorption increases the time required for aeration after fumigation.

[43] Oil, fats or porous or finely ground materials may be highly sorptive materials. Highly sorptive commodities or packaging should not be fumigated unless concentration readings can be taken to ensure that the required minimum concentration is achieved.

4.4 Determination of fumigation temperature

[44] Temperature is a factor in achieving the required efficacy of fumigation, in particular because it affects the respiration rate of the target pest. In general, the lower the temperature, the lower the respiration rate of the pest and the greater the dose of the fumigant or the duration of exposure needed to achieve the required efficacy.

[45] The temperatures of the commodity and the atmosphere within the enclosure should be measured and recorded. The lowest temperature recorded in the enclosure or the commodity should be taken as the temperature at which the fumigation is undertaken.

4.5 Gas tightness test

[46] The required gas tightness of an enclosure should be based on the fumigant being used. If necessary, before fumigation (preferably immediately before), a gas tightness test should be performed. However, if the enclosure is of sufficiently resistant construction and in regular use, the testing may only be necessary at intervals of, for example, 6 or 12 months, or after a number of treatments, as specified by the NPPO.

[47] Where the gas tightness of an enclosure may not be sufficient to ensure that adequate gas concentrations are maintained throughout the fumigation period, the gas tightness should be determined by measuring the pressure half time.

4.6 Introduction of the fumigant

[48] The minimum temperature that the enclosure or commodity (whichever is less) is expected to experience over the duration of the treatment should be used when determining the dosage.

[49] The total amount of fumigant to be applied is a product of the required dosage (dose rate) and the volume of the enclosure. Correct measurement of the enclosure volume is therefore important. Excess sorption or leakage from the fumigation enclosure should be taken into consideration.

[50] A sufficient amount of fumigant should be introduced into the enclosure to ensure that the required minimum concentration, as stated in the treatment schedule, is achieved. The required amount of fumigant should be calculated with an appropriate formula: for examples, see Appendix 2.

[51] The volume of the enclosure is the internal volume and should be calculated separately for each differently shaped enclosure (see Appendix 3 for examples of shapes and formulae for calculations). The volume of containers (e.g. drums or boxes) within the enclosure that are airtight and non-absorbent to the fumigant can be subtracted from the enclosure volume.

[52] If it is required that the fumigant is introduced into the enclosure in a gaseous state, the liquid fumigant may be applied through a vaporizer (see section 3.2.2). However, some fumigants can be introduced as solids that then turn into a gaseous state (see section 3.2.1).

4.7 Measuring and recording

[53] When fumigant concentration is measured and recorded, the measurements should be used to verify whether the concentration of fumigant in the enclosure is correct and that there has been no excessive leakage or sorption of the fumigant. Fumigant concentration should be measured and recorded with sufficient frequency to provide confidence that the required dose has been achieved and maintained and to allow adequate calculations of the concentration–time product (CT) to be made (if required). Concentration readings should also be taken according to the treatment schedule to ensure that the fumigant is evenly distributed in the enclosure over the duration of the treatment.

4.7.1 Measuring and recording the fumigant concentration

[54] Where possible, sampling lines should be positioned in the places that are expected to be the most difficult for the fumigant to reach. The number of sampling lines required to adequately measure the fumigant concentration throughout the enclosure depends on the volume and nature of the enclosure. Purpose-built fumigation chambers may require fewer sampling lines than tarpaulin tent enclosures.

[55] Depending on the commodity and the treatment schedule, it may be necessary to place further sampling lines within the commodities within the enclosure. For example, a minimum of three sampling lines may be used for the first 300 m^3 of commodity, with additional lines for commodities that are tightly packed or difficult to penetrate.

4.7.2 Concentration–time product calculation

[56] The CT can be calculated in different ways (Appendix 4). The CT values obtained from a contiguous series of readings can be used to calculate the cumulative CT for the whole exposure period for that location, taking into account the interval in between the readings. The number of contiguous measurements required to obtain a suitable estimate of the CT depends on the shape of the dose curve over the duration of the treatment.

[57] If the sampling lines provide different readings of the fumigant concentration, the cumulative CT should be calculated using the lowest readings.

4.8 Completion of the fumigation

[58] Once the treatment time has been completed and the required CT, temperature and minimum concentration have been achieved, the fumigation should be considered as completed. In circumstances where a minimum CT is not initially achieved, an extension to the fumigation period or application of additional fumigant may be permitted for some fumigant types and fumigation conditions if the treatment schedule allows.

[59] Indications of fumigation success can be obtained by inspection or testing, after aeration, to verify target pest mortality. For many fumigations, an extended post-fumigation period may be required before pest mortality at the stated efficacy is achieved.

5. Adequate Systems for Treatment Facilities

[60] Confidence in the adequacy of fumigation as a phytosanitary measure is primarily based on assurance that the treatment is effective against the target pest under specific conditions and that the treatment has been properly applied. Systems for treatment delivery should be designed, used and monitored to ensure that treatments are properly conducted and commodities are protected from infestation and contamination after treatment.

[61] The NPPO of the country in which the treatments are conducted or initiated is responsible for ensuring that the system requirements are met.

5.1 Authorization of treatment providers

[62] The NPPO of the country in which the phytosanitary treatment is conducted or initiated (the latter when fumigation takes place during transport) is responsible for the authorization of treatment providers. This authorization normally includes approval of both treatment facilities and treatment providers. The NPPO should set requirements for treatment provider authorization, including training of personnel, fumigation procedures, adequate equipment and storage conditions. Specific procedures appropriate for each facility, provider and commodity treatment should also be approved by the NPPO.

[63] NPPOs should maintain a list of authorized treatment providers capable of undertaking fumigation, including, where appropriate, approved facilities.

5.2 Monitoring and auditing

[64] The NPPO of the country in which the fumigation is conducted or initiated is responsible for the monitoring and auditing of treatment facilities and providers. The NPPO should maintain an audit schedule and ensure that such audits are performed by appropriately trained personnel. Continuous supervision of fumigations should not be necessary, provided treatment procedures are properly designed and can be verified to ensure a high degree of system integrity for the facility, process and commodity in question. The monitoring and auditing should be sufficient to detect and correct deficiencies promptly.

[65] Treatment providers should meet monitoring and auditing requirements set by the NPPO. These requirements may include:

- access for the NPPO for audit, including unannounced visits
- a system to maintain and archive treatment records and provide NPPOs with access to these
- corrective action to be taken in the event of nonconformity.

5.3 Prevention of infestation after fumigation

[66] The consignment owner is responsible for prevention of infestation and contamination after fumigation and may cooperate with the treatment provider on how to achieve this. Measures should be implemented to prevent possible infestation or contamination of the commodity after fumigation. The following measures may be applied:

- keeping the commodity in a pest free enclosure
- packing the commodity immediately in pest-proof packaging
- segregating and identifying treated commodities
- dispatching the commodity as soon as possible.

5.4 Labelling

[67] Commodities may be labelled with fumigation lot numbers or other features of identification (e.g. locations of packing and the treatment facility, dates of packing and fumigation) allowing trace-back for non-compliant consignments. When used, labels should be easily identifiable and placed on visible locations.

6. Documentation

[68] The NPPO of the country in which the fumigation is conducted or initiated is responsible for ensuring that treatment providers use approved fumigants, document procedures and keep appropriate records, such as raw data on fumigant concentration and temperature recorded during treatments. Accurate record keeping is essential to allow for trace-back capability.

6.1 Documentation of procedures

[69] Procedures should be documented to ensure that commodities are fumigated consistently in accordance with the treatment schedule. Process controls and operational parameters should be established to provide the operational details necessary for the authorization of a treatment provider. Calibration and quality control procedures should be documented by the treatment provider. A written document on procedures should include the following:

- commodity handling procedures before, during and after fumigation
- critical process parameters and the means for measuring them
- temperature and gas sensor calibration and recording, and calibration and recording for humidity sensors or moisture meters
- contingency plans and corrective actions to be taken in the event of fumigation failure or problems with critical treatment processes
- procedures for handling rejected lots

- labelling (if required), record keeping and documentation requirements
- training of personnel.

6.2 Record keeping

[70] The treatment provider should keep appropriate records for each treatment application. These records should be made available to the NPPO of the country in which the fumigation is conducted or initiated for auditing and verification purposes or trace-back.

[71] Appropriate records for fumigation as a phytosanitary measure should be retained by the treatment provider for at least one year to enable the trace-back of treated lots. Information on individual fumigation records may include data on:

- name of fumigant
- identification of enclosure and treatment provider
- enclosure leakage testing records
- equipment calibration records
- commodity fumigated and key characteristics (e.g. moisture content, presence of bark, type of packaging, etc.)
- target regulated pest
- packer, grower and place of production of the commodity
- fumigation lot number and other identifying markings or characteristics
- lot size and volume, including number of articles or packages
- date and duration of fumigation and name of individual performing the fumigation
- position and number of gas sample lines within enclosure
- any observed deviation from the treatment schedule
- the lowest air and commodity temperature
- humidity levels
- fumigant dosage and concentration records, including time of reading
- fumigant volumes (dose rate) calculated and added throughout fumigation.

6.3 Documentation by the NPPO

[72] All NPPO procedures should be appropriately documented and records, including those of monitoring inspections made and phytosanitary certificates issued, should be maintained for at least one year. In cases of non-compliance or new or unexpected phytosanitary situations, documentation should be made available upon request as described in ISPM 13 (*Guidelines for the notification of non-compliance and emergency action*).

7. Inspection

[73] Inspection should be carried out by the NPPO of the exporting country, and may be carried out by the NPPO of the importing country, to determine compliance with phytosanitary import requirements. Where live non-target pests are found after fumigation, the NPPO should consider if their survival indicates a fumigation failure and whether additional phytosanitary measures may be necessary.

The NPPO of the importing country may also inspect documentation and records for treatments conducted during transport to determine compliance with phytosanitary import requirements.

8. Responsibilities

[74] The NPPO of the country in which the fumigation is conducted or initiated is responsible for the evaluation, approval and auditing of the application of fumigation as a phytosanitary measure, including fumigation performed by the NPPO itself and by other authorized treatment providers. However, when

fumigation is conducted or completed during transport, the NPPO of the exporting country is usually responsible for authorizing the treatment provider applying the fumigation during transport and the NPPO of the importing country is responsible for verifying if the fumigation schedule has been met.

[75] To the extent necessary, the NPPO should cooperate with other national regulatory agencies concerned with the development, approval and safety of the fumigation, including the training and certification of personnel conducting the fumigation, the authorization of treatment providers and the approval of treatment facilities. The respective responsibilities of the NPPO and the other regulatory agencies should be identified to avoid requirements that are overlapping, conflicting, inconsistent or unjustified.

This appendix is for reference purposes only and is not a prescriptive part of the standard.

APPENDIX 1: Chemical properties of some common fumigants (at 25 °C)

Fumigant active substance	Formula	Molecular weight (g/mol)	Boiling point (°C) (@ 1 atm)	Specific gravity (gas) (air = 1.0)	Flammability limits in air (v/v %)	Solubility in water	Conversion factor (mg/litre to ppm, v/v @ 1 atm)
Carbonyl sulphide	COS	60	−50.2	2.07	12–29	0.125 g/100 ml	408
Ethane dinitrile	C_2N_2	52	−21.2	1.82	6–32	Highly soluble	470
Ethyl formate	$CH_3.CH_2.COOH$	74.08	54.5	2.55	2.7–13.5	11.8 g/100 ml	330
Hydrogen cyanide	HCN	27	26	0.9	5.6–40	Miscible	906
Methyl bromide	CH_3Br	95	3.6	3.3	10–15	3.4 v/v %	257
Methyl iodide	CH_3I	141.94	42.6	4.89	non	1.4 g/100 ml	172
Methyl isothiocyanate	C_2H_3NS	73.12	119	2.53	non	0.82 g/100 ml	334
Phosphine	PH_3	34	−87.7	1.2	>1.7	0.26 v/v %	719
Sulphur dioxide	SO_2	64.066	−10	2.26	non	9.4 g/100 ml	382
Sulphuryl fluoride	SO_2F_2	102	−55.2	3.72	non	Slight	240

This appendix is for reference purposes only and is not a prescriptive part of the standard.

APPENDIX 2: Examples of formulae to calculate the amount of fumigant required

[76] Examples of formulae to calculate fumigants by weight and by volume are provided below.

By weight:

[77] Amount of fumigant (g) = $\frac{Volume\ of\ Enclosure\ (m^3) \times Target\ Dosage\ (g/m^3) \times 100}{\%\ Fumigant\ Purity}$

[78] The fumigant purity is the percentage of active substance in the chemical product as indicated on the label.

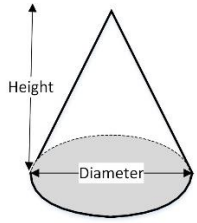
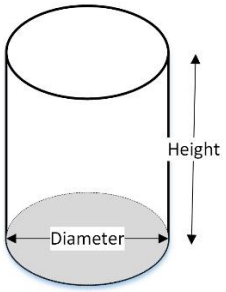
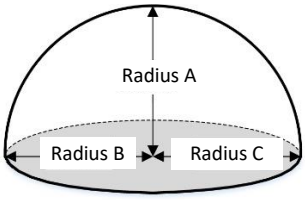
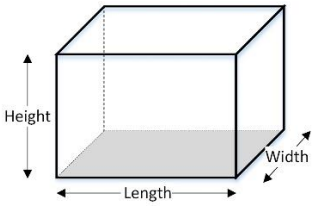
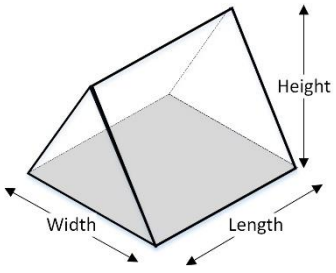
By volume:

[79] Amount of fumigant (ml) = $(273\ (K) + Temperature\ (°C)) \times \left(\frac{\text{Gas Constant (R) } (62.363\ L.mmHg.K^{-1}.mol^{-1}) \times Volume\ of\ Enclosure\ (L) \times Target\ Dosage\ (mg/L) \times 100}{Atmospheric\ Pressure\ (mmHg) \times Molecular\ Weight\ of\ Fumigant\ (g/mol) \times \%\ Fumigant\ Purity}\right)$

[80] The fumigant purity is the percentage of active substance in the chemical product as indicated on the label.

This appendix is for reference purposes only and is not a prescriptive part of the standard.

APPENDIX 3: Formulae for calculating volume of geometrical shapes

Type of geometrical shape	Geometrical structure	Formula for calculating volume
Cone		$Volume = \frac{\pi \times Radius^2 \times Height}{3}$
Cylinder		$Volume = \pi \times Radius^2 \times Height$
Dome†		$Volume = \frac{2 \times \pi \times Radius\ \ A \times Radius\ \ B \times Radius\ \ C}{3}$
Rectangular prism		$Volume = Length \times Width \times Height$
Triangular prism		$Volume = \frac{Length \times Width \times Height}{2}$

† The formula used provides an approximate volume only.

This appendix is for reference purposes only and is not a prescriptive part of the standard.

APPENDIX 4: Examples of formulae to calculate concentration–time product (CT)

[81] Examples of formulae to calculate the concentration–time product are provided below.

Example 1: $CT_{n,n+1} = (T_{n+1} - T_n) \times \sqrt{C_n \times C_{n+1}}$

Example 2: $CT_{n,n+1} = (T_{n+1} - T_n) \times (C_n + C_{n+1})/2$

where:

T_n is the time the first reading was taken, in hours

T_{n+1} is the time the second reading was taken, in hours

C_n is the concentration reading at T_n, in g/m^3

C_{n+1} is the concentration reading at T_{n+1}, in g/m^3

$CT_{n,n+1}$ is the calculated CT between T_n and T_{n+1}, in $g \cdot h/m^3$

DRAFT 2017 AMENDMENTS TO ISPM 5: *GLOSSARY OF PHYTOSANITARY TERMS* (1994-001)

Publication history

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Notes	Note to Secretariat formatting this paper: formatting in definitions and explanations (strikethrough, bold, italics) needs to remain. **NOTE: The explanations for each proposal are presented only in the version of the draft Amendments presented to consultation and to the SC. For CPM, only the proposals will be presented. For full details on the discussions related to the specific terms, please refer to the meeting reports on the IPP.**

1. REVISIONS

1.1 "growing period" (2016-004)

Current definition

growing period (of a **plant** species)	Time period of active growth during a **growing season** [ICPM, 2003]

Proposed revision

growing period ~~(of a **plant** species)~~	~~Time p~~Period ~~of active growth during a **growing season**~~ when a **plant** species actively grows in an **area**, **place of production** or **production site** [ICPM, 2003]

1.2 "survey" (2013-015)

Current definition

survey	An **official** procedure conducted over a defined period of time to determine the characteristics of a **pest** population or to determine which species are present in an **area** [FAO, 1990; revised CEPM, 1996; CPM, 2015]

Proposed revision

survey (of pests)	An **official** procedure conducted over a defined period ~~of time~~ to determine the presence or absence of **pests**, or the boundaries or characteristics of a **pest** population~~, or to determine which species are present~~ in an **area**, **place of production** or **production site** [FAO, 1990; revised CEPM, 1996; CPM, 2015]

2. DELETIONS

2.1 "confinement (of a regulated article)" (2016-002)

Proposed deletion

confinement (of a **regulated article**)	Application of **phytosanitary measures** to a **regulated article** to prevent the escape of **pests** [CPM, 2012]

2.2 "growing season" (2016-004)

Proposed deletion

growing season	Period or periods of the year when **plants** actively grow in an **area**, **place of production** or **production site** [FAO, 1990; revised ICPM, 2003]

2.3 "mark" (2013-007)

Proposed deletion

mark	An **official** stamp or brand, internationally recognized, applied to a **regulated article** to attest its phytosanitary status [ISPM 15, 2002]

This diagnostic protocol was adopted by the Standards Committee on behalf of the Commission on Phytosanitary Measures in August 2018.

The annex is a prescriptive part of ISPM 27.

ISPM 27
Diagnostic protocols for regulated pests

DP 2: *Plum pox virus*

Adopted 2018; published 2018

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1. Pest Information

Sharka (plum pox) is one of the most serious viral diseases of stone fruit. The disease was first reported in *Prunus domestica* in Bulgaria in 1917–1918, and was described as a viral disease in 1932. Since then, the virus has spread progressively to a large part of Europe, around the Mediterranean basin and the Near East. It has been found with a restricted distribution in South and North America and Asia (EPPO, 2006; CABI, 2018).

Sharka, caused by *Plum pox virus* (PPV), affects plants of the genus *Prunus* (family Rosaceae). It is particularly detrimental in *Prunus armeniaca*, *P. domestica*, *P. persica* and *P. salicina* because it reduces quality and causes early fruit drop. It is estimated that the costs of managing sharka worldwide since the 1970s exceed 10 billion euros (Cambra *et al*., 2006b).

Plum pox virus is a member of the genus *Potyvirus* in the family *Potyviridae*. The virus particles are flexuous rods of approximately 700 nm × 11 nm, and are composed of a single-stranded RNA molecule consisting of almost 10 000 nucleotides coated by up to 2 000 subunits of a single coat protein (García *et al*., 2014). PPV is transmitted in the field by aphids in a non-persistent manner, but movement of infected propagative plant material is the main way in which PPV is spread over long distances. Transmission via seed and pollen have not been confirmed (Pasquini and Barba, 2006; Ilardi and Tavazza, 2015). PPV can be transmitted mechanically, under experimental conditions, to numerous *Prunus* species and to several herbaceous species such as *Arabidopsis thaliana*, *Chenopodium foetidum*, *Nicotiana benthamiana*, *Nicotiana clevelandii*, *Nicotiana glutinosa* and *Pisum sativum* (Barba *et al*., 2011).

Plum pox virus isolates can currently be classified into nine monophyletic strains: D (Dideron), M (Marcus), C (Cherry), EA (El Amar), W (Winona), Rec (Recombinant), T (Turkish), CR (Cherry Russian) and An (Ancestor Marcus) (James *et al*., 2013). The strains have specific genome sequences and may vary in their symptomatology, pathogenicity, host range, epidemiology and aphid transmissibility. Most PPV isolates belong to the D and M strains. PPV-D and -M strains can easily infect *P. armeniaca* and *P. domestica* but differ in their ability to infect *P. persica* cultivars. These two strains also differ in their pathogenicity, with M isolates generally causing faster epidemics and more severe symptoms than D isolates in *P. armeniaca*, *P. domestica*, *P. persica* and *P. salicina*. EA isolates are geographically restricted to Egypt and little information is available about their epidemiology and biological properties. PPV isolates infecting *Prunus avium* and *Prunus cerasus* have been identified in several European countries. These isolates form two distinct strains that have been defined as PPV-C and PPV-CR. An atypical PPV was detected in *P. domestica* in Canada (PPV-W), representing a distinct PPV strain. PPV-W has since been detected in several countries in Europe (James *et al.*, 2013). In addition, natural recombinants between the D and M strains of PPV have been described as PPV-Rec, these show an epidemiological behaviour similar to the D strain. A second type of recombinant strain has been reported in Turkey and defined as a T strain (Ulubaş Serçe *et al*., 2009). A single isolate of PPV-An has been described and it has been proposed as a potential ancestor of PPV-M (Palmisano *et al*., 2012). A novel sour cherry-adapted putative strain (Tat), neither C nor CR, has also been proposed (Chirkov *et al*., 2016).

Further information about PPV, including illustrations of disease symptoms, can be found in Barba *et al*. (2011), CABI (2018), EPPO (2004, 2006, 2018b), García *et al*. (2014) and PaDIL (2018).

2. Taxonomic Information

Name: *Plum pox virus* (acronym PPV)

Synonym: *Sharka virus*

Taxonomic position: *Potyviridae*, *Potyvirus*

Common names: Plum pox, sharka

3. Detection and Identification

Detection of PPV can be achieved using a biological, serological or molecular method, while identification requires use of either a serological or molecular method. A test using a serological or molecular method is the minimum requirement to detect and identify PPV, especially during routine diagnosis if the pest is known to be widely established in a country. In instances where the national plant protection organization (NPPO) requires additional confidence in the identification of PPV (e.g. detection in an area where the virus is not known to be present or detection in a consignment originating in a country where the pest is declared to be absent), further tests may be carried out to confirm the identification. Where the initial identification was made using a molecular method, the confirmation should preferably be made using a method with a higher analytical sensitivity or, if possible, using a molecular method targeting a different genome region or sequence analysis. Further tests may also be carried out, including the use of serological methods that target protein elements or methods used to identify the strain of PPV present. In all cases, positive and negative controls must be included in the tests. The recommended techniques are described in the following sections.

This diagnostic protocol describes well-established methods for the detection and identification of PPV. Some new and advanced techniques have been used to detect PPV such as loop-mediated isothermal amplification (Varga and James, 2006b) and next-generation sequencing (Rodamilans *et al*., 2014). However, since next-generation sequencing and loop-mediated isothermal amplification (LAMP)[1] have not yet been fully validated as tools for routine detection of PPV, with published protocols described, these techniques have not been included in this diagnostic protocol.

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. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

3.1 Host range

Under natural conditions, PPV readily infects fruit trees of the genus *Prunus* (family Rosaceae) used as commercial varieties or rootstocks. Major hosts include *Prunus armeniaca*, *P. cerasifera*, *P. davidiana*, *P. domestica*, *P. mahaleb*, *P. marianna*, *P. mume*, *P. persica*, *P. salicina* and interspecific hybrids between these species. There are cherry-adapted strains of PPV (C and CR) that naturally infect *P. avium* and *P. cerasus* (James *et al*., 2013). Occasionally, *Prunus dulcis* may be infected by PPV (Llácer and Cambra, 2006). The virus also infects many wild and ornamental *Prunus* species such as *P. besseyi*, *P. cistena*, *P. glandulosa*, *P. insititia*, *P. laurocerasus*, *P. spinosa*, *P. tomentosa* and *P. triloba* (James and Thompson, 2006). Under experimental conditions, PPV can be transmitted mechanically to numerous *Prunus* spp. and several herbaceous plants (see section 1).

3.2 Symptoms

Plum pox virus symptoms may appear on leaves, shoots, bark, petals, fruits and stones in the field. They are usually distinct on leaves early in the growing season and include: mild light-green discoloration; chlorotic spots, bands or rings; vein clearing or yellowing; or leaf deformation. Some of these leaf symptoms are similar to those caused by other viruses, such as *American plum line pattern virus*. *P. cerasifera* 'GF 31' shows rusty-brown corking and cracking of the bark.

[1] When using LAMP on a regular basis in an area which has a patent system such as Japan (Patent Nos. 3,313,358, 3,974,441 and 4,139,424), the United States of America (US6,410,278, US6,974,670 and US7,494,790), the European Union (Nos. 1,020,534, 1,873,260, 2,045,337 and 2,287,338), China (ZL008818262), the Republic of Korea (Patent No, 10-0612551), Australia (No. 779160), and the Russian Federation (No. 2,252,964), it is necessary for users to receive a license from Eiken Chemical Co., Ltd. before use.

Flower symptoms may include discoloration (pinkish streaks) on flower petals and flower breaking symptoms (Barba *et al*., 2011). These flower symptoms can occur on some *P. persica* cultivars when infected with PPV-M or in *P. glandulosa* infected with PPV-D.

Infected fruits show chlorotic spots or lightly pigmented yellow rings or line patterns. Fruits may become deformed or irregular in shape and develop brown or necrotic areas under the discoloured rings. Some fruit deformations, especially in *P. armeniaca* and *P. domestica*, are similar to those caused by *Apple chlorotic leaf spot virus*. Diseased fruits may show internal browning and gummosis of the flesh and reduced quality. In severe cases, the diseased fruits drop prematurely from the tree. In general, the fruits of early maturing cultivars show more marked symptoms than those of late maturing cultivars. Stones from diseased fruits of *P. armeniaca* typically show pale rings or spots. The alcohol or spirits produced from diseased fruits are unmarketable owing to an undesirable flavour.

Symptom development and severity depend strongly on the host plant and climatic conditions. The virus may be latent for several years in cold climates.

Symptoms on various hosts can be seen, for example, on the EPPO (European and Mediterranean Plant Protection Organization) Global database website (https://gd.eppo.int/taxon/PPV000).

3.3 Biological detection

The main indicator plants used for PPV indexing are seedlings of *P. cerasifera* 'GF 31', *P. persica* 'GF 305', *P. persica* × *P. davidiana* 'Nemaguard', or *P. tomentosa*. Indicator plants are raised from seed, planted in a well-drained soil mixture and maintained in an insect-proof greenhouse between 18 °C and 25 °C until they are large enough to graft (usually 25–30 cm high with a diameter of 3–4 mm). Alternatively, seedlings of other *Prunus* species may be grafted with indicator plant scions. The indicators must be graft-inoculated according to conventional methods such as bud grafting (Desvignes, 1999), using at least four replicates per indicator plant. The grafted indicator plants are maintained in the same conditions and, after three weeks, are pruned to a few centimetres above the top graft (Gentit, 2006). The grafted plants should be inspected for symptoms for at least six weeks. Symptoms, in particular chlorotic banding and patterns, are observed on the new growth after 3–4 weeks and must be compared with positive and healthy controls. Illustrations of symptoms caused by PPV on indicator plants can be found in Damsteegt *et al*. (1997, 2007) and Gentit (2006).

There are no quantitative data published on the specificity, sensitivity or reliability of grafting. The method is used widely in certification schemes and is considered a sensitive method of detection. However, it is not a rapid test (symptom development requires several weeks post-inoculation), it can only be used to test budwood, it requires dedicated facilities such as temperature-controlled greenhouse space, and the symptoms observed may be confused with those of other graft-transmissible agents. Moreover, there are asymptomatic strains that do not induce symptoms and thus are not detectable on indicator plants.

Herbaceous plants can also be used for biological detection of PPV (Barba *et al*., 2011). PPV can be transmitted mechanically to several herbaceous species (see section 1).

3.4 Sampling for serological and molecular tests

General guidance on sampling methodologies is provided in ISPM 31 (*Methodologies for sampling of consignments*). In some circumstances (e.g. during the routine diagnosis of a pest widely established in a country), multiple plants may be tested simultaneously using a bulked sample derived from a number of plants. The decision to test individual or multiple plants depends on the virus concentration in the plants and the level of confidence required by the NPPO. *Prunus* plant material is often shipped as dormant cuttings. In this case, only buds or phloem tissue (bark scrapings) can be used directly for testing.

Appropriate sample selection is critical for PPV detection. Sampling should take into account virus biology and local climatic conditions, in particular the weather conditions during the growing season. If

typical symptoms are present, samples should be collected from flowers, leaves or fruits showing symptoms. In symptomless plants, samples should be taken from shoots that are at least one year old and have mature or fully expanded leaves, collected from the middle of each of the main branches (detection is not reliable in shoots less than one year old). Samples should be collected from at least four different sites (e.g. four branches or four leaves) in each plant; this is critical because of the uneven distribution of PPV. Sampling should not be carried out during months with the highest temperatures. Tests on samples collected in the autumn are less reliable than tests carried out on samples collected earlier in the spring. Plant material should preferably be collected from the internal parts of the tree canopy. In springtime, samples can be flowers, shoots with fully expanded leaves, or fruits. In summer and autumn, mature leaves and the skin of mature fruits collected from the field or packing houses can be used for analysis. In summer, buds from dormant cuttings can be tested using reverse transcription-polymerase chain reaction (RT-PCR) or real-time PCR, these also being the preferred techniques for testing mature leaves. Flowers, leaves, shoots and fruit skin can be stored at 4 °C for not more than 10 days before processing. Fruits can be stored for one month at 4 °C before processing. In winter, dormant buds or bark tissues from the basal part of twigs, shoots or branches, or complete spurs can be used for testing.

3.5 Serological detection and identification

Enzyme-linked immunosorbent assays (ELISA) are highly recommended for screening large numbers of samples.

For sample processing, approximately 0.2–0.5 g of fresh plant material is cut into small pieces and placed in a suitable tube or plastic bag. The sample is homogenized in approximately 4–10 ml (1:20 w/v) of extraction buffer (or as recommended by the ELISA kit manufacturer) using an electrical tissue homogenizer, or a manual roller, hammer or similar tool. The extraction buffer is phosphate-buffered saline (PBS) pH 7.2–7.4, containing 2% polyvinylpyrrolidone and 0.2% sodium diethyl dithiocarbamate (Cambra *et al.*, 1994), or an alternative suitable buffer. Plant material should be homogenized thoroughly and used fresh.

3.5.1 Double-antibody sandwich indirect enzyme-linked immunosorbent assay

Double-antibody sandwich indirect enzyme-linked immunosorbent assay (DASI-ELISA), also called triple-antibody sandwich (TAS)-ELISA, should be performed according to Cambra *et al*. (1994) using a specific monoclonal antibody such as 5B-IVIA, following the manufacturer's instructions.

The monoclonal antibody (MAb) 5B-IVIA has been shown to detect most if not all strains of PPV (Cambra *et al.*, 2006a). MAb 5B-IVIA will detect isolates of strain CR, but the extracts for analysis must be adjusted to pH 6.0 for enhanced MAb 5B-IVIA recognition (Glasa *et al*., 2013; Chirkov *et al*., 2013). The putative cherry-adapted strain (Tat) can also be detected by MAb 5B-IVIA (Chirkov *et al*., 2016). However, there has been no report of the detection of PPV-An using MAb 5B-IVIA (Palmisano *et al.*, 2012).

In a DIAGPRO ring test (Harju *et al.*, 2000) conducted by 17 laboratories using a panel of ten samples, including both PPV-infected (PPV-D, PPV-M and PPV-D+M) and healthy samples from France and Spain, DASI-ELISA using MAb 5B-IVIA was 95% accurate (number of true negatives and true positives diagnosed by the technique, divided by the number of samples tested). This accuracy was greater than that achieved with either immunocapture RT-PCR (IC-RT-PCR), which was 82% accurate, or co-operational RT-PCR (Co-RT-PCR), which was 94% accurate (Olmos *et al*., 2007; Cambra *et al*., 2008). The proportion of true negatives (number of true negatives diagnosed by the technique, divided by the number of healthy plants) identified by DASI-ELISA using MAb 5B-IVIA was 99.0%, compared with real-time RT-PCR using purified nucleic acid (89.2%) or spotted samples (98.0%), or IC-RT-PCR (96.1%). Capote *et al.* (2009) also reported that there is a 98.8% probability that a positive result obtained in winter with DASI-ELISA using MAb 5B-IVIA was a true positive. Antibodies may exhibit variation between batches, therefore verification of performance should be carried out before routine use.

The 5B-IVIA monoclonal antibody detects all tested PPV strains specifically, sensitively and reliably (Cambra *et al.*, 1994; Cambra *et al.*, 2006a; Glasa *et al*., 2013; Chirkov *et al*., 2013; Chirkov *et al*., 2016). Several commercial kits using polyclonal antibodies are available along with some validation data (Gougherty *et al.*, 2015; EPPO, 2018a). However, these have been shown to be less specific and to lack homogeneity among different batches (Cambra *et al.*, 2006a) and should therefore be validated before use. The use of additional methods is recommended in situations where polyclonal antibodies have been used in a test and the NPPO requires additional confidence in the identification of PPV.

3.5.2 Double-antibody sandwich enzyme-linked immunosorbent assay

The conventional or biotin–streptavidin system of double-antibody sandwich (DAS)-ELISA utilizes kits based on the specific monoclonal antibody 5B-IVIA or on polyclonal antibodies that have been demonstrated to detect most strains of PPV, including the most widely distributed strains D, M and Rec, without cross-reacting with other viruses or healthy plant material (Cambra *et al*., 2006a; Capote *et al*., 2009). The test should be carried out according to the manufacturer's instructions.

3.6 Molecular detection and identification

Molecular methods such as RT-PCR may be more expensive than serological methods, especially for large-scale testing. However, molecular methods, especially real-time RT-PCR, are generally more sensitive than serological methods. The use of real-time RT-PCR also avoids the need for any post-amplification processing (e.g. gel electrophoresis) and is therefore quicker and less prone to contamination (with the target DNA) than conventional PCR.

With the exception of IC-RT-PCR (for which RNA isolation is not required), RNA extraction should be conducted using appropriately validated protocols. The samples should be placed in individual plastic bags to avoid cross-contamination during extraction. Alternatively, for real-time RT-PCR, spotted plant extracts, printed tissue sections or squashes of plant material can be immobilized on blotting paper or nylon membranes for analysis (Olmos *et al.*, 2005; Osman and Rowhani, 2006; Capote *et al.*, 2009). It is recommended that spotted or tissue-printed samples be tested using real-time RT-PCR rather than conventional PCR because of its higher sensitivity.

Each of the following methods describes the volume of extracted sample that should be used as a template. Depending on the sensitivity of the method, the minimum concentration of template required to detect PPV varies as follows: RT-PCR, 100 fg RNA template/ml; Co-RT-PCR, 1 fg RNA template/ml; and real-time RT-PCR, 2 fg RNA template/ml.

3.6.1 RNA purification, immunocapture and cDNA synthesis

3.6.1.1 RNA purification

RNA purification should be carried out using appropriately validated protocols or using an RNA purification kit according to the manufacturer's instructions. The extracted RNA should be stored at −70 °C (preferably) or at −20 °C until its use as a template and for less than one year. Storage should be in small quantities to avoid degradation of RNA due to repeated freeze–thaw cycles.

3.6.1.2 Immunocapture

Immunocapture is an alternative option to RNA purification. For this procedure, a diluted antibody mixture is prepared and used to coat the microtubes used for the reverse transcription reaction. See section 3.6.2 for details of the procedure.

3.6.1.3 cDNA synthesis

Because the preservation of RNA during storage is problematic, it is recommended that complementary (c)DNA be synthesized, as this can be preserved for long periods and with fewer temperature requirements compared with RNA.

3.6.2 Reverse transcription-polymerase chain reaction

The primers used in the RT-PCR methods described below have been well validated and are considered as references for general PPV detection even though there may be other broad-spectrum primers available (Olmos *et al.*, 2006). No false positive results were observed in the studies describing the development and validation of these methods (Wetzel *et al*., 1991; Levy and Hadidi, 1994). Another benefit of the Wetzel *et al*. (1991) primers is that they also allow identification of the two most common strains of PPV, when combined with analysis of the 243 base pair (bp) product using restriction fragment length polymorphism.

The RT-PCR primers used in this method are either the primers of Wetzel *et al.* (1991):

P1 antisense (5′-ACC GAG ACC ACT ACA CTC CC-3′)

P2 sense (5′-CAG ACT ACA GCC TCG CCA GA-3′)

or the primers of Levy and Hadidi (1994):

3′NCR sense (5′-GTA GTG GTC TCG GTA TCT ATC ATA-3′)

3′NCR antisense (5′-GTC TCT TGC ACA AGA ACT ATA ACC-3′)

The 25 µl reaction mixture is composed as follows: 1 µM of each primer (P1 and P2, or the 3′NCR primer pair), 250 µM dNTPs, 1 unit *Avian myeloblastosis virus* (AMV) reverse transcriptase[2], 0.5 units Taq DNA polymerase, 2.5 µl 10× Taq polymerase buffer, 1.5 mM $MgCl_2$, 0.3% Triton X-100 and 5 µl RNA template. The reaction is performed with the following thermocycling parameters: 45 min at 42 °C, 2 min at 94 °C, 40 cycles of 30 s at 94 °C, 30 s at either 60 °C (P1 and P2 primers) or 62 °C (3′NCR primers), and 60 s at 72 °C, followed by a final extension for 10 min at 72 °C. The PCR products are analysed by gel electrophoresis. The P1/P2 pair of primers produces a 243 bp amplicon and the 3′NCR primers produce a 220 bp amplicon.

The method of Wetzel *et al.* (1991) was evaluated by testing PPV isolates from Mediterranean areas (Cyprus, Egypt, France, Greece, Spain and Turkey). It was able to detect 10 fg of viral RNA, corresponding to 2 000 viral particles (Wetzel *et al.*, 1991). Levy and Hadidi (1994) evaluated their method using PPV isolates from Egypt, France, Germany, Greece, Hungary, Italy, Spain and Romania.

3.6.3 Immunocapture reverse transcription-polymerase chain reaction

The immunocapture phase may be performed according to Wetzel *et al.* (1992), using plant sap extracted as in section 3.5 and using individual tubes or plastic bags to avoid contamination. Any suitably validated antibody may be used. This test has been validated only for isolates of the widely distributed strains D and M.

A dilution (1 µg/ml) is prepared of polyclonal antibodies or a PPV-specific monoclonal antibody (e.g. 5B-IVIA) in carbonate buffer pH 9.6. Aliquots of 100 µl diluted antibody are dispensed into PCR tubes and incubated at 37 °C for 3 h. The tubes are then washed twice with 150 µl sterile PBS-Tween (washing buffer), and rinsed twice with RNase-free water. Plant extract (100 µl; see section 3.5) is clarified by centrifugation (5 min at 15 500 *g*), and the supernatant added to the coated PCR tubes. The tubes are incubated for 2 h on ice or at 37 °C, and then washed three times with 150 µl sterile PBS-Tween. The RT-PCR reaction mixture is prepared as described in section 3.6.2 using the primers of Wetzel *et al.*

[2] The 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.

(1991), and added directly to the coated PCR tubes. The amplification is performed as described in section 3.6.2.

In general, IC-RT-PCR requires the use of specific antibodies, although direct-binding methods may eliminate this requirement. IC-RT-PCR using the 5B-IVIA monoclonal antibody has been validated in a DIAGPRO ring test showing an accuracy of 82% for PPV detection (Olmos *et al.*, 2007; Cambra *et al.*, 2008). Capote *et al.* (2009) reported that there is a 95.8% probability that a positive result obtained in winter with IC-RT-PCR using the 5B-IVIA monoclonal antibody was a true positive.

3.6.4 Co-operational reverse transcription-polymerase chain reaction

The RT-PCR primers used in this Co-RT-PCR are the primers of Wetzel *et al.* (1991; P1 and P2) and Olmos *et al.* (2002; P10 and P20):

Internal primer P1 (5′-ACC GAG ACC ACT ACA CTC CC-3′)

Internal primer P2 (5′-CAG ACT ACA GCC TCG CCA GA-3′)

External primer P10 (5′-GAG AAA AGG ATG CTA ACA GGA-3′)

External primer P20 (5′-AAA GCA TAC ATG CCA AGG TA-3′)

The 25 µl reaction mixture is composed as follows: 0.1 µM each of P1 and P2 primers, 0.05 µM each of P10 and P20 primers, 400 µM dNTPs, 2 units AMV reverse transcriptase, 1 unit Taq DNA polymerase, 2.5 µl 10× reaction buffer, 3 mM $MgCl_2$, 5% dimethyl sulphoxide, 0.3% Triton X-100 and 5 µl RNA template. The RT-PCR is performed with the following thermocycling parameters: 45 min at 42 °C, 2 min at 94 °C, 60 cycles of 15 s at 94 °C, 15 s at 50 °C and 30 s at 72 °C, followed by a final extension for 10 min at 72 °C.

The RT-PCR reaction is coupled to a colorimetric detection of amplicons using a 3′digoxigenin (DIG)-labelled PPV universal probe (5′-TCG TTT ATT TGG CTT GGA TGG AA-DIG-3′) as follows. The amplified cDNA is denatured at 95 °C for 5 min and immediately placed on ice. A 1 µl aliquot of sample is placed on a nylon membrane. The membrane is then dried at room temperature and UV cross-linked in a transilluminator for 4 min at 254 nm. For prehybridization, the membrane is placed in a hybridization tube at 60 °C for 1 h using a standard hybridization buffer. The solution is discarded and the hybridization performed by mixing the 3′DIG-labelled probe with standard hybridization buffer at a final concentration of 10 pmol/ml, before incubating for 2 h at 60 °C. The membrane is washed twice for 15 min at room temperature with 2× washing solution, and twice for 15 min at room temperature with 0.5× washing solution. The membrane is then equilibrated for 2 min in washing buffer before soaking for 30 min in sterilized 1% blocking solution (1 g blocking reagent dissolved in 100 ml maleic acid buffer). The membrane is incubated at room temperature with anti-DIG-alkaline phosphatase conjugate antibodies at a working concentration of 1:5 000 in 1% blocking solution (w/v) for 30 min. The membrane is then washed twice for 15 min with washing buffer, and equilibrated for 2 min with detection buffer (100 mM Tris-HCl, 100 mM NaCl, pH 9.5). The substrate solution is prepared by mixing 45 µl NBT solution (75 mg/ml nitro blue tetrazolium salt in 70% (v/v) dimethylformamide) and 35 µl BCIP solution (50 mg/ml 5-bromo-4chloro-3indolyl phosphate toluidinium salt in 100% dimethylformamide) in 10 ml detection buffer. After incubation with the substrate, the reaction is stopped by washing with water.

This method has been found to be 100 times more sensitive than the RT-PCR method of Wetzel *et al.* (1991) (Olmos *et al.*, 2002). The method was validated in the DIAGPRO ring test, where it had an accuracy of 94% (Olmos *et al.*, 2007; Cambra *et al.*, 2008).

3.6.5 Real-time reverse transcription-polymerase chain reaction

Real-time RT-PCR can be performed using either TaqMan or SYBR Green I[2]. Two TaqMan methods have been described for universal detection of PPV (Schneider *et al.*, 2004; Olmos *et al.*, 2005). The primers and TaqMan probe used in the first method are those reported by Schneider *et al.* (2004):

Forward primer (5′-CCA ATA AAG CCA TTG TTG GAT C-3′)

Reverse primer (5′-TGA ATT CCA TAC CTT GGC ATG T-3′)

TaqMan probe (5′-FAM-CTT CAG CCA CGT TAC TGA AAT GTG CCA-TAMRA-3′)

The 25 µl reaction mixture is composed as follows: 1× reaction mix (0.2 mM of each dNTP and 1.2 mM $MgSO_4$), 200 nM each of forward and reverse primers, 100 nM TaqMan probe, 4.8 mM $MgSO_4$, 0.5 µl RT/Platinum Taq mix (Superscript One-Step RT-PCR with Platinum Taq DNA polymerase; Invitrogen)[2] and 5 µl RNA template. The RT-PCR is performed with the following thermocycling parameters: 15 min at 52 °C, 5 min at 95 °C, and 60 cycles of 15 s at 95 °C and 30 s at 60 °C. The PCR products are analysed in real time according to the equipment manufacturer's instructions.

The method of Schneider *et al.* (2004) was evaluated by testing PPV D isolates from the United States of America, and isolates of strains PPV-C, PPV-D, PPV-EA and PPV-M, and eight other viral species. The method was specific and able to detect consistently 10–20 fg of viral RNA (Schneider *et al.*, 2004). The method could also detect PPV in a number of hosts and in the leaves, stems, buds and roots of *P. persica*.

The primers and TaqMan probe used in the second method are those reported by Olmos *et al.* (2005):

P241 primer (5′-CGT TTA TTT GGC TTG GAT GGA A-3′)

P316D primer (5′-GAT TAA CAT CAC CAG CGG TGT G-3′)

P316M primer (5′-GAT TCA CGT CAC CAG CGG TGT G-3′)

PPV-DM probe (5′-FAM-CGT CGG AAC ACA AGA AGA GGA CAC AGA-TAMRA-3′)

The 25 µl reaction mixture is composed as follows: 1 µM P241 primer, 0.5 µM each of P316D and P316M primers, 200 nM TaqMan probe, 1× TaqMan Universal PCR Master Mix (Applied Biosystems)[2], 1× MultiScribe and RNase Inhibitor Mix (Applied Biosystems)[2], and 5 µl RNA template. The RT-PCR is performed with the following thermocycling parameters: 30 min at 48 °C, 10 min at 95 °C, and 40 cycles of 15 s at 95 °C and 60 s at 60 °C. The PCR products are analysed in real time according to the equipment manufacturer's instructions.

The method was evaluated by Olmos *et al.* (2005) using three isolates each of PPV-D and PPV-M, and was 1 000 times more sensitive than DASI-ELISA using the 5B-IVIA monoclonal antibody. The proportion of true positives (number of true positives diagnosed by the technique, divided by the number of PPV-infected plants) identified correctly by real-time RT-PCR using TaqMan (Olmos *et al.*, 2005) and purified nucleic acid was 97.5%, compared with real-time RT-PCR using spotted samples (93.6%), IC-RT-PCR (91.5%) or DASI-ELISA using the 5B-IVIA monoclonal antibody (86.6%) (Capote *et al.*, 2009).

Varga and James (2005) described a SYBR Green I[2] method for the simultaneous detection of PPV and identification of D and M strains:

P1 (5′-ACC GAG ACC ACT ACA CTC CC-3′)

PPV-U (5′-TGA AGG CAG CAG CAT TGA GA-3′)

PPV-FD (5′-TCA ACG ACA CCC GTA CGG GC-3′)

PPV-FM (5′-GGT GCA TCG AAA ACG GAA CG-3′)

PPV-RR (5′-CTC TTC TTG TGT TCC GAC GTT TC-3′)

The following internal control primers (Menzel *et al.*, 2002) may be included to ensure the validity of the test results:

Nad5-F (5′-GAT GCT TCT TGG GGC TTC TTG TT-3′)

Nad5-R (5′-CTC CAG TCA CCA ACA TTG GCA TAA-3′)

A two-step RT-PCR protocol is used. The RT reaction mixture is composed as follows: 2 µl 10 µM P1 primer, 2 µl 10 µM Nad5-R primer, 4 µg total RNA and 5 µl water. The mixture is incubated at 72 °C for 5 min, then placed on ice. The following are then added: 4 µl 5× first strand buffer (Invitrogen)[2], 2 µl 0.1 M dithiothreitol (DTT), 1 µl 10 mM dNTPs, 0.5 µl RNaseOUT (40 units/µl) (Invitrogen)[2], 1 µl

Superscript II reverse transcriptase (Invitrogen)[2] and 2.5 µl water. The mixture is incubated at 42 °C for 60 min followed by 99 °C for 5 min. The 24 µl PCR reaction mixture is composed as follows: 400 nM PPV-U primer, 350 nM PPV-FM primer, 150 nM PPV-FD primer, 200 nM PPV-RR primer, 100 nM Nad5-F primer, 100 nM Nad5-R primer, 200 µM dNTPs, 2 mM $MgCl_2$, 1× Karsai buffer (Karsai *et al.*, 2002), 1:42 000 SYBR Green I[2] and 0.1 µl Platinum Taq DNA high fidelity polymerase (Invitrogen)[2]. The PCR reaction mixture and 1 µl diluted cDNA (1:4) are added to a sterile PCR tube. The PCR is performed with the following thermocycling parameters: 2 min at 95 °C, and 39 cycles of 15 s at 95 °C and 60 s at 60 °C. Melting curve analysis is performed by incubation at 60 °C to 95 °C with 0.1 °C/s melt rates and a smooth curve setting averaging 1 point. Under the conditions of Varga and James (2005), the melting temperatures for each product are:

Universal PPV detection (74 bp fragment): 80.08–81.52 °C

D strains (114 bp fragment): 84.3–84.43 °C

M strains (380 bp fragment): 85.34–86.11 °C

Internal control (181 bp fragment): 82.45–82.63 °C

Varga and James (2005) evaluated their method using isolates of PPV-C, PPV-D, PPV-EA, PPV-M and an uncharacterized strain in *Nicotiana* and *Prunus* species.

4. Identification of Strains

This section describes additional steps for the identification of PPV strains (using DASI-ELISA, RT-PCR, Co-RT-PCR and real-time RT-PCR) (see Figure 1). Strain identification is not an essential component of PPV identification but an NPPO may wish to determine the identity of the strain to assist in predicting its epidemiological behaviour.

Given the variability of PPV, techniques other than sequencing or some PCR-based techniques (see below) may provide erroneous results with a small percentage of isolates. However, it is generally possible to discriminate the D and M strains of PPV using the serological or molecular methods described (Cambra *et al.*, 2006a; Candresse and Cambra, 2006; Capote *et al.*, 2006). Techniques for the identification of strains such as An and T are not provided as methods for their identification have not been validated and published or as yet too few isolates have been characterized.

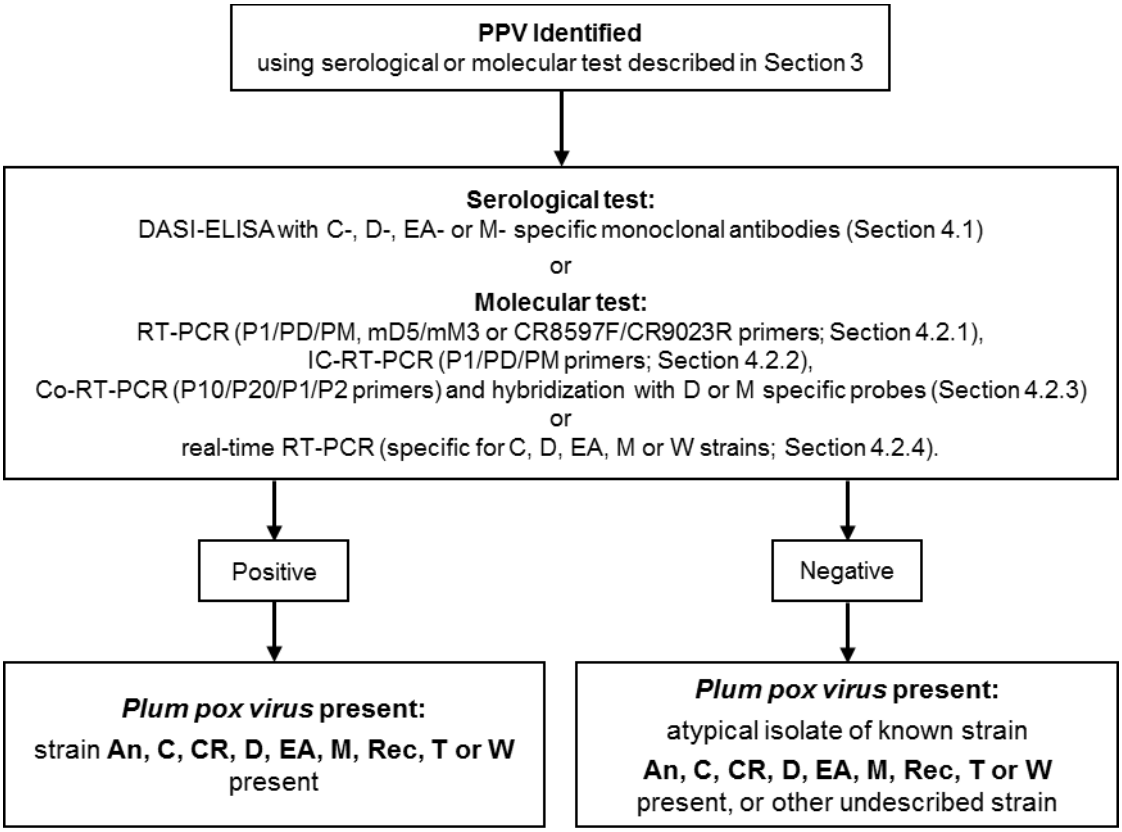

Figure 1. Steps in the methods for the identification of strains of *Plum pox virus*.

Further tests may be conducted in instances where the NPPO requires additional confidence in the identification of the PPV strain. Sequencing of the complete PPV genome, or complete or partial coat protein, P3-6K1 and cytoplasmic inclusion protein genes should also be carried out where atypical or undescribed strains are present.

4.1 Serological identification of strains

DASI-ELISA for differentiation between the two main PPV strains (D and M) should be performed according to Cambra *et al.* (1994), using D- and M-specific monoclonal antibodies (Cambra *et al.*, 1994; Boscia *et al.*, 1997), according to the manufacturer's instructions.

This method has been validated in the DIAGPRO ring test, showing an accuracy of 84% for PPV-D detection and 89% for PPV-M detection (Olmos *et al.*, 2007; Cambra *et al.*, 2008). The 4D monoclonal antibody is PPV-D specific but does not react with all PPV-D isolates. Furthermore, the AL monoclonal antibody used for PPV-M detection reacts with isolates belonging to strains M, Rec and T because these groups share the same coat protein sequence. A molecular test is therefore required to differentiate between M, Rec and T strains detected using an M-specific monoclonal antibody.

Serological identification of PPV isolates from EA and C groups may be carried out by DASI-ELISA using the EA- or the C-specific monoclonal antibodies described by Myrta *et al.* (1998, 2000). However, these methods need to be validated.

4.2 Molecular identification of strains

4.2.1 Reverse transcription-polymerase chain reaction

PPV-D and PPV-M are identified using the primers described by Olmos *et al.* (1997):

P1 (5′-ACC GAG ACC ACT ACA CTC CC-3′)

PD (5′-CTT CAA CGA CAC CCG TAC GG-3′)
PM (5′-CTT CAA CAA CGC CTG TGC GT -3′)

The 25 µl reaction mixture is composed as follows: 1 µM P1 primer, 1 µM of either PD or PM primer, 250 µM dNTPs, 1 unit AMV reverse transcriptase (10 units/µl), 0.5 units Taq DNA polymerase (5 units/µl), 2.5 µl 10× Taq polymerase buffer, 1.5 mM $MgCl_2$, 0.3% Triton X-100, 2% formamide and 5 µl RNA template. The RT-PCR is performed with the following thermocycling parameters: 45 min at 42 °C, 2 min at 94 °C, 40 cycles of 30 s at 94 °C, 30 s at 60 °C and 60 s at 72 °C, followed by a final extension for 10 min at 72 °C. The PCR products are analysed by gel electrophoresis. The P1/PD pair of primers, and the P1/PM pair of primers, both produce a 198 bp amplicon. Olmos *et al.* (1997) evaluated their method using six isolates of PPV-D and four PPV-M isolates.

The real-time RT-PCR with SYBR Green I[2] by Varga and James (2005), described in detail in section 3.6.5, is also suitable for the identification of D and M strains of PPV.

PPV-Rec is identified using the mD5 and mM3 Rec-specific primers described by Šubr *et al.* (2004):

mD5 (5′-TAT GTC ACA TAA AGG CGT TCT C-3′)
mM3 (5′-CAT TTC CAT AAA CTC CAA AAG AC-3′)

The 25 µl reaction mixture is composed as follows (modified from Šubr *et al.*, 2004): 1 µM of each primer, 250 µM dNTPs, 1 unit AMV reverse transcriptase (10 units/µl), 0.5 units Taq DNA polymerase (5 units/µl), 2.5 µl 10× Taq polymerase buffer, 2.5 mM $MgCl_2$, 0.3% Triton X-100 and 5 µl extracted RNA (see section 3.6). Reverse transcription is carried out with random hexanucleotide primers, at 42 °C for 45 min (Glasa *et al*., 2002). PCR is carried out using an initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 45 s, 60 °C for 30 s and 72 °C for 60 s, and final elongation at 72 °C for 7 min (Šubr *et al.*, 2004). The PCR product of 605 bp is analysed by gel electrophoresis.

PPV-CR is identified using the CR8597F and CR9023R primers described by Glasa *et al.* (2013):

CR8597F (5′-ATG ATG TGA CGT TAG TGG AC-3′)
CR9023R (5′-TCG TGT GTT AGA CAG GTC AAC-3′)

A two-step RT-PCR protocol is used for specific detection of PPV-CR isolates (Glasa *et al*., 2013). Complementary (c)DNA is synthesized from total RNA extracts (NucleoSpin RNA Plant Kit, Macherey-Nagel)[2] using random hexamer primers and AMV reverse transcriptase. An aliquot of cDNA is then added to the PCR reaction mixture containing EmeraldAmp GT PCR Master Mix (TaKaRa Bio Inc.)[2]. The PCR is performed with the following thermocycling parameters: 60 s at 98 °C, 35 cycles of 98 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, followed by a final extension at 72 °C for 5 min. The PCR products are analysed by gel electrophoresis. The CR-specific primers amplify a product 427 bp in size, targeting the 5′ terminal CP coding region. The specificity of the CR primers was validated using isolates of PPV strains D, M, Rec, T, W, EA and C (Glasa *et al*., 2013).

4.2.2 Immunocapture reverse transcription-polymerase chain reaction

The immunocapture phase should be performed as described in section 3.6.3. The PCR reaction mixture is added directly to the coated PCR tubes. Identification of PPV-D and PPV-M is carried out as described in section 4.2.1.

4.2.3 Co-operational reverse transcription-polymerase chain reaction

Identification of PPV-D or PPV-M should be carried out as described in section 3.6.4 using 3′DIG-labelled probes specific for D and M strains (Olmos *et al.*, 2002):

PPV-D Specific Probe: 5′-CTT CAA CGA CAC CCG TAC GGG CA-DIG-3′
PPV-M Specific Probe: 5′-AAC GCC TGT GCG TGC ACG T-DIG-3′

The prehybridization and hybridization steps are performed at 50 °C with standard prehybridization and hybridization buffers + 30% formamide (for PPV-D identification) and + 50% formamide (for PPV-M identification). The blocking solution is used at 2% (w/v).

4.2.4 Real-time reverse transcription-polymerase chain reaction

PPV-D and PPV-M are specifically identified using either SYBR Green I[2] chemistry according to the method of Varga and James (2005) (see section 3.6.5) or the TaqMan method described by Capote *et al.* (2006).

The primers and TaqMan probes used in the method of Capote *et al*. (2006) are:

PPV-MGB-F primer (5′-CAG ACT ACA GCC TCG CCA GA-3′)

PPV-MGB-R primer (5′-CTC AAT GCT GCT GCC TTC AT-3′)

MGB-D probe (5′- FAM-TTC AAC GAC ACC CGT A-MGB-3′)

MGB-M probe (5′-FAM-TTC AAC AAC GCC TGT G-MGB-3′)

The 25 µl reaction mixture is composed as follows: 1 µM of each primer, 150 nM MGB-D or MGB-M FAM probe, 1× TaqMan Universal PCR Master Mix (Applied Biosystems)[2], 1× MultiScribe and RNase Inhibitor Mix (Applied Biosystems)[2] and 5 µl RNA template (see section 3.6). The RT-PCR is performed with the following thermocycling parameters: 30 min at 48 °C, 10 min at 95 °C, and 40 cycles of 15 s at 95 °C and 60 s at 60 °C. The PCR products are analysed in real time according to the manufacturer's instructions. Capote *et al*. (2006) evaluated the method using 12 isolates each of PPV-D and PPV-M, and 14 samples co-infected with both strains.

PPV-C, PPV-EA and PPV-W are specifically identified using SYBR Green I[2] chemistry according to the method of Varga and James (2006a). The primers used in this method are:

P1 (5′-ACC GAG ACC ACT ACA CTC CC-3′)

PPV-U (5′-TGA AGG CAG CAG CAT TGA GA-3′)

PPV-RR (5′-CTC TTC TTG TGT TCC GAC GTT TC-3′)

The following internal control primers (Menzel *et al.*, 2002) may be included to ensure the validity of the test results:

Nad5-F (5′-GAT GCT TCT TGG GGC TTC TTG TT-3′)

Nad5-R (5′-CTC CAG TCA CCA ACA TTG GCA TAA-3′)

The 25 µl RT-PCR reaction mixture is composed as follows: 2.5 µl of a 1:10 (v/v) water dilution of extracted RNA (see section 3.6) and 22.5 µl master mix. The master mix has the following composition: 2.5 µl Karsai buffer (Karsai *et al.*, 2002); 0.5 µl each of 5 µM primers PPV-U, PPV-RR, Nad5R and Nad5F; 0.5 µl 10 mM dNTPs; 1 µl 50 mM $MgCl_2$; 0.2 µl RNaseOUT (40 units/µl; Invitrogen)[2]; 0.1 µl Superscript III reverse transcriptase (200 units/µl; Invitrogen)[2]; 0.1 µl Platinum Taq DNA high fidelity polymerase (5 units/µl, Invitrogen)[2]; and 1 µl of 1:5 000 (in Tris-ethylenediaminetetraacetic acid (TE), pH 7.5) SYBR Green I[2] in 16.1 µl water. The reaction is performed with the following thermocycling parameters: 10 min at 50 °C, 2 min at 95 °C, and 29 cycles of 15 s at 95 °C and 60 s at 60 °C. Melting curve analysis is performed by incubation at 60 °C to 95 °C with 0.1 °C/s melt rates and a smooth curve setting averaging 1 point. Under the conditions of Varga and James (2006a), the melting temperatures for each product are:

C strain (74 bp fragment): 79.84 °C

EA strain (74 bp fragment): 81.27 °C

W strain (74 bp fragment): 80.68 °C

Varga and James (2006a) evaluated their method using one isolate each of PPV-C, PPV-D, PPV-EA and PPV-W.

5. Controls for Molecular Tests

For the test result 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 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 test method (apart from the extraction) and, in RT-PCR, the amplification. Total plant or viral RNA, or PPV-infected plant material printed on a membrane, may be used. The stored RNA or PPV preparations should be verified periodically to determine the quality of the control with increased storage time.

Internal control. For RT-PCR, mRNA of the mitochondrial gene *NADH dehydrogenase* 5 (*nad5*, Menzel *et al.*, 2002) could be incorporated into the RT-PCR protocol as an internal control to eliminate the possibility of RT-PCR false negatives due to nucleic acid extraction failure or degradation or the presence of RT-PCR inhibitors.

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 (with the target DNA) during the preparation of the reaction mixture. RNase-free 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 the target nucleic acid extracted is of sufficient quantity and quality for RT-PCR and that the target virus is detectable. Nucleic acid is extracted from PPV infected host tissue, or healthy plant or insect tissues that have been spiked with PPV.

For RT-PCR, care needs to be taken to avoid cross-contamination due to aerosols from the positive control or from positive samples.

Negative extraction control. This control is used to monitor both contamination during nucleic acid extraction and cross-reaction with the host tissue. The control comprises nucleic acid that is extracted from uninfected host tissue and subsequently amplified. It is recommended that multiple controls be included in random order when large numbers of positive samples are expected.

In the case of IC-RT-PCR where no nucleic extraction is performed, plant sap from a known PPV positive should be used as a positive control, and plant sap from a healthy plant should be used as a negative control. A negative amplification control may also be included. The latter control is used to rule out false positives due to contamination during the preparation of the reaction mixture. RNase-free PCR-grade water that was used to prepare the reaction mixture is added at the amplification stage for use as a negative amplification control.

6. Records

Records and evidence should be retained as described in section 2.5 of ISPM 27 (*Diagnostic protocols for regulated pests*).

In cases where other contracting parties may be affected by the results of the diagnosis, in particular in cases of non-compliance (ISPM 13 (*Guidelines for the notification of non-compliance and emergency action*)) and where the virus is found in an area for the first time, the following additional material should be kept for at least one year in a manner that ensures traceability:

- The original sample (labelled appropriately) should be kept frozen, if possible, at −80 °C or freeze-dried and kept at room temperature.
- If relevant, RNA extracts should be kept at −80 °C and spotted plant extracts or printed tissue sections (paper on paper or nylon membranes) should be kept at room temperature.
- If relevant, RT-PCR amplification products should be kept at −20 °C.

7. Contact Points for Further Information

Further information on this protocol can be obtained from:

United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Science and Technology Beltsville Laboratory, Building 580 BARC-East, Powder Mill Road, Beltsville, MD 20705, United States of America (Vessela Mavrodieva; email: vessela.a.mavrodieva@aphis.usda.gov; tel.: +1 3013139208; fax: +1 3023139232).

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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), who will in turn forward it to the Technical Panel on Diagnostic Protocols (TPDP).

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9. References

The present annex may refer to ISPMs. ISPMs are available on the International Phytosanitary Portal (IPP) at https://www.ippc.int/core-activities/standards-setting/ispms.

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2012-03 CPM-7 adopted Annex 2 to ISPM 27.

ISPM 27. Annex 2. *Plum pox virus* (2012). Rome, IPPC, FAO.

2015-07 IPPC Secretariat incorporated editorial amendments and reformatted standards following revoking of standards procedure from CPM-10 (2015).

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2017-11 Draft revised by the Lead based on consultation comments.

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ISPM 27
Diagnostic protocols for regulated pests

DP 25: *Xylella fastidiosa*

Adopted 2018; published 2018

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1. Pest Information

Xylella fastidiosa Wells *et al.*, 1987 is a xylem-limited bacterium and is the causal agent of many economically important plant diseases of agronomic and horticultural crops such as *Vitis vinifera*, *Prunus domestica*, *Prunus dulcis*, *Citrus sinensis*, *Olea europaea*, *Ulmus* spp. and *Quercus* spp. *X. fastidiosa* has a wide, expanding host range and comprehensive lists of susceptible hosts are available (EFSA, 2016; European Commission, 2018). *X. fastidiosa* is also expanding its geographical range. Until recently, it was mainly distributed throughout the Americas (Almeida and Nunney, 2015), but there have now been reports of outbreaks in Asia and Europe (EPPO, 2018a).

X. fastidiosa is genetically diverse and consists of several subspecies. *X. fastidiosa* subsp. *fastidiosa* causes Pierce's disease and infects a large host range including *V. vinifera*, *P. dulcis*, *Medicago sativa* and *Acer* spp. (Schuenzel *et al.*, 2005). *X. fastidiosa* subsp. *multiplex* is associated with scorch diseases of a range of trees that include *P. dulcis*, *Prunus persica*, *Quercus* spp. and *Platanus occidentalis*. *X. fastidiosa* subsp. *sandyi* causes oleander leaf scorch in *Nerium oleander* (Schuenzel *et al.*, 2005). *X. fastidiosa* subsp. *tashke* has been isolated from the ornamental tree *Chitalpa tashkentensis* (Randall *et al.*, 2009). *X. fastidiosa* subsp. *morus* (Nunney *et al.*, 2014) infects *Morus* spp. Finally, *X. fastidiosa* subsp. *pauca* (Schadd *et al.*, 2004) infects most *Citrus* and *Coffea* species, and *O. europaea.*

X. fastidiosa is a Gram-negative bacterium with fastidious growth requirements. The bacterial cells are non-motile, non-flagellate, rod-shaped cells, with rounded or tapered ends and numerous irregular ridges or folds on the cell wall surface (Wells *et al.*, 1987). The bacterium is inoculated into the water-transporting xylem elements of its host plants by xylem sap-feeding insects. The colonization of the xylem blocks the transport of mineral nutrients and water in the infected plants. Many diseases caused by *X. fastidiosa* are characterized by leaf scorch, defoliation, foliage wilt and a general decline in vigour, but expression of symptoms is heterogeneous, depending on the host plant species, *X. fastidiosa* genotype and the climatic conditions. Many host plants infected with *X. fastidiosa* show no symptoms (Almeida and Purcell, 2003). The bacterium proliferates in the xylem of an infected host and invades the plant's shoot and root system systemically (Aldrich *et al.*, 1992; He *et al.*, 2000; Li *et al.*, 2003). The pathogen overwinters in the xylem of the host plant. Insect transmission is considered the main factor for localized spread of *X. fastidiosa*. The vectors belong to the order Hemiptera, sub-order Auchenorrhyncha, families Cicadellidae (sharpshooter leafhopper), Cercopidae (spittlebugs) (Redak *et al*., 2004; Chatterjee *et al*., 2008), Aphrophoridae and Cicadidae. The transmission of *X. fastidiosa* by insects is persistent. Nymphs and adults are able to acquire the bacteria by feeding on the xylem fluid of an infected plant, and they then transmit the pathogen to other healthy plant hosts. While nymphs are able to acquire (and transmit) the bacterium, they lose it at each moult, so only continue to be infected if they reacquire the bacterium by feeding on infected plants after moulting (Almeida *et al.*, 2014). Once adults acquire the bacterium, they have it for life (as they do not moult). Once infected, adults can transmit throughout their whole lifetime, as the bacterium multiplies and persists in the vector foregut (cibarium and precibarium) (Brlansky *et al.*, 1983; Almeida *et al.*, 2005). There is no evidence of transovarial transmission (transmission from a female to her eggs) (Redak *et al.*, 2004). The movement of infected plants and planting material (e.g. budwood, seedlings) is assumed to be responsible for the long distance spread of the disease and its entry into new areas.

2. Taxonomic Information

Name: *Xylella fastidiosa* Wells *et al*., 1987

Synonyms: None

Taxonomic position: Bacteria, Proteobacteria, Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae

Common names: Alfalfa dwarf, bacterial leaf scorch disease, dwarf lucerne, citrus variegated chlorosis, olive quick decline syndrome, periwinkle wilt phony peach disease, Pierce's disease of grapevines, plum leaf scald. The leaf scorch diseases are

named in relation to their host plants; for example, almond leaf scorch, oleander leaf scorch, olive leaf scorch, pear leaf scorch.

Recent studies have split *X. fastidiosa* into several subspecies (Schaad *et al.*, 2004; Scally *et al.*, 2005; Schuenzel *et al.*, 2005; Randall *et al.*, 2009; Yuan *et al.*, 2010; Nunney *et al.*, 2014). Currently, only the subspecies *fastidiosa* and *multiplex* are considered valid names by the International Society of Plant Pathology Committee on the Taxonomy of Plant Pathogenic Bacteria (Bull *et al.*, 2012). Other additional *X. fastidiosa* subspecies proposed are "pauca" (Schaad *et al.*, 2004), "sandyi" (Schuenzel *et al.*, 2005), "morus" (Nunney *et al.*, 2014) and "taskhe" (Randall *et al.*, 2009). The *Xylella* species associated with pear leaf scorch in Taiwan Province of China (Leu and Su, 1993) is a new species, *X. taiwanensis* (Su *et al*., 2016). Recently, a revision of the *X. fastidiosa* subspecies has been proposed (Marceletti and Schortichini, 2016) based on comparative genomic analysis.

3. Detection

Plants infected with *X. fastidiosa* may be asymptomatic (Almeida and Purcell, 2003) or the symptoms may be similar to those associated with water stress or physiological disorders. Isolation methods are not recommended for detection due to the difficulty in isolating *X. fastidiosa* from plant tissue. Therefore, detection is based on inspection for symptoms and the use of specific serological and molecular tests on symptomatic plant material. There is limited information available on testing asymptomatic plants and the concentration of *X. fastidiosa* is likely to be lower than in symptomatic plants (Almeida & Nunney, 2015). Therefore, it is advisable to include molecular methods for testing asymptomatic plant material.

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. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

3.1 Symptoms

The presence of *X. fastidiosa* can have a broad impact on its host: from symptomless to plant death. Most host plants infected with *X. fastidiosa* do not show any symptoms, while some display symptoms that include leaf scorching, defoliation, chlorosis or bronzing along the leaf margin, and dwarfing. The bronzing may intensify before browning and drying. Symptoms are usually more pronounced in stressed plants (e.g. stressed by high or low temperature, or by drought) and they can vary according to the plant species or cultivar and environmental conditions (Janse and Obradovic, 2010; CABI, 2016).

Symptoms can be confused with other biotic (e.g. several fungal diseases) or abiotic causes (environmental stresses, water deficiency, salt, air pollutants, nutritional problems, etc.). Pictures of symptoms on various hosts can be found at https://gd.eppo.int/taxon/XYLEFA/photos and https://nature.berkeley.edu/xylella. Symptoms may vary depending on the host and *X. fastidiosa* subspecies combination. The host range can be markedly different between subspecies; however, there is some uncertainty with regards to the potential host range for each subspecies. Each subspecies can be found in multiple host plants. For example, *X. fastidiosa* subspecies *fastidiosa* not only infects grapes but it also causes alfalfa dwarf and overlaps with *X. fastidiosa* subsp. *multiplex* in causing almond leaf scorch (Yuan *et al.*, 2010). The following descriptions provide examples of the more characteristic symptoms observed on some key hosts, and the associated subspecies of *X. fastidiosa*, that are widely acknowledged in the current literature.

3.1.1 Pierce's disease of grapevines

Symptoms of Pierce's disease vary depending on the *Vitis* species, cultivar and local climatic conditions. *X. fastidiosa* subsp. *fastidiosa* has been the only subspecies reported to cause disease in grapevines (Nunney *et al.*, 2010). Muscadinia and native American cultivars display milder symptoms than those of *V. vinifera*. On *V. vinifera*, the initial symptoms are chlorotic spots on areas of the leaf lamina, in particular along the margins, with a sudden drying of leaf edges often surrounded by a yellowish or a

reddish halo (Hopkins and Purcell, 2002). In late summer and autumn, the necrotic leaf edges coalesce to form concentric rings that extend from the outer edge towards the centre. Subsequently, the leaf turns dry on the edges, but the leaf remains turgid and the whole lamina may shrivel and drop; the petiole remains attached to the branch (as so-called "match sticks"). The latter is a characteristic symptom of Pierce's disease late in the season. Fruit clusters shrivel or turn into raisins; branches and twigs usually start wilting from the tip; and infected stems mature irregularly showing patches of green tissue called "green islands". Buds on infected plants sprout later than those on healthy plants, and the new shoots grow slowly and are stunted. Severely affected plants may die within one or two years, although in several species and cultivars they may continue to live considerably longer. Symptoms are rarely seen in one-year-old plants. Symptoms on the twigs can be confused with those of fungal diseases such as rotbrenner and esca (EPPO, 2018b).

3.1.2 Citrus variegated chlorosis

The first symptoms of citrus variegated chlorosis (CVC) to appear on leaves are mottled variegations, with small chlorotic spots on the upper surface that correspond to small gummy brown spots on the underside of the leaf. Isolates within the *X. fastidiosa* subsp. *pauca* complex have been reported to cause CVC (Schaad *et al.*, 2004; Almeida *et al.*, 2008). Symptoms are most obvious on three- to six-year-old trees and mainly on *C. sinensis* cultivars. Affected trees show foliar interveinal chlorosis resembling zinc deficiency, but the symptoms are not symmetrical on opposite sides of the leaf. Symptoms of CVC can also be distinguished from zinc chlorosis by the presence of the gummy, brown necrotic regions on the underside of the leaf, which coincide with the chlorosis on the upper leaf surface (CABI, 2016). Sectoring of symptoms in the canopy occurs on newly affected trees. However, the CVC syndrome generally develops throughout the entire canopy on older infected trees. Affected trees are stunted and the canopy has a thin appearance because of defoliation and dieback of twigs and branches. Flowering is abnormal; fruits ripen earlier and do not fill, being much smaller than normal and very firm. The growth rate of affected trees is greatly reduced and twigs and branches may wilt. The plants do not usually die, but the yield and quality of the fruit are severely reduced (Donadio and Moreira, 1998).

3.1.3 Coffee leaf scorch

Symptoms of coffee leaf scorch appear on young flushes of field plants as large marginal and apical scorched zones on recently matured leaves (EPPO, 2018b). Affected leaves drop prematurely, shoot growth is stunted, and apical leaves are small and chlorotic. Symptoms may progress to shoot dieback and overall plant stunting. Fruit size and yield are generally reduced (De Lima *et al.*, 1998). Side branches have no leaves and fruits except for a tuft of leaves at the branch tip. Infection of coffee plants by *X. fastidiosa* can also lead to the "crespera" disease, which has been reported from Costa Rica (Montero-Astúa *et al.*, 2008). Symptoms range from mild to severe curling of leaf margins, chlorosis and deformation of leaves, asymmetry (Bergsma-Vlami *et al.*, 2015), stunting of plants, shortening of internodes and dieback of branches (Montero-Astúa *et al.*, 2008). *Coffea* plants may remain asymptomatic (De Lima *et al.*, 1998; Montero-Astúa *et al.*, 2008).

3.1.4 Olive leaf scorching and quick decline

In three different distant regions around the world (the southern region of Italy, Argentina and Brazil), leaf scorching symptoms on *O. europaea* trees have been associated with *X. fastidiosa* (Saponari *et al.*, 2013; Haelterman *et al.*, 2015; Coletta-Filho *et al.*, 2016). The strains associated with this disease in Italy are recombinants of strains within *X. fastidiosa* subsp. *pauca* (Loconsole *et al.*, 2014). The olive quick decline syndrome is characterized by leaf scorching and randomly distributed desiccation of twigs and small branches, which, in the early stages of the infection, are mainly observed in the upper part of the canopy. Leaf tips and margins turn dark yellow to brown, eventually leading to desiccation. Over time, symptoms become increasingly severe and extend to the rest of the crown, which acquires a blighted appearance. Desiccated leaves and mummified drupes remain attached to the shoots. Trunks, branches and twigs viewed in cross-section show irregular discoloration of the vascular elements, sapwood and vascular cambium (Nigro *et al.*, 2013). Rapid dieback of shoots, twigs and branches may

be followed by death of the entire tree. *X. fastidiosa* has also been detected in young olive trees with leaf scorching and quick decline (EPPO, 2018b).

3.1.5 Almond leaf scorch disease

The most characteristic symptoms of almond leaf scorching disease are leaf scorching followed by decreased productivity and general decline. Strains of *X. fastidiosa* subsp. *fastidiosa* and subsp. *multiplex* have been reported to cause almond leaf scorch disease (Yuan *et al.*, 2010). In early summer, leaves appear with marginal leaf scorch (brown, necrotic (dead) leaf tissue). Usually, a narrow band of yellow (chlorotic) tissue occurs between the dead tissue and the part of the leaf that is still green, but when the sudden appearance of leaf scorch symptoms is prompted by hot weather the narrow chlorotic band may not develop. As the disease progresses, affected twigs on limbs die back from the tip (Mircetich *et al.*, 1976). Even highly susceptible varieties take many years to die completely, but nut production is severely reduced within a few years in most varieties.

3.1.6 Bacterial leaf scorch of shade trees

Symptoms of bacterial leaf scorch of shade trees are similar on different shade tree hosts (e.g. *Acer* spp., *Platanus* spp., *Quercus* spp., *Ulmus americana* (Gould and Lashomb, 2007)). In most cases, the disease is identified by a characteristic marginal leaf scorch. Symptoms first appear in late summer to early autumn. Affected leaves have marginal necrosis, which may be surrounded by a chlorotic (yellow) or red halo. Generally, symptoms progress from older to younger leaves as the diseased branches die and the tree declines.

3.1.7 Bacterial leaf scorch of blueberry

The first symptom of bacterial leaf scorch of blueberry is a marginal leaf scorching, and the scorched leaf zone may be bordered by a darker band (Brannen *et al.*, 2016; EPPO, 2018b). In the early stages of disease progression, symptoms may be localized, but over time, symptoms can become uniformly distributed throughout the foliage. Newly developed shoots can be abnormally thin with a reduced number of flower buds. Leaf drop occurs, and twigs and stems have a distinct “skeletal” yellow appearance. Following leaf drop, the plant dies, this typically occurring during the second year after symptoms are observed (Chang *et al.*, 2009).

3.1.8 Phony peach disease and plum leaf scald

In phony peach disease and plum leaf scald, young shoots are stunted and bear greener, denser foliage than those on healthy trees. Strains associated with *X. fastidiosa* subsp. *multiplex* have been associated with phony peach disease. Lateral branches grow horizontally or droop, so that the tree seems uniform, compact and rounded. Leaves and flowers appear early, and remain on the tree longer than on healthy trees. Affected trees yield increasingly fewer and smaller fruits, becoming economically worthless after three to five years (Mizell *et al.*, 2015).

3.1.9 Alfalfa dwarf

The main symptom of alfalfa dwarf is stunted regrowth after cutting. This stunting may not be apparent until many months after initial infection. Leaflets on affected plants are smaller and often slightly darker in colour than those on uninfected plants, but not distorted, cupped, mottled or yellow. The tap-root is of a normal size, but the lignified tissue has an abnormally yellowish colour, with fine dark steaks of dead tissue scattered throughout. In recently infected plants, the yellowing is mostly in a ring beginning under the bark, with a normal white-coloured cylinder of tissue inside the yellowed outer layer of wood (EPPO, 2018b). The inner bark is not discoloured, nor do large brown or yellow patches appear as in bacterial wilt (caused by *Clavibacter michiganensis* subsp. *insidiosus*). Alfalfa dwarf progressively worsens over the first one to two years after the symptoms appear, and eventually kills infected plants.

3.1.10 Other hosts

X. fastidiosa has been detected on a number of different hosts in the recent European outbreaks. Most symptomatic plants display typical leaf scorching symptoms. On *N. oleander*, necrosis develops on the

leaf margin and infection may lead to death of entire plants (EPPO, 2018b). *Polygala myrtifolia* has been found to be one of the most susceptible hosts in the recent European outbreaks. Infected plants show scorched leaves, with desiccation starting from the tip and progressing to the entire blade (EPPO, 2018b). Symptoms can be seen at https://gd.eppo.int/taxon/XYLEFA/photos.

3.2 Sampling and sample preparation for symptomatic and asymptomatic material

Samples of necrotic and dead tissue or sections of the plant at an advanced stage of infection are unsuitable for *X. fastidiosa* diagnosis as saprophytes quickly colonize necrotic or dead tissue, interfering with the isolation or detection of the pathogen. Samples should be taken from close to the symptoms and preferably consist of stems that have mature symptomatic leaves with petioles and woody twigs. Individual leaves with petioles can also be sampled. The best plant material to test for the presence of *X. fastidiosa* is the leaf petiole and the midrib of mature leaves from either asymptomatic or symptomatic plant material. Guidance on the number of leaves (including their petioles) and approximate weights to be used in the laboratory sample is provided in Table 1 (EPPO, 2018b).

Table 1. Number of leaves (including their petioles) to be used and approximate weight of the laboratory sample. Data from EPPO (2018b).

Type of sample	Host plants and type of tissue	Minimum number of leaves per laboratory sample	Approximate weight of laboratory sample
Sample from individual plant with leaves	Petioles or midribs, or both, of leaves of large size (e.g. from *Coffea* spp., *Ficus* spp., *Vitis* spp., *Nerium* spp.)	5	0.5–1 g
	Petioles or midribs, or both, of leaves of small size (e.g. *Polygala myrtifolia* and *Olea* spp.)	25	0.5–1 g
	Plant species without petioles or with small petiole and midrib	25	0.5–1 g
Dormant plant or cuttings	Xylem tissue	n/a[†]	0.5–1 g
Composite sample from several coffee plants from a single lot with leaves	Samples of asymptomatic plants (e.g. collected from imported consignments or nursery monitoring)	100–200	10–50 g

[†] n/a, not applicable.

3.2.1 Sampling period for symptomatic or asymptomatic plants

The distribution and concentration of *X. fastidiosa* within the plant can be variable and is dependent upon plant species type, seasonal and environmental factors. To maximize the likelihood of detection, sampling should be performed during the period of active growth of the plants (Hopkins, 1981). This is usually from late spring to autumn in temperate zones. For asymptomatic plants, sampling is also possible during the period of active growth. However, sampling after warm periods (e.g. late summer–early autumn) increases the probability of accurate bacterial detection (European Commission, 2015).

In temperate zones of the world where *V. vinifera* or deciduous trees (e.g. *Prunus cerasus*, *P. dulcis*) have been infected for some time, the bacteria do not move into the new season's growth until the middle of summer, when symptoms may also become visible. For example, the most suitable time for searching for symptoms in grapevine is late summer to early autumn when weather conditions are predominately hot and dry or when grape plants are exposed to drought stress (Galvez *et al.*, 2010). For tropical plant species grown indoors such as coffee plants, sampling may be performed all year round when plants are exhibiting periods of active growth (EPPO, 2018b).

3.2.2 Plant sample collection

X. fastidiosa is confined to the xylem tissue of its hosts. The petiole and the midrib recovered from leaf samples are therefore the best sources for diagnosis, as they contain a greater number of xylem vessels (Hopkins, 1981). Other sources of tissue can include small twigs and roots of *P. persica* (Aldrich *et al.*, 1992), stem and roots of *Vaccinium* (Holland *et al.*, 2014) and *Citrus* fruit petioles (Rossetti *et al.*, 1990). Samples of branches or canes with attached leaves that include mature leaves generally provide the most reliable results. Young growing shoots should be avoided. For small plants, the entire plant can be sent to the laboratory.

3.2.3 Sampling of symptomatic plants

The sample should consist of branches or cuttings representative of the symptoms seen on the plant or plants and containing at least 10 to 25 leaves depending on leaf size. The approximate weight needed for laboratory samples is between 0.5 g and 1 g leaf petioles or midribs from each individual plant (EPPO, 2018b). Symptomatic plant material should preferably be collected from a single plant; however, a pooled sample may also be collected. It is recommended that, when testing pooled samples, the limit of detection for each detection test should be confirmed.

3.2.4 Sampling of asymptomatic plants

For asymptomatic plants, the sample should be representative of the entire aerial part of the plant. Recent experimental data on detection of *X. fastidiosa* in monumental and ancient *O. europaea* trees showed that detection was more reliable when sampling the medium–upper part of the canopy (Valentini and Porcelli, 2016). For testing individual asymptomatic plants, the number of branches to be collected is at least four to ten, depending on the host and plant size. There is limited experience of testing samples composed of leaves (including their petioles) collected from several asymptomatic plants. Further information on number of samples to be collected per lot can be found in ISPM 31 (*Methodologies for sampling of consignments*).

3.2.5 Plant sample transport and storage in the laboratory

Once samples are collected, they should be kept cool (e.g. 4–15 °C) and transported to the laboratory as quickly as possible. Lower temperatures can reduce sample deterioration. However, *X. fastidiosa* does not survive well in cold temperatures and for culture isolation work it is better to process samples immediately rather than refrigerate. Samples should be processed as soon as possible after arrival at the laboratory. If necessary, however, samples for isolation (see section 4.1) may be kept refrigerated (e.g. 4 °C) for up to three days. For other tests, samples may be refrigerated for up to one week. For longer term storage, samples may be stored at −20 °C or −80 °C for molecular or serological detection.

3.2.6 Sampling of vectors

Vectors should preferably be collected with sweeping nets (adults) or aspirators. Sticky traps are usually not effective for xylem feeders (Purcell *et al.*, 2014), but insects may be trapped accidentally and specimens collected from sticky traps can be used for testing. Vectors can be removed from the traps using small forceps (pincers) and a suitable solvent. After removal from the traps, insects should be rinsed in ethanol or acetone. Sampling for insects should preferably be carried out from late spring until early autumn to maximize the likelihood of detecting the bacterium. If insects cannot be processed immediately, they should be stored in 95–99% ethanol, or at −20 °C or −80 °C with or without ethanol. Sticky traps with captured insects can also be stored at −20 °C.

Insects collected from the field or from the wild can be analysed by polymerase chain reaction (PCR) to detect *X. fastidiosa*. Enzyme-linked immunosorbent assay (ELISA: see section 3.3) is not sensitive enough, as the bacterium only colonizes the insect foregut where, in spite of its multiplication, it is generally present at low levels (Purcell *et al.*, 2014). On the aphrophorid *Philaenus spumarius*, the population size of *X. fastidiosa* may be limited to fewer than 10^3 cells (Cornara *et al*., 2016).

3.3 Serological detection

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. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

A number of serological methods have been developed for the detection of *X. fastidiosa*, including methods using ELISA (Sherald and Lei, 1991), membrane entrapment immunofluorescence (Hartung *et al.*, 1994), dot immunobinding assay (Lee *et al.*, 1992), western blotting (Chang *et al.*, 1993) and immunofluorescence (Carbajal *et al*., 2004). More recently, direct tissue blot immunoassay has been reported as an alternative means of rapidly screening *O. europaea* samples for *X. fastidiosa* (Djelouah *et al*., 2014). Instructions for performing an ELISA (including tissue print, squash or dot ELISA) or an immunofluorescence test can be found in EPPO (2009, 2010). Serological methods are not sensitive enough for use early in the growing season, when no symptoms of the disease are observed, due to the low concentration of bacteria likely to be present in young asymptomatic tissue.

3.3.1 Preparation of material

ELISA works well for samples with symptoms and tissue that contains high concentrations of *X. fastidiosa*. The leaf petiole and mid-veins of symptomatic leaves are the best sources of tissue for ELISA. The technique can also be used on twigs and canes but is unsuitable for use on necrotic or dead tissue.

3.3.2 Double-antibody sandwich ELISA (DAS-ELISA)

Positive and negative controls should be included in each test and these are normally provided in commercial kits. Positive controls should consist of a reference *X. fastidiosa* 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 plant materials, the healthy plant extract control should be of the same species, variety or cultivar to allow for comparison with the test samples and to check for potential background- or cross-reactions.

Samples should be processed following the general procedure recommended for the specific serological method being used. In general, plant tissue is macerated in extraction 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; $Na_2HPO_4 \cdot 12\ H_2O$, 2.9 g; KH_2PO_4, 0.2 g; distilled water to 1 litre; pH 7.2) (1:10 w/v) using either a mortar and pestle or a tissue homogenizer (e.g. Polytron[1], Homex[1]) or is pulverized in liquid nitrogen (Loconsole *et al*., 2014; EPPO, 2018a). Further information on using DAS-ELISA to detect plant pathogenic bacteria is available in EPPO (2010).

Kits for the serological detection of *X. fastidiosa* are commercially available from Agritest[1], Agdia[1] and Loewe Biochemica[1]. These kits detect a wide range of *X. fastidiosa* strains isolated from different hosts. When using them, the manufacturer's instructions should be followed. The sensitivity of detection when using DAS-ELISA is approximately 10^4 colony-forming units (cfu)/ml; however, test sensitivity can vary depending on the plant species matrix being tested (Loconsole *et al.*, 2014; EPPO, 2018b).

The specificity and sensitivity of DAS-ELISA to detect *X. fastidiosa* on *O. europaea*, using a kit from Loewe[1], were evaluated by Loconsole *et al.* (2014). Additionally, a test performance study performed at the Institute for Sustainable Plant Protection (Bari, Italy) was conducted on serological kits from Agritest[1], Agdia[1] and Loewe[1]. These studies showed that these kits achieved 100% diagnostic sensitivity

[1] The 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.

and specificity when testing naturally infected samples. The data on the test performance study are available in the EPPO database on diagnostic expertise (EPPO, 2018c).

3.3.3 Interpretation of ELISA results

The reactions of the controls should be verified. Negative ELISA readings in positive control wells indicate that the test has not been performed correctly or that it has been inhibited. Positive ELISA readings in negative control wells indicate that cross-contamination or non-specific antibody binding has occurred. In these cases, the test should be performed again.

Once the reactions of the controls have been verified, the results for each sample are interpreted as follows:

- The ELISA is negative if the average absorbance readings of duplicate wells containing tissue macerate is <2× the average absorbance of the negative control wells containing healthy host tissue macerate.
- The ELISA is positive if the average absorbance readings of duplicate sample wells is ≥2× the average absorbance readings of the negative control wells containing healthy host tissue macerate.
- It is also recommended that the manufacturer's instructions be checked for interpretation of test results.

3.4 Molecular detection

Various molecular methods have been developed for the detection and identification of *X. fastidiosa* directly on pure cultures, plant tissue and insect vectors (Firraro and Bazzi., 1994; Minsavage *et al*., 1994; Pooler and Hartung, 1995; Schaad *et al.*, 2002; Rodrigues *et al.*, 2003; Francis *et al.*, 2006; Harper *et al.*, 2010, erratum 2013; Li *et al.*, 2013; Ouyang *et al.*, 2013). The conventional PCR developed by Minsavage *et al.* (1994) and Rodrigues *et al*. (2003), and two real-time PCRs (Harper *et al.*, 2010, erratum 2013, and Li *et al.*, 2013) are described in this protocol for the detection and identification of *X. fastidiosa*. The PCR methods described hereafter are as described in the original publications; however, some modifications (e.g. variations in PCR conditions or the use of other mixes) can be applied for optimization purposes.

3.4.1 DNA extraction from plant material

A number of methods have been described for the extraction of the DNA of *X. fastidiosa* from bacterial colonies and from plant tissue (Minsavage *et al.*, 1994; Pooler and Hartung, 1995; Francis *et al.*, 2006; Huang *et al.*, 2006; Harper *et al.*, 2010, erratum 2013; Li *et al.*, 2013). Extraction can be achieved using a number of standard commercial kits (e.g. Bextine and Child, 2007; Huang, 2009). The following methods are a selection of those widely used in several laboratories. There are many other similar DNA extraction kits that will also readily extract *Xylella* DNA from plant material. Validation data on the sensitivities associated with the different nucleic acid extraction methods can be found in the EPPO database on diagnostic expertise (EPPO, 2018c). A PCR can be readily conducted on boiled or heated preparations (e.g. suspensions of 10^8 cfu/ml heated at 95 °C for 15 min or 100 °C for 5 min) of bacterial colonies, or on DNA extracts purified using the methods below.

CTAB-based extraction. 0.5–1 g midrib, petiole or twig tissue is placed into an extraction bag with 5 ml CTAB buffer (100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 10 mM ethylenediaminetetraacetic acid (EDTA); 2% hexadecyltrimethylammonium bromide (CTAB); 3% polyvinylpyrrolidone (PVP)-40) and homogenized using a homogenizer (e.g. Homex[1], Polytron[1]). The homogenate (1 ml) is transferred to a microcentrifuge tube and incubated at 65 °C for 30 min. After cooling, the tube is centrifuged at 16 000 *g* for 5 min. The supernatant (1 ml) is transferred to a new tube and mixed with the same volume of chloroform:isoamylalcohol (24:1, v/v), vortexed and then centrifuged at 3000 *g* for 15 min. The aqueous layer (the upper layer – approximately 700 µl) is carefully transferred to a new tube and mixed with 490 µl ice-cold isopropanol. The suspension is mixed gently and incubated for at least 30 min at −20 °C. After this DNA precipitation step, the suspension is centrifuged at 16 000 *g* for 15 min and the supernatant is then discarded, taking care not to disturb the pellet. The pellet is washed with 1 ml ethanol

(70%) by repeating the last centrifugation step. After washing and decanting the supernatant, the pellet is air dried and suspended in 100 µl deoxyribonuclease-free water.

DNeasy Plant Mini Kit (Qiagen)[1]. DNA is extracted from 0.5–1.0 g plant tissue (leaf midrib, petiole or twig tissue) and macerated in lysis buffer using homogenizing equipment (e.g. Homex[1], Polytron[1]). Alternatively, plant tissue can be ground to a fine powder in liquid nitrogen prior to extraction. These extracts are then treated according to the manufacturer's instructions.

QuickPick SML Plant DNA Kit (Bio-Nobile)[1]. Plant tissue (200 mg leaf midrib, petiole or twig tissue) is homogenized using any of the available methods (e.g. mechanical grinding with bead mills or with liquid nitrogen, tissue grinder). The plant material should be sufficiently homogenized before starting the purification procedure. Appropriate volumes of plant DNA lysis buffer and proteinase K solution, as specified in the manufacturer's instructions, are added to the plant tissue. The sample is thoroughly vortex-mixed and then incubated at 65 °C for 15–30 min. After the lysis step, DNA purification is performed according to the manufacturer's instructions. Alternatively, a larger sample size can be processed by crushing 0.5–1 g fresh small pieces of midribs, petioles, basal leaf parts or twigs in 5 ml sterile water and leaving to soak for 15 min with gentle shaking. The plant extract (250 µl) is centrifuged for 20 min at 20 000 *g*. The pellet is then suspended in 75 µl lysis buffer with 5 µl proteinase K and the manufacturer's instructions followed. This method can be performed either manually or with the KingFisher mL[1] (15 samples) or KingFisher Flex[1] (96 samples) purification system (Thermo Scientific)[1]. Validation data are available in the EPPO database on diagnostic expertise (EPPO, 2018c). Caution is needed for users who are not familiar with this method, if performing manually, because the risk of cross-contamination between samples is high.

KingFisher (Thermo Scientific)[1] **using InviMAG Plant DNA Kit** (Stratec Molecular)[1]. This automated magnetic bead extraction procedure is ideal for high-throughput testing and uses the InviMAG Plant DNA Mini Kit (Stratec Molecular)[1] with the KingFisher 96 system (Thermo Scientific)[1]. Samples are homogenized in the lysis kit buffer (or CTAB buffer) at a tissue to buffer ratio of 1:5. The plant extracts are incubated at 60 °C for 30 min and then treated according to the manufacturer's instructions.

3.4.2 DNA extraction from insect vectors

DNA may be extracted from a single insect head or a pool of five heads (Bextine *et al.*, 2004; Purcell *et al.*, 2014; EPPO, 2018b). Only the heads of insects are used because they contain the foregut and mouthparts where *X. fastidiosa* resides (Bextine *et al.*, 2004). For DNA extraction from insects with big heads (e.g. *Cicadella viridis*, *Cicada orni*), only a single head should be used. The removal of the eye tissue, a potential source of PCR inhibitors, is recommended as it has been reported that this increases sensitivity (Bextine *et al.*, 2004; Purcell *et al*., 2014). Insect tissue can be ground in lysis buffer, or homogenized using a bead-beater system such as MagNA Lyser (Roche)[1] or by vacuum application and release (Bextine *et al.*, 2004, 2005; Huang *et al.*, 2006). A number of DNA extraction methods have been evaluated for the detection of *X. fastidiosa* in insect vectors. The following methods are a selection of those widely used in several laboratories.

DNeasy Tissue Kit (Qiagen)[1]. A DNA extraction method using this kit has been shown to reliably detect 50–500 *X. fastidiosa* cells in *Homalodisca coagulata* (Bextine *et al.*, 2004, 2005; Huang *et al.*, 2006).

QuickPick SML Plant DNA Kit (Bio-Nobile)[1] **for insects**. The homogenization of individual insect heads can be performed in 200 µl sterile distilled water using a bead-beater system such as the Retsch MM400[1]. Samples are homogenized for 2 min at 30 Hertz using ten stainless steel beads (diameter 3 mm) per 2 ml microtube. The microtube is placed on a magnet and the supernatant is transferred to a new microtube. The extract is centrifuged for 20 min at 20 000 *g*. The pellet is then suspended in 37.5 µl lysis buffer with 2.5 µl proteinase K, and the manufacturer's instructions followed. This kit can be used either manually or with the KingFisher mL[1] (15 samples) or KingFisher Flex[1] (96 samples) system (Thermo Scientific)[1].

CTAB-based extraction for insects. The homogenization of the insect heads can be performed in a microcentrifuge tube using a microhomogenizer or tungsten carbide beads. For the DNA extraction of

insect samples, 500 µl CTAB buffer is used. The incubation and centrifugation steps are similar to those used for plant samples (see section 3.4.1), but with adapted volumes.

3.4.3 Conventional polymerase chain reaction (PCR) using the primers of Minsavage *et al*. (1994)

This PCR was designed by Minsavage *et al*. (1994) to target part of the *rpoD* gene, producing an amplicon of 733 base pairs (bp). It is widely used in many laboratories for the detection of *X. fastidiosa* in different host plants and vectors. Analytical specificity was validated by Harper *et al.* (2010, erratum 2013) with 22 different *X. fastidiosa* strains from 11 different hosts and 12 closely related or host related non-target bacterial strains. In their study, American *X. fastidiosa* strains from red oak and turkey oak and several strains from grapevines were not detected with this PCR. The analytical sensitivity of the method as stated by Minsavage *et al.* (1994) is 1×10^2 cfu/ml on *V. vinifera* and *P. persica*. Further validation data on other hosts are available in the EPPO database on diagnostic expertise (EPPO, 2018c).

The oligonucleotide primers used are:

RST31 (forward): 5′-GCG TTA ATT TTC GAA GTG ATT CGA TTG C-3′

RST33 (reverse): 5′-CAC CAT TCG TAT CCC GGT G-3′

The master mix used for this PCR developed by Minsavage *et al.* (1994) is described in Table 2.

Table 2. Master mix composition, cycling parameters and amplicons for conventional PCR using the primers of Minsavage *et al.* (1994)

Reagents	Final concentration
PCR grade water	–[†]
PCR buffer (Invitrogen)[1]	1×
dNTPs	200 µM
$MgCl_2$	1.5 mM
Primer RST31 (forward)	0.5 µM
Primer RST33 (reverse)	0.5 µM
Taq DNA polymerase (Invitrogen)[1]	1.25 U
DNA volume	2 µl bacterial suspension or DNA extract
Cycling parameters	
Initial denaturation	95 °C for 1 min
Number of cycles	40
- Denaturation	95 °C for 30 s
- Annealing	55 °C for 30 s
- Elongation	72 °C for 45 s
Final elongation	72 °C for 5 min
Expected amplicons	
Size	733 bp

[†] For a final reaction volume of 20 µl.

bp, base pairs; PCR, polymerase chain reaction.

3.4.4 Conventional polymerase chain reaction (PCR) using the primers of Pooler and Hartung (1995)

This PCR was designed by Pooler and Hartung (1995) by developing PCR primers that target a specific randomly amplified polymorphic DNA fragment present in *X. fastidiosa*. The primers 272-1-int and

272-2-int are known to detect all known strains of *X. fastidiosa*. Analytical specificity has been validated with 57 different *X. fastidiosa* strains collected from different regions of Brazil and the United States of America (Huang, 2009; Reisenzein, 2017).

The oligonucleotide primers used are:

272-1-int (forward): 5′-CTG CAC TTA CCC AAT GCA TCG-3′

272-2-int (reverse): 5′-GCC GCT TCG GAG AGC ATT CCT-3′

The master mix used for this PCR is described in Table 3.

Table 3. Master mix composition, cycling parameters and amplicons for conventional PCR using the primers of Pooler and Hartung (1995)

Reagents	Final concentration
PCR grade water	–†
PCR buffer (Invitrogen)[1]	1×
dNTPs	200 µM
$MgCl_2$	1.5 mM
Primer 272-1-int (forward)	0.4 µM
Primer 272-2-int (reverse)	0.4 µM
Taq DNA polymerase (Invitrogen)[1]	1.0 U
DNA volume	2 µl bacterial suspension or DNA extract
Cycling parameters	
Initial denaturation	94 °C for 1 min
Number of cycles	40
- Denaturation	94 °C for 1 min
- Annealing	67 °C for 1 min
- Elongation	72 °C for 1 min
Final elongation	72 °C for 10 min
Expected amplicons	
Size	500 bp

† For a final reaction volume of 20 µl.

bp, base pairs; PCR, polymerase chain reaction.

3.4.5 Conventional PCR using the primers of Rodrigues *et al.* (2003)

The PCR based on primers for the 16S ribosomal (r)RNA and *gyrB* genes was developed by Rodrigues *et al.* (2003). The 16S rRNA gene-targeted primers (sets A, B, C), the *gyrB* gene-targeted primers (FXYgyr499 and RXYgyr907) and the multiplex PCR (16SrRNA and *gyrB* primers combined) were evaluated using 30 *X. fastidiosa* strains from different plant hosts and 36 closely related or host related non-target bacterial strains. The specific sets of primers for the 16S rRNA or *gyrB* genes can be used as either single or multiplex PCR. The analytical sensitivity for the multiplex PCR is similar to the singleplex reactions, which is approximately 10^2 cfu/ml.

The 16S rRNA gene-targeted primers are as follows.

Set A:

S-S-X.fas-0067-a-S-19 (forward): 5′-CGG CAG CAC ATT GGT AGT A-3′

S-S-X.fas-1439-a-A-19 (reverse): 5′-CTC CTC GCG GTT AAG CTA C-3′

Primer set A amplifies a product of 1348 bp.

Set B:

S-S-X.fas-0067-a-S-19 (forward): 5′-CGG CAG CAC ATT GGT AGT A-3′

S-S-X.fas-0838-a-A-21 (reverse): 5′-CGA TAC TGA GTG CCA ATT TGC-3′

Primer set B amplifies a product of 745 bp.

Set C:

S-S-X.fas-0838-a-S-21 (forward): 5′-GCA AAT TGG CAC TCA GTA TCG-3′

S-S-X.fas-1439-a-A-19 (reverse): 5′-CTC CTC GCG GTT AAG CTA C-3′

Primer set C amplifies a product of 603 bp.

The master mix and PCR conditions for the Rodrigues *et al*. primers (sets A, B, C) are described in Table 4. Multiplex PCR conditions are maintained as described except with 0.2 and 0.4 µM concentrations of each 16S rRNA and *gyrB* primer, respectively.

Table 4. Master mix composition, cycling parameters and amplicons for conventional PCR using the 16S rRNA gene-targeted primers of Rodrigues *et al.* (2003)

Reagents	**Final concentration**
PCR grade water	–[†]
PCR buffer	1×
dNTPs	200 µM
$MgCl_2$	1.5 mM
Primer (forward set A, or B or C)	0.2 µM
Primer (reverse set A, or B or C)	0.2 µM
Taq DNA polymerase (Invitrogen)[1]	2.0 U
DNA volume	2 µl bacterial suspension or DNA extract
Cycling parameters	
Initial denaturation	94 °C for 3 min
Number of cycles	30
- Denaturation	94 °C for 1 min
- Annealing	55 °C for 30 s
- Elongation	72 °C for 2 min
Final elongation	72 °C for 7 min
Expected amplicons	
Size	Primer set A: 1348 bp Primer set B: 745 bp Primer set C: 603 bp

[†] For a final reaction volume of 20 µl.

bp, base pairs; PCR, polymerase chain reaction.

The *gyrB* primers used are:

FXYgyr499 (forward): 5′-CAG TTA GGG GTG TCA GCG-3′

RXYgyr907 (reverse): 5′-CTC AAT GTA ATT ACC CAA GGT-3′

The *gyrB* primer set produces an amplicon of 429 bp.

The master mix for the *gyr*B gene-targeting primers is described in Table 5.

Table 5. Master mix composition, cycling parameters and amplicons for conventional PCR using the *gyr*B gene-targeting primers of Rodrigues *et al.* (2003)

Reagents	**Final concentration**
PCR grade water	–[†]
PCR buffer	1×
dNTPs	200 µM
$MgCl_2$	1.5 mM
Primer FXYgyr499 (forward)	0.4 µM
Primer RXYgyr907 (reverse)	0.4 µM
Taq DNA polymerase (Invitrogen)[1]	2.5 U
DNA volume	2 µl bacterial suspension or DNA extract
Cycling parameters	
Initial denaturation	94 °C for 3 min
Number of cycles	30
- Denaturation	94 °C for 1 min
- Annealing	60 °C for 1 min
- Elongation	72 °C for 2 min
Final elongation	72 °C for 7 min
Expected amplicons	
Size	429 bp

[†] For a final reaction volume of 20 µl.

bp, base pairs; PCR, polymerase chain reaction.

3.4.6 Real-time PCR using the primers and probes of Harper *et al.* (2010, erratum 2013)

This PCR, developed by Harper *et al.* (2010, erratum 2013), is designed to amplify part of the 16S rRNA processing protein *rimM* gene. DNA can be amplified from bacterial cultures, infected leaves, cane tissue or insect vectors.

Harper *et al.* (2010, erratum 2013) evaluated analytical specificity with 95 strains of *X. fastidiosa* from 20 different hosts and 26 non-target bacterial strains. Only *X. fastidiosa* was detected. *Xylella taiwanensis* from Taiwan Province of China was not detected. The PCR was further validated by Li *et al.* (2013). Diagnostic specificity and sensitivity, as determined using citrus and grape hosts, are 100% (EPPO, 2018b). For *O. europaea* hosts when using CTAB extraction methods, diagnostic specificity is 100% and diagnostic sensitivity is 91% (EPPO, 2018b). Further validation data are available in the EPPO database on diagnostic expertise (EPPO, 2018c). The analytical sensitivity (detection limit) is between 10^2 cfu/ml for *Citrus* spp. and *V. vinifera* and 10^5 cfu/ml for *O. europaea*.

The oligonucleotide primers and probes used are:

XF-F (forward primer): 5′-CAC GGC TGG TAA CGG AAG A-3′

XF-R (reverse primer): 5′-GGG TTG CGT GGT GAA ATC AAG-3′

XF-P (hydrolysis probe): 5′-6-FAM-TCG CAT CCC GTG GCT CAG TCC-BHQ-1-3′

The master mix for the Harper *et al.* (2010, erratum 2013) primers and probes is described in Table 6.

Table 6. Master mix composition and cycling parameters for real-time PCR using the primers and probes of Harper *et al.* (2010, erratum 2013)

Reagents	Final concentration
PCR grade water	–[†]
PCR mix (2× Supermix – UDG Invitrogen)[1]	1×
$MgCl_2$ (to a final concentration of)	4 mM
BSA	300 ng/µl
Primer XF-F (forward)	0.3 µM
Primer XF-R (reverse)	0.3 µM
Probe XF-P	0.1 µM
DNA volume	2 µl bacterial suspension or DNA extract
Cycling parameters	
Pre-incubation	50 °C for 2 min
Initial denaturation	94 °C for 2 min
Number of cycles	40
Heating ramp speed	5 °C/s
Denaturation	94 °C for 10 s
Annealing and elongation	62 °C for 40 s

[†] For a final reaction volume of 20 µl.

BSA, bovine serum albumin; PCR, polymerase chain reaction.

3.4.7 Real-time PCR using the primers and probes of Li *et al.* (2013)

This PCR, developed by Li *et al.* (2013), is designed to amplify part of the 16S rDNA gene. DNA can be amplified from bacterial cultures, infected leaves, cane tissue or insect vectors.

Li *et al.* (2013) evaluated analytical specificity with 77 strains of *X. fastidiosa* from 15 different hosts and 14 non-target bacterial strains. Only *X. fastidiosa* was detected. Diagnostic specificity and sensitivity, as determined using *Citrus* hosts, were both 100%. The analytical sensitivity (detection limit) is between 2 and 10 cells of *X. fastidiosa* per reaction for *Citrus* samples.

The oligonucleotide primers and probes used are:

XF16Sf (forward primer): 5′-CGG CAG CAC GTT GGT AGT AA-3′

XF16Sr (reverse primer): 5′-CCG ATG TAT TCC TCA CCC GT-3′

XF16Sp (hydrolysis probe): 5′-6-FAM-CA TGG GTG GCG AGT GGC-BHQ-1-3′

The master mix for the Li *et al.* (2013) real-time PCR is described in Table 7.

Table 7. Master mix composition and cycling parameters for real-time PCR using the primers and probes of Li *et al.* (2013)

Reagents	Final concentration
PCR grade water	–[†]
PCR buffer (Invitrogen)[1]	1×
dNTPs	240 µM
$MgCl_2$	6 mM
Primer XF16Sf (forward)	0.240 µM
Primer XF16Sr (reverse)	0.240 µM
Probe XF16Sp	0.12 µM
Platinum Taq (Invitrogen)[1]	1 U
DNA volume	2 µl bacterial suspension or DNA extract
Cycling parameters	
Initial denaturation	95 °C for 20 s
Number of cycles	40
Heating ramp speed	5 °C/s
Denaturation	95 °C for 1 s
Annealing and elongation	60 °C for 40 s

[†] For a final reaction volume of 25 µl.

PCR, polymerase chain reaction.

3.4.8 LAMP[2] using the primers of Harper *et al.* (2010, erratum 2013)

3.4.8.1 The LAMP[2] of Harper et al. *(2010, erratum 2013)*

This loop-mediated isothermal amplification (LAMP[2]) method was developed by Harper *et al.* (2010, erratum 2013) and can be used on crude plant tissue and insect extracts or with the DNA extraction methods described in section 3.4.1. Hydroxynaphthol blue can be used as a means of detecting the endpoint (Harper *et al.*, 2010, erratum 2013). Hydroxynaphthol blue or other dyes that can be added prior to amplification are recommended as they allow the LAMP[2] to be performed as a closed-tube system. This avoids the risk of opening tubes post amplification, which could lead to aerosol contamination due to the high titre of the LAMP[2] amplicon.

Analytical specificity using hydroxynaphthol blue for endpoint detection is similar to that reported for the real-time PCR (Harper *et al.*, 2010, erratum 2013). In validation, only *X. fastidiosa* was detected among 95 strains of *X. fastidiosa* from 20 different hosts and 26 non-target bacterial strains. All strains of *X. fastidiosa* were detected.

The primers used are:

XF-F3 (external primer): 5′-CCG TTG GAA AAC AGA TGG GA-3′

XF-B3 (external primer): 5′-GAG ACT GGC AAG CGT TTG A-3′

XF-FIP (internal primer): 5′-ACC CCG ACG AGT ATT ACT GGG TTT TTC GCT ACC GAG AAC CAC AC-3′

[2] When using LAMP on a regular basis in an area which has a patent system such as Japan (Patent Nos. 3,313,358, 3,974,441 and 4,139,424), the United States of America (US6,410,278, US6,974,670 and US7,494,790), the European Union (Nos. 1,020,534, 1,873,260, 2,045,337 and 2,287,338), China (ZL008818262), the Republic of Korea (Patent No, 10-0612551), Australia (No. 779160), and the Russian Federation (No. 2,252,964), it is necessary for users to receive a license from Eiken Chemical Co., Ltd. before use.

XF-BIP (internal primer): 5′-GCG CTG CGT GGC ACA TAG ATT TTT GCA ACC TTT CCT GGC ATC AA-3′

XF-LF (loop primer): 5′-TGC AAG TAC ACA CCC TTG AAG-3′

XF-LB (loop primer): 5′-TTC CGT ACC ACA GAT CGC T-3′

The master mix for the Harper *et al.* (2010, erratum 2013) LAMP[2] is described in Table 8.

Table 8. Master mix composition and test conditions for LAMP[2], according to Harper *et al.* (2010, erratum 2013)

Reagents	Final concentration
PCR grade water	–[†]
ThermoPol buffer (New England Biolabs)[1]	1×
$MgSO_4$ (additional to a final concentration)	8 mM
Betaine	0.8 M
BSA	300 ng/µl
Each dNTP	1.4 mM
External primer XF-F3	0.2 µM
External primer XF-B3	0.2 µM
Internal primer XF-FIP	1.6 µM
Internal primer XF-BIP	1.6 µM
Loop primer XF-LF	0.8 µM
Loop primer XF-LB	0.8 µM
Hydroxynaphthol blue (Sigma Aldrich)[1]	150 µM
Bst DNA polymerase	8 U
DNA volume	2 µl DNA extract
Incubation parameters	
Incubation	65 °C for 60 min
Enzyme inactivation	80 °C for 2 min

[†] For a final reaction volume of 25 µl.

BSA, bovine serum albumin; PCR, polymerase chain reaction.

A colour change from purple to a light blue is considered a positive result. Negative samples in which no amplification occurs remain violet.

3.4.8.2 Real-time LAMP[2]

This method is based on the above LAMP[2] primers developed by Harper *et al*. (2010, erratum 2013), and was modified by Yaseen *et al*. (2015). The modifications consist of a simplified extraction method and reduced incubation times. Ready-to-use kits for the method are commercially available and they are performed in real-time on a specific device or by using a standard real-time thermocycler (e.g. Enbiotech[1], Qualiplante[1], Optigene[1]). The kits should be used as per the manufacturer's instructions. Diagnostic sensitivity and specificity using the Enbiotech[1] and Qualiplante[1] kits have been determined as being between 83% and 92%. The analytical sensitivity (detection limit) of these kits is between 10^2 and 10^3 cfu/ml for *Citrus* spp., *V. vinifera* and *O. europaea*. Validation data are available in the EPPO database on diagnostic expertise (EPPO, 2018c).

3.4.9 Controls for molecular testing

For the test result 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 isolations and amplifications of the target pest or target nucleic acid.

For PCR, a positive nucleic acid (*X. fastidiosa*) control, an internal (host gene) control and a negative amplification control (no template control) are the minimum controls that should be used.

For LAMP[2], a positive nucleic acid (*X. fastidiosa*) control and a negative amplification control (no template control) are the minimum controls that should be used.

Additional controls may be used for both LAMP[2] and PCR as described below.

Positive nucleic acid control. This control is used to monitor the efficiency of PCR amplification. Pre-prepared (stored) nucleic acid, whole genomic DNA or a synthetic control (e.g. cloned PCR product) may be used. For this protocol, genomic DNA (50 ng/µl) extracted from either a culture of *X. fastidiosa* or naturally infected tissue is recommended as a positive nucleic acid control.

Internal control. For conventional and real-time PCR, a plant housekeeping gene such as *COX* (Weller *et al.*, 2000; Li *et al*., 2006), the 16S rDNA gene (Weisburg *et al.*, 1991) or *GADPH* (Mafra *et al.*, 2012) should be used as an internal control, to eliminate the possibility of PCR false negatives resulting either from nucleic acid extraction failure or degradation or from the presence of PCR inhibitors.

Negative amplification control (no template control). This control is necessary for conventional and real-time PCR to rule out false positives resulting from contamination during preparation of the reaction mixture. PCR-grade water that was used to prepare the reaction mixture, or sterile PBS, is added at the amplification stage.

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 from healthy plant tissue that has been spiked with the target near the concentration considered the detection limit of the test.

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 resulting from aerosols from the positive control or from positive samples. If required, the positive control used in the laboratory should be sequenced so that this 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 that, again, can be compared with PCR amplicons of the correct size.

Negative extraction control. This control is used to monitor both contamination during nucleic acid extraction and cross-reaction with the host tissue. The control comprises nucleic acid that is extracted from uninfected host tissue and subsequently amplified, or a tissue macerate sample extract previously tested negative for *X. fastidiosa*. It is recommended that multiple controls be included when large numbers of positive samples are expected.

3.4.10 Interpretation of results from conventional and real-time PCR

3.4.10.1 Conventional PCR

The pathogen-specific PCR will be considered valid only if both these 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), the positive control, and each of the test samples must produce an approximately 1.6 kilobase (kb) band (amplicon size will depend on which 16S rDNA primers are used (Weisburg *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.

3.4.10.2 Real-time PCR

The real-time PCR will be considered valid only if both these criteria are met:

- the positive control produces an amplification curve with the pathogen-specific primers and probe
- no amplification curve is seen 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), the 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 nucleic acid 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 method for the first time. Guidance on how to determine the cycle cut-off value can be found in Chandelier *et al.* (2010).

3.4.10.3 Real time LAMP

The real time PCR LAMP will be considered valid only if both these criteria are met:

- the positive nucleic acid control produces a specific reaction (the type of reaction varies with the technology of the LAMP test (e.g. fluorescence, coloration, amplification curve); the specific reaction is described in the instructions of the kit providers or in the specific section of the protocol describing the LAMP test)
- the negative amplification control does not produce a specific reaction.

A test will be considered positive if it produces a specific reaction as defined for the control reactions (see above). A test will be considered negative if it produces no specific reaction. Tests should be repeated if any contradictory or unclear results are obtained.

4. Identification

The minimum requirements for identification are positive results from two tests based on different biological principles or from two molecular tests that amplify different genetic loci. However, if the outcome is critical (e.g. post-entry quarantine samples, new host record, new country record), it is recommended that the bacterium is isolated and the requirements for Koch's postulates fulfilled.

Further tests may be done in instances where the NPPO requires additional confidence in the identification of the *X. fastidiosa* subspecies or strain type. Sequencing of the complete genome (Simpson *et al.*, 2000; Van Sluys *et al.*, 2003), or multilocus sequence analysis (MLSA or MLST) (Scally *et al.*, 2005; Yuan *et al.*, 2010), is recommended for subspecies identification or when atypical or undescribed strains are suspected (section 4.5.1).

4.1 Isolation

X. fastidiosa strains are difficult to isolate, even from symptomatic plants, and difficult to grow in axenic culture. They do not grow on most common bacterial media, and require specialized media such as PD2 (Davis *et al.*, 1980), BCYE (Wells *et al.*, 1981) or PWG (modified from Hill and Purcell, 1995; EPPO, 2018b). It is recommended that at least two different media be used for isolation.

Midrib and petiole tissue from symptomatic leaf samples are considered the best sources for reliable isolation of *X. fastidiosa*. However, other sources of infected plant tissue from which the bacterium can be isolated include small twigs, stem and root sections (Hopkins, 2001). *X. fastidiosa* can also be isolated from insect vectors (Hill and Purcell, 1995).

It is very important to surface sterilize the sample in order to avoid contaminants, because *X. fastidiosa* grows very slowly (up to 30 days) and can be readily overgrown by other microorganisms. Petiole or midrib samples are surface sterilized by immersion in 70% ethanol for 1 min and flaming, or in 1% bleach for 2 min, followed by two rinses in sterile distilled water. Surface-sterilized plant tissue segments are cut in the middle, squeezed with flame-sterilized needle-nose pliers, and the sap that exudes can be blotted directly onto media (Hopkins, 2001). Alternatively, tissue is ground in PBS at ratios of 1:10 and 1:100 with a mortar and pestle or a homogenizer (e.g. Homex)[1] and then plated onto two different types of specific media (e.g. PD2, BCYE, PWG). The application of ultrasonication during the extraction process has been shown to improve isolation from asymptomatic *Coffea arabica* plants (Bergsma-Vlami *et al.*, 2017). After tissue is ground in PBS, the crushed plant material is ultrasonicated for 30–60 s at 40 kHz.

Insect vectors are surface sterilized as above and the heads are severed from the body and homogenized in 2 ml PBS. Drops of the insect tissue are plated onto specific media as above.

The plates should be incubated at 28 °C for 8–30 days, in plastic bags or sealed with parafilm[1] to prevent desiccation. Plates are observed regularly for colony development using a binocular microscope. Colonies visible to the unaided eye within two days should be regarded as contaminants.

4.1.1 Culture media

The culture media described in this protocol are as described in the original publications. There are other modifications of these culture media available that have been observed to produce reliable results (EPPO, 2018b). All media are autoclaved at 121 °C for 15 min.

PD2 medium (Table 9). All components except BSA (bovine serum albumin) and hemin chloride stock solution are added to 980 ml of distilled water prior to autoclaving. The pH is adjusted to 7.0 after dissolving the agar. After autoclaving, the BSA (dissolved in distilled water) and hemin chloride stock solution are filter sterilized (0.2 µm membrane) and added to the cooled (45–50 °C) sterile basal medium.

Table 9. PD2 medium (Davis *et al.*, 1980)

Reagents	Per litre
Phytone peptone (BD BBL)[1]	2.0 g
Bacto tryptone (Oxoid)[1]	4.0 g
Trisodium citrate	1.0 g
Disodium succinate	1.0 g
Hemin chloride stock solution (0.1% in 0.05 N NaOH)	10 ml
BSA (20% w/v) (Sigma)[1]	10 ml
$MgSO_4 \cdot 7H_2O$	1.0 g
K_2HPO_4	1.5 g
KH_2PO_4	1.0 g
Bacto agar (e.g. BD Difco)[1]	15 g
Distilled water to a final volume of 1 litre	

BSA, bovine serum albumin.

BYCE medium (Table 10). Due to the difficulty of dissolving and re-suspending the individual components it is recommended that ingredients are dissolved in the following order. ACES buffer is first rehydrated in 500 ml distilled water at 50 °C before addition of the yeast extract, activated charcoal and agar. Before adding the agar, the pH is adjusted to 6.9 by the addition of approximately 40 ml 1 M KOH. The medium is autoclaved and then cooled to 50 °C. Both the cysteine hydrochloride (0.4 g) and ferric pyrophosphate (0.25 g) are resuspended in 10 ml distilled water, filter sterilized and added to the

cooled sterile medium. The ferric pyrophosphate needs to be heated, under agitation, at 75 °C for approximately 15–20 min (EPPO, 2018b).

Table 10. BCYE medium (Wells *et al.*, 1981)

Reagents	Per litre
ACES buffer (Sigma)[1]	10.0 g
Yeast extract	10.0 g
Activated charcoal (Norit)[1]	2.0 g
L-cysteine hydrochloride-1-hydrate (Sigma)[1]	0.4 g
Ferric pyrophosphate (Sigma)[1]	0.25 g
Bacto agar (e.g. BD Difco)[1]	17 g
Distilled water to a final volume of 1 litre	

Modified PWG medium (Table 11). All constituents except L-glutamine, hemin chloride stock solution and BSA are added prior to autoclaving. Bovine serum albumin (3 g) is dissolved in 15 ml distilled water, and 4 g L-glutamine is dissolved in 50 ml distilled water over a low heat (c. 50 °C). Hemin chloride stock is 0.1 % bovine hemin chloride dissolved in 0.05 N NaOH. These three solutions are filter sterilized (0.2 µm membrane) and added to the cooled sterile basal medium.

Table 11. Modified PWG medium (based on Hill and Purcell (1995) and information provided in EPPO (2018b))

Reagents	Per litre
Gelrite gellan gum (Sigma)[1]	9.0 g
Phytone peptone (e.g. BD BBL)[1]	4.0 g
Bacto tryptone (e.g. Oxoid)[1]	1.0 g
Phenol red stock solution (0.2%)	10 ml
L-glutamine (Sigma)[1]	4 g
Hemin chloride stock solution (0.1% in 0.05 N NaOH)	10 ml
BSA (Sigma)[1]	3.0 g
$MgSO_4 \cdot 7H_2O$	1.0 g
K_2HPO_4	1.5 g
KH_2PO_4	1.0 g
Distilled water to a final volume of a 1 litre	

BSA, bovine serum albumin.

4.1.2 Colony morphology

X. fastidiosa colony morphology can be variable (Davis *et al.*, 1981; Chen *et al.*, 2005), but on most selective media colonies are convex, either smooth or rough, and with entire or finely undulate margins (Bradbury, 1991). The comparison of colony morphology with a reference culture of *X. fastidiosa* (Table 12) may help a correct identification to be reached.

Table 12. Reference *X. fastidiosa* strains

Strain	Source
CFBP 7969, 8073	International Center for Microbial Resources – French Collection for Plant-associated Bacteria, Beaucouze, France
LMG 17159	Belgium Co-ordinated Collection of Micro-organisms, Ghent, Belgium
ICMP 11140, 15197	International Collection of Microorganisms from Plants, Auckland, New Zealand
NCPPB 4432	National Collection of Plant Pathogenic Bacteria, York, United Kingdom of Great Britain and Northern Ireland
DSM 10026	Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

4.1.3 Interpretation of isolation results

The isolation is negative if no bacterial colonies with growth characteristics and morphology similar to *X. fastidiosa* are observed after 14–30 days on any medium and typical *X. fastidiosa* colonies are found in the positive controls.

The isolation is positive if bacterial colonies with growth characteristics and morphology similar to *X. fastidiosa* are observed after 14–30 days on at least one medium. In some cases, the incubation time can be up to 30 days due to the fastidious growth requirements of *X. fastidiosa*. The presumptive identification of *X. fastidiosa* colonies should be confirmed by serological- or molecular-based methods.

4.2 Description and biochemical characteristics

X. fastidiosa is a fastidious Gram-negative, straight, rod-shaped bacterium measuring 0.25–0.35 µm by 0.9–3.5 µm. It is strictly aerobic, non-flagellate, non-motile, and does not form spores (Davis *et al.*, 1978; Wells *et al.*, 1987; Bradbury, 1991). Some of the key biochemical and physiological characteristics for *X. fastidiosa* are listed in Table 13.

The reference *X. fastidiosa* strains available from different collections are listed in Table 12. These strains are suggested for use as positive controls in biochemical and molecular tests.

Table 13. Key biochemical and physiological characteristics of *X. fastidiosa* (Davis ***et al.***, 1978; Wells ***et al.***, 1987; Bradbury, 1991)

Test	Result
Catalase	+
Oxidase reaction	−
Gelatin liquefaction	+
Indol production	−
H_2S production	−
DL-lactate	+
Glucose fermentation	−
Temperature optimum	26 to 28 °C
pH optimum (*X. fastidiosa* is very sensitive to variations in pH)	6.5 to 6.9

4.3 Pathogenicity tests

Pathogenicity testing is recommended when requiring additional information on strain aggressiveness, potential host range, or to fulfil the requirements of Koch's postulates.

Actively growing, susceptible plants need to be maintained in a greenhouse or growth chamber at 26–28 °C. Inoculation techniques should deliver inoculum directly into the xylem vessels for development

of symptoms. The most widely used method for plant inoculation is by needle puncture into the stem at the insertion of the petiole (Hill and Purcell, 1995; Almeida *et al.*, 2001). A general inoculation procedure is described below.

Pathogenicity tests should use plants of the same host from which the suspect *X. fastidiosa* was isolated. Where possible, the most susceptible cultivars should be used. Some recommended examples include: for *V. vinifera*, the cultivars 'Chardonnay', 'Cabernet sauvignon', 'Chenin Blanc' and 'Pinot Noir'; for *C. sinensis*, 'Pera', 'Hamlin', 'Natal' and 'Valencia'; and for *O. europaea*, 'Cellina di Nardo', 'Frantoio' and 'Leccino' (EPPO, 2018b). *Catharanthus roseus* (Madagascar periwinkle) is a herbaceous plant that is easily grown in a greenhouse and is susceptible to *X. fastidiosa* (Monteiro *et al.*, 2001).

To facilitate the rapid uptake of the inoculum by the transpiration system, inoculated plants should be young and should be grown in pots with dry soil. Cultures of bacteria grown for 8–10 days on suitable media should be used for pathogenicity tests. Bacteria are removed from solid media and suspended in PBS to produce a turbid suspension of approximately 10^8–10^9 cfu/ml ($Abs_{600nm} = 0.2$). A drop (20–50 µl) of inoculum is placed in a leaf axil and punctured through several times with a fine needle until the liquid is completely absorbed. Control plants are treated in the same way except that the suspending medium (PBS) is used instead of bacterial suspension. Plants must be maintained in the greenhouse or growing chambers at 26–28 °C.

An alternative method of inoculation is to raise a flap of stem tissue by cutting upward with a razor blade to expose the wood. A few drops of bacterial suspension are placed under the flap and the flap replaced and wrapped with grafting tape.

Symptom development usually appears 60–80 days after inoculation; however, this is known to be variable and could be up to 24 months depending on host and strain combination (Hopkins, 2001).

For both methods of inoculation, if possible the bacterium should be re-isolated to fulfil the requirements for Koch's postulates.

In addition, a bioassay can be performed on *Nicotiana tabacum* (tobacco) plants by inoculating the petioles with suspensions of *X. fastidiosa* (Francis *et al*., 2008). Leaf scorch symptoms develop 10–14 days after inoculation.

4.4 Serological identification

ELISA (described in section 3.3) can be used for the identification of suspect *X. fastidiosa* strains isolated from diseased plant material.

4.5 Molecular identification

PCR (described in section 3.4) can be used for the identification of suspect *X. fastidiosa* strains isolated from diseased plant material. If only PCR is being performed, to allow rapid diagnosis, it is recommended that identification is confirmed by using two different sets of primers targeting two different genes. For interpretation of conventional and real-time PCR results see section 3.4.9. For conventional PCR tests, the amplicons can be sequenced to further support the identification. Sequence data can be analysed using the Standard Nucleotide Basic Local Alignment Search Tool (BLASTN), available at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/).

4.5.1 Multilocus sequence typing

A multilocus sequence typing (MLST) approach has been described for the identification of *X. fastidiosa* subspecies and is recommended for the characterization of new strains (Scally *et al.*, 2005; Yuan *et al.*, 2010; Jacques *et al.*, 2016; Bergsma-Vlami *et al.*, 2017). This approach can be used on DNA extracted from either bacterial cultures or infected plants tested positive for *X. fastidiosa* (Loconsole *et al.*, 2016). For amplification of DNA direct from plant tissue, it has been observed that the quality of the target DNA may not always be suitable for obtaining all amplicons (EPPO, 2018b). Primers and conditions for the sequencing and analysis of seven housekeeping genes (*cysG*, *gltT*, *holC*, *leuA*, *malF*, *nuoL* and

petC) are described by Yuan *et al.* (2010) and further details regarding analysis can be found on the *X. fastidiosa* MLST website (http://pubmlst.org/xfastidiosa/). If erratic amplification occurs, the following PCR parameters can be adjusted: the DNA extract can be diluted (to limit inhibition) or the amount of DNA added to the PCR can be increased, different Taq polymerases or master mixes can be used, the annealing temperature can be decreased from 65 °C to 60 °C or 58 °C, or the primer concentration can be increased from 0.3 to 0.5 µM (EPPO, 2018b).

Expected amplicon sizes for the different housekeeping genes are: 708 bp for *leuA*, 533 bp for *petC*, 600 bp for *cysG*, 654 bp for *gltT*, 379 bp for *holC*, 730 bp for *malF*, and 557 bp for *nuoL*.

The targeted regions are amplified by PCR, and if the amplicons are of good quality and the expected size they should be sequenced directly using forward and reverse primers. Sequences are concatenated by following the alphabetical order of the genes and analysis should be performed as per advice on the MLST website (http://pubmlst.org/xfastidiosa/). The results of the sequencing should be compared with reference sequences for the different housekeeping genes that can be found on the MLST website.

Although different methods are available for subspecies identification (see section 4.5.2), it is recommended that MLST be used to analyse *X. fastidiosa* strains detected in new areas or on new host associations.

4.5.2 Subspecies- and strain-specific PCR

There are a number of specific methods using PCR that enable *X. fastidiosa* subspecies determination (Pooler and Hartung 1995; Hernandez-Martinez *et al.*, 2006; Li *et al.*, 2013). The PCR described by Hernandez-Martinez *et al.* (2006) can allow the identification of cultures of subspecies *fastidiosa*, *multiplex* and *sandyi*. The methods described above have mainly been developed on pure cultures but can be used on DNA extract from plants except for the multiplex PCR by Hernandez-Martinez *et al.* (2006). However, it is recognized that the quantity and quality of target DNA, or the occurrence of possible mixed infections, may mean that not all amplicons are obtained or may prevent clear assignment of subspecies. Pooler and Hartung (1995) developed a conventional PCR that identifies subspecies *pauca*. The CVC strains of *X. fastidiosa* can be identified by using either a conventional PCR (Pooler and Hartung, 1995) or a real-time PCR (Li *et al.*, 2013). The oleander leaf scorch strains of *X. fastidiosa* can be specifically detected and differentiated from other strains by PCR (Huang, 2009) or real-time PCR (Guan *et al*., 2013). Recently, a PCR has been developed to allow specific detection and identification of American mulberry-infecting strains of *X. fastidiosa* and the newly discovered strains of *X. fastidiosa* associated with Italian olive trees (Guan *et al*., 2015).

5. Records

Records and evidence should be retained as described in section 2.5 of ISPM 27 (*Diagnostic protocols for regulated pests*).

In cases where other contracting parties may be affected by the results of the diagnosis, in particular in cases of non-compliance (ISPM 13 (*Guidelines for the notification of non-compliance and emergency action*)) and where the bacterium is found in an area for the first time, the following additional material should be kept for at least one year in a manner that ensures traceability:

- Photographs of symptoms and signs, printouts of ELISA plate results, and photographs of DNA agarose gels should be retained.
- Cultures can be stored at −80 °C or stored in an international culture collection.
- The original sample (labelled appropriately) should be kept frozen if possible at −80 °C, or freeze-dried and kept at room temperature.
- If relevant, DNA extracts should be kept at −80 °C and PCR amplification products at −20 °C.

6. Contact Points for Further Information

Further information on this protocol can be obtained from:

Austrian Agency for Health and Food Safety (AGES), Plant Health Laboratory, Spargelfeldstraße 191, 1220 Vienna, Austria (Helga Reisenzein; email: Helga.reisenzein@ages.at).

Ministry for Primary Industries, Plant Health and Environment Laboratory, PO Box 2095, Auckland 1140, New Zealand (Robert Taylor; email: Robert.taylor@mpi.govt.nz).

United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Molecular Plant Pathology Laboratory, Beltsville Agriculture Research Center-West, 10300 Baltimore Avenue, Beltsville, MD 20705, United States of America (John Hartung; email: John.hartung@ars.usda.gov).

USDA Animal Plant Health and Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Phytosanitary Issues Management, 4700 River Road, Riverdale, MD 20737, United States of America (Wenbin Li; email: Wenbin.li@aphis.usda.gov).

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), which will forward it to the Technical Panel on Diagnostic Protocols (TPDP).

7. Acknowledgements

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8. Figures

No figures are included in the protocol itself. Pictures of symptoms are accessible on the EPPO global database website at https://gd.eppo.int/taxon/XYLEFA/photos.

9. References

The present annex may refer to ISPMs. ISPMs are available on the International Phytosanitary Portal (IPP) at https://www.ippc.int/core-activities/standards-setting/ispms.

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ISPM 27
Diagnostic protocols for regulated pests

DP 26: *Austropuccinia psidii*

Adopted 2018; published 2018

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1. Pest Information

Austropuccinia psidii (G. Winter) Beenken (2017) (formerly *Puccinia psidii*, Beenken, 2017), a rust pathogen first described from *Psidium guajava* in Brazil (Winter, 1884), is considered to be native to South and Central America and possibly the Caribbean (Coutinho *et al.*, 1998). It was first reported outside its native range in the late 1970s, in the United States of America, and has subsequently been reported in Australia, China, Japan, New Caledonia, South Africa (da S. Machado *et al.*, 2015) and Indonesia (McTaggart *et al.*, 2016). The fungus infects young actively growing tissues of plants within the family Myrtaceae (Morin *et al.*, 2012). The most economically important hosts are *Eucalyptus* species. The impact in susceptible species is branch death and dieback, a significant reduction of canopy density, and tree death (Pegg *et al.*, 2014).

A. psidii is considered to be a threat to plants of the family Myrtaceae worldwide (Coutinho *et al.*, 1998). Since the rust spread out of its native region, its host range has expanded rapidly (Maier *et al.*, 2016). As of September 2014, the global host list comprised more than 300 species from 73 genera in this family (Giblin and Carnegie, 2014), but it is likely that the majority of the thousands of Myrtaceae species have the potential to be infected (Carnegie and Lidbetter, 2012; Morin *et al.*, 2012). *A. psidii* is not known to infect host plants that are not Myrtaceae. There is evidence of physiological specialization within *A. psidii* (Graça *et al.*, 2013), which may have quarantine implications (Roux *et al.*, 2016).

A. psidii is an obligate biotroph with an autoecious, but incomplete, life cycle, producing urediniospores, teliospores and basidiospores on an infected host (Glen *et al.*, 2007). Under natural conditions, *A. psidii* can reproduce quickly and simply through asexual reproduction whereby urediniospores are produced in pustules known as uredinia. These spores are dispersed to leaves on the same plant or to other hosts, which in turn are infected and on which the pathogen produces pustules with more urediniospores. In some circumstances, the uredinia may switch to producing teliospores, which can germinate *in situ* to produce basidiospores. Teliospores may also be produced by another type of spore producing body, telia. Teliospore and basidiospore production were initially considered rare stages of the life cycle, but in some regions are often observed along with urediniospore production within a single sorus (Pegg *et al.*, 2014). While the production of all three types of spores in a host is considered to be a strategy for survival in adverse conditions, the role of teliospores and basidiospores in the life cycle of *A. psidii* has not been understood (Morin *et al.*, 2012; Giblin, 2013). Spermagonia and aecia have never been observed.

A. psidii prefers wet tropical and subtropical regions where moist conditions and warm temperatures prevail, but a spread to cool regions has been reported (Kriticos *et al.*, 2013) and the optimum temperature for survival of the fungus is unknown. Disease development is favoured following periods of rainfall or in high humidity or fog. Extended periods of leaf wetness promote urediniospore germination and infection of the host. For infection to occur, urediniospores must encounter a host plant during stages of active growth or flush, which can occur throughout the year depending on the host species and climatic conditions (Pegg *et al.*, 2014).

Although urediniospores of *A. psidii* can be dispersed over long distances by wind, far reaching dispersal into new geographical regions is believed to result from human activities (Giblin, 2013). Modes of spread include: infected or contaminated planting material, nursery stock, plant cuttings, flowers and germplasm; animals and insects such as bees, birds, bats and possums that have been in contact with urediniospores; contaminated plant waste, timber, and wood packaging material; contaminated equipment and tools used on or around plants (e.g. chainsaws, secateurs); and contaminated clothing, shoes and other personal effects (Giblin, 2013). Once an initial infection occurs, urediniospores are readily dispersed naturally to nearby susceptible hosts.

2. Taxonomic Information

Name: *Austropuccinia psidii* (G. Winter) Beenken (2017)

Basionym: *Puccinia psidii* G. Winter, 1884

Synonyms: *Caeoma eugeniarum* Link, 1825

Uredo neurophila Speg., 1884

Uredo subneurophila Speg., 1884

Uredo flavidula G. Winter, 1885

Uredo myrtacearum Pazschke, 1890

Uredo eugeniarum Henn., 1895

Aecidium glaziovii Henn., 1897

Dicaeoma psidii (G. Winter) Kuntze, 1898

Uredo pitangae Speg., 1899 (published as "*pitanga*")

Puccinia jambosae Henn., 1902

Uredo puttemansii Henn., 1902

Uredo goeldiana Henn., 1903

Uredo rochaei Puttemans, 1906

Puccinia rompelii Magnus, 1907

Puccinia jambolani Rangel, 1912

Uredo myrciae Mayor, 1913

Puccinia barbacensis Rangel, 1916

Puccinia brittoi Rangel, 1916

Puccinia cambucae Puttemans, 1916

Puccinia eugeniae Rangel, 1916

Puccinia grumixamae Rangel, 1917

Bullaria psidii (G. Winter) Arthur and Mains, 1922

Puccinia camargoi Puttemans, 1930

Puccinia actinostemonis H.S. Jacks. and Holw., 1931

Uredo rangelii Simpson *et al.*, 2006

Taxonomic position: Eukaryota, Fungi, Basidiomycota, Pucciniomycotina, Pucciniomycetes, Pucciniales, Sphaerophragmiaceae, *Austropuccinia*

Common names: Eucalyptus rust, guava rust, myrtle rust, ohia rust

Reference: Mycobank MB#819171 (*P. psidii* MB#213865)

Because of the age and poor quality of the original type specimen, this species has been epitypified for precise application of this taxon name (da S. Machado *et al.*, 2015).

3. Detection

All plants in the family Myrtaceae should be considered potentially susceptible to infection by *A. psidii*, and rust infecting any plants in this family should be investigated to rule out *A. psidii* infection.

Site characteristics and climatic conditions that favour lengthy periods of leaf wetness, combined with susceptible new growth on the myrtaceous host plant, provide ideal conditions for sign manifestation and detection. The use of a hand lens (10× magnification) is recommended for examining suspect lesions.

This diagnostic protocol describes established methods for the detection and identification of *A. psidii.* It is not a comprehensive review of all methods available for the diagnosis of *A. psidii*. Detection of *A. psidii* can be achieved using the biological and molecular methods shown in the flow chart in Figure 1. It is important to first determine whether the host of the sample belongs to the family Myrtaceae. If it does, the diagnosis may start with morphological observation of rust structures on the plant host material. Observation may lead to a diagnosis or highlight the need for a further study with molecular methods. In the case of a first detection in a country, confirmation with DNA sequencing is recommended. When plant samples are received with signs or symptoms of a suspected rust, and the host is unknown, conclusive identification of *A. psidii* can be achieved only with DNA sequencing.

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. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

3.1 Signs and symptoms of infection

Symptoms of infection by *A. psidii* range from minor leaf spots to severe foliage and stem blight, and in some species, flowers and fruits are infected (Figures 2–4). Since it established in Australia in 2010, the rapid expansion of the fungus' host range has resulted in a wide range of symptoms being observed which vary depending on the host species, the level of host susceptibility within a host species and the age of the host tissue. Symptoms become more obvious during rust epidemics.

The primary sign is the appearance of yellow pustules (uredinia) on the upper and lower leaf surfaces of Myrtaceae hosts, with a higher prevalence on the lower leaf surfaces (abaxial). Pustules can also be found on stems, fruits and flowers.

The first symptoms of infection are often chlorotic flecks and young sori on leaves, shoots and fruits, which appear two to four days after infection. These early signs and symptoms are similar to those caused by many other pests and disorders. Hence, identification based on signs and symptoms alone may not be sufficient for diagnosis. Further testing to confirm *A. psidii* infection can be carried out using molecular diagnostic methods.

The early symptoms are followed by the production of masses of bright yellow urediniospores. Teliospores, which are brown, can be produced in the same sori intermingled with urediniospores. The infected area on the host spreads radially outwards and with age, multiple pustules eventually merge and coalesce. From this stage onwards, it is not difficult to distinguish rust lesions from insect damage or necrosis from various causes. Lesions tend to be angular in shape, extending through the leaf (Coutinho *et al.*, 1998; Pegg *et al.*, 2014). Lesions can turn reddish-purple then grey with age, and often have a purple or dark-brown margin. For example, older symptoms on leaves and shoots of many *Eucalyptus*, *Melaleuca* and *Callistemon* show purpling on their margins. Lesions on fleshy fruits of *Eugenia*, *Psidium* and *Syzygium* may not have obvious margins because the symptoms are covered with heavy spore masses in an early stage of disease development or because they develop fruit rot, caused by secondary pathogens, as the fruits ripen.

Secondary infections can occur within days but are confined to new young tissue, shoots and expanding foliage. Prolonged infection can cause deformed leaves, heavy defoliation of branches, dieback, stunted

growth and even plant death. Severe rust infections in young trees may kill shoot tips, causing loss of leaders and a bushy habit. Prolific branching (witches' brooms), galling, persistent localized lesions, and stem swellings may be a sign of previous rust infection; however, these symptoms may also result from other causes.

On resistant hosts, *A. psidii* may induce a hypersensitive reaction expressed as flecks, chlorotic halos or brown spots but with no urediniospores or teliospores produced (Junghans *et al.*, 2003). However, depending on the level of resistance, punctiform pustules may be formed over the brown necrotic lesions (Glen *et al.*, 2007).

3.2 Sampling and sample preparation

Samples of suspected rust or suspicious lesions on plant stems, flowers, fruits, shoots or leaves should be collected. Where possible, triplicate samples from each infected host species should be collected, with each sample comprising several lesions. The likelihood of obtaining enough material for DNA extraction and of finding features for a robust morphological diagnosis increases with increasing number of collected lesions. In the event of an older infection, woody twigs and branches with swellings or galls or other evidence of infection should be sampled.

Direct handling of the plant material should be avoided to prevent spread of the disease, as rust spores are readily dispersed by wind and carried on clothing and other equipment, and can survive for up to three months. A new pair of disposable gloves should be worn for each sampling. A paper bag should be placed around the sample before snipping the infected part from the plant. Collecting tools should be thoroughly dipped and lightly shaken in bleach (a solution of sodium hypochlorite (NaOCl) diluted to at least 5% active chlorine) for about 1 min, followed by draining and drying with paper towelling before and after each sampling. Each sample should be sealed in a single paper bag and labelled.

The rust samples need to be transported on the collected plant material (stems, flowers, fruits, shoots or leaves) and the spores should remain *in situ* in the pustules for all pertinent diagnostic information to be retained.

Samples should be sent to the diagnostic laboratory in sealed plastic containers to avoid damage to the paper bags and contamination among samples. Storage and transport at cool temperatures (e.g. 4 °C) is recommended to prolong sample life, but even at this temperature samples can deteriorate if stored for more than seven days.

In the diagnostic laboratory, the sample should be allowed to dry inside the paper bag at room temperature (20–25 °C under low humidity conditions). All plant material should be preserved as air-dried samples in sealed paper bags to minimize the growth of saprophytic organisms.

3.3 Morphological detection

Because rust spores have a high potential for dispersal, samples should be handled with care during the diagnostic process. A large sheet of paper can be placed under the work area and changed between samples. Use of a laminar flow cabinet is not advisable as this may disperse the spores. Gloves should be changed and equipment (scalpels, forceps, etc.) disinfected between samples. At the end of the work period, all packaging, gloves, benchcoating, paper, tissues, etc. should be considered contaminated, double-bagged and disposed of as required for quarantineable waste (e.g. by autoclaving or incineration). Equipment should be surface sterilized with 70% ethanol or a similar disinfectant known to be effective against rust spores.

A. psidii, like other rust pathogens, cannot be cultured *in vitro*; therefore, morphological identification is based on the symptoms of infected host material. The lesions should be investigated for rust sori and spores using appropriate magnification without destroying the sori or contaminating them with histological fluids.

The samples should be examined under a dissecting microscope for rust sori. Rust spores should be picked up carefully with a disposable scalpel or needle, mounted in a drop of clear mountant such as

lactic acid on a microscope slide and covered with a coverslip. The slide should be heated gently to remove air bubbles, then observed under a compound light microscope fitted with high quality optics. Urediniospores and teliospores of *A. psidii* are readily distinguishable by light microscopy (Figure 5).

Examination of cross-sections or squash preparations of uredinia is necessary to confirm the absence of paraphyses (sterile accessory hymenial structures), as well as to confirm urediniospore morphological characters (see section 4.1).

Samples of early infections may not show the morphological characters required for the identification of *A. psidii*. Incubation for 10–14 days at 25°C in 80% relative humidity (in a humid chamber) may elicit these characters. For a faster diagnosis or to avoid the potential increase of inoculum, the pathogen may be identified using polymerase chain reaction (PCR) (section 3.4).

3.4 Molecular detection

Molecular methods have been developed to detect *A. psidii* directly from different types of infected plant material. These methods are a nested PCR (Langrell *et al.*, 2008) and a real-time PCR (Baskarathevan *et al.*, 2016). The real-time PCR is faster and more sensitive than the nested PCR as well as having a reduced risk of the cross-contamination that is inherent in nested PCR (Baskarathevan *et al.*, 2016). These methods may be useful for the rapid screening of samples after the first detection has been confirmed. Definitive identification requires comparison of fungal barcoding regions with those published for the epitype (da S. Machado *et al.*, 2015; Rodas *et al.*, 2015). This requires DNA extracted from fungal spores (section 4.2).

3.4.1 Preparation of plant material

DNA for PCR analysis can be extracted from individual sori or, if sori are not yet erumpent, from small pieces (10–100 mm^2) of infected plant tissue excised from the sample. If spores are abundant, they should be used in preference to other plant material, and should be placed into a microcentrifuge tube using a clean brush for each sample.

The sample is placed into a tube or clean sterile mortar bowl. Different grinding methods can be used such as mortar and pestle with liquid nitrogen, plastic pestles and microcentrifuge tubes with liquid nitrogen, or a TissueLyser (Qiagen[1]). New tubes or clean mortars and pestles must be used for each sample. Mortars and pestles should be washed in soapy water, rinsed with ethanol and autoclaved prior to and after use. The spore wall is very strong and all methods for DNA extraction and purification depend on its adequate disruption. For the TissueLyser, the addition of two 3 mm tungsten carbide beads, the pre-freezing of tubes in liquid nitrogen for 1 min, and two 2 min sessions in the TissueLyser at 30 Hz is sufficient for adequate grinding. For other methods, adequate grinding can be checked by microscopic examination of the ground material: if >50% of the urediniospores have lost their contents and are hyaline rather than yellow, grinding is sufficient.

3.4.2 Nucleic acid extraction

Various commercially available kits, such as the DNeasy Plant Mini Kit (Qiagen[1]) or the Wizard Genomic DNA Purification Kit (Promega[1]), are suitable for DNA extraction and purification (following the manufacturer's instructions), as are standard manual DNA extraction procedures usually used for the extraction of DNA from plants or fungi. Quantification of DNA is inexact, varying with the procedure used, and the DNA obtained may be a mixture of plant and fungal DNA. Therefore, a range of DNA concentrations should be tested using the fungal-specific primers ITS1-F and ITS4 (section 3.4.3) to confirm the quality of the DNA and whether sufficient fungal DNA can be amplified. The DNA obtained should be stored at −20 °C.

[1] The 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.

3.4.3 Conventional PCR and sequencing

This method does not require species-specific primers or probes and many diagnostic laboratories may have the required primers and other reagents in stock. The ribosomal (r)DNA internal transcribed spacer (ITS) region should be amplified using the primer combination ITS1-F/ITS-Rust1 (Kropp *et al.*, 1995). The primer combination ITS1-F/ITS4 (Gardes and Bruns, 1993) can also be used, but with these primers there is the possibility that contaminating fungi may be co-amplified, resulting in an illegible chromatogram, or preferentially amplified, resulting in a legible chromatogram that gives no information about the rust species and may cause some confusion. Primers have also been designed to amplify fragments of the *β-tubulin* (primer combination Ppsi-BtubF/Ppsi-BtubR) and *elongation factor 1α* (primer combination PPEFF/PPEFR) genes in *A. psidii* (da S. Machado *et al.*, 2015), but the specificity of these primers has not been evaluated against other rust fungi. The PCR products should be sequenced (in-house or sent to a sequencing facility) and the sequence data compared with the reference data described in section 4.2.

Table 1 lists the primer sequences and Table 2 outlines the PCR.

Table 1. Primers for conventional PCR

Gene region	Primer name	Primer sequence (5′-3′)	Reference
rDNA ITS including 5.8S rDNA	ITS1-F	CTT GGT CAT TTA GAG GAA GTA A	Gardes and Bruns (1993)
	ITS4	TCC TCC GCT TAT TGA TAT GC	White *et al.* (1990)
rDNA ITS including 5.8S rDNA and ~500 bp LSU rDNA	ITS1-F	CTT GGT CAT TTA GAG GAA GTA A	Gardes and Bruns (1993)
	ITS-Rust1	GCT TAC TGC CTT CCT CAATC	Kropp *et al.* (1995)
β-tubulin	Ppsi-BtubF	CTT TTG GTT CAC TCT TCA GAC C	da S. Machado *et al.* (2015)
	Ppsi-BtubR	AGA TGA TAA AAG ACT ACT GAC TCC	
elongation factor 1α	PPEFF	AAG GAT GCT GCT GAC ATG GGC	da S. Machado *et al.* (2015)
	PPEFR	ATC CCG AAA TGG GGA CAA AAG G	

bp, base pairs; ITS, internal transcribed spacer; LSU, large subunit.

Table 2. Conventional PCR master mix composition, cycling parameters and amplicons

Reagent	Final concentration
PCR-grade water	–[†]
PCR buffer	1×
$MgCl_2$	2.0 mM
dNTPs (each)	200 µM
BSA[‡]	0.2 mg/ml
Forward primer	0.25 µM
Reverse primer	0.25 µM
DNA polymerase	1 U
DNA (volume)	5 µl
Cycling parameters	
For ITS1-F/ITS4, Ppsi-BtubF/Ppsi-BtubR, PPEFF/PPEFR	
Initial denaturation	94 °C for 2 min
Number of cycles	30
- Denaturation	94 °C for 30 s
- Annealing	55 °C for 30 s
- Extension	72 °C for 30 s
Final extension	72 °C for 10 min
For ITS1-F/ITS-Rust1	
Initial denaturation	95 °C for 3 min
Number of cycles	35
- Denaturation	94 °C for 30 s
- Annealing	44 °C for 30 s
- Extension	72 °C for 2 min
Final extension	72 °C for 10 min
Expected amplicons	
ITS1-F/ITS4	700 bp
Ppsi-BtubF/Ppsi-BtubR	816 bp
PPEFF/PPEFR	635 bp
ITS1-F/ITS-Rust1	1 240 bp

[†] For a final reaction volume of 25 µl.

[‡] Addition of bovine serum albumin is recommended but not essential.

bp, base pairs; BSA, bovine serum albumin; PCR, polymerase chain reaction.

3.4.4 Species-specific real-time PCR of Baskarathevan *et al.* (2016)

This method is fast, sensitive and is suitable for high throughput and for detection of the pathogen in its early infection stages, but it requires species-specific primers and a dual-labelled probe. Species specificity has been demonstrated against *Puccinia* species including *P. coronata*, *P. graminis*, *P. hemerocallidis*, *P. hordei*, *P. myrsiphylla*, *P. oxalidis* and *P. striiformis* (Baskarathevan *et al.*, 2016). *A. psidii* is so phylogenetically remote from other rust species that infect Myrtaceae that cross-amplification is considered unlikely. *In silico* analysis using the Standard Nucleotide Basic Local Alignment Search Tool (BLASTN) confirmed that these primers do not bind to DNA sequences of other rust species, including those found on Myrtaceae. The TaqMan real-time PCR can detect as little as 0.011 pg of *A. psidii* genomic DNA (Baskarathevan *et al.*, 2016). An internal control based on

amplification of the host cytochrome oxidase (*COX1*) gene (Weller *et al.*, 2000) should be included unless the PCR is performed on fungal material. When the internal control is included, an additional PCR reagent "COX Block" (dNature Diagnostics & Research Ltd[1]) is required in the reaction to delay the amplification of the *COX* gene, so that the internal control does not outcompete the *A. psidii* DNA and prevent it from being detected. This PCR can be run as a duplex reaction, provided the spectrum of the probe dye for COX does not overlap with that of the target probe (Table 3).

The method is repeatable and reproducible with a coefficient of variation when repeated (for cycle threshold (Ct)) between 0.8 and 1.6 (Baskarathevan *et al*., 2016). Three combinations of primers/probes have been developed – two targeting the rDNA ITS and one targeting *β-tubulin* – but only the most sensitive primer combination is outlined here.

Table 3 lists the primer sequences and Table 4 outlines the PCR.

Table 3. Primers and probe for real-time PCR

Gene region	Primer or probe name	Primer or probe sequence (5′-3′)	Reference
A. psidii rDNA ITS1	PpsiITS1F	GTA GCT TTA TTG AAA CAT AGT AA	Baskarathevan *et al.* (2016)
	PpsiITS1R	TGA TTT TAG ACA ATA ATA ATA AGG G	
	PpsiITS1P	FAM-AGA TTA ATA TCT TTG CCA CGT ATA CCA-BHQ1	
Host cytochrome oxidase†	COX-F	CGT CGC ATT CCA GAT TAT CCA	Weller *et al.* (2000)
	COX-R	CAA CTA CGG ATA TAT AAG AGC CAA AAC TG	
	COX-P	CAL Fluor Red 610-TGC TTA CGC TGG ATG GAA TGC CCT-BHQ2	Amended from Weller *et al.* (2000)

† Optional positive control.

ITS, internal transcribed spacer; P, probe; PCR, polymerase chain reaction.

Table 4. Real-time PCR master mix composition, cycling parameters and amplicon

Reagent	Final concentration
PCR-grade water	–[†]
PCR buffer	1×
$MgCl_2$	4.2 mM
dNTPs (each)	200 µM
BSA	0.5 mg/ml
Primer PpsiITS1F	0.30 µM
Primer PpsiITS1R	0.30 µM
Probe PpsiITS1P	0.12 µM
Primer COX-F[‡]	0.30 µM
Primer COX-R[‡]	0.30 µM
Probe COX-P[‡]	0.10 µM
COX Block[‡]	750 nM
DNA polymerase	1 U
DNA (volume)	5 µl
Cycling parameters	
Initial denaturation	95 °C for 3 min
Number of cycles	40
- Denaturation	95 °C for 5 s
- Annealing and extension	59 °C for 30 s
Expected amplicon (size)	
PpsiITS1F/PpsiITS1R	91 bp

[†] For a final reaction volume of 20 µl.

[‡] For internal control (host material).

bp, base pairs; BSA, bovine serum albumin; PCR, polymerase chain reaction.

3.4.5 Species-specific nested PCR of Langrell *et al.* (2008)

A species-specific nested PCR targeting the rDNA ITS region is available for laboratories that lack the facilities to perform the TaqMan real-time PCR (section 3.4.4). The nested PCR is sensitive, detecting as little as one or two urediniospores, but it is not recommended for laboratories that lack extensive experience with nested PCR as it is prone to cross-contamination and care must be taken to avoid this.

The first round of PCR is carried out with the primer combination Ppsi1/Ppsi6 and the product of this amplification is diluted 1:5 in Tris-ethylenediaminetetraacetic acid (EDTA) (TE) buffer and used as a template in the second round of PCR, with internal primers Ppsi2/Ppsi4. The cycling parameters in Table 6 have been amended from Langrell *et al.* (2008).

PCR products can be visualized on a transilluminator after electrophoresis on agarose gel and staining with a compatible DNA-binding dye such as ethidium bromide or gel red.

Table 5 lists the primer sequences and Table 6 outlines the PCR.

Table 5. Primers for nested PCR

Gene region†	Primer name	Primer sequence (5′-3′)
First round	Ppsi1	TTC TAC CTT ATT ACA TGT AGC T
	Ppsi6	GTC ATA TTG ACA GGT TAG AAG C
Second round	Ppsi2	ATA GTA ATT TGG TAT ACG TGG C
	Ppsi4	GTC AAT CCA AAT CAA AGT ATG

Source: Langrell *et al.* (2008).

† Ribosomal (r)DNA internal transcribed spacer (ITS) including 5.8S rDNA.

PCR, polymerase chain reaction.

Table 6. Nested PCR master mix composition, cycling parameters and amplicons

Reagent	Final concentration
PCR-grade water	–†
PCR buffer	1×
$MgCl_2$	1.5 mM
dNTPs (each)	100 µM
BSA	0.2 mg/ml
Forward primer	0.10 µM
Reverse primer	0.10 µM
DNA polymerase	1.25 U
DNA (volume)	5 µl
Cycling parameters‡	
Initial denaturation	95 °C for 3 min
Number of cycles	30
- Denaturation	95 °C for 1 min
- Annealing	57 °C for 1 min
- Extension	72 °C for 1 min
Final extension	72 °C for 7 min
Expected amplicons (size)	
Ppsi1/Ppsi6	508 bp
Ppsi2/Ppsi4	379 bp

† For a final reaction volume of 25 µl.

‡ For both rounds of the nested PCR.

bp, base pairs; BSA, bovine serum albumin; PCR, polymerase chain reaction.

3.4.6 Controls for molecular tests

For the test result 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 DNA sequence of the target pest. For PCR, a positive nucleic acid 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 test method (apart from the extraction), and specifically the amplification. Pre-prepared (stored) genomic DNA, whole genome amplified DNA or a synthetic control (e.g. cloned PCR product) may be used. Any fungal DNA will be a suitable positive control for the ITS1-F/ITS4 primers, and any rust DNA for the ITS1-F/Rust1 primers. The other primer pairs (PpsiBtubF/PpsiBtubR, PPEFF/PPEFR, PpsiITS1F/PpsiITS1R,

Ppsi1/Ppsi6 and Ppsi2/Ppsi4) require *A. psidii* DNA (genomic DNA or suitable plasmid or amplicon) as a positive control. In the absence of a positive control, it may be possible to confirm the presence of *A. psidii*, but not its absence. To obtain *A. psidii* genomic DNA to use as a positive control, a request should be made to countries that have *A. psidii*, such as Brazil and Australia.

Internal control. The efficiency of the extraction method is confirmed with amplification of the rDNA ITS using the primers ITS1-F/ITS4.

Negative amplification control (no template control). This control is necessary 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 in place of template DNA at the amplification stage.

Negative extraction control. This control is used to monitor both contamination during nucleic acid extraction and cross-reaction with the host tissue. The control comprises nucleic acid that is extracted from uninfected host tissue and subsequently amplified. Alternatively, extraction blanks may be processed with the samples to be tested if sufficient uninfected host tissue is not available.

3.4.7 Interpretation of results

3.4.7.1 Species-specific real-time PCR

The real-time PCR will be considered valid only if both these criteria are met:

- the positive control produces an amplification curve with the pathogen-specific primers and probe
- no amplification curve is seen 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), the 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 nucleic acid 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 method for the first time. Guidance on how to determine the cycle cut-off value can be found in Chandelier *et al.* (2010).

Baskarathevan *et al.* (2016) were able to detect 0.011 pg of *A. psidii* DNA at a Ct of 35, which represents less than one genome copy for an expected genome size of 100–150 mega base pairs. The infected plant samples had a Ct ranging from 17 to 35, depending to some extent on the severity of infection.

3.4.7.2 Species-specific nested PCR

The species-specific nested PCR will be considered valid only if both these criteria are met:

- the positive control produces the correct size amplicon (379 base pair (bp)) after the second round of PCR for *A. psidii*.
- no amplicons of the correct size are produced in the negative extraction control and the negative amplification control.

4. Identification

Any rusts detected on Myrtaceae species should be regarded as suspect and reported for prompt identification. In most cases, *A. psidii* can be identified by morphological characters (section 4.1). A final morphological diagnosis is based on the absence of marginal paraphyses and on urediniospore characters. However, given that the newly described *Phakopsora myrtacearum* from *Eucalyptus* in Africa has morphological characters that overlap with *A. psidii* (Maier *et al.*, 2016), the identity of the pathogen on *Eucalyptus* must be confirmed by using molecular methods (Figure 1). All detections that comprise a new record for a country should be confirmed by a second official laboratory. In these cases,

it is also recommended that identification be confirmed by DNA sequencing to support the morphological identification.

Although more than 40 species of rust fungi have been reported from Myrtaceae species, most have since been synonymized with *A. psidii* (Maier *et al.*, 2016). There are six currently accepted rust species on Myrtaceae (Maier *et al.*, 2016) (Table 7). These are *A. psidii*, *Puccinia cygnorum*, *Phakopsora juelii*, *Ph. myrtacearum*, *Uredo seclusa* and *Uredo xanthostemonis*. *Ph. myrtacearum* is phylogenetically distinct from *A. psidii* (Maier *et al.*, 2016) and while no sequence data are available for *Ph. juelii* (=*Ph. rossmaniae*), it is also expected to be phylogenetically distinct. *P. cygnorum* is phylogenetically distinct from *A. psidii* (Carnegie *et al.*, 2010) and not expected to amplify with the species-specific primers for *A. psidii*. No sequence data are available for *U. seclusa* or *U. xanthostemonis.* The former species is known only from the type collection, and the latter has only been recorded from *Xanthostemon* spp. and is a member of the Phakopsoraceae so should be quite distinct from *A. psidii*.

4.1 Morphological identification

Microscopic examination of suspect rust samples can be used to look for key morphological characters of *A. psidii* (Table 7).

Table 7. Morphological characters of the six rust species currently accepted as infecting Myrtaceae

Rust species	Urediniospores (µm)	Teliospores (µm)	Paraphyses	Comment
Austropuccinia psidii	15–26 × 14–22	23–50 × 14–28 Pedicel fragile	Absent	Wide host range
Puccinia cygnorum	Unknown	35–60 × 12–20 Pedicel persistent	n/a	Known only on *Kunzea ericifolia* from near Perth, Western Australia
Phakopsora juelii	14–23 × 12–18	10–14 × 6–9 Subepidermal, aseptate	Present	Known on *Campomanesia* spp. from Brazil
Phakopsora myrtacearum	20–30 × 14–20	Unknown	Absent	Known only on *Eucalyptus* spp. from southern and eastern Africa
Uredo seclusa	24–32 × 15–20	Unknown	Absent	Known only from type specimen from Brazil
Uredo xanthostemonis	17–28 × 15–20	Unknown	Present	Known only on *Xanthostemon* spp. from Australia

Source: Maier *et al.* (2016).
n/a, not available.

The following key (Maier *et al.*, 2016) can be used to distinguish the two described rust fungi on eucalyptus:

1. Uredinia forming bright yellow–orange pustules on leaves, petioles, shoots, flowers or fruits. Urediniospores mainly globose to subpyriform, 15–26 × 14–22 µm ..... ***Austropuccinia psidii***

– Uredinia pale yellow to light brown, on discoloured areas of leaves. Urediniospores mainly pyriform to ellipsoid, 20–30 × 14–20 µm ..... ***Phakopsora myrtacearum***

4.1.1 Morphological characters of *A. psidii*

Sori are scattered on green tissue, leaf spots, stems, flowers and fruits, and are mainly hypophyllous. **Uredinia**, which are bright yellow to orange yellow, are usually more common than **telia**, which are dark brown; both are aparaphysate. Both teliospores and urediniospores may occur together in the same sorus (Pegg *et al.*, 2014).

The following descriptions are taken from Pegg *et al.* (2014) and are a composite morphological description based on samples from 11 host genera.

Uredinia on chlorotic, red–purple or greyish leaf spots with a darker margin up to 1 mm diameter, amphigenous, mostly abaxial, subepidermal, erumpent, round, up to 500 µm, bright yellow to orange to yellowish brown (depending on host genus).

Urediniospores globose to subglobose or ellipsoidal to ovoid or obpyriform, yellowish brown, 15−26 × 14–22 µm; wall 1.0–3.0 µm thick, finely echinulate, germ pore absent or inconspicuous (Figures 5(a), (b), (d)). The presence of a tonsure (smooth patch) on urediniospores is often observed, but its presence or absence is not consistent even in the same sorus.

Telia on fruit, leaves or stems, up to 0.5 mm diameter, abaxial, erumpent, pulvinate, yellow to yellowish brown.

Teliospores cylindrical to ellipsoidal, apex rounded, pale yellowish brown, 23–50 × 14–28 µm; wall 1.0–2.0 µm thick, smooth, two-celled, pedicel up to 15 µm long (Figures 5(c), (d), (e)).

Basidia cylindrical, up to 110 µm long and 6–8 µm wide, hyaline, four-celled, produced from each cell of the teliospores, apically in upper cell and laterally in lower cell.

Basidiospores globose to pyriform, 8–11 µm diameter, hyaline, smooth, germinate *in situ* without dormancy from an apical pore (Figure 5(f)).

4.2 Molecular identification

Morphological variation has in the past led to erroneous identification and classification of *A. psidii*. In 2015, an epitype was designated for *A. psidii* for the precise application of the taxon name, providing DNA characterization for stability and comparison. The epitype and 17 *A. psidii* specimens from collections from Australia, Hawaii, New Caledonia and South America had identical DNA sequences for three genetic regions: ITS, *β-tubulin* and *elongation factor 1α* (da S. Machado *et al.*, 2015). The epitype sequences are deposited in GenBank as KM282154 (ITS), KM282123 (*β-tubulin*) and KM282143 (*elongation factor 1α*). They should be referred to for comparison when identifying samples.

For a definitive identification, the preferred method is to extract DNA from rust spores (section 3.4.1 and 3.4.2), amplify the selected region or regions (section 3.4.3), and compare the sequence data of the fungal barcoding region, the rDNA ITS region, with GenBank KM282154. Sequence similarity should be ≥99%. DNA sequencing of secondary regions such as *β-tubulin* and *elongation factor 1α* genes and the rDNA large sub-unit (LSU) region provides support for initial diagnoses. All regions have very low intraspecific variation (<1%), and they have barcode gaps of 10% (ITS), 17% (*β-tubulin*) or 20% (*elongation factor 1α*).

DNA sequencing of the primary fungal barcode region, rDNA ITS, supported by one or more secondary barcode regions, provides the most robust form of molecular identification (Schoch *et al.*, 2012). Any combination of the rDNA ITS plus *β-tubulin* and *elongation factor 1α* regions described in section 3.4 is suitable for identification. The DNA sequences of species-specific PCR products are acceptable if they meet the minimum length of 400 bp indicated as desirable for DNA barcodes (Kress and Erickson, 2008). Chromatograms should be edited to trim the background or "noise" peaks and the sequence used to search the International Nucleotide Sequence Databases (GenBank, European Molecular Biology Laboratory, and DNA Data Bank of Japan) using the BLAST algorithm (Altschul *et al.*, 1997).

4.2.1 Interpretation of results

Appropriate reference sequences from the *A. psidii* epitype are available – GenBank accession numbers KM282154 (ITS), KM282123 (*β-tubulin*) and KM282143 (*elongation factor 1α*). These regions all have less than 1% intraspecific variation and >10% interspecific variation, so a sequence similarity of >98% to any of these *A. psidii* reference sequences over an alignment longer than 400 bp can be taken as confirmation of identification as *A. psidii* (Rodas *et al.*, 2015).

5. Records

Records and evidence should be retained as described in section 2.5 of ISPM 27 (*Diagnostic protocols for regulated pests*).

In cases where other contracting parties may be affected by the results of the diagnosis, in particular in cases of non-compliance (ISPM 13 (*Guidelines for the notification of non-compliance and emergency action*)) and where *A. psidii* is found in an area for the first time, the records and evidence and additional material should be kept for at least one year in a manner that ensures traceability:

- After samples have been detected as positive, the DNA should be labelled and stored frozen at −20 or −80 °C.
- The remaining infected plant material should be pressed and dried, packaged and labelled, and stored as appropriate for herbarium specimens.
- Microscopic slides should be sealed and stored with the plant specimens.

6. Contact Points for Further Information

Further information on this protocol can be obtained from:

Agriculture Victoria, Department of Economic Development, Jobs, Transport and Resources, Australia, AgriBio Centre for AgriBioscience, La Trobe University, 5 Ring Road, Bundoora, Victoria 3083, Australia (Jacqueline Edwards; email: jacky.edwards@ecodev.vic.gov.au).

School of Land and Food, University of Tasmania, Private Bag 98, Hobart, Tasmania 7001, Australia (Morag Glen; email: Morag.Glen@utas.edu.au).

Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), Laboratoire de la Santé des Végétaux [Plant Health Laboratory], Unité de mycologie [Mycology Unit], Domaine de Pixérécourt – Bâtiment E, C.S. 40009, 54220 Malzéville, France (Jacqueline Hubert; email: jacqueline.hubert@anses.fr).

General Research and Biotechnology Unit, Nigeria Agriculture Quarantine Service, Post-Entry Quarantine Station, Moor Plantation (NCRI Compound), Apata, Ibadan, Oyo State, Nigeria (Kazeem Shakiru Adewale; email: kazeems2001@yahoo.com).

United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Regulations, Permits and Manuals, 4700 River Rd. Unit 133, Riverdale, MD 20737, United States of America (José R. Hernández; email: Jose.R.Hernandez@aphis.usda.gov).

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), which will in turn forward it to the Technical Panel on Diagnostic Protocols (TPDP).

7. Acknowledgements

The first draft of this protocol was written by J. Edwards (Agriculture Victoria, Department of Economic Development, Jobs, Transport and Resources, Australia (see preceding section)), M. Glen (School of Land and Food, University of Tasmania, Australia (see preceding section)), J. Hubert (Mycology Unit, ANSES, France (see preceding section)), J. Hernandez (USDA-APHIS, United States of America (see preceding section)) and K. Shakiru Adewale (General Research and Biotechnology Unit, Nigeria Agricultural Quarantine Service, Nigeria (see preceding section)). In addition, the following experts were significantly involved in the development of this protocol: M. Piepenbring (Department of Mycology, Goethe University Frankfurt am Main, Germany), C. Rodriguez-Delgado (Department of Agriculture and Water Resources, Australia), F. Sorgoni (Ministero delle Politiche Agricole Alimentari e Forestali and Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Italy) and H. Shirato (Ministry of Agriculture, Forestry and Fisheries, Yokohama Plant Protection Station, Japan).

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9. Figures

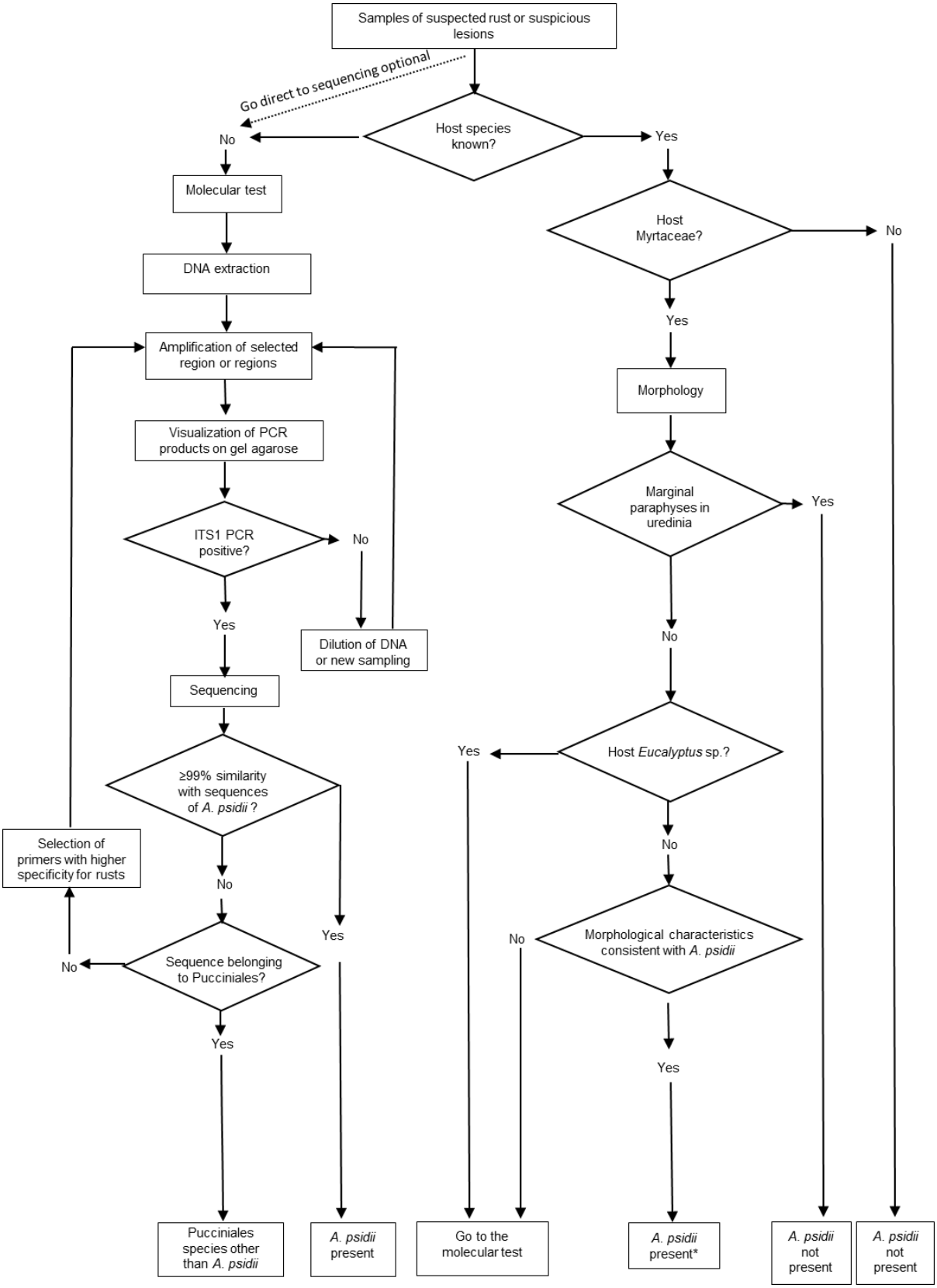

Figure 1. Flow chart of methods to confirm the identity of *Austropuccinia psidii*.
* Confirm with DNA sequencing for a first detection in a country.
ITS, internal transcribed spacer; PCR, polymerase chain reaction.

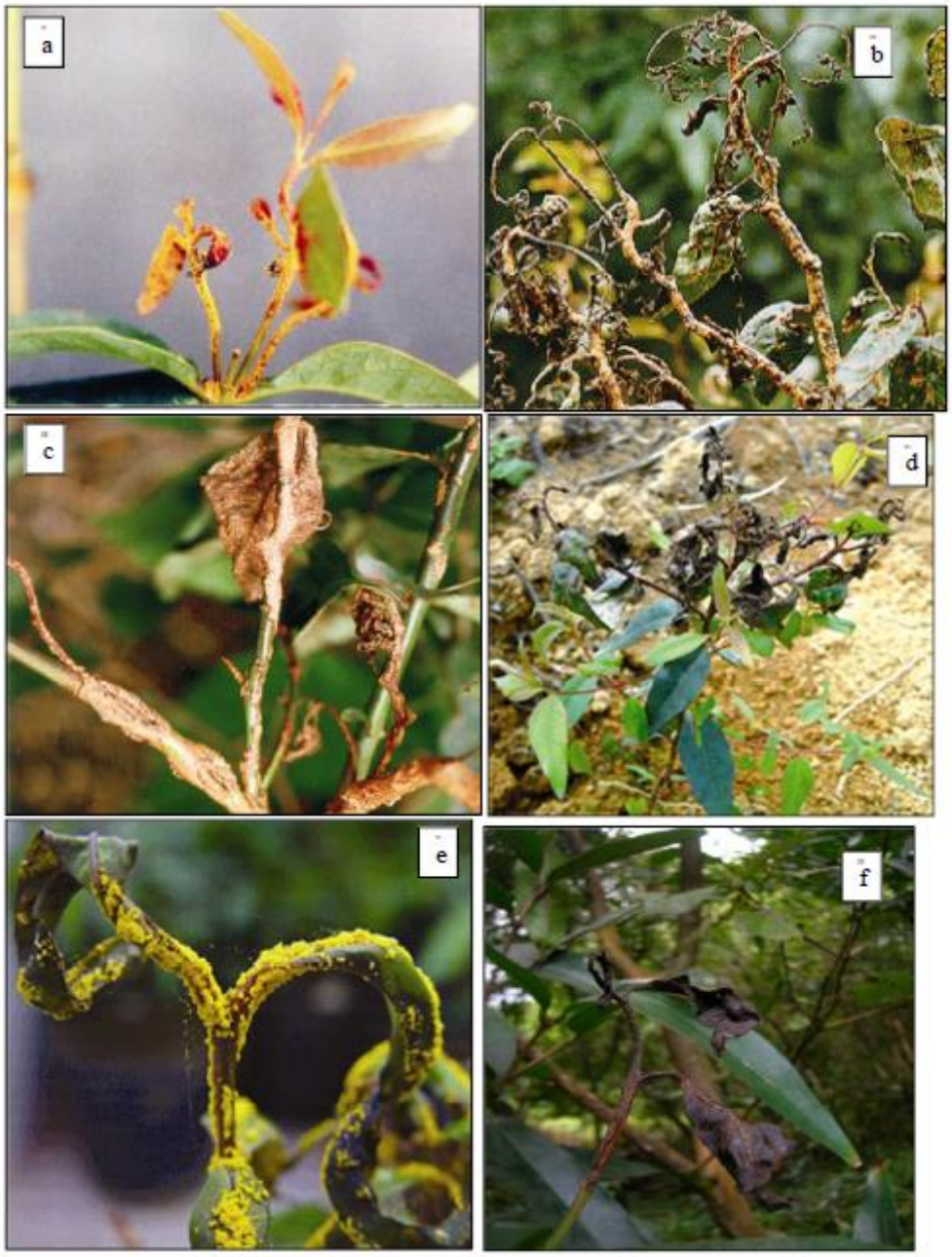

Figure 2. *Austropuccinia psidii* on *Eucalyptus grandis* causing (a) infection on leaves and stems, (b) defoliation and shoot death, (c) stem galls on a previous infection and (d) defoliation and shoot death on young leaves and stems. *A. psidii* on *Syzygium jambos* showing (e) uredinia on young leaves and stems and (f) leaf and shoot death.
Source: Photos courtesy of Forest Pathology and Genetics of Plant Pathogen Interactions Laboratory, Federal University of Viçosa, Brazil.

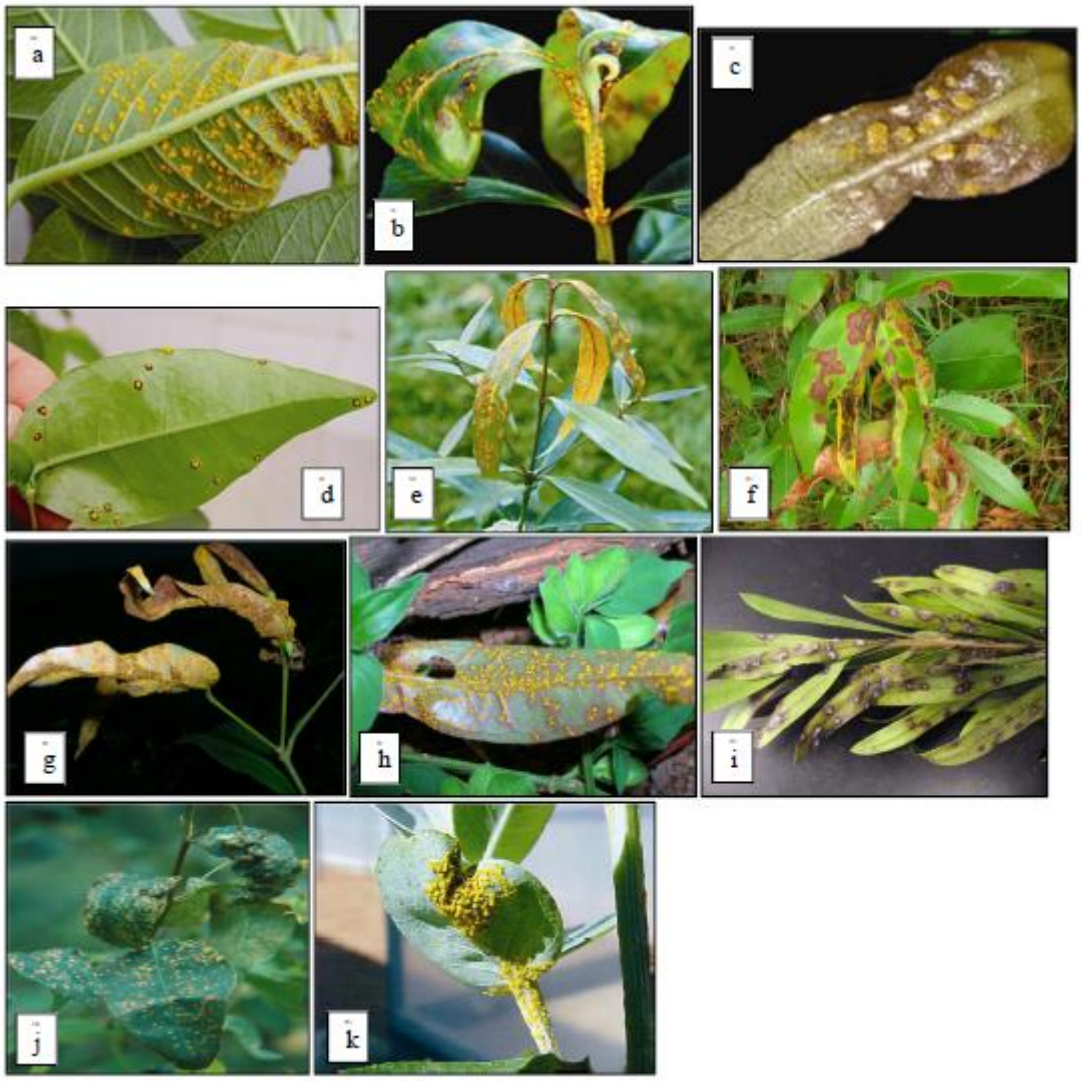

Figure 3. *Austropuccinia psidii* causing infection on leaves of (a) *Psidium guajava*, (b)–(c) *Heteropyxis natalensis*, (d) *Eugenia uniflora*, (e)–(h) *Syzygium jambos*, (i) *Callistemon citrinus*, (j) *Eucalyptus grandis* hybrid and (k) *Eucalyptus globulus*.
Source: Photos courtesy of Forest Pathology and Genetics of Plant Pathogen Interactions Laboratory, Federal University of Viçosa, Brazil.

Figure 4. *Austropuccinia psidii* infection on fruit of (a) *Psidium guajava*, (b) *Eugenia uniflora* and (c) *Myrciaria cauliflora*, and on flower buds of (d) *Syzygium jambos*.
Source: Photos courtesy of Forest Pathology and Genetics of Plant Pathogen Interactions Laboratory, Federal University of Viçosa, Brazil.

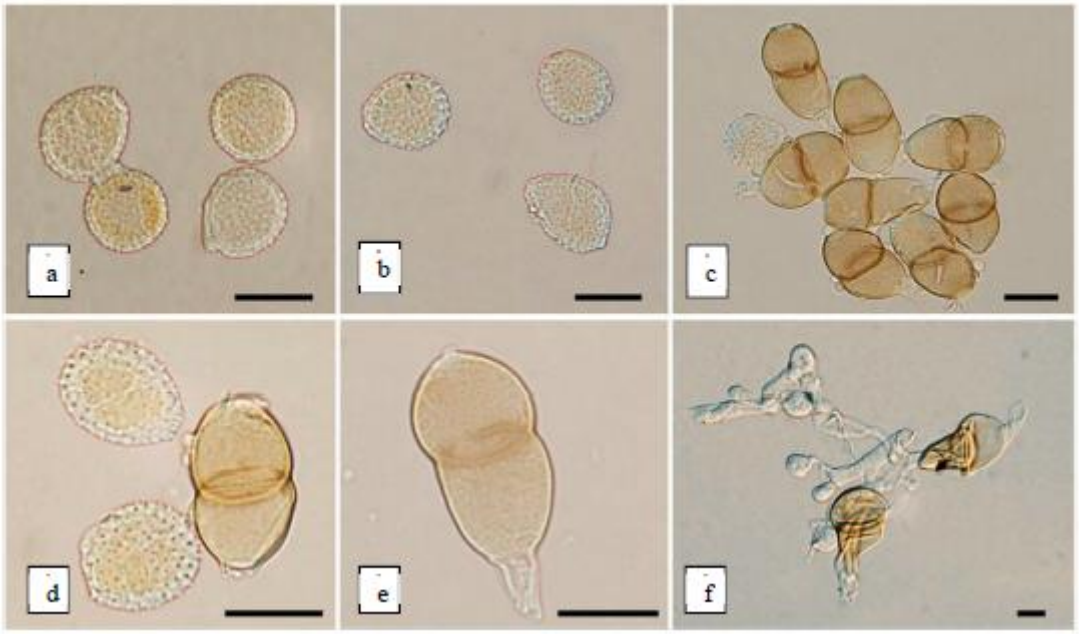

Figure 5. *Austropuccinia psidii*: (a)-(b) urediniospores; (c)–(d), teliospores and urediniospores; (e) teliospore; and (f), germinated teliospores and basidiospores. Scale bars: 20 µm.
Source: da S. Machado et al. *(2015).*

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ISPM 27
Diagnostic protocols for regulated pests

DP 27: *Ips* spp.

Adopted 2018; published 2018

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1. Pest Information

Ips species (Coleoptera: Curculionidae: Scolytinae: Ipini), commonly known as bark beetles, are sub-cortical phloem feeders in Pinaceae (conifer trees), especially *Pinus* (pine), *Picea* (spruce) and *Larix* (larch or tamarack) species (Cognato, 2015). In non-outbreak times, *Ips* beetles mainly inhabit weak or dead trees (Cognato, 2015). Adults and larvae kill healthy trees during outbreaks (Cognato, 2015) by destroying the phloem and cambium in tree trunks and limbs when feeding and tunnelling (Furniss and Carolin, 1977). Outbreaks can destroy thousands of hectares of healthy trees (Cognato, 2015). Some or all *Ips* bark beetles also transmit pathogenic fungi (Krokene and Solheim, 1998; Meng *et al.*, 2015), in particular blue stain fungi (genera *Grosmannia* and *Ceratocystis*, Ascomycota: Sordariomycetes, Figure 1). *Ceratocystis* fungi from *Ips* beetles also interfere with biological control of the conifer pest *Sirex noctilio* Fabricius (Hymenoptera: Siricidae) (Yousuf *et al.*, 2014). Certain climatic conditions may promote *Ips* outbreaks (Wermelinger, 2004; Breshears *et al.*, 2005; Marini *et al*., 2017). Trees injured in outbreaks are sometimes later killed by *Dendroctonus* bark beetles (Furniss and Carolin, 1977).

Native *Ips* species are present in all countries where *Pinus* and *Picea* occur naturally (Cognato, 2015). Two *Ips* species (*I. apache* and *I. grandicollis*) also occur as exotic species, especially in temperate southern hemisphere regions (Knizek, 2011; Cognato, 2015) where *Pinus* has been planted. Some *Ips* species use *Larix* as the principal host genus in their native range (Table 1). A few species use *Abies* (fir) and *Cedrus* (true cedar) as hosts during outbreaks (Wood and Bright, 1992). *Ips* species are not limited to the principal host genera provided in Table 1, as other conifers could be attacked when a principal host is not available.

There are 37 valid *Ips* species worldwide (Table 1). Phylogenetic analyses of the Ipini prompted transfer of several species to the genera *Pseudips* (Cognato, 2000) and *Orthotomicus* (Cognato and Vogler, 2001). Cognato (2015) reviews the phylogeny, taxonomy, diagnosis and biology of all *Ips* species.

Table 1. Worldwide list of *Ips* species with distribution and principal host genera (from Cognato, 2015). Principal host genera refer to hosts from which *Ips* species are most commonly collected in native range. Species targeted by this protocol are underlined.

Species	Authority	Native Distribution*	Principal host genera
Ips acuminatus	(Gyllenhal, 1827)	Eurasia	*Pinus*
Ips amitinus	(Eichhoff, 1872)	Eurasia (west)	*Picea*, *Pinus*
Ips apache	Lanier, 1991	North America (south)	*Pinus*
Ips avulsus	(Eichhoff, 1868)	North America (east)	*Pinus*
Ips bonanseai	(Hopkins, 1905)	North America (south)	*Pinus*
Ips borealis	Swaine, 1911	North America (north)	*Picea*
Ips calligraphus	(Germar, 1824)	North America, Caribbean	*Pinus*
Ips cembrae	(Heer, 1836)	Eurasia (widespread)	*Larix*
Ips chinensis	Kurenzov & Kononov, 1966	Eurasia (southeast)	*Pinus*
Ips confusus	(LeConte, 1876)	North America (west)	*Pinus*
Ips cribricollis	(Eichhoff, 1869)	North America (south), Central America, Caribbean	*Pinus*
Ips duplicatus	(Sahlberg, 1836)	Eurasia (widespread)	*Picea*
Ips emarginatus	(LeConte, 1876)	North America (west)	*Pinus*
Ips grandicollis	(Eichhoff, 1868)	North America (east, south)	*Pinus*
Ips hauseri	Reitter, 1894	Eurasia (central)	*Picea*
Ips hoppingi	Lanier, 1970	North America (southwest)	*Pinus*

(Table 1 continued on next page)

(Table 1 continued)

Species	Authority	Native Distribution*	Principal host genera
Ips hunteri	Swaine, 1917	North America (west)	*Picea*
Ips integer	(Eichhoff, 1869)	North America (west, south)	*Pinus*
Ips knausi	Swaine, 1915	North America (west)	*Pinus*
Ips lecontei	Swaine, 1924	North America (south)	*Pinus*
Ips longifolia	(Stebbing, 1909)	Eurasia (central)	*Pinus*
Ips montanus	(Eichhoff, 1881)	North America (west)	*Pinus*
Ips nitidus	Eggers, 1933	China	*Picea*
Ips paraconfusus	Lanier, 1970	North America (west)	*Pinus*
Ips perroti	Swaine, 1915	North America (north)	*Pinus*
Ips perturbatus	(Eichhoff, 1869)	North America (north)	*Picea*
Ips pilifrons	Swaine, 1912	North America (west)	*Picea*
Ips pini	(Say, 1826)	North America (widespread)	*Pinus*
Ips plastographus	(LeConte, 1868)	North America (west)	*Pinus*
Ips schmutzenhoferi	Holzschuh, 1988	Asia (Himalayas)	*Larix*, *Picea*, *Pinus*
Ips sexdentatus	(Boerner, 1767)	Eurasia (widespread)	*Pinus*, *Picea*
Ips shangrila	Cognato & Sun, 2007	Asia (east)	*Picea*
Ips stebbingi	Strohmeyer, 1908	Eurasia (central)	*Picea*, *Pinus*
Ips subelongatus	(Motschulsky, 1860)	Eurasia (east)	*Larix*
Ips tridens	(Mannerheim, 1852)	North America (west)	*Picea*
Ips typographus	(Linnaeus, 1758)	Eurasia (north and west)	*Picea*
Ips woodi	Thatcher, 1965	North America (west)	*Pinus*

* South = tropical and subtropical parts of North America. North America refers to the North American continent including countries north of Colombia. Widespread may not include all countries in the continent.

Most attacks are initiated by male beetles, who create a nuptial chamber under the bark and release semiochemicals to attract males and females to colonize the same tree. The polygynous males attract up to six females to the nuptial chamber (diameter: 7–15 mm). Females mate with the resident male and then create radiating egg galleries along the inner bark (Cognato, 2015; Figures 2 and 3). Females each lay eggs along their tunnel, these hatching after about seven days (Chararas, 1962). Larval galleries radiate from the "Y"- or "H"-shaped egg galleries (Figures 2 and 3), spreading over a span of 10–30 cm. Development requires six weeks in warm temperatures, allowing up to five generations per year in warm areas. In cooler areas, development requires up to two years (Furniss and Carolin, 1977). Adult beetles overwinter within parental breeding galleries, in forest litter, or in living wood tissue (Chansler, 1964; Lanier, 1967).

2. Taxonomic Information

Name: *Ips* DeGeer, 1775

Synonyms: *Cumatotomicus* Ferrari, 1867

Cyrtotomicus Ferrari, 1867

Taxonomic Position: Insecta, Coleoptera, Curculionidae, Scolytinae, Ipini

Table 2. Common names and synonyms of target *Ips* species, sorted by subgenera. Synonymy follows Knizek (2011).

Subgenus	*Ips* species	Common name	Synonyms
Bonips	*Ips pini* (Say, 1826)	pine engraver beetle	*Bostrichus dentatus* Sturm, 1826 *Bostrichus pallipes* Sturm, 1826 *Bostrichus pini* Say, 1826 *Tomicus praefrictus* Eichhoff, 1868 *Tomicus rectus* LeConte, 1868 *Tomicus oregonis* Eichhoff, 1869 *Ips laticollis* Swaine, 1918
	Ips plastographus (LeConte, 1868)*	California pine engraver	*Tomicus plastographus* LeConte, 1868
Cumatotomicus	*Ips sexdentatus* (Boerner, 1767)	six-toothed bark beetle	*Dermestes sexdentatus* Boerner, 1767 *Ips pinastri* Bechstein, 1818 *Ips stenographus* Duftschmid, 1825 *Ips junnanicus* Sokanovskiy, 1959
Granips	*Ips calligraphus* (Germar, 1824)	sixspined *Ips*, coarsewriting engraver	*Bostrichus calligraphus* Germar, 1824 *Bostrichus exesus* Say, 1826 *Tomicus praemorusus* Eichhoff, 1868 *Tomicus interstitialis* Eichhoff, 1869 *Ips ponderosae* Swaine, 1925
	Ips confusus (LeConte, 1876)	piñon *Ips*	*Tomicus confusus* LeConte, 1876
	Ips grandicollis (Eichhoff, 1868)	southern pine engraver	*Ips chagnoni* Swaine, 1916 *Tomicus cacographus* LeConte, 1868 *Tomicus grandicollis* Eichhoff, 1868 *Ips cloudcrofti* Swaine, 1924
	Ips lecontei Swaine, 1924	Arizona fivespined *Ips*	none
	Ips paraconfusus Lanier, 1970	California fivespined *Ips*	none
Ips	*Ips amitinus* (Eichhoff, 1872)	small spruce bark beetle, eight-toothed spruce bark beetle	*Tomicus amitinus* Eichhoff, 1872 *Ips amitinus* var. *montanus* Fuchs, 1913
	Ips cembrae (Heer, 1836)	large larch bark beetle	*Bostrichus cembrae* Heer, 1836 *Ips cembrae* var. *engadinensis* Fuchs, 1913 *Ips fallax* Eggers, 1915 *Ips shinanoensis* Yano, 1924
	Ips duplicatus (Sahlberg, 1836)	northern bark beetle	*Bostrichus duplicatus* Sahlberg, 1836 *Tomicus rectangulus* Ferrari, 1867 *Tomicus judeichi* Kirsch, 1871 *Tomicus infucatus* Eichhoff, 1877 *Tomicus infucatus* Eichhoff, 1878
	Ips subelongatus (Motschulsky, 1860)	larch bark beetle, oblong bark beetle	*Tomicus subelongatus* Motschulsky, 1860
	Ips typographus (Linnaeus, 1758)	eight-toothed spruce bark beetle	*Dermestes typographus* Linnaeus, 1758 *Bostrichus octodentatus* Paykull, 1800 *Ips japonicus* Niisima, 1909
No subgenus (*Incertae sedis*)	*Ips hauseri* Reitter, 1894	Kyrgyz mountain engraver, Hauser's engraver	*Ips ussuriensis* Reitter, 1913

**Ips plastographus* has two subspecies, *I. p. plastographus* (LeConte) and *I. p. maritimus* Lanier.

3. Detection

Ips bark beetles can be found in boles and limbs of the tree genera *Pinus*, *Picea*, *Larix* and *Cedrus*. *Pinus* and *Picea* wood are of primary economic importance to the world lumber trade. If bark is present, round wood, handicrafts, dunnage, crates or pallets suspected of originating from these tree genera could harbour *Ips*. Flying adult beetles are collected using a well-developed system of semiochemical lure-based traps (Fettig and Hilszczański, 2015).

Larvae and pupae are found in the host plant or wood products only immediately underneath the bark or in the phloem, not deeper in the wood or xylem (although some overwintering adults tunnel into the xylem, Lanier, 1967). Trees can be examined externally for symptoms of infestation (circular holes and red-brown boring dust, Figure 4).

3.1 Symptoms of infestation in living trees

Four general symptoms indicating possible attack in living Pinaceae trees are as follows:

- Yellowing, dying needles on the crown, a branch or all of the tree.
- Appearance of red-brown or yellow-brown boring dust on the bark or near the tree (Figure 4). *Ips* beetles often cause resin leakage, but only rarely cause appearance of resinous pitch tubes on the surface of the bark as in *Dendroctonus* colonization.
- Presence of intersecting maternal galleries up to 30 cm long, with lateral larval galleries, under the bark (Figures 2 and 3).
- Appearance of many small holes on the bark (e.g. ten or more 3–5 mm diameter holes in a 10 cm × 10 cm area). This is consistent with the post-emergence stage of *Ips* infestation. At this time the progeny has emerged from the tree to find unexploited bark tissue in which to establish new galleries.

Several months or more after successful colonization, the attacked tree may change leaf colour to yellow-green or red as the tree dies. *Ips* beetles sometimes kill healthy trees when beetle populations are high, although some trees recover even after the beetles have successfully reproduced in their tissues.

3.2 Collecting specimens from plants and wood products

The bark can be removed from affected trees or wood products using a sharp, strong knife or a small axe. The wood underneath the bark layer and the inner bark can be inspected for “H”- or “Y”-shaped galleries (or similar, Figures 2 and 3). A 40× magnifying lens can be used to inspect galleries for adults, larvae and eggs. If gallery engravings are present, some of the bark or affected material should be collected and photographed. Infested materials can be transported using a sealed bag or container. Double bagging of samples is useful for preventing escape.

Detected adults, larvae, pupae or eggs can be removed using forceps. Larvae can be placed for 30 to 60 seconds in near boiling water (90 °C to 100 °C) to fix for long-term preservation. Specimens should then be stored in a glass vial containing 70% to 80% ethanol. Adults can be killed in ethanol or by placement into a dry tube and then in a freezer at either −20 °C for at least 24 h or −80 °C for at least 6 h before card- or point-mounting on a pin. If specimens are to be saved for DNA analysis it is recommended that they be stored in a preservative such as a high percentage (>95%) of ethanol or propylene glycol.

It is necessary to collect any adults present because adults have important diagnostic morphological characters. It is not possible to identify juveniles to genus or species level based on morphology. In the laboratory, adult specimens should be mounted for examination while larvae, pupae or eggs should be examined in ethanol. See sections 4.1 and 4.2 for details on preparation of specimens for identification.

4. Identification

The genus *Ips* can be recognized and identified to species level by adult external morphology. Adult structures are illustrated in Figures 5 and 6. Descriptions and regional keys to the species of *Ips* based

on morphology are available (Balachowsky, 1949; Kurenzov and Kononov, 1966; Grüne, 1979; Schedl, 1981; Wood, 1982; Holzschuh, 1988; Lanier *et al.*, 1991; Pfeffer, 1995; Cognato and Sun, 2007). A generic key to Scolytinae larvae of eastern Canada is available (Thomas, 1957) but juvenile stages cannot be used for reliable identification on a global scale. Although *Ips* species have been discovered and identified using DNA sequence data (Cognato and Sun, 2007), validated protocols for universal DNA identification of *Ips* species have not yet been developed (Chang *et al.*, 2012). Additional work is needed to demonstrate that DNA sequence records provide accurate identification of the target species and to determine how to interpret DNA similarity between the target and non-target species.

4.1 Morphological identification of beetle adults

4.1.1 Preparation of adults for morphological examination

Ethanol preserved specimens (section 3.2) are transferred to a dish filled with 70% to 80% ethanol, to be cleaned of dirt, debris and frass. Specimens can be cleaned by gently brushing with a fine-hair artist's paint brush. The integument must be clean to show the surface texture and setal punctures. Adult specimens preserved in ethanol to be point-mounted on a pin should first be dried by removing the specimen from ethanol, blotting it with paper towel and allowing it to air-dry for 2–5 min. Specimens removed from −20 or −80 °C freezers should be placed on blotting paper and thawed for 10–20 min or until any visible condensation evaporates from the specimen. A triangular point mount can be used, attaching the beetle to the point along the right side of its thorax. Specimens may, alternatively, be glued ventrally to the middle of an 11 × 4.5 mm mounting card. Ideally the left lateral, dorsal and ventral views should be free and visible for examination. Once adults are pinned, they may be examined under a dissecting microscope capable of 40× magnification or higher (a higher magnification may be preferable). Strong, diffuse lighting is important for examination of adult bark beetles to see the surface sculpturing. Because adult bark beetles are shiny, light reflected from specimens may make it difficult to see surface structures. The sheen can be reduced by placing tracing paper or translucent drafting film between the light source and the specimen.

4.1.2 Identification of adults in the subfamily Scolytinae

Wood (1986) provides a key to the world genera of Scolytinae. Rabaglia (2002) provides an updated key to the North American genera of Scolytinae. The Scolytinae can be recognized by the following set of adult morphological characters (Hulcr *et al*., 2015):

- Body cylindrical (nearly circular in cross-section).
- Head width in dorsal view at least half of pronotal width.
- Legs and antennae (Figure 7) short (shorter than maximum body width in most, hind legs up to two-thirds of body length in a few Xyleborini), and flattened in cross-section in most.
- Tarsi of legs with four visible tarsomeres (tiny fourth tarsomere is hidden between the third and fifth).
- Antennae (Figures 5, and 8(e) to (g)) are geniculate (bent or elbowed) with: a long basal segment (the scape); an angled junction with a series of one to seven bead-like antennomeres (the funicle); and a compressed 3-segmented apical club (intersegmental sutures visible or not).
- The head anterior to the eyes is not elongated into a snout (Figures 6, and 8(a) to (d)). A snout or rostrum is present in most other Curculionidae (weevils).

Additional confirmatory characters for use in diagnosing damaged specimens are as follows:

- Eyes flush (level) with surface of head (Figures 8(a) to (d)). Eyes of many similar-shaped Bostrichidae protrude.
- Ventrally, the pregular sclerite (= submentum) is visible with a pregular suture present (Figure 6: pregula).
- Anterior legs of *Ips* and most other Scolytinae have socketed denticles on their apical and posterior edges (Figure 9(a)). Such spine-like hairs are also present in three other weevil

subfamilies. Magnification greater than 100× is required to separate socketed denticles from nearby non-socketed spines.

- The first tarsomere (the basal tarsal "segment") is approximately as long as the second and third tarsomeres combined (Figure 5).

4.1.3 Identifying adults of the tribe Ipini Bedel, 1888

Ips belongs to the tribe Ipini and can be distinguished from most other Scolytinae by the concave elytral declivity surrounded by large spines. The following tribal-level diagnostic characters are modified from Wood (1986):

- Compound eye (Figures 5, and 8(a) to (d)) sinuate (narrowed at mid-height), ventral half narrower than dorsal part.
- Antennal scape (basal segment) slender elongate, funicle 5-segmented, club either obliquely truncate or sutures on posterior face strongly displaced toward apex (Figures 5, and 8(e) to (g)).
- Pronotum (Figures 5 and 10) strongly declivous on anterior half (posterior half approximately horizontal, anterior half descends abruptly), with large asperities (broad spines).
- Procoxae contiguous, intercoxal piece deeply notched or absent.
- Protibia with three or four socketed denticles (Figure 9(a)).
- Scutellum visible in dorsal view (Figures 5 and 11(a)).
- Elytral declivity moderately sulcate to strongly excavated, sides with tubercles or spines in most (Figures 7 and 12).
- Vestiture hair-like (not scale-like or wider at midlength than at base), except for branched hairs at anterior opening of prothorax (Figure 8(g), row of hairs to right of antennal club).

Also: Frons sexually dimorphic in most.

4.1.4 Identification of *Ips* adults

Ips can be separated from other members of the tribe Ipini by features of the antennal club and elytral declivity, combined. The following diagnostic characters are modified from Wood (1986), as modified by Cognato (2000) and Cognato and Vogler (2001):

- Body length 2.1–8.0 mm (most are larger than 3 mm). Other Ipini are 1.0–4.3 mm long.
- Antennal club flattened (thickness less than one-third maximum width) and marked by sutures (Figures 8(e) to (g)). Sutures nearly straight to strongly bisinuate (not procurved).
- Elytral declivity broadly and deeply excavated, with sides acutely elevated and armed by three or more pairs of spines (Figures 7, 10 and 12). Apices of spines aligned with edge of declivity. Second spine (beginning from dorsal part of declivity) acute in lateral profile. Lower edge of concavity with an acutely elevated, explanate transverse ridge separating declivital excavation from apical edge (Figure 12(f)). Apex of declivity is not visible in dorsal view.

Ips is most similar in appearance to two other Ipini genera that also inhabit Pinaceae: *Orthotomicus* Ferrari, 1867 and *Pseudips* Cognato, 2000. *Ips* can be distinguished from *Orthotomicus* by the pointed second spine of its elytral declivity (right-angled in many *Orthotomicus*) and the broader explanate edge of its elytral declivity (Figure 12(f) vs 12(g)). *Ips* can be distinguished from *Pseudips* by its straight, bisinuate or acutely angulate antennal club sutures (Figures 8(e) to (g)). These sutures are broadly procurved (curved away from the antennal base at the midline of the club) in *Pseudips*, and also in the tropical, angiosperm feeding *Acanthotomicus* Blandford, 1894 and the warm-climate, ambrosia feeding *Premnobius* Eichhoff, 1878. *Pityogenes* Bedel, 1888 and *Pityokteines* Fuchs, 1911 are conifer-feeding Ipini, recognized by their small size (1.8–3.7 mm) and the rounded edges of their elytral declivity. The tropical, ambrosia fungus feeding *Premnophilus* Brown, 1962 lacks visible antennal sutures.

Most *Ips* species are grouped into subgenera, based on phylogenetic results by Cognato and Vogler (2001) and Cognato and Sun (2007). Diagnostic characteristics (external morphology only) of subgenera are as follows: *Cumatotomicus* Ferrari, body length >5 mm, spines on first and second elytral intervals

on declivity; *Bonips* Cognato, elytral declivity with four spines per side, elytral disc without punctures on intervals; *Granips* Cognato, elytral declivity with five to six spines per side; *Ips* DeGeer, elytral declivity with four spines per side, elytral disc without punctures on intervals; *Incertae sedis*, several *Ips* species outside any named subgenus. It is not necessary to identify to subgenus level in order to identify *Ips* species.

4.1.5 Key to distinguish *Ips* adults from other Scolytinae

The following key is modified from Wood (1986).

1. Anterior edge of elytra procurved or armed with spines or asperities (Figure 11(b)).....**not *Ips***

– Anterior edge of elytra straight or transverse, without asperities (Figure 11(a)) **2**

2. Apex of protibiae and dorsal (outer) ridge with only a single spine (Figure 9(b), circled part), or mesotibiae wider at midlength than apex .....**not *Ips***

– Apex of protibiae with multiple spines and denticles (Figure 9(a)), and mesotibiae widest at apex (as in Figures 9(a) and (b)).....**3**

3. Eye sharply, deeply emarginate, lower half usually almost equal in width to upper half; elytral declivity flattened to convex, unarmed by spines or large tubercles.....**not *Ips***

– Eye shallowly sinuate (Figure 8(a)), its lower half distinctly narrower than above; elytral declivity elaborately excavated, with lateral edges armed by three to six pairs of spines (Figures 7, 10 and 12)
.....
.....**4**

4. Elytral declivity narrowly bisulcate, sides broadly elevated, rounded, and armed by three or fewer pairs of spines; posterior margin of declivity rounded; most shorter than 3 mm.....**not *Ips***

– Elytral declivity broadly, deeply excavated, sides acutely elevated and armed by three or more pairs of spines (Figures 7, 10 and 12), posterior edge of declivity with an acutely elevated (Figures 12(f) and (g), circled), transverse ridge separating declivital excavation from elytral apex; most longer than 3 mm
.....**5**

5. With one or more of the following characteristics: sutures of antennal club absent, or procurved; elytral declivity with spines between the edge of the declivity and the elytral suture; second declivital spine obtuse or right-angled in lateral profile, or explanate apex of declivity absent or narrower or wider than length of second declivital spine (Figure 12(g)). Body length 1.4–4.3 mm.....**not *Ips***

– Sutures of antennal club weakly to strongly bisinuate (Figures 8(e) to (g)); elytral declivity with all spines in line with edge of declivity (Figures 7, 10 and 12), second declivital spine acute in lateral profile; explanate apex of declivity wider than length of second declivital spine (Figure 12(f)). Body length 2.1–8.0 mm..... ***Ips***

4.1.6 Species identification of *Ips* adults

Diagnostic characters of *Ips* spp. adults are based on key characters and diagnostic notes in Cognato (2015). The closely-related (Cognato and Sun, 2007) species *I. confusus* and *I. paraconfusus*, and also *I. cembrae* and *I. subelongatus*, are not fully distinguished from each other in the key to species. This may be important as these species may differ in their biology and distribution and whether they are a regulated pest or not (Stauffer *et al*., 2001). Additional examination by *Ips* specialists with appropriate reference collections is required to identify these beetles to species level using morphology (Cognato, 2015). DNA studies have been published to support identification of *I. confusus* and *I. paraconfusus* (Cognato *et al*., 1995; Cognato and Sun, 2007) and *I. cembrae* and *I. subelongatus* (Stauffer *et al*., 2001; Cognato and Sun, 2007) but these studies have not yet been developed into identification tests. In this protocol, 14 species are treated as target species (section 4.1.8) based on their known pest status

according to CABI and EPPO (1997). However, other *Ips* can also cause tree mortality, especially if introduced outside their native ranges.

Ips species are distinguished primarily by characters of the elytra and frons. Experts usually begin identifications by counting declivital spines. Here the following characters are useful: the number of spines on the declivity (not including small denticles on the first elytral interval); the distance from the first spine to the elytral suture relative to its height or to its distance from the second spine (Figures 13(a) and (b)); and the shininess of the declivity compared to the elytral dorsal surface (Figure 12(a) vs (b)). Several characters come from the third declivital spine (Figure 14): its pointedness (acute, subacute or nearly right-angled, and obtuse or rounded) and its profile (simple (triangular); straight-sided with acute apex; pedunculate (narrower near base than near apex); hooked (with second point on ventral side); double-pointed (appearing like two basally fused spines)). On the elytral disc (the horizontal part of the elytra), the presence or absence of punctures on the interstriae (elevated smooth surfaces between striae) are important (Figures 13(c) and (d)), especially on the second and third interstriae midway between the anterior edge of the elytra and the declivity.

On the frons (Figures 8(a) to (d)) the following presence or absence characters are used: presence or absence of a median tubercle; of a median carina (between median tubercle and labrum if both present); of a median fossa or pit (above median tubercle if present); of scattered circular tubercles; of setae; of dense setal brushes obscuring integument; or of setal punctures. A few species pairs can only be distinguished by the number of ridges on the pars stridens (Lanier *et al.*, 1991), a stridulatory organ at the posterior of the head capsule. However, this technique is not included in this protocol because it is only required for a few localized non-target species and because it requires removal of the head.

4.1.7 Key to diagnose adults of target *Ips* species

NT = non-target species.

1. Elytral declivity with three spines (Figure 12(c)); or frons with dense setae hiding part of integument; or frons protruding near epistoma; or frons without tubercles above level of eyes ...**non-target species: *I. acuminatus* (Gyllenhal), some *I. borealis* Swaine, some *I. pilifrons* Swaine, some *I. tridens* Swaine**

– Elytral declivity with four to six spines (Figures 12(a), (b), and (d) to (g)); frons evenly convex, not partly hidden by dense setae, and with tubercles above level of eyes (Figure 8(c)) **2**

2. Elytral declivity with six spines per side (Figure 12(a), counts do not include small spines on the first interval) **3**

– Elytral declivity with four to five spines per side (Figures 12(b), and (d) to (g)) **5**

3. Elytral disc without punctures between striae (Figure 13(c), on interstriae 2–3 between basal quarter and apical third); elytral declivity with fourth spine largest (Figure 10(d)); and frons with transverse carina .....***I. sexdentatus* (Boerner)**

– Elytral disc with punctures between striae (Figure 13(d), as restricted above); elytral declivity with third spine largest in most (Figure 12(a), although fourth spine is largest in some ♀ *I. calligraphus*); frons without transverse carina.....**4**

4. Pronotal width 1.7 mm or less.....***I. apache* Lanier** (NT)

– Pronotal width 2.0 mm or more.....***I. calligraphus* (Germar)**

5. First suture of antennal club nearly straight (Figure 8(e)).....**6**

– First and second suture of antennal club sinuate or acutely angulate (Figures 8(f) and (g)).....**8**

6. Elytral declivity with third spine tapered (Figure 14(a)) or straight-sided with tapered apex (Figure 14(b)) ..... ***I. borealis* Swaine, part** (NT)

– Elytral declivity with third spine pedunculate (Figure 14(c)) **7**

7. Frons with median tubercle (Figure 8(a)); body length 3.5–4.8 mm (Palaearctic)
..... ***I. amitinus* (Eichhoff)**

– Frons without median tubercle; body length 2.7–3.5 mm (Nearctic)..... ***I. perroti* Swaine** (NT)

8. Sutures of antennal club acutely angulate (Figure 8(g)); elytral declivity with five spines in most (Figures 12(b) and (d)) **9**

– Sutures of antennal club sinuate (Figure 8(f)); elytral declivity with four spines (Figures 12(e) and (f)) **19**

9. Elytral declivity with four spines (Figures 12(e) and (f))..... **10**

– Elytral declivity with five spines (Figures 12(b) and (d)) **11**

10. Frons with median epistomal tubercle connected to frontal tubercle by a vertical carina (Figure 8(b), requires magnification >50× and diffuse light)..... ***I. integer* (Eichhoff)** (NT)

– Median epistomal tubercle not connected to frontal tubercle..... ***I. plastographus* (LeConte)**

11. Frons with median tubercle split (Figure 8(d)) (or transverse pair of tubercles)
..... ♂ ***I. lecontei* Swaine**

– Frons with median tubercle entire (Figure 8(a)) or absent **12**

12. Frons with median tubercle absent (females only)..... **13**

– Frons with single entire median tubercle (males & females) **15**

13. Distance between first and second declivital spines greater than from suture to first spine (Figure 13(a)) ..... ***I. lecontei* Swaine and *I. grandicollis* (Eichhoff)**

– Distance between first and second declivital spines not greater than from suture to first spine (Figure 13(b)) **14**

14. Frons with central fovea impressed (circular impression mid frons, above tubercle if present)..... ***I. confusus* (LeConte) and *I. paraconfusus* Lanier; *I. hoppingi* Lanier** (NT)

– Frons with central fovea not impressed..... ***I. montanus* (Eichhoff)** (NT)

15. Distance between first and second declivital spines greater than from suture to first spine (Figure 13(a)) ..... ***I. grandicollis* (Eichhoff) part; *I. paraconfusus* Lanier, part; *I. cribricollis* (Eichhoff)** (NT)

– Distance between first and second declivital spines not greater than from suture to first spine (Figure 13(b)) **16**

16. Frons with central fovea weak (shallow concavity) or absent
..... **non-target species, including *I. hoppingi* Lanier**

– Frons with central fovea impressed..... **17 (diagnostically difficult species)**

17. Elytral declivity with third spine pointed (Figures 14(a) to (c), acute or subacute) **18**

– Elytral declivity with third spine rounded (Figure 14(e), not at arrow) ..... ♂ ***I. confusus*** **(LeConte)**

18. Frons with median tubercle separated from epistoma by distance less than half its diameter, central carina absent ..... ♂ ***I. paraconfusus*** **Lanier, part;** ***I. montanus*** **(Eichhoff), part** (NT)

– Frons with median tubercle (Figure 8(a), circled) separated from epistoma (Figure 8(a), arrow) by distance equal to or more than half its diameter, central carina present (Figure 8(b)) or absent ..... ***I. montanus*** **(Eichhoff), part** (NT)

19. Frons with median tubercle absent **20**

– Frons with single entire median tubercle (Figure 8(a)) **27**

20. Elytral disc without punctures between striae (Figure 13(c)) **21**

– Elytral disc with punctures between striae (Figure 13(d)) **24**

21. Elytral declivity with third spine simple: tapered from base (Figure 14(a)), or straight-sided with tapered apex (Figure 14(b)) **22**

– Elytral declivity with third spine apex emarginate (Figure 14(d)) ..... ***I. emarginatus*** **(LeConte)** (NT)

22. Body length 2.0–3.0 mm ..... **non-target species:** ***I. avulsus*** **(Eichhoff),** ***I. bonanseai*** **(Hopkins) (some ♀)**

– Body length 3.0–4.5 mm **23**

23. Frons with central fovea impressed ..... **non-target species:** ***I. nitidus*** **Eggers,** ***I. bonanseai*** **(Hopkins),** ***I. perturbatus*** **(Eichhoff)**

– Frons with central fovea absent ..... ♀ ***I. pini*** **(Say), part, also non-target species:** ***I. bonanseai*** **(Hopkins) (♀) and** ***I. perturbatus*** **(Eichhoff)**

24. Elytral declivity with distance between first and second spines less than height of first spine ..... ***I. cembrae*** **(Heer) and** ***I. subelongatus*** **(Motschulsky)**

– Elytral declivity with distance between first and second spines greater than or equal to height of first spine **25**

25. Elytral declivity with third spine tapered and acute (Figure 14(a)), or straight-sided with tapered or rounded apex (Figure 14(b)) **26**

– Elytral declivity with third spine straight-sided with acute apex, pedunculate (Figure 14(c)), or bidentate (Figure 14(d)) ..... **non-target species:** ***I. nitidus*** **Eggers,** ***I. chinensis*** **Kurentsov & Kononov,** ***I. knausi*** **Swaine,** ***I. stebbingi*** **Strohmeyer**

26. Elytral declivity with distance between bases of spines 2 and 3 similar to (at least 85%) distance between spines 1 and 2 ..... ***I. hauseri*** **Reitter**

– Elytral declivity with spine 2 closer to spine 3 than to spine 1 (distance between 2 and 3 is less than 75% of distance between 1 and 2) ..... ***I. duplicatus*** **(Sahlberg) (some ♀)**

27. Elytral disc without punctures between striae (Figure 13(c)) **28**

– Elytral disc with punctures on interstriae (Figure 13(d)) **31**

28. Elytral declivity with third spine evenly tapered (Figure 14(a)) or emarginate at apex (Figure 14(d)) ..... **some ♀ of *I. pini* (Say), and non-target species: *I. avulsus* (Eichhoff), *I. bonanseai* (Hopkins), *I. emarginatus* (LeConte)**

– Elytral declivity with third spine pedunculate (Figure 14(c)) or straight-sided with tapered apex (Figure 14(b)) **29**

29. Elytral declivity with matt appearance (Figure 12(c)); if declivity shiny then frons with median tubercle up to three times tubercle diameter from base of epistomal setae, frons median tubercle not connected to epistoma by carina, elytral declivity with third spine pedunculate
..... ***I. typographus* (Linnaeus) and *I. nitidus* Eggers** (NT)

– Elytral declivity shiny (Figure 12(b)) and frons with median tubercle two to three times tubercle diameter from base of epistomal setae, frons median tubercle connected to epistoma by carina or not, and elytral declivity with third spine pedunculate or not **30**

30. Head with median frontal tubercle connected to epistomal tubercle (Figure 8(a), requires magnification >50× and diffuse light) ..... ***I. bonanseai* (Hopkins)** (NT)

– Head with median frontal tubercle not connected to epistomal tubercle ..... ***I. pini* (Say), part**

31. Head without median epistomal carina; frons median tubercle separated from base of epistomal setae by at least twice its diameter (Figure 8(a)), median fovea present, vertex with many coarse punctures; elytral declivity with third spine straight-sided with acute apex, or pedunculate (Figure 14(c)) ..... ***I. duplicatus* (Sahlberg) (♂ & most ♀)**

– Any of above not true: head without epistomal carina; frons median tubercle separated from base of epistomal setae by only its diameter, median fovea absent, vertex with few scattered punctures; elytral declivity with third spine evenly tapered (Figure 14(a)) or apically emarginate (Figure 14(d))
..... **non-target species: *I. borealis* Swaine and *I. knausi* Swaine**

4.1.8 Diagnostic notes on target species (Modified from Cognato, 2015)

Notes on diagnosis, distributions and hosts are provided below to supplement information presented in the species key. Body lengths are rounded to the nearest 0.5 mm (except for *I. pini* and *I. montanus*).

Subgenus *Bonips*

I. pini (Say, 1826) (Figure 7). Principal hosts: *Pinus* spp. Diagnosis: *I. pini* has four spines on the elytral declivity, and lacks punctures on elytral intervals 2 and 3 near the midlength of the disc. Body length: 3.0 to 4.5 mm. *I. pini* should be diagnosed using the key or a full description that includes interspecific variation and sexual dimorphism. This species differs from the related species *I. avulsus* and *I. bonanseai* as follows:

- *I. avulsus* (Eichhoff, 1869). Principal hosts: *Pinus* spp. Differs from *I. pini* in the non-pedunculate profile of the third spine of the male declivity, the short expansion of the declivital apex, and its smaller size, 2.1–2.8 mm (Wood, 1982).
- *I. bonanseai* (Hopkins, 1905). Principal hosts: *Pinus* spp. Differs from *I. pini* in that the median frontal tubercle is connected to the epistomal tubercle, and it is a smaller size, 2.9–3.4 mm.

I. plastographus (Eichhoff, 1868), (*I. p. plastographus* (LeConte) and *I. p. maritimus* Lanier), (Figures 8(a) and 12(j)). Principal hosts: *Pinus contorta* and *Pinus muricata*. Diagnosis: This species has four spines on the elytral declivity and is similar to *I. pini* (Figure 7). Body length: 3.5–6.5 mm.

I. plastographus lacks a frontal carinate elevation and differs from the related species *I. integer* as follows:

- *I. integer* (Eichhoff, 1869). Principal hosts: *Pinus* spp. Sibling species to *I. plastographus*, diagnosable by the connection of the median epistomal and frontal tubercles by a carinate elevation or by molecular phylogenetics (Cognato and Sun, 2007). These species are potentially sympatric in northwestern North America. However, *I. plastographus* is mostly restricted to two hosts, *P. contorta* and *P. muricata*.

Subgenus *Cumatotomicus*

I. sexdentatus (Boerner, 1767) (Figure 10(d)). Principal hosts: *Pinus* spp. and *Picea* spp. Diagnosis: *I. sexdentatus* has six spines on the elytral declivity. This species differs from all other *Ips* spp. in having the largest spine in the fourth position (Figure 10(d)). Body length: 4.5–8.0 mm. This Palaearctic species is not closely related to the North American six-spined species *I. calligraphus* (Figure 12(a)) and *I. apache*, which have the largest spine in the third position.

Subgenus *Granips*

I. calligraphus (Germar, 1824) (Figure 12(a)). Principal hosts: *Pinus* spp. Diagnosis: *I. calligraphus* has six spines on the elytral declivity (Figure 12(a)) and its general appearance is like *I. apache*. Body length: 3.5–7.0 mm. This species differs from *I. sexdentatus* in that the third declivital spine of *I. calligraphus* is the largest. It is distinguished from other *Ips* spp. by the presence of three spines beyond the third declivital spine. It differs from *I. apache* (Lanier *et al.*, 1991) in the distance between the ridges of the pars stridens and by being a larger size, with a pronotal width of 2.0–2.1 mm (1.6 mm in *I. apache*).

I. confusus (LeConte, 1876) (Figure 10(b)). Principal hosts: *Pinus edulis* and *Pinus monophylla*. Diagnosis: *I. confusus* has five spines on the elytral declivity. Body length: 3.0–5.5 mm. This protocol does not reliably distinguish *I. confusus* from *I. paraconfusus*. *Ips confusus* differs from *I. paraconfusus* in the distance between the ridges of the pars stridens.

- *I. hoppingi* Lanier, 1970. Principal hosts: Pinyon pines including *Pinus cembroides* and *P. discolor*. Sibling species to *I. confusus*, from which it is diagnosed by the distance between the ridges of the pars stridens (Lanier, 1970) or by molecular phylogenetics (Cognato and Sun, 2007).
- *I. montanus* (Eichhoff, 1881). Differs from *I. confusus* and *I. paraconfusus* in the absence of the frontal fovea; the male major median frontal tubercle displaced from the epistoma; and some specimens are larger, 4.6–5.4 mm.

I. paraconfusus Lanier, 1970. Principal hosts: *Pinus attenuata*, *Pinus coulteri*, *Pinus jeffreyi*, *Pinus lambertiana* and *Pinus ponderosa*. Diagnosis: Body length: 3.5–5.0 mm. This species has five spines on the elytral declivity and is most like *I. confusus* (Figure 10(b)). The *Ips* species that are most similar to *I. paraconfusus* differ from it as follows: *I. confusus* differs in characters of the pars stridens (not presented here); *I. montanus* has more and larger frontal punctures, lacks a median frontal fovea, the male major median frontal tubercle is displaced from the epistoma, and some specimens are larger, 4.6−5.4 mm; and *I. hoppingi* is only partly distinguishable from *I. paraconfusus* by methods presented here.

I. grandicollis (Eichhoff, 1868) (Figures 8(g), 12(b), 15). Principal hosts: *Pinus* spp. Diagnosis: Body length: 2.5–5.0 mm. There are five spines on the elytral declivity and its general appearance is like *I. confusus* (Figure 10(b)). This species differs from *I. confusus* in that declivital spine 1 is closer to the second spine than to the suture, and from *I. cribricollis* in the width of the female pars stridens and the presence of a central fovea on the male frons in *I. grandicollis* (Lanier, 1987).

I. lecontei Swaine, 1924 (Figure 12(i)). Principal hosts: *Pinus ponderosa* and *Pinus pseudostrobus*. Diagnosis: Body length: 3.5–5.0 mm. This species has five spines on the elytral declivity and is most like *I. confusus* (Figure 10(b)). This species differs from all other species with five declivital spines in having a pair of median frontal tubercles on the epistoma (Figure 8(d)).

Subgenus *Ips*

I. amitinus (Eichhoff, 1872) (Figure 10(a)). Principal hosts: *Picea* spp. and *Pinus* spp. Diagnosis: *I. amitinus* has four spines on the elytral declivity. Body length: 3.5–5.0 mm. This species differs from all other Eurasian *Ips* spp. in that the antennal club sutures are nearly straight (as in Figure 8(e)). Body length: 3.5–5.0 mm. It differs from the morphologically similar North American *I. perroti* (2.5–3.5 mm) in its larger size.

I. cembrae (Heer, 1836) (Figure 12(l)). Principal hosts: *Larix* spp. Diagnosis: Body length: 4.0–6.5 mm. *I. cembrae* has four spines on the elytral declivity and is most like *I. typographus* (Figure 10(e)). This species differs from *I. typographus* by having a shiny elytral declivity and interstrial punctures of the elytral disc. It differs from the morphologically similar North American *Picea*-feeding species and *I. woodi* in the space between the first and second spines, which is less than the length of the first spine in *I. cembrae*. It differs from its sister species *I. subelongatus* in its less setose elytral declivity, but these species are best diagnosed using DNA data (Stauffer *et al.*, 2001).

I. subelongatus (Motschulsky, 1860) (Figure 12(k)). Principal hosts: *Larix* spp. Diagnosis: There are four spines on the elytral declivity. Body length: 4.0–6.5 mm. This species differs from *I. typographus* (Figure 10(e)) in having a shiny elytral declivity and interstrial punctures of the elytral disc. This species differs morphologically from *I. cembrae* only slightly, in having a more densely setose elytral declivity. DNA methods have been reported for distinguishing between these two species (Stauffer *et al*., 2001). It differs from the morphologically similar North American *Picea*-feeding species and *I. woodi* in the space between the first and second spines, which is less than the length of the first spine in *I. subelongatus*.

I. duplicatus (Sahlberg, 1836) (Figure 10(c)). Principal hosts: *Picea* spp. Diagnosis: *I. duplicatus* has four spines on the elytral declivity. Body length: 2.5–4.5 mm. This species differs from many other *Ips* spp. in the position of the first spine of the elytral declivity, which is closer to the elytral suture than to the second spine. It differs from the morphologically similar Himalayan species, North American *Picea*-feeding species and *I. woodi*, in having a sparsely granulate frons. This species differs from the similar *I. hauseri* (Figure 12(h)) in the close proximity of the bases of spines 2 and 3 in *I. duplicatus* (less than the distance between the first and second spines).

I. typographus (Linnaeus, 1758) (Figure 10(e)). Principal hosts: *Picea* spp. Diagnosis: *I. typographus* has four spines on the elytral declivity. Body length: 3.5–5.5 mm. This species differs from most other species in its dull elytral declivity (in most specimens) and impunctate interstriae on the basal half of the elytral disc. *I. nitidus* can be distinguished from most *I. typographus* specimens by its shiny declivity, and all specimens can be distinguished by phylogenetic analysis of DNA (Cognato and Sun, 2007). It differs from the morphologically similar Himalayan species, North American *Picea*-feeding species and *I. woodi* in having a major median frontal tubercle.

No subgenus: *Incertae sedis*

I. hauseri Reitter, 1894 (Figure 12(h)). Principal hosts: *Picea* spp. Diagnosis: Body length: 3.5–5.5 mm. There are four spines on the elytral declivity and its general appearance is like *I. duplicatus* (Figure 10(c)). This species differs from all other European *Ips* spp. in the position of the first spine of the elytral declivity, which is closer to the elytral suture than to the second spine. It differs from the morphologically similar Himalayan species, North American *Picea*-feeding species and *I. woodi* by having a sparsely granulate frons. This species differs from its sister species *I. duplicatus* in the separation of the bases of the second and third spines (nearly equal to the distance between the first and second spines in *I. hauseri*).

4.2 Morphological identification of larvae in the subfamily Scolytinae

While adult specimens are needed to confirm the genus-level identification of *Ips* species, it is useful to examine larvae if no adults are available. However, they may be confused with other similar Scolytinae larvae.

Ips larvae are indistinguishable from some species in other genera. Morphological examination of larvae will not allow positive identification but may allow elimination of some candidate genera. Methods are provided to indicate if a larva is either not *Ips* or suspected to be *Ips*.

4.2.1 Preparation of larvae for morphological examination

The ethanol preserved specimens can be transferred to a small Petri dish filled with 70% ethanol for morphological examination. Specimens should be clean of debris and frass prior to examination (especially the head). Specimens can be cleaned by gently brushing with a fine camel-hair brush. They may be examined under a dissecting microscope capable of 40× magnification or higher (higher magnification is better).

4.2.2 Identifying larvae in the subfamily Scolytinae

Mature larvae are 2–6 mm long. Larvae of this subfamily have no legs (Figure 15). The body is soft with three thoracic segments and ten abdominal segments. The mouthparts and head capsule are sclerotized, and are pale brown in most specimens. The head capsule is globular and not retracted into the first thoracic segment; the antennae have one segment; and the cranium has a "Y"-shaped ecdysial suture. The thorax has three pairs of pedal lobes (where legs would be), each with two to four setae. Each abdominal segment has two or three tergal (dorsal) folds. The prothorax and the first eight abdominal segments bear spiracles (Bright, 1991).

Ips larvae are difficult to distinguish from larvae of weevils (and of other Curculionidea in general). They are mainly recognizable as Scolytinae because of their presence in complex gallery systems with multiple larvae. Other non-Scolytinae beetle larvae that may co-occur in such galleries have thoracic legs allowing them to actively colonize bark beetle galleries.

4.2.3 Key to distinguish final instar *Ips* larvae from some other Scolytinae

Ips larvae in their final instar stage may be distinguished from some other Nearctic and Palaearctic conifer-feeding genera. The key below is based on work by Thomas (1957), with only 15 genera examined from mostly North American fauna. This key may help determine that some larvae are not *Ips*, but it should not be used for positive identification of *Ips*. *Ips* larvae cannot be identified to species level using morphology.

1. Posterior part of the premental sclerite rectangular, lightly pigmented (Figure 16(c)) ..... **Not *Ips***

– Posterior part of the premental sclerite of the labium acute, and dark at midline (Figures 16(a) and (b)) **2**

2. The three postlabial setae (ventral side of head capsule) arranged in a triangle (middle pair most distant from each other) (Figure 16(b)), or posterior pair not the most distant from each other across midline of head ..... **Not *Ips***

– The three postlabial setae arranged in a line (Figure 16(a)), and posterior pair furthest apart **3**

3. Six or more dorsal epicranial setae on head capsule ..... **Not *Ips***

– Five or fewer dorsal epicranial setae on head capsule **4**

4. Labial palps 1-segmented, or appearing 1-segmented ..... **Not *Ips***

– Labial palps 2-segmented (Figure 16(a), near midline) **5**

5. Epipharynx with three pairs of median setae ..... **Not *Ips***

– Epipharynx with more than three pairs of median setae **6**

6. Labium with two anteromedian setae ..... **Not *Ips***

– Labium with four anteromedian setae, outer pair smaller ..... ***Ips*** **(and some other genera)**

5. Records

Records and evidence should be retained as described in section 2.5 of ISPM 27 (*Diagnostic protocols for regulated pests*).

In cases where other contracting parties may be adversely affected by the results of the diagnosis, the following records and evidence and additional material should be kept for at least one year in a manner that ensures traceability: preserved pinned or slide-mounted specimens and photographs of distinctive taxonomic structures.

6. Contact Points for Further Information

Further information on this protocol can be obtained from:

Michigan State University, 288 Farm Lane, Room 243 Natural Science Building, East Lansing, MI 48824, United States of America (Anthony I. Cognato; email: cognato@msu.edu; tel.: +1 517 432 2369).

NPPO–NL, Ministry of Economic Affairs, NVWA (Dutch Food and Consumer Product Safety Authority), National Reference Centre, Geertjesweg 15, 6706 EA, Wageningen, Netherlands (Brigitta Wessels-Berk; email: b.f.wessels@nvwa.nl; tel.: +31 3 17 49 68 35, +31 8 82 23 29 41).

Canadian National Collection of Insects, Arachnids and Nematodes, Agriculture and Agri-Food Canada, K.W. Neatby Building, 960 Carling Avenue, Ottawa, Ontario, K1A0C6, Canada (Hume Douglas; email: hume.douglas@canada.ca; tel.: +1 613 759 7128).

Norwegian Institute of Bioeconomy Research, Division of Biotechnology and Plant Health, Box 115, N-1431 Ås, Norway (Torstein Kvamme; email: Torstein.Kvamme@nibio.no; tel.: +47 915 73 942).

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), which will in turn forward it to the Technical Panel on Diagnostic Protocols (TPDP).

7. Acknowledgements

The first draft of this protocol was written by Hume Douglas (Agriculture and Agri-food Canada, Canada (see preceding section)), with content from Anthony I. Cognato (Michigan State University, United States of America (see preceding section)), and editing by Brigitta Wessels-Berk (Netherlands Food and Consumer Product Safety Authority, Netherlands (see preceding section)) and Norman Barr (United States Department of Agriculture, Animal and Plant Health Inspection Service, United States of America). K. Savard (Agriculture and Agri-Food Canada, Canada) provided additional images.

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8. References

The present annex refers to ISPMs. ISPMs are available on the International Phytosanitary Portal (IPP) at https://www.ippc.int/core-activities/standards-setting/ispms.

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9. Figures

Figure 1. Blue stain fungus (*Ceratocystis* sp.) affecting wood of *Pinus* sp. Scale bar: 5 cm.
Source: Ronald F. Billings, Texas Forest Service, United States of America, Bugwood.org.

Figure 2. Partial *Ips calligraphus* maternal galleries in *Pinus* wood with radiating and intersecting larval galleries. The central “H”-shaped gallery was built by one male and four females. One adult female (black) and two pupae (white) are shown with arrows. Scale bar: 5 cm.
Source: William M. Ciesla, Forest Health Management International, Bugwood.org.

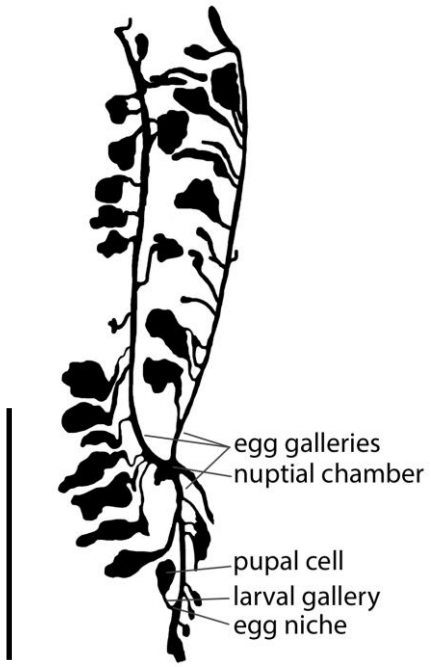

Figure 3. Partial *Ips pini* gallery system. The central "Y"-shaped gallery was built by one male and three females. Scale bar: 5 cm.
Drawing courtesy of K. Savard, Agriculture and Agri-Food Canada, Ottawa, Canada.

Figure 4. Bark of fallen *Pinus* sp. tree with boring dust from dense population of *Ips pini*. Scale bar: 5 cm.
Source: Brytten Steed, United States Department of Agriculture Forest Service, Bugwood.org.

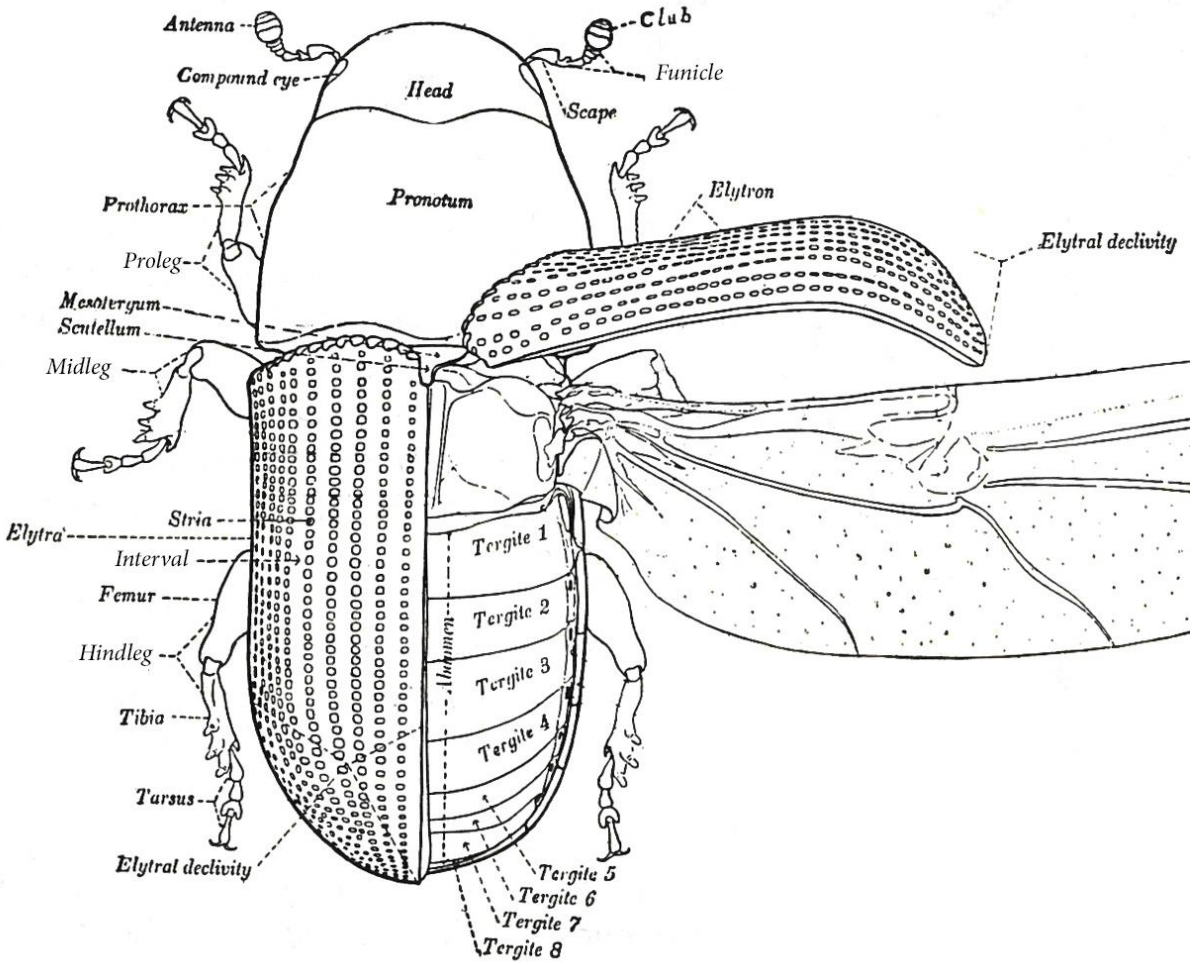

Figure 5. Morphology of an adult bark beetle (*Dendroctonus valens*) in dorsal view.
Modified from Hopkins (1909).

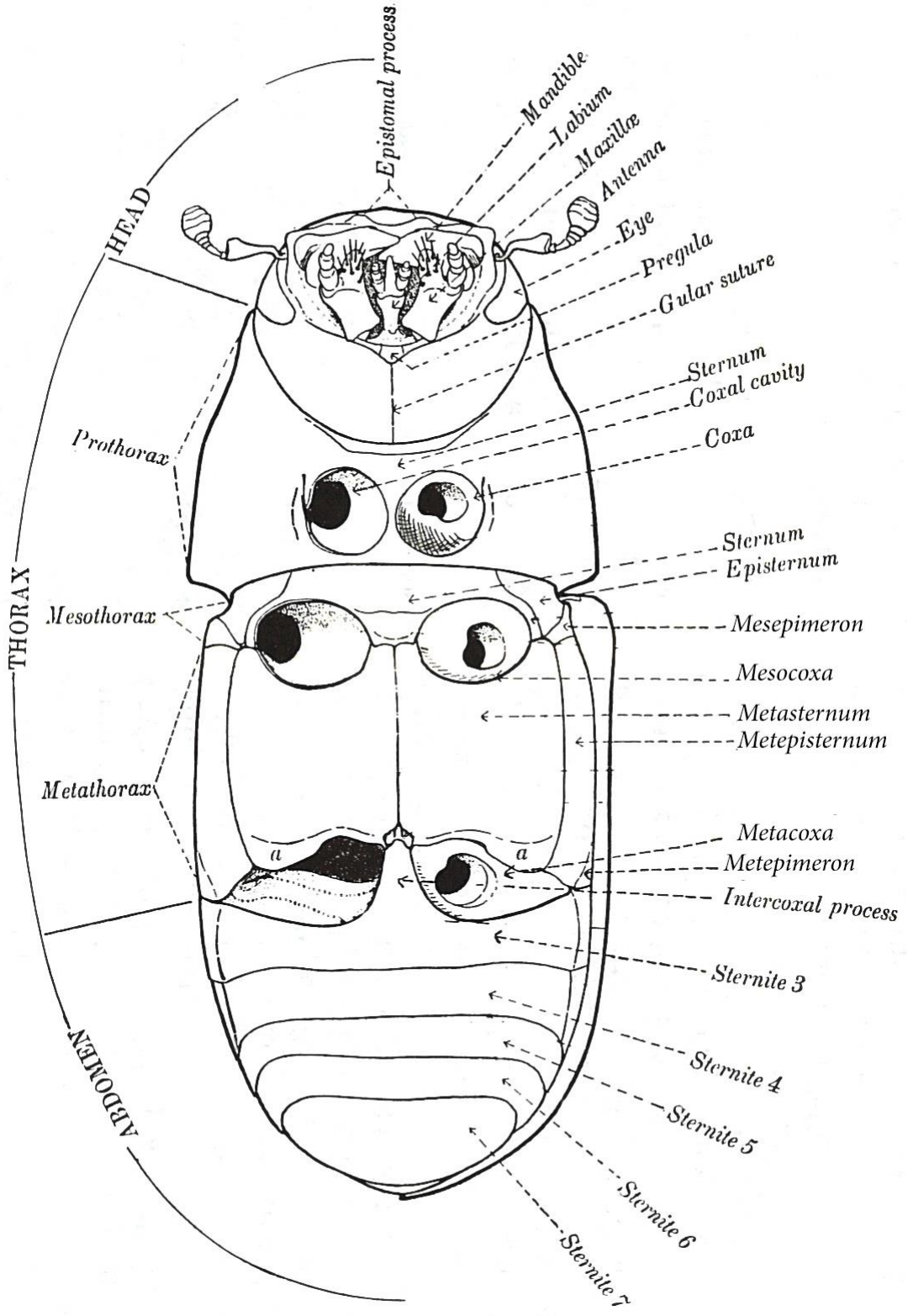

Figure 6. Morphology of an adult bark beetle (*Dendroctonus valens*) in ventral view.
Modified from Hopkins (1909).

Figure 7. *Ips pini*: dorsal habitus of adult.
Photo courtesy of K. Bolte, Canadian Forest Service, Ottawa, Canada.

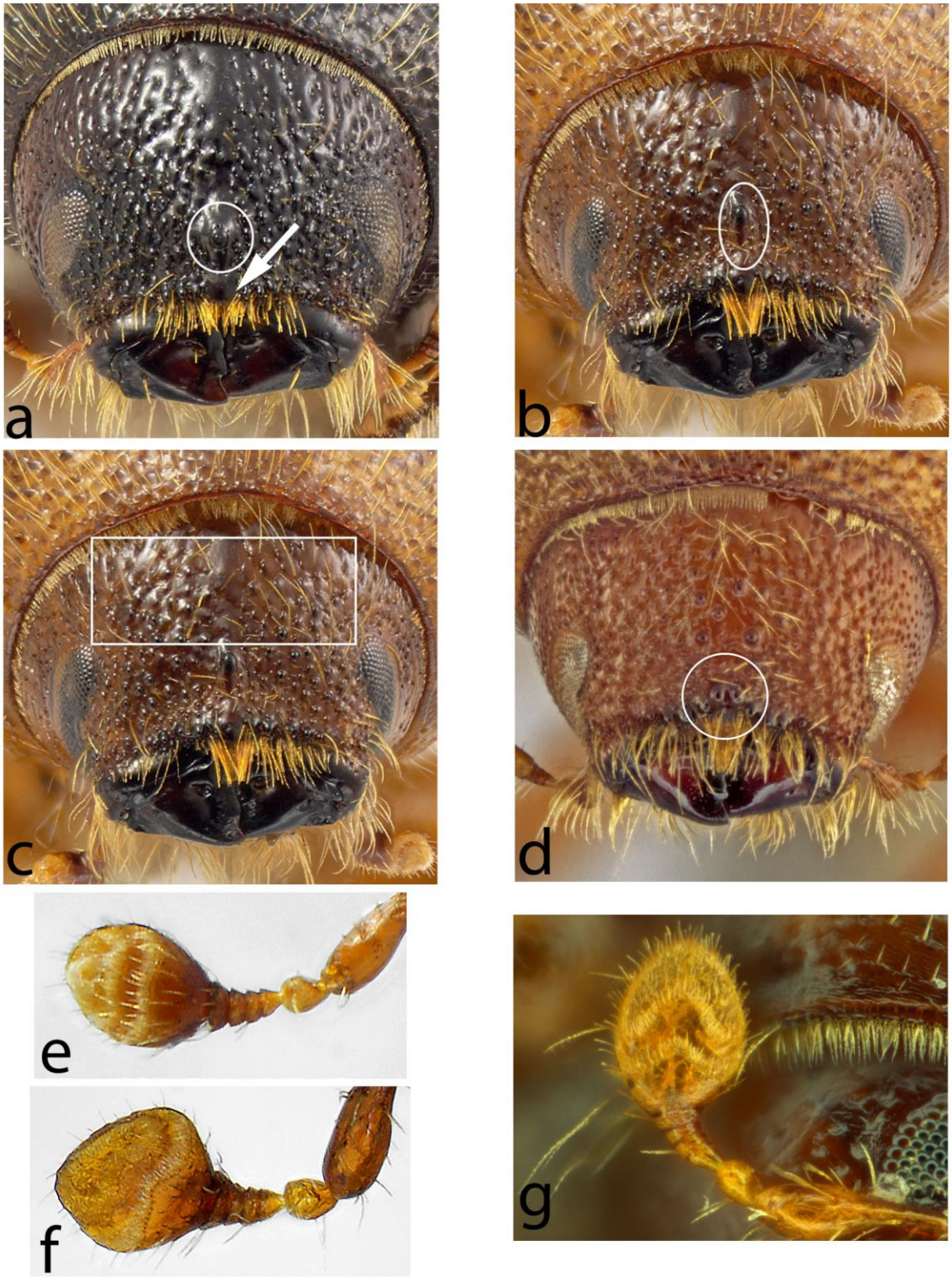

Figure 8. (a)–(d) Head of *Ips* spp.: (a) *I. plastographus* with round median tubercle (circled) and epistoma marked with an arrow; (b) *I. integer* with elongate frontal tubercle (in vertical white oval); (c) *I. integer* with tubercles on frons above eyes highlighted; (d) *I. lecontei* with split frontal tubercle. (e)–(g) Antenna of *Ips* spp.: (e) *I. perroti* (straight sutures); (f) *I. tridens* (bisinuate sutures); (g) *I. grandicollis* (angulate sutures).
Photos courtesy of (a)–(c), (e) and (f) K. Bolte, Canadian Food Inspection Agency; (d) K. Savard, Agriculture and Agri-Food Canada, Ottawa, Canada (AAFC); (g) H. Douglas (AAFC).

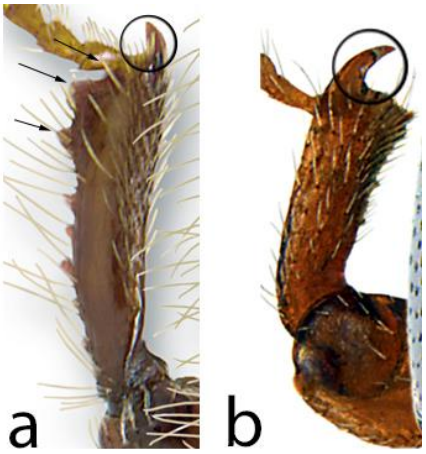

Figure 9. Front tibia of Scolytinae spp.: (a) *Ips pini*, (b) *Scolytus multistriatus*. Arrows indicate socketed denticles; circle surrounds apical non-socketed spine.
Photos courtesy of K. Bolte, Canadian Forest Service, Ottawa, Canada.

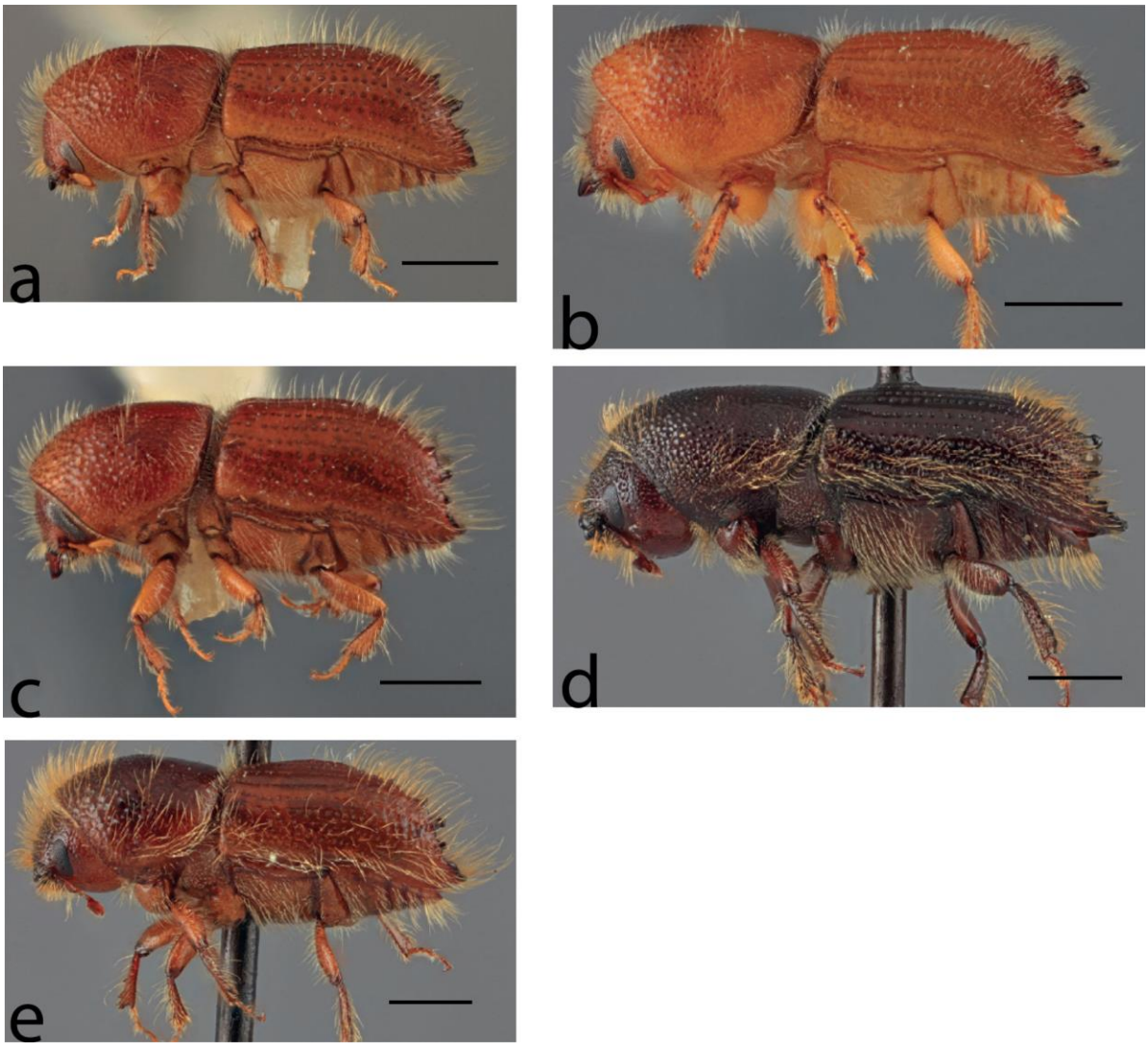

Figure 10. Lateral habitus of *Ips* spp.: (a) *I. amitinus* (four spines); (b) *I. confusus* (five spines); (c) *I. duplicatus* (four spines); (d) *I. sexdentatus* (six spines); (e) *I. typographus* (four spines). Scale bars: 1 mm.
Source: Cognato (2015).

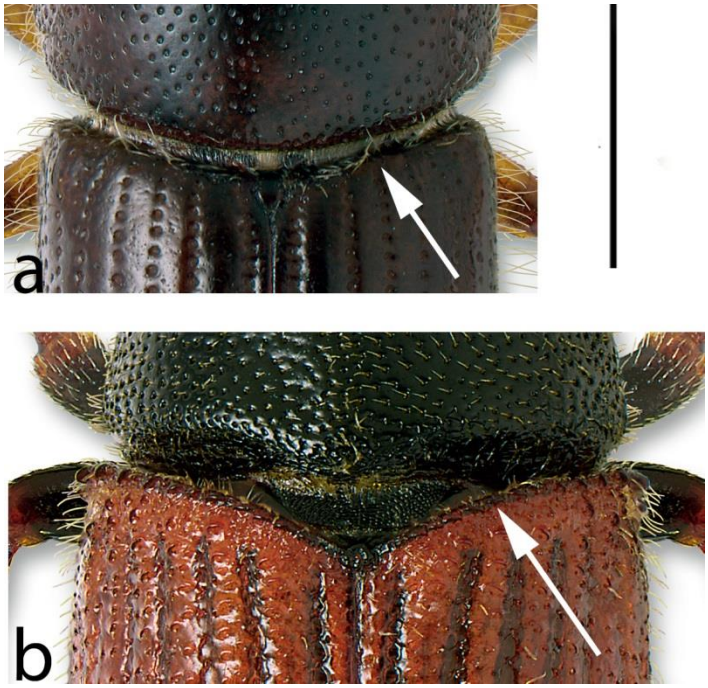

Figure 11. Shape of elytral base (arrow): (a) not procurved and smooth, *Ips pini*; (b) procurved and asperate (with spines), *Phloeosinus punctatus*. Scale bar: approximately 1 mm.
Photos courtesy of K. Bolte, Canadian Forest Service, Ottawa, Canada.

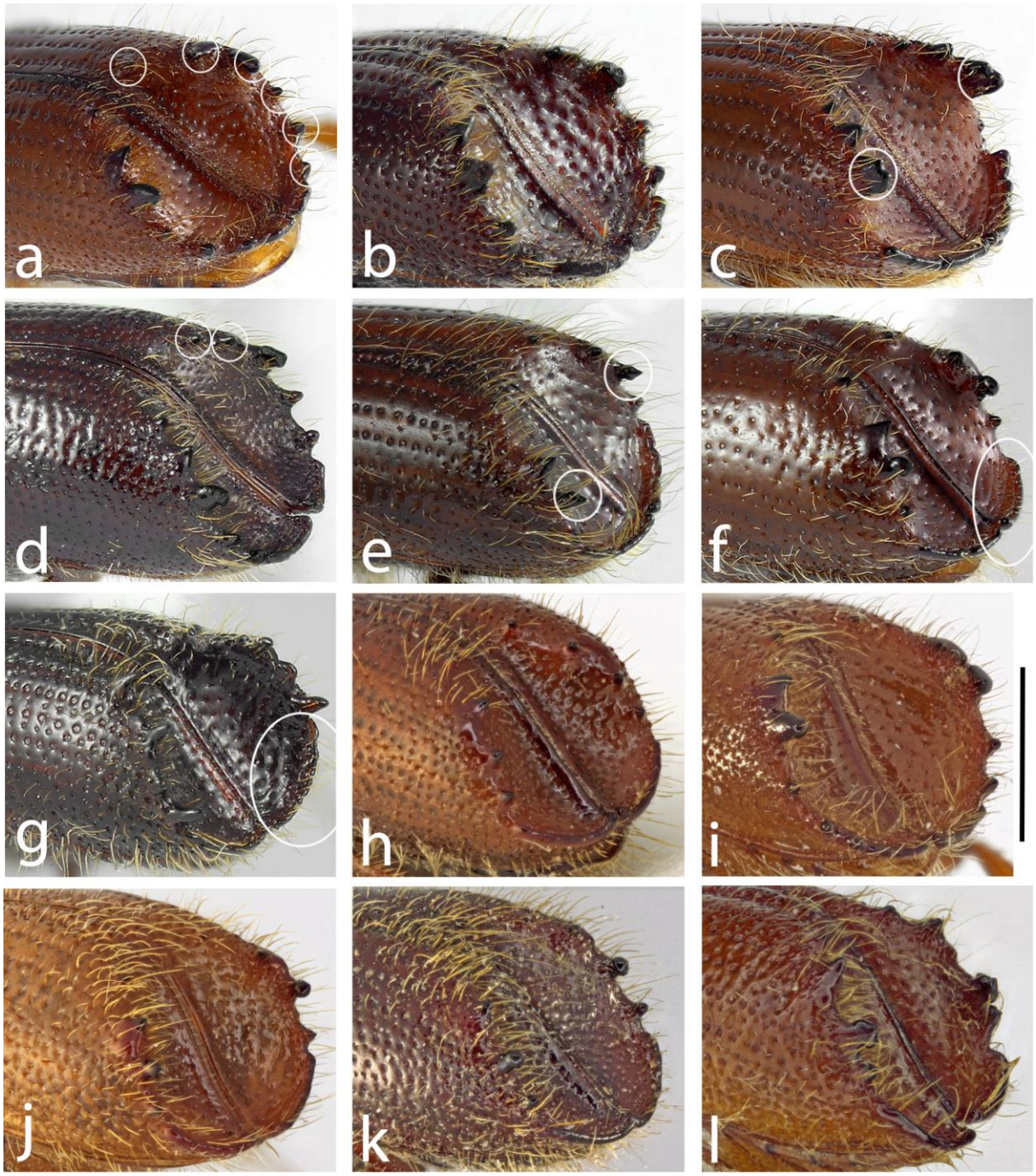

Figure 12. Elytral declivity of Ipini spp.: (a) *I. calligraphus* (six spines); (b) *I. grandicollis* (five spines); (c) *I. emarginatus* (bidentate third spine); (d) *I. montanus* (five spines); (e) *I. perturbatus* (third spine pedunculate and acute); (f) *I. tridens* (explanate apex of declivity); (g) *Orthotomicus latidens* (smaller explanation of apex of declivity); (h) *I. hauseri* (third spine tapered and acute); (i) *I. lecontei* (third spine hooked and obtuse); (j) *I. plastographus* (third spine pedunculate and subacute); (k) *I. subelongatus* (third spine pedunculate and subacute); (l) *I. cembrae* (third spine pedunculate and subacute). Scale bar: 1 mm.
Photos courtesy of (a to g) K. Bolte, Canadian Food Inspection Agency, Ottawa, Canada; (h to l) K. Savard, Agriculture and Agri-Food Canada, Ottawa, Canada.

Figure 13. (a)–(b) Elytral declivity of Ipini spp. showing relative distances between first spine and suture vs first and second spines: (a) *I. pini* (first spine closer to suture); (b) *Pseudips mexicanus* (first spine closer to second spine). Scale bar: 1 mm. (c)–(d) Elytral disc of Ipini spp. showing punctation of elytral intervals (between major strial rows of punctures): (c) *I. pini* (without punctures); (d) *Pseudips mexicanus* (punctate). Scale bar: 1.5 mm.
Photos courtesy of K. Bolte, Canadian Food Inspection Agency, Ottawa, Canada.

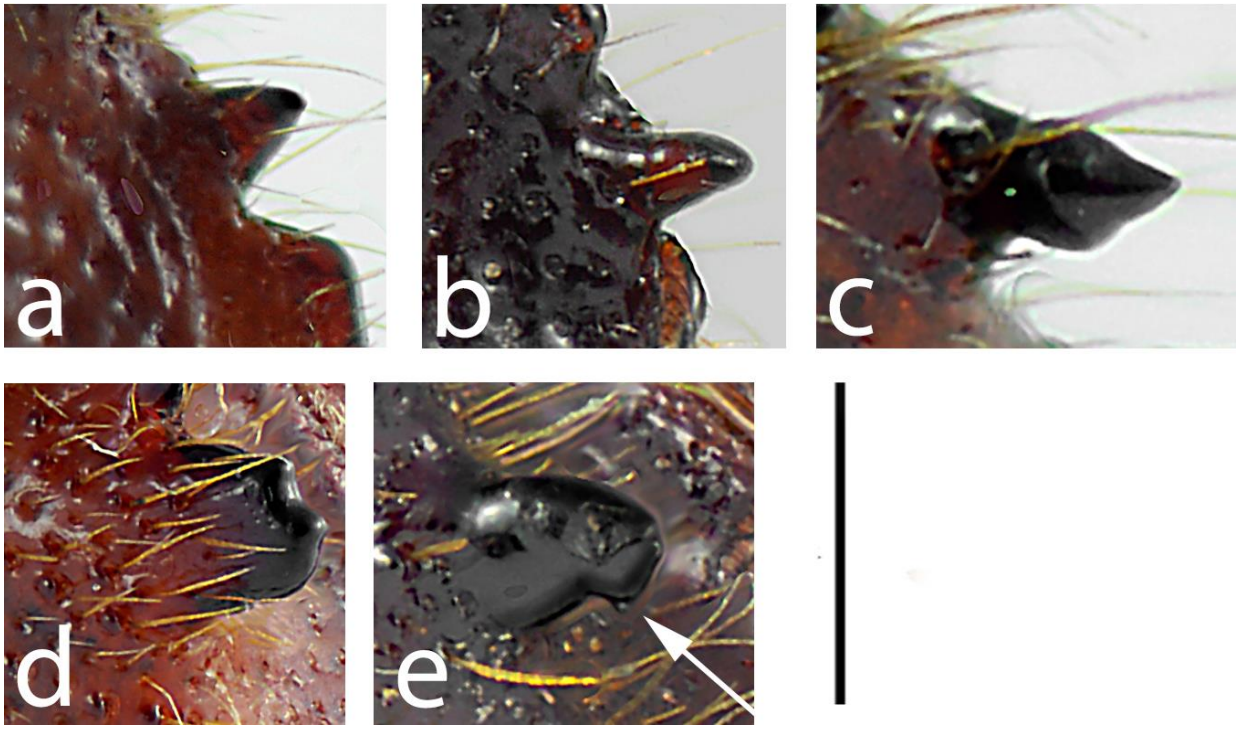

Figure 14. Shape of spines of elytral declivity of *Ips* spp.: (a) tapered; (b) straight-sided with tapered apex; (c) pedunculate (narrowed near base); (d) bidentate (two apices); (e) hooked (point on posterior edge shown with arrow). Scale bar: 0.5 mm.
Photos courtesy of K. Bolte, Canadian Food Inspection Agency, Ottawa, Canada.

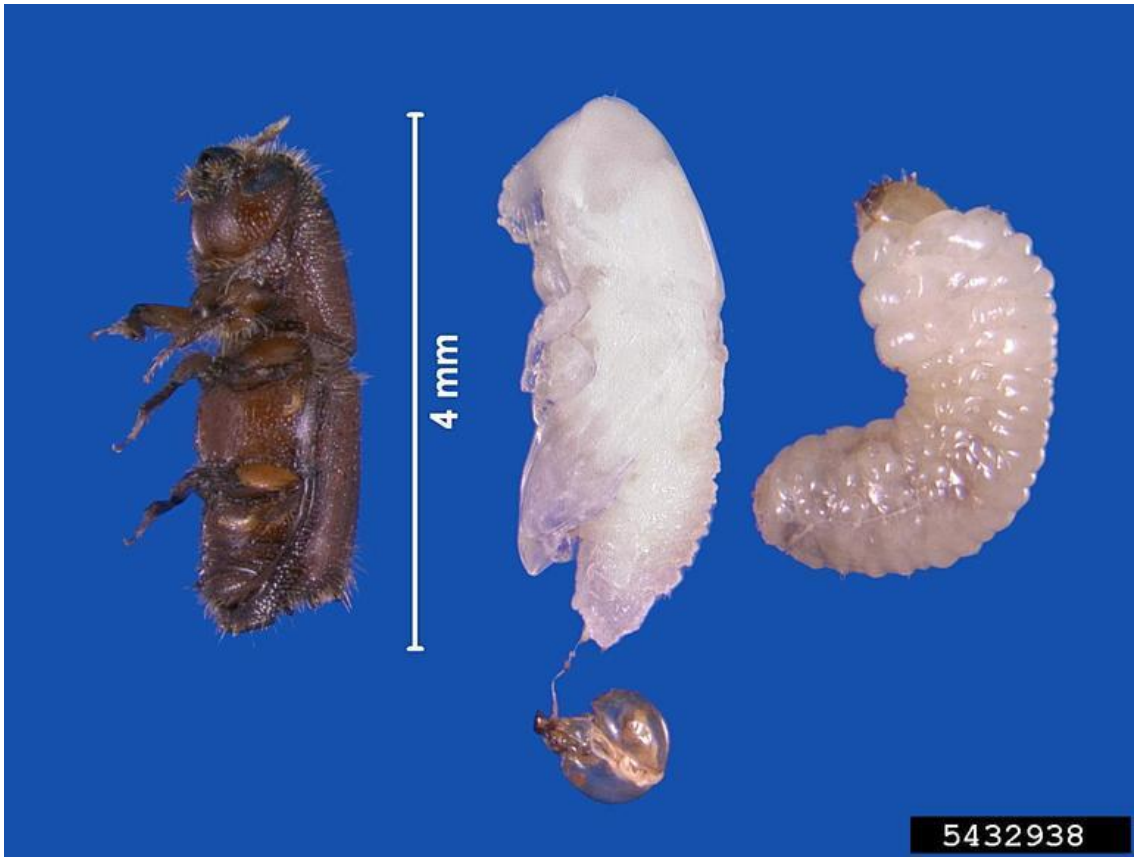

Figure 15. *Ips grandicollis*: from left to right, adult, pupa (with larval head capsule attached) and larva.
Source: Erich G. Vallery, USDA Forest Service - SRS-4552, Bugwood.org.

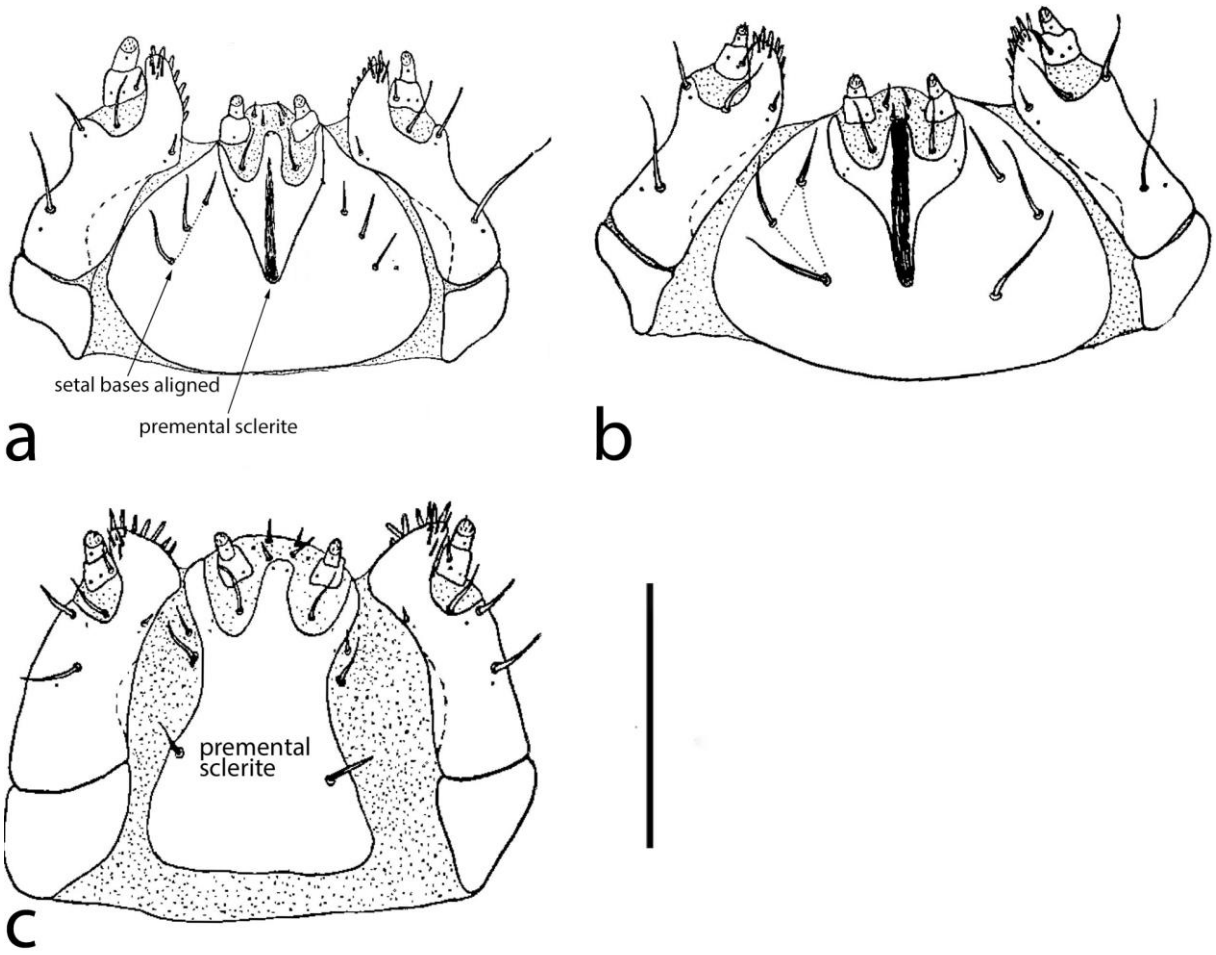

Figure 16. Scolytinae larvae, ventral view of mouthparts: (a) *Ips pini*, showing triangular premental sclerite and aligned postlabial setal bases; (b) *Polygraphus rufipennis* with postlabial setal bases arranged in triangle; (c) *Trypodendron lineatum* with premental sclerite rectangular. Scale bar: 0.5 mm.
Source: Thomas (1957).

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ISPM 27
Diagnostic protocols for regulated pests

DP 28: *Conotrachelus nenuphar*

Adopted 2018; published 2018

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1. Pest Information

The weevil *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae) is a native pest of fruits in eastern North America. Its main hosts include native and exotic rosaceous plant species (Chapman, 1938; CABI, 2018). It is an agricultural pest of orchard fruit – especially *Prunus* spp. (apricots, plums, peaches, nectarines, cherries), *Malus pumila* (apples), *Pyrus communis* (pears) and *Vaccinium corymbosum* (blueberries) (Quaintance and Jenne, 1912; Chapman, 1938). The adult beetles will feed on the fruits of many other kinds of rosaceous plants (including *Cydonia oblonga*, *Fragaria ×ananassa*, *Sorbus aucuparia*, *Amelanchier arborea* and *Crataegus* spp.), non-rosaceous plants (including *Diospyros kaki*, *Ribes* spp. and *Vitis* spp.) and, if given the opportunity, tropical fruits (Quaintance and Jenne, 1912; Chapman, 1938; Hallman and Gould, 2004). *C. nenuphar* discriminates among these potential food sources and prefers *Prunus* spp., *M. pumila* and *P. communis* (Jenkins *et al*., 2006; Leskey and Wright, 2007). Females will oviposit in these fruits, and larvae can successfully develop in any of them. Larvae have been known to develop in fungal black knot (*Plowrightia morbosa*) on *Prunus avium* (Quaintance and Jenne, 1912; Jenkins *et al.*, 2006).

The adults feed on the fruit and the larvae develop within the fruit of these plants. Crop damage arises as a result of oviposition sites on the fruit, and adult as well as larval feeding on fruits. The adult feeding punctures often deform the fruit and open the skin to further damage by other insect pests or to fungal attacks. The developing larvae consume the flesh of the fruit and cause the fruit to drop from the tree before ripening. Fully developed larvae leave the dropped fruit through exit holes and burrow into the soil to pupate. All forms of fruit damage are problems for fresh market fruits, and premature drop prevents the fruit from being used as a processed food item.

There are two phenological strains of *C. nenuphar* in its native range: a northern strain and a southern strain (Chapman, 1938; Zhang and Pfeiffer, 2008). The number of generations per year is a defining characteristic of the strains. The northern strain of *C. nenuphar* must diapause to become reproductively mature (obligate diapause) and has a single generation per year, with adults entering diapause in the late summer and early autumn before female reproductive features have developed. The *C. nenuphar* southern strain usually has only one generation per year but can develop reproductively and have a second, or even in rare cases, a third generation in a single season (facultative diapause) (Smith and Salkeld, 1964). For this reason, summer- and autumn-harvested fruit may have viable larvae in them in the southeastern United States of America, although this is rare. The two strains can successfully mate but unidirectional reproductive incompatibility between strains has been observed under laboratory conditions (Zhang and Pfeiffer, 2008). DNA analysis of *C. nenuphar* in the eastern United States of America supports the concept of genetic separation in the species between the northern and southern populations (Zhang *et al.*, 2008). However, the voltinism of several populations included in the Zhang *et al.* (2008) study was not known, precluding a comprehensive analysis of the genetic separation between strains. The *C. nenuphar* populations distinguished by the genetic data were not the same as the strain distributions reported in Chapman (1938).

2. Taxonomic Information

Name: *Conotrachelus nenuphar* (Herbst, 1797)

Synonyms: *Curculio nenuphar* Herbst, 1797

Rhynchaenus argula Fabricius, 1801

Rhynchaenus cerasi Peck, 1819

Taxonomic position: Insecta, Coleoptera, Curculionidae, Molytinae

Common names: Plum curculio, plum weevil

See Schoof (1942) for more taxonomic details.

3. Detection

Conotrachelus nenuphar can be detected in or near plants on which it feeds. It is commonly found on the following plant parts and in plant-associated media, depending on the life stage:

- eggs – found within immature fruit tissue and mature fruit tissue (if from the southern strain)
- larvae – found within immature fruit tissue and mature fruit tissue (Figure 1)
- pupae – found in the soil
- adults – found on leaves, branches, flowers and fruits.

The larvae are the life stage most likely to be transported in late-season fruit, especially from the southern extent of the *C. nenuphar* range. Pupae may be transported in soil along with tree seedlings or transplants. Adults may be transported in nursery material, rootstocks, branches, flowers and fresh packed fruit.

3.1 Symptoms

In immature fruit, a small crescent-shaped cut and scar are indicative of oviposition (Figure 2). To lay an egg, a female must puncture the skin of the developing fruit with her mouthparts and excavate a small, shallow cavity. A single egg is deposited in the centre of this cavity, after which the female cuts a crescent-shaped slit which extends beneath the egg cavity. A single female may lay multiple eggs on a single fruit. Adults also feed on fruits. Adult feeding punctures on immature fruits are circular (not crescent shaped) and extend up to 3 mm into the fruit.

In mature fruit, the oviposition scar becomes more diffuse and takes on a corky appearance. These can look like mottled fans with a small scar at the base of the fan (Figure 3). Adult feeding punctures on mature fruits also appear circular and tend to cluster around the calyx of the fruit.

3.2 Collection of insects from plants and plant products

Eggs and larvae. Eggs can be detected by observing fresh fruit for signs of oviposition and examining the plant tissue beneath the scar. Larvae can be recovered from fruit by splitting the fruit and looking for signs of larval feeding and larvae (Figure 1). Larvae will exit the fruit only after fruit drop, so any fruit still on the stem may yield live larvae.

Pupae. Pupae can be recovered from soil by sifting and examining the soil associated with plant products. Pupae tend to be found within 20 mm from the soil surface.

Adults. Adults (Figure 4) can be found by examining plant parts, including fruits, flowers, leaves, branches and trunks. Adults are well camouflaged and tend to appear as small pieces of bark. The weevil exhibits thanatosis (a state of paralysis or tonic immobility) when disturbed. Collection of insects in the field is achieved by placing a white sheet underneath the plant, then jarring or disturbing the branches and small tree trunks on which the adults are found, and examining the sheet for immobile adults which fold their legs under their body, bend their rostrum under the prothorax and appear as an oblong shape.

4. Identification

Identification of *C. nenuphar* by morphological examination is limited to adult specimens because there are no adequate keys for the identification of eggs, larvae or pupae. A guide to identification of adult *C. nenuphar* is given below.

Larval and pupal life stages are particularly at risk of being misidentified, because of the lack of reliable identification diagnostics or keys for them. Molecular methods of diagnosis for this species are still in development (Lin *et al.*, 2008; Crane, 2011) and are not included in this protocol. Methods for molecular identification of the two phenological strains are not included in this protocol because additional studies are needed to verify that they are accurate (Zhang *et al.*, 2008).

4.1 Preparation of adult beetles for microscopic examination

Adult weevils in the genus *Conotrachelus* are generally less than 9 mm in body length and should be examined for morphological identification under magnification of at least 20×. Most diagnostic characters can be observed at this level of magnification. For routine identification, dissection of genitalia is not usually necessary.

The typical size for adult *C. nenuphar* specimens is between 4 mm and 6 mm body length, allowing for pin mounting directly through the right elytron. There are several important diagnostic characters on the legs, so spreading the legs, or moving them to the side and down, while mounting is recommended to facilitate identification.

4.2 External morphological characters used to identify adult weevils in the family Curculionidae

The weevil family, Curculionidae, is very large, with more than 50 000 described species (Anderson, 2002; Oberprieler *et al*., 2007). Keys to identify this family are available in general entomology references and not provided in the current protocol. The best external morphological characters for the recognition of the weevils are associated with their rostrum (snout or beak) (Figure 5), although some weevils have a very short or truncate rostrum (as seen in the subfamily Enteminae) and some have no rostrum at all (especially in the Scolytinae and Platypodinae). The length of the rostrum, its curvature, or lack of curvature, the degree of punctation or sculpturing, and the type and density of vestiture are all used in classification. Another set of diagnostic characters are those of the antennae (Figure 5). The first article (the scape) is elongate and inserted away from the base, usually near the middle and at times near the apex. It can be directed in many ways (e.g. dorsally, ventrally), has various lengths and shapes, and often rests in a lateral groove (scrobe). The number of funicular articles varies from four to eight articles, and the last three antennal articles normally form a compact club (Figure 5).

4.3 Morphological identification of adults of the genus *Conotrachelus*

Conotrachelus Dejean 1835 is a New World beetle genus with approximately 1 200 named species (O'Brien and Wibmer, 1982; Wibmer and O'Brien, 1986). The highest species diversity is concentrated in South America, where there are many endemic species. The *Conotrachelus* diversity found in the United States of America and Canada (where *Conotrachelus nenuphar* is endemic) is limited to approximately 63 of 1 200 described species. Identification of the genus *Conotrachelus* is possible using the adult characters provided in Table 1. For additional information, a key to North American genera in the tribe Conotrachelini, including *Conotrachelus*, is available (Anderson, 2002).

Table 1. Diagnostic characters of the genus *Conotrachelus*

Body Part	Characteristic
Antennae	• Funicle seven-segmented (Figure 5)
Rostrum	• In repose, received into ventral groove on prosternum (Figure 6)
Prothorax	• With postocular lobe at anterior lateral margin (Figure 5) • Prosternum with groove for reception of the rostrum, the groove not extending beyond the apical portion of the prosternum (Figure 6)
Elytra	• All or alternate intervals carinate or at least swollen throughout most of their length (Figure 7(A))
Legs	• Tibia of hind leg with metaunci (Figure 7(B)) • Procoxae contiguous or approximate • Femora with one or two distinct teeth on ventral surface (Figure 7(B)) • Tarsal claw with basal tooth, claws not contiguous • Tarsal formula a modified 5-5-5, with the third tarsomere broadly bilobed and fourth tarsomere small and partly hidden in base of third tarsomere, formula often appearing to be 4-4-4

4.4 Morphological identification of adult *Conotrachelus nenuphar*

Of the *Conotrachelus* species found in the United States of America and Canada, 46 species are broadly sympatric with *C. nenuphar*, being found in the eastern portion of North America, here defined as north of Mexico and east of the Rocky Mountains. Of those *Conotrachelus* species found in the same geographical regions as *C. nenuphar*, only three are known to use commercial fruit trees as hosts (Schoof, 1942). Two of these are *C. anaglypticus* (Say) and *C. carolinensis* Schoof, which are closely related, and the third is *C. crataegi* Walsh.

Critically important in the identification of many *Conotrachelus* species is the postmedian elytral band (Figure 7(A)). This is a region just behind the middle of the elytra, and there are diagnostic differences between species. Among species related to *C. nenuphar*, the most important diagnostic characters are the presence or absence of carinate elytral intervals and elytral crests, and the type of vestiture and its pattern. The minimum requirements to reliably identify *C. nenuphar* and separate it from *C. anaglypticus*, *C. carolinensis* and *C. crataegi* are contained in Table 2. For reliable identification, a *C. nenuphar* adult specimen must have all the characteristics described; the identification is strengthened further if the specimen has been collected from one of the known host fruits of the species. In addition, it is important to consider sexually dimorphic differences within the species: male *C. nenuphar* have broad dentate metaunci (uncus of the hind leg), whereas females have narrow, non-dentate metaunci (Figure 10).

Four species related to *C. nenuphar* and from the same region from North America are also discussed and illustrated in this protocol, although they are not associated with stone fruits (Table 3). These are *Conotrachelus juglandis* Leconte associated with species of *Juglans*, *Conotrachelus corni* Brown on *Cornus stolonifera* Michx., *Conotrachelus buchanani* Schoof on *Celtis occidentalis*, and *Conotrachelus iowensis* Schoof , which to date has no known host. Of these species, *C. corni* is the most similar in appearance to *C. nenuphar* but is much smaller in size, with body length 2.9–3.9 mm. The prothorax of *C. corni*, at most, has a scarcely evident anterior median carina, and the scutellum is not sloping and is prominent on all sides; compare characters in Table 2 and Table 3 for other differences (Brown, 1966).

Table 2. Diagnostic characters for *C. nenuphar* and three *Conotrachelus* species that are found in the same geographical regions as *C. nenuphar* and use stone fruit trees as hosts: *C. anaglypticus*, *C. carolinensis* and *C. crataegi*

Character	*Conotrachelus* species			
	C. nenuphar (Figures 8–10)	***C. anaglypticus*** (Figure 11)	***C. carolinensis*** (Figure 12)	***C. crataegi*** (Figure 13)
Prothorax	No median ridge or furrow. With four submedian tubercles (Figure 8(A)).	With weak median furrow bordered rarely with distinct carinae. Lacking submedian tubercles (Figure 11(A)).	With strong median furrow bordered by distinct carinae (Figure 12 (A)), with posterior margin of carina sometimes tuberculate. Lacking submedian tubercles.	With median carinate crest extending from near the apex to beyond middle of prothorax. Lacking submedian tubercles (Figures 13(A) and (B)).
Thorax	Scutellum gently sloping, depressed and flat on basal margin and not prominent along both side margins (Figure 8(A)).	Scutellum prominent on all margins and not sloping (Figure 11(A)).	Scutellum prominent on all margins and not sloping (Figure 12 (A)).	Scutellum prominent on all margins and not sloping (Figure 13(A)).
Elytra	Humeri obliquely rounded, prominent (Figure 8(A)). Two distinct costae (or crests), one on each elytron on interval 3 (Figures 8(B) and 9(A)). Region between and around costae and costae themselves devoid of vestiture; smooth and black (Figures 9(A) and (B)). Postmedian band distinctly reddish brown to reddish yellow, vestiture with distinct lines of white recumbent setae (Figures 9(A) and (B)).	Humeri not denticulate (Figure 11(B)). Lacking distinct costae (or crests), odd-numbered intervals carinate, none interrupted. With distinct oblique posthumeral bar of yellowish vestiture (Figure 11(A)). With usually narrow postmedian band of white and tan setae, at times split into two or three separate bands (Figure 11(A)).	Humeri not denticulate. Lacking distinct costae (or crests), odd-numbered intervals carinate, none interrupted. Lacking oblique posthumeral bar of yellowish vestiture. With distinct narrow postmedian band of evenly distributed dense recumbent white and fewer brown scale-like setae (Figure 12(A)).	Humeri projecting, usually strongly denticulate (Figure 13(A)). Lacking distinct costae (or crests), odd-numbered intervals carinate, 3 and 5 interrupted but not crested. Vestiture with evenly distributed, dense recumbent white and brown scale-like setae. Lacking distinct postmedian band (Figure 13(A)).
Venter	Abdominal sterna 1 and 2 very strongly densely punctate, with many punctures more oblong than circular. Punctures each with fine reddish-brown setae. Lateral setae broader and reddish yellow (Figure 9(C)).	Abdominal sterna 3 and 4 coarsely, moderately densely punctate. Vestiture of lateral sterna composed of broader setae and forming small patches (Figure 11(B)).	Abdominal sterna deeply coarsely punctate. Sterna 1 and 5 densely punctate, others vary from sparse to dense. Vestiture of broader lateral setae on sterna 3 and 4 and on apical portion of sternum 1 (Figure 12(B)).	Abdominal sterna 3 and 4 weakly sparsely punctate, often smooth. Vestiture moderately dense to dense on all sterna (Figure 13(B)).

(Table 2 continued on next page)

(Table 2 continued)

Character	*Conotrachelus* species			
Legs	Femora with two teeth, proximal tooth larger (Figure 7(B)). Metaunci of male broad and dentate, in female narrow and non-dentate (Figure 10). Tarsal claws divergent, not close together, with prominent teeth.	Femur with tooth and denticle. Tarsal claws divergent, not close together, with prominent teeth.	Femur with tooth and denticle. Tarsal claws divergent, not close together, with prominent teeth.	Femora with one tooth. Tarsal claws divergent, not close together, with prominent teeth.
Host	Stone fruits (*Prunus* spp.), etc. (see section 1 of this protocol).	Cambium and inner bark of *Prunus persica* (peach), *Malus* spp. (apple) and various other fruit and shade trees including *Acer rubrum* (red maple), *Amelanchier* spp. (serviceberry), *Betula lenta* (sweet birch), *Carpinus caroliana* (American hornbeam), *Castanea dentata* (American chestnut), *Conopodium majus* (pignut), *Cornus florida* (flowering dogwood), *Fagus grandifolia* (American beech), *Liriodendron* spp. (tulip tree), *Nyssa sylvatica* (tupelo), *Oxydendrum* spp. (sourwood), *Pyrus* spp. (pear), *Quercus alba* (white oak), *Quercus prinus* (chestnut oak) and *Quercus rubra* (red oak), and breed in stems of *Aquilegia* spp. (columbine).	*Prunus persica*.	*Crataegus* spp. (hawthorns), *Prunus persica*.

Table 3. Diagnostic characters for four species of *Conotrachelus* not associated with stone fruit: *C. juglandis*, *C. corni*, *C. buchanani* and *C. iowensis*

Character	***Conotrachelus* species**			
	C. juglandis (Figure 14)	***C. corni*** (Figure 15)	***C. buchanani*** (Figure 16)	***C. iowensis*** (Figure 17)
Prothorax	No median ridge or furrow. With four submedian tubercles (Figure 14(A)).	No median ridge or furrow. With faint indication of median carina anteriorly and median pair of tubercles (Figure 15(A)).	No median furrow. Often with four distinct submedian tubercles (Figure 16(A)). Often with a feeble median longitudinal carina extending apically from between median tubercles.	No median ridge or furrow. Often with six submedian tubercles, middle pair always evident (Figure 17(A)), other pairs at times obsolete or at times with one tubercle of the pair feeble and obscure. At most with feeble median longitudinal carina extending apically from between median tubercles.
Thorax	Scutellum prominent on all margins and not sloping (Figure 14(A)).	Scutellum prominent on all margins and not sloping (Figure 15(A)).	Scutellum gently sloping, depressed and flat on basal margin and not prominent along both side margins (Figure 16(A)).	Scutellum prominent on all margins and not sloping (Figure 17(A)).
Elytra	Two distinct costae (or crests), one on each elytron on interval 3 (Figure 14(B)). Region between and around costae and costae themselves not devoid of vestiture; black with sparse brown and white setae. Postmedian band broad, usually white, occasionally with mix of tan setae (Figure 14(A)).	Two distinct costae (or crests), one on each elytron on interval 3. Region between and around costae and costae themselves devoid of vestiture; smooth and black (Figures 15(A) and (B)). Postmedian band distinctly reddish brown and patch of white vestiture, lacking distinct lines of white recumbent setae (Figure 15(A)).	Two moderately distinct costae (or crests), one on each elytron on interval 3 (Figures 16(A) and (B)). Region between and around costae and costae themselves with uniform vestiture of relatively dense brown and white setae. Postmedian band broad, of imbricate white setae (Figure 16(A)).	Two distinct costae (or crests), one on each elytron on interval 3. Region between and around costae with at most sparse vestiture and costae themselves devoid of vestiture; smooth and black (Figures 17(A) and (B)). Postmedian band distinctly reddish brown with white recumbent setae, latter concentrated behind and between crests on interval 3 (Figure 17(B)).
Venter	Abdominal sternum 1 usually finely punctate, at most with course punctures along anterior margin. Sternum 2 sparsely and finely punctate. Punctures each with fine reddish-brown setae, lateral setae broader and light brownish yellow and white, forming patches on sterna 2, 3, 4 and 5 (Figure 14(B)).	Abdominal sterna moderately coarsely and moderately sparsely punctate. Vestiture of sterna fine; white, widely scattered setae (Figure 15(B)).	Abdominal sternum 1 coarsely rugulose and densely punctate, and sterna 2 to 5 moderately to densely punctate, each puncture with fine amber to reddish-brown setae, with lateral setae broader and white and yellow, forming small patches on sterna 2 and 5 (Figure 16(B)).	Abdominal sterna 1 to 4 coarsely and densely punctate, with punctures round. Punctures each with fine pale amber setae; lateral setae usually white and broader, sometimes reddish yellow (Figure 17(B)).

(Table 3 continued on next page)

(Table 3 continued)

Character	***Conotrachelus* species**			
Legs	Femora with two teeth, proximal tooth larger. Metaunci of male narrow and dentate. Tarsal claws divergent, not close together, with prominent teeth.	Femora with two teeth. Tarsal claws divergent, not close together, with prominent teeth.	Femora with two teeth, proximal tooth broader at base. Metaunci of male narrow and not dentate. Tarsal claws divergent, not close together, with prominent teeth.	Femora with two teeth, small or with proximal tooth larger. Metaunci of male and female narrow and not dentate. Tarsal claws divergent, not close together, with prominent teeth.
Host	Numerous species of *Juglans* spp., with breeding in nuts and attacking cambium of young trees.	*Cornus stolonifera*.	*Celtis occidentalis*.	Collected frequently in *Quercus* (oak) woodlands, but no breeding host is known.

5. Records

Records and evidence should be retained as described in section 2.5 of ISPM 27 (*Diagnostic protocols for regulated pests*).

In cases where other contracting parties may be affected by the results of the diagnosis, in particular in cases of non-compliance (ISPM 13 (*Guidelines for the notification of non-compliance and emergency action*)) and where *Conotrachelus nenuphar* is found in an area for the first time, the following records and evidence and additional material should be kept for at least one year in a manner that ensures traceability: preserved pinned or slide-mounted specimens, and photographs of distinctive taxonomic structures.

6. Contact Points for Further Information

Further information on this protocol can be obtained from:

Caribbean Agricultural Health and Food Safety Agency, Suriname (Juliet Goldsmith; email: Juliet.goldsmith@cahfsa.org).

University of Arizona, Tucson, AZ, United States of America (Charles W. O'Brien; email: cobrien6@cox.net).

United States Department of Agriculture, Systematic Entomology Laboratory, MRC 168, National Museum of Natural History, Washington, DC 200137012, United States of America (Lourdes Chamorro; email: lourdes.chamorro@ars.usda.gov).

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), which will in turn forward it to the Technical Panel on Diagnostic Protocols (TPDP).

7. Acknowledgements

This protocol was drafted by Charles W. O'Brien (University of Arizona, United States of America (see preceding section)) and Juliet Goldsmith (Caribbean Agricultural Health and Food Safety Agency, Suriname (see preceding section)) from a preliminary draft by Samuel Crane (Amplify, New York, United States of America), all with assistance from Norman Barr (Animal and Plant Health Inspection Service, United States Department of Agriculture, United States of America).

In addition, the following experts were significantly involved in the development of this protocol: Nico Franz (Arizona State University, United States of America), Hiroaki Shirato (Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan) and Raphaëlle Mouttet (Plant Health Laboratory, ANSES-LSV, France).

8. References

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9. Figures

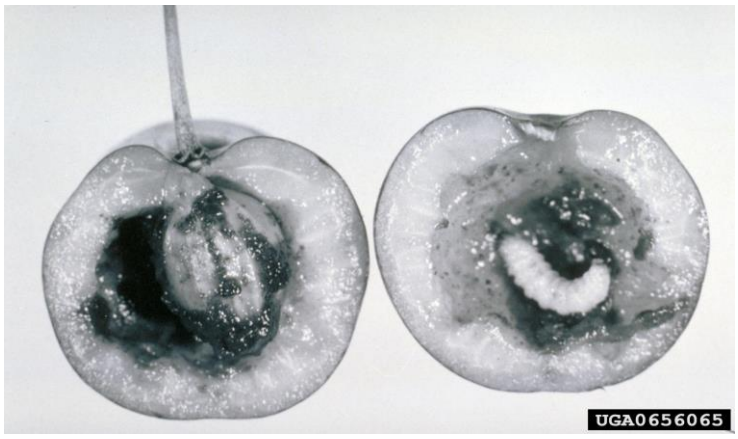

Figure 1. *Conotrachelus nenuphar* larva in cherry fruit.
Photo courtesy of P.J. Chapman, New York State Agricultural Experiment Station, Bugwood.org.

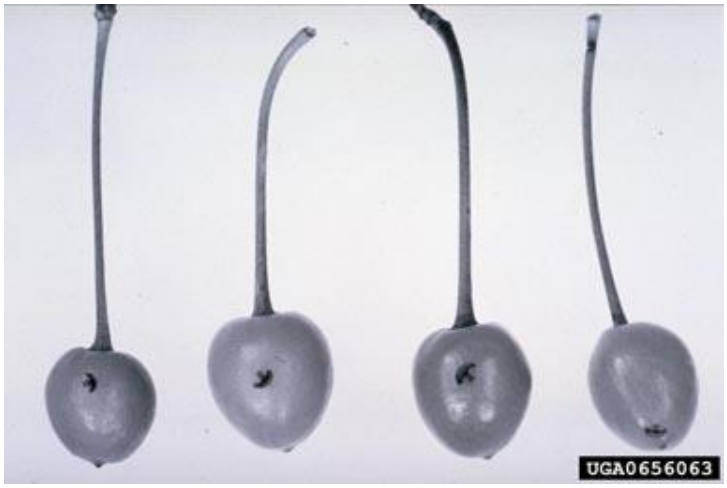

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Photo courtesy of P.J. Chapman, New York State Agricultural Experiment Station, Bugwood.org.

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Photo courtesy of P.J. Chapman New York State Agriculture Experiment Station, Bugwood.org.

Figure 4. *Conotrachelus nenuphar* adult on fruit.
Photo courtesy of E. Levine, The Ohio State University, Bugwood.org.

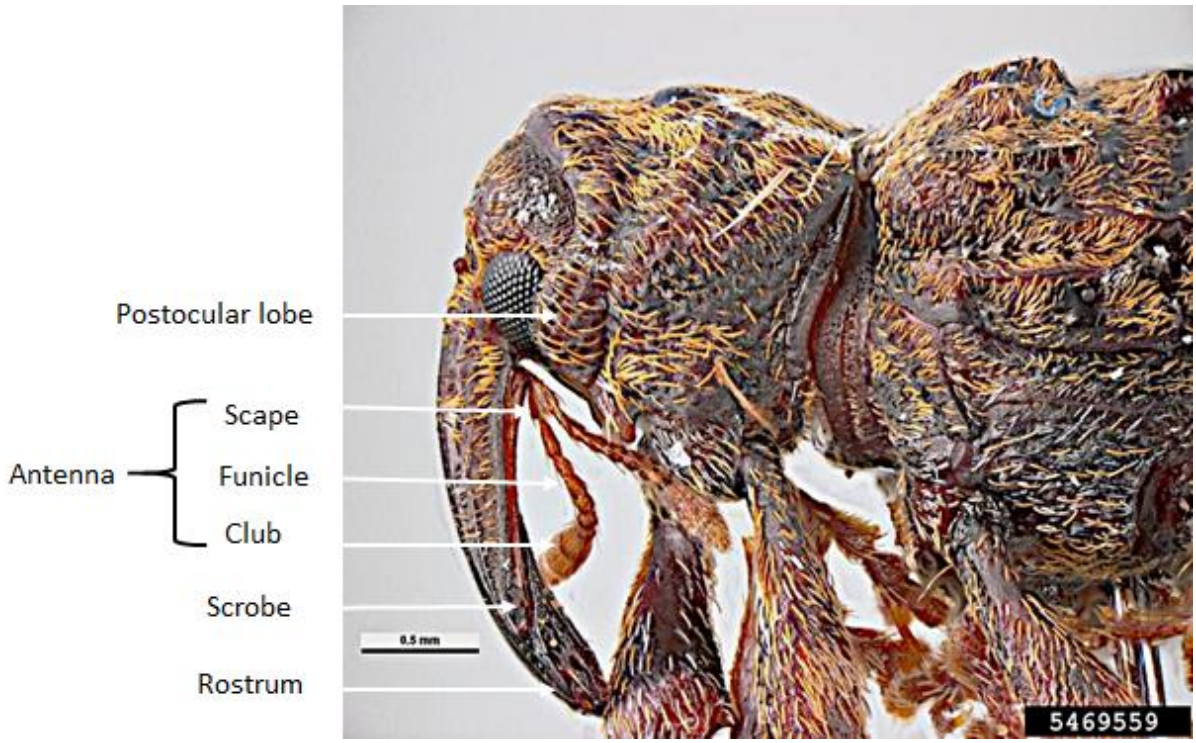

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Photo Pest and Diseases Image Library, Bugwood.org.

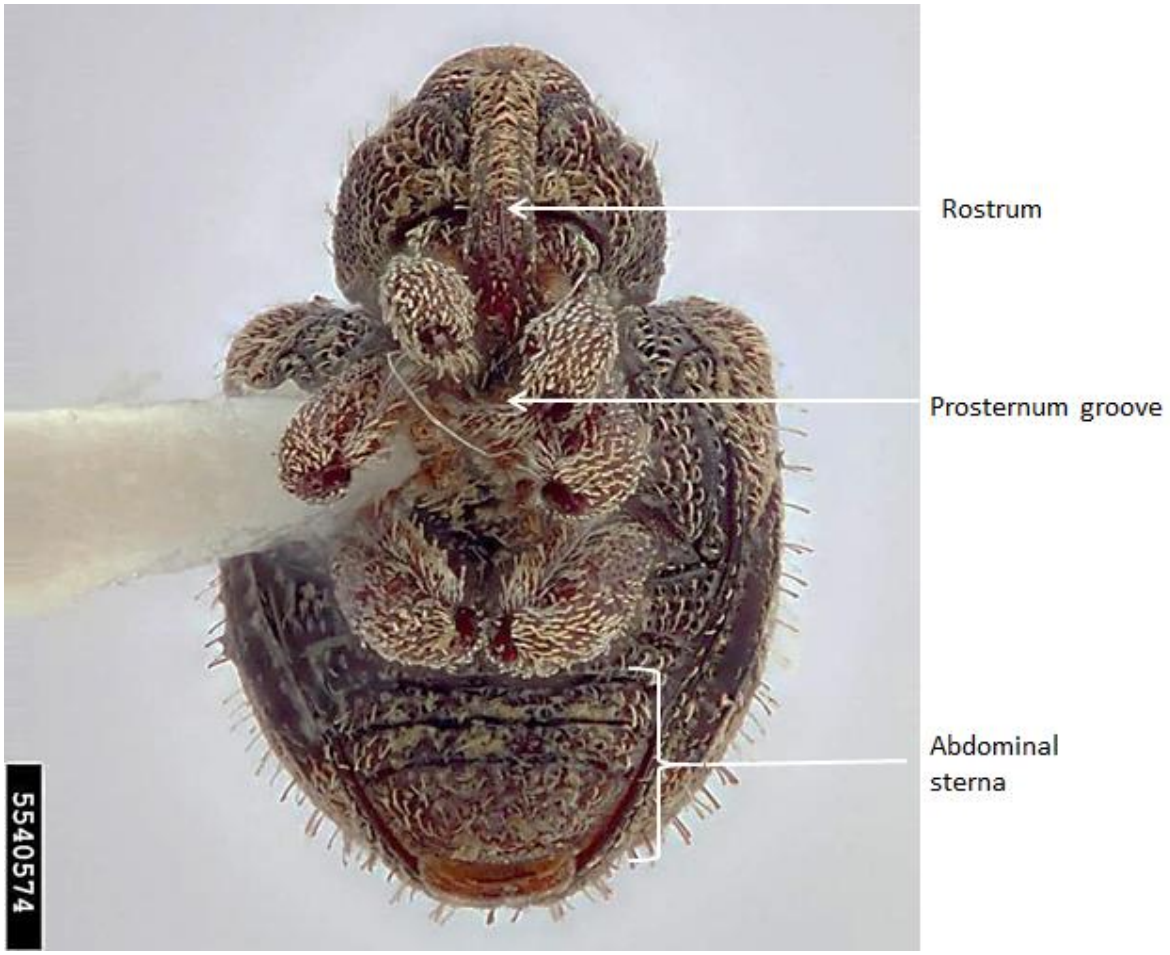

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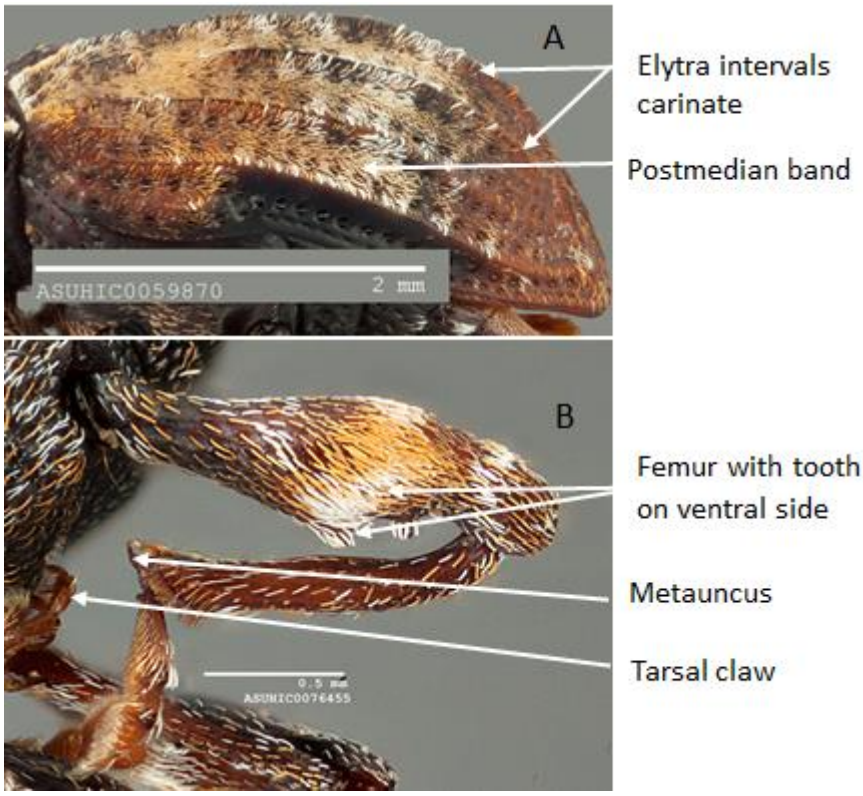
Photo courtesy of Hanna Royals, Museum Collections: Coleoptera, USDA APHIS ITP, Bugwood.org.



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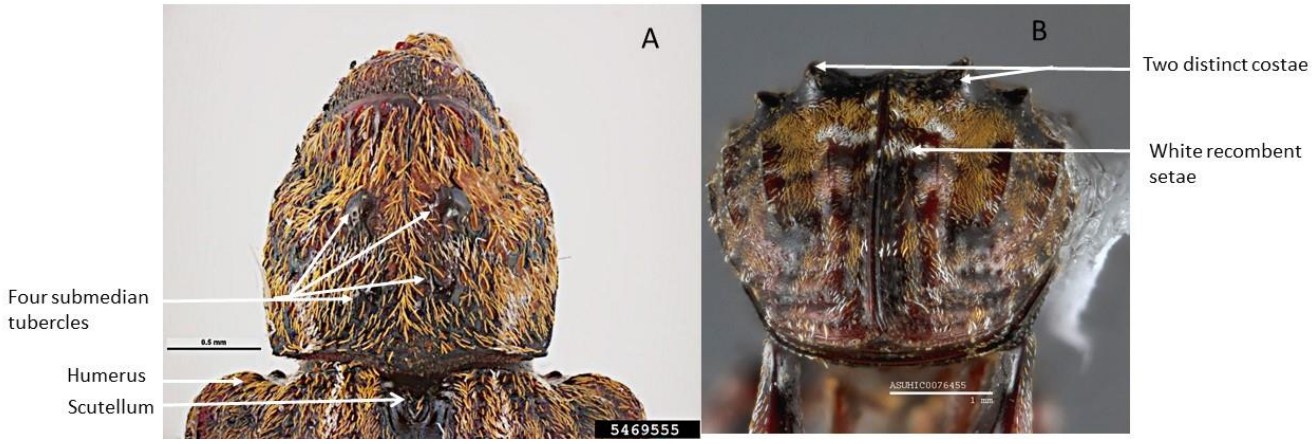
Photos courtesy of Nico Franz, Arizona State University Hasbrouck Insect Collection, AZ, United States of America.



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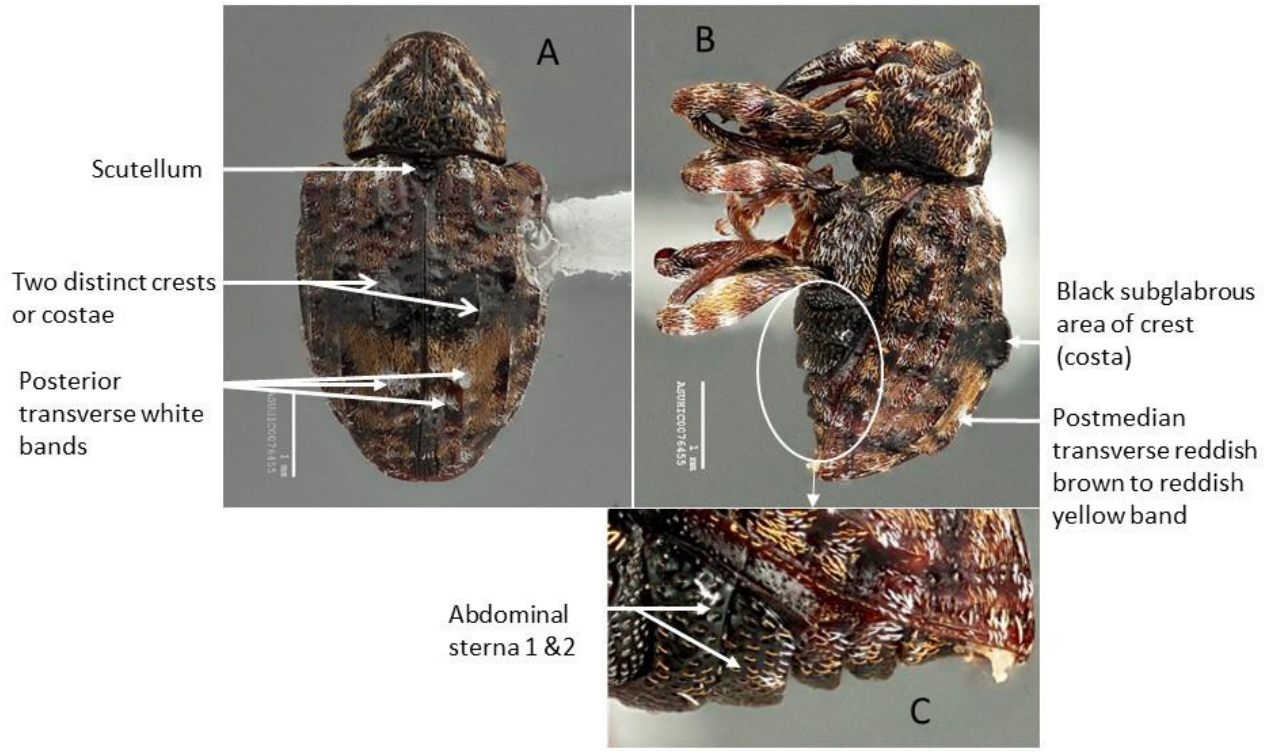
Photos (A) Pest and Diseases Image Library, Bugwood.org; (B) courtesy of Nico Franz, Arizona State University Hasbrouck Insect Collection, AZ, United States of America.



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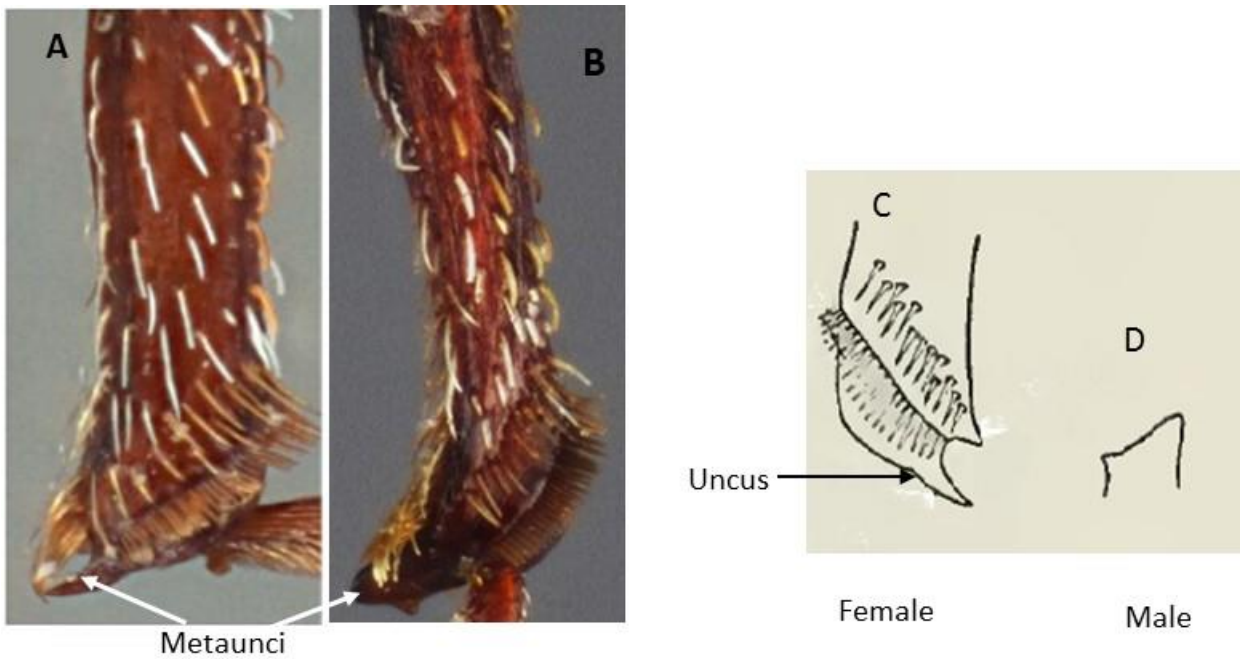
Photos courtesy of Nico Franz, Arizona State University Hasbrouck Insect Collection, AZ, United States of America.



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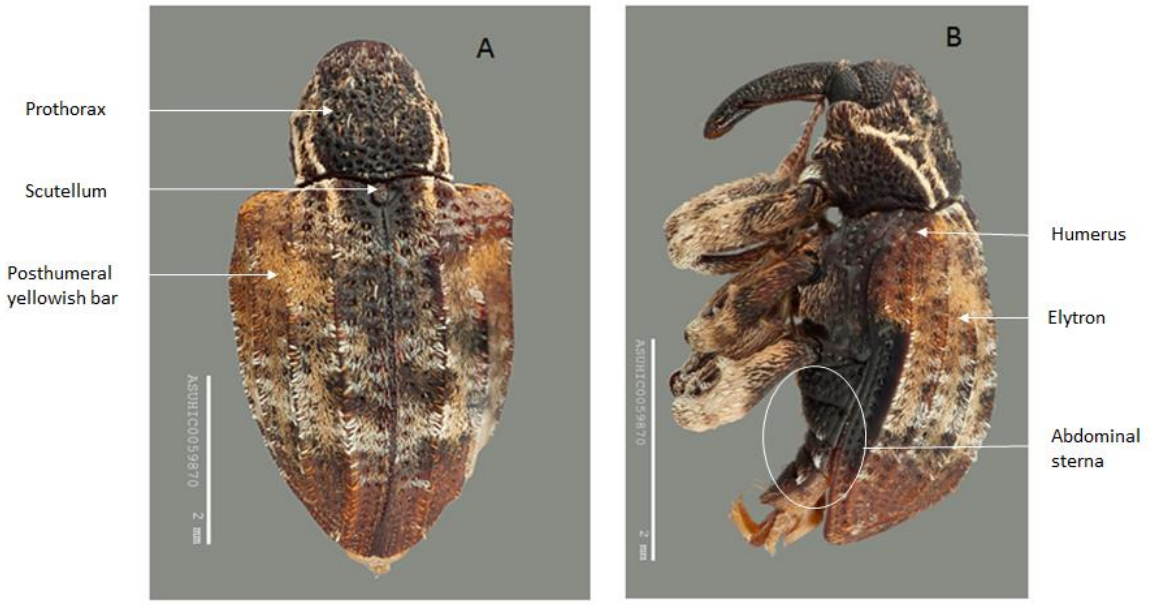
Photos (A) and (B) courtesy of Nico Franz, Arizona State University Hasbrouck Insect Collection, AZ, United States of America; (C) and (D) from Schoof (1942).



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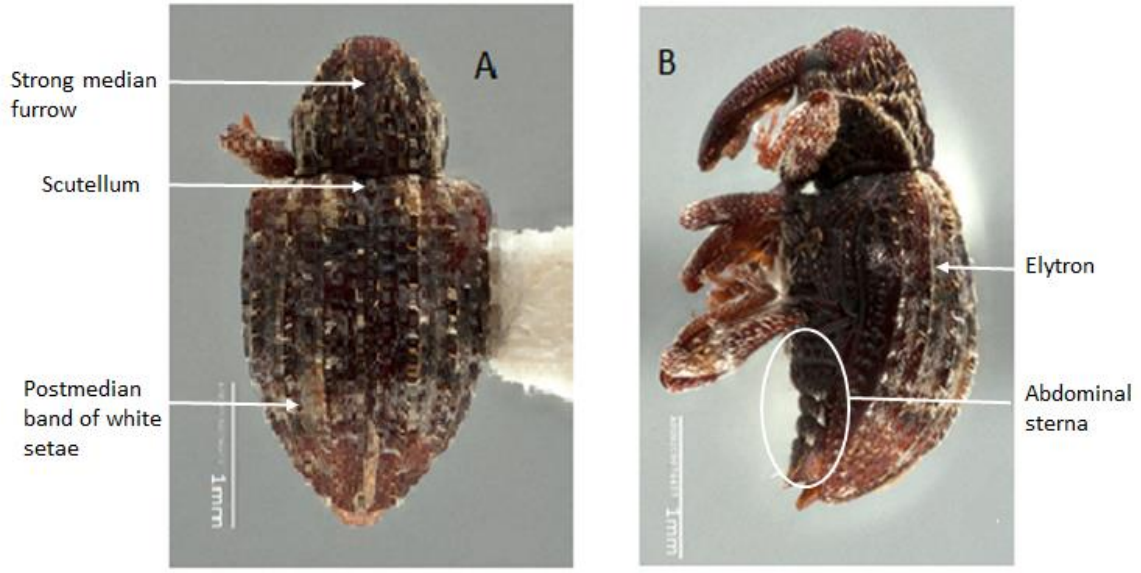
Photos courtesy of Nico Franz, Arizona State University Hasbrouck Insect Collection, AZ, United States of America.



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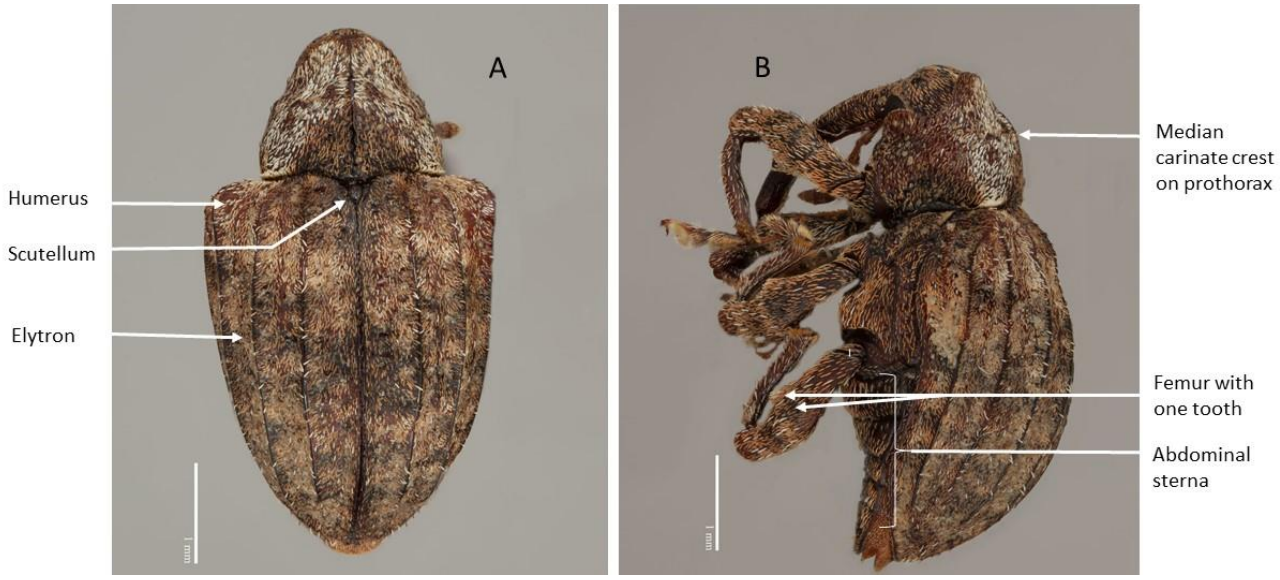
Photos courtesy of Nico Franz, Arizona State University Hasbrouck Insect Collection, AZ, United States of America.



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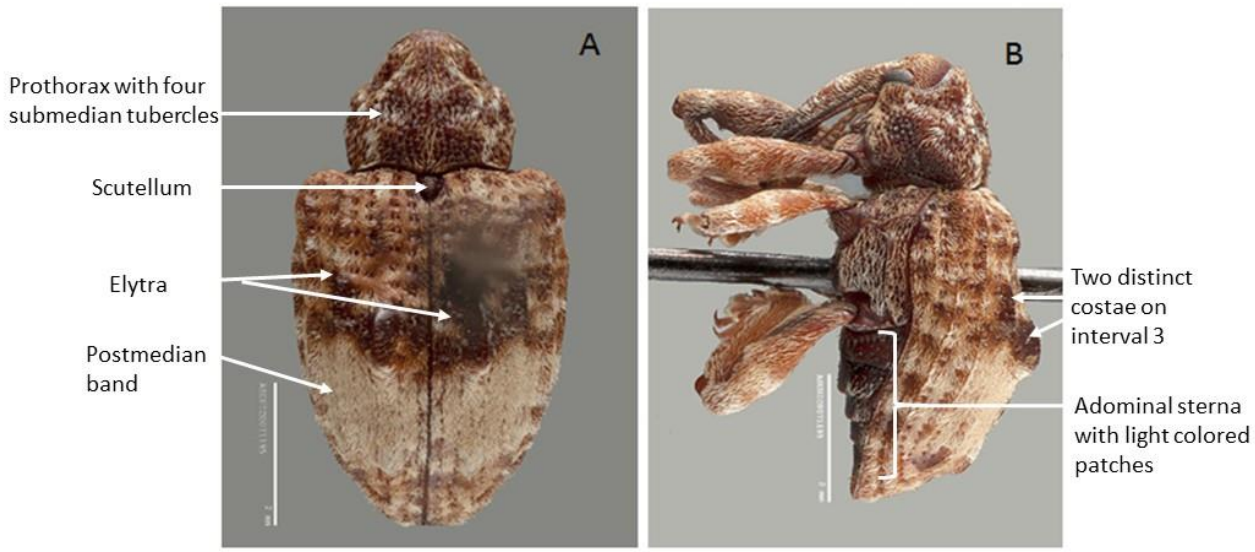
Photos courtesy of Nico Franz, Arizona State University Hasbrouck Insect Collection, AZ, United States of America.



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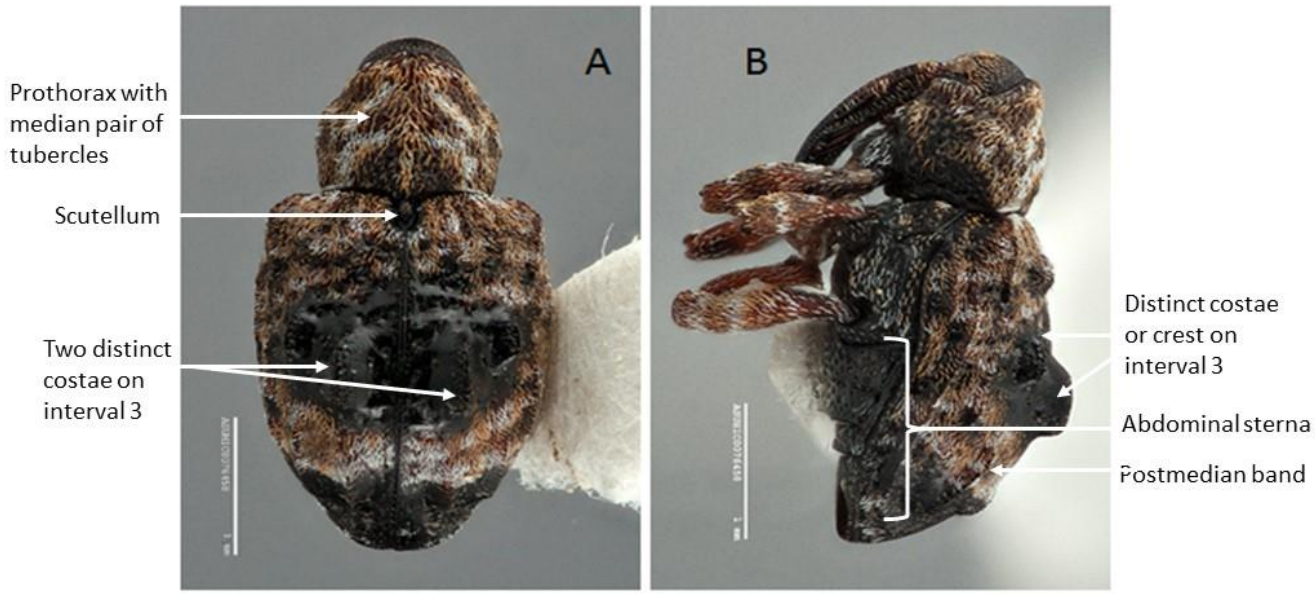
Photos courtesy of Nico Franz, Arizona State University Hasbrouck Insect Collection, AZ, United States of America.



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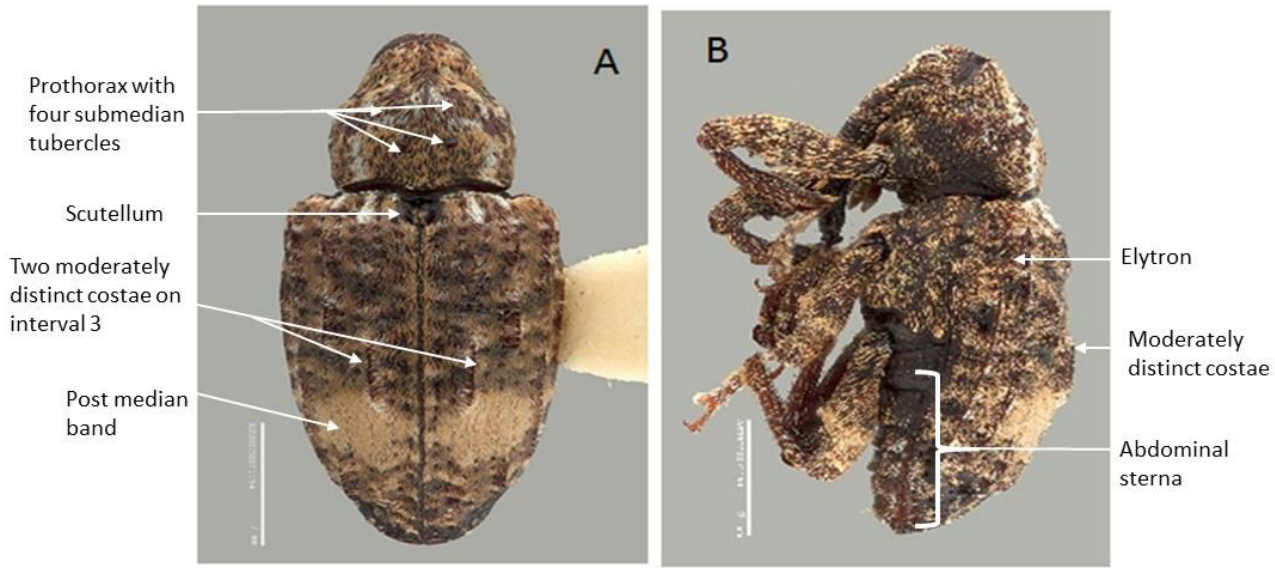
Photos courtesy of Nico Franz, Arizona State University Hasbrouck Insect Collection, AZ, United States of America.



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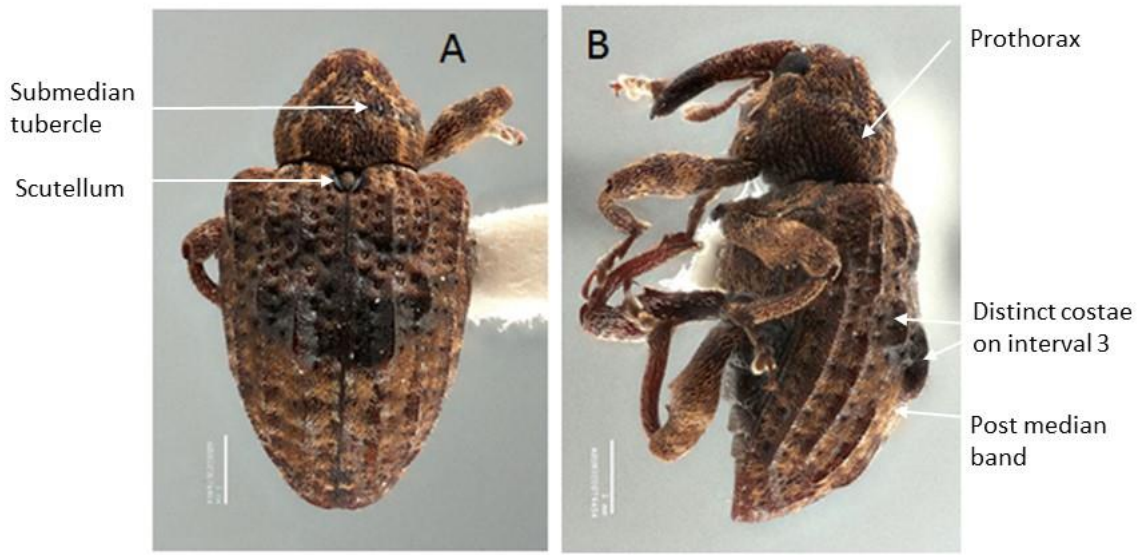
Photos courtesy of Nico Franz, Arizona State University Hasbrouck Insect Collection, AZ, United States of America.



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Photos courtesy of Nico Franz, Arizona State University Hasbrouck Insect Collection, AZ, United States of America.

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This is not an official part of the standard

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2018-02 TPDP approved draft to submit to SC for approval for adoption.

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ISPM 27. Annex 28. *Conotrachelus nenuphar* (2018). Rome, IPPC, FAO.

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The annex is a prescriptive part of ISPM 27.

ISPM 27
Diagnostic protocols for regulated pests

DP 29: *Bactrocera dorsalis*

Adopted 2019; published 2019

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1. Pest Information

Fruit flies of the family Tephritidae represent an economically important insect group with a worldwide distribution. The biology of these fruit flies is dependent on host plants that can serve as mating locations, oviposition sites for eggs, and nutrient resources for developing larvae. The genus *Bactrocera* Macquart consists of over 460 described species that are distributed mostly in regions of Asia and Australasia and subtropical islands of the southern Pacific Ocean (Drew and Romig, 2013; Doorenweerd *et al*., 2018). A few *Bactrocera* species are native to Africa and several pest species were introduced to that continent. Within the genus is a group of flies named the *Bactrocera dorsalis* complex (Drew and Hancock, 1994; Drew, 2004; Clark *et al*., 2005). This complex comprises 85 described species (Vargas *et al.*, 2015) that share a very similar appearance, but the complex as a whole does not represent a monophyletic lineage and is merely a group of convenience (Leblanc *et al.*, 2015). The complex is named after one of its member species, *Bactrocera dorsalis* (Figure 1), which is a polyphagous pest of commercial fruits. Several other species in the complex are also recognized as pests, based on plant host use and pest records (White and Elson-Harris, 1992; Clarke *et al*., 2005; Vargas *et al.*, 2015; Plant Health Australia, 2016).

The scope of the current protocol is to diagnose adult *B. dorsalis* fruit flies. Five economically important species of the *Bactrocera dorsalis* complex that are found in commercial fruits and vegetables associated with international trade and that can be confused with *B. dorsalis* during an identification are also included in the protocol. These five species are: *B. carambolae*, *B. caryeae*, *B. kandiensis*, *B. occipitalis* and *B. pyrifoliae*. Distributions of these species are mapped with their pest status and invasion history by Vargas *et al.* (2015).

A lack of characters that can be used reliably to distinguish *B. dorsalis* from two other species (i.e. *B. papayae* Drew and Hancock, 1994 and *B. invadens* Drew *et al*., 2005) has resulted in debate regarding the valid taxonomy of the species (Clarke *et al.*, 2005; Chen and Hui, 2007; Schutze *et al.*, 2015a, b; Drew & Romig, 2016; Schutze *et al.*, 2017). These three species have been treated as members of a sibling species complex, not to be confused with the *Bactrocera dorsalis* complex (Clarke and Schutze, 2014). It is not possible to reliably distinguish among these three species because an accurate identification requires both evaluation of species distribution information and analysis of morphological characters that are not discrete for the species. Species distribution information may not be reliable when examining specimens collected outside the species' known range. Published molecular data cannot distinguish these species (Schutze *et al.*, 2015a). In a review of available evidence, Schutze *et al*. (2015a) concluded that these three species are in fact a single biological species called *Bactrocera dorsalis*. Drew and Romig (2016) disagree with that revision and reversed the synonymy; however, Schutze *et al.* (2017) published a rebuttal to Drew and Romig (2016) that supports the synonymy by Schutze *et al.* (2015a). In this protocol, the three species are collectively treated as *B. dorsalis sensu lato*.

Evidence has been reported of hybridization among some of these six *Bactrocera* species under laboratory conditions (McInnis *et al*., 1999; Ebina and Ohto, 2006; Schutze *et al*., 2013) and of morphological intermediates in the wild (Delomen *et al*., 2013; Jalani *et al*. 2014). The frequency of hybrids between these species in nature has not been estimated. Although methods for detecting hybrids between *B. dorsalis* and *B. carambolae* have been reported (Ebina and Ohto, 2006) it is currently not possible to measure impacts of hybridization events over time, such as genome introgression or detection of progeny of backcrossed populations.

B. carambolae attacks a wide range of fruits from 20 plant families, particularly *Averrhoa carambola* (carambola) (CABI, n.d.). It is found in the southern peninsular area of southeast Asia through Indonesia and several islands in the Bay of Bengal (Drew and Romig, 2013). It is also present in some South American countries (CABI, n.d.).

B. caryeae is known to attack *Mangifera* spp. (mango), *Malpighia emarginata* (acerola), *Psidium* spp. (guava), *Citrus* spp. and *Pouteria* spp. (mamey sapote), and is endemic to southern India (CABI, n.d.).

B. dorsalis s.l. attacks over 270 plant species (Vargas *et al*., 2015) in over 50 families of commercial fruits and wild fruits (CABI, n.d.). It has the largest species range of the six pests included in this protocol, and is found on some islands in the Pacific Ocean and most of continental Africa (sub-Saharan countries) in addition to its original Asian range (Drew and Hancock, 1994; Drew *et al*., 2005; White, 2006; Drew and Romig, 2013; Schutze *et al*., 2015a, b).

B. kandiensis attacks a wide range of fruits including *Mangifera indica* (mango), *Garcinia* spp., *Carica papaya* (papaya), *Persea americana* (avocado) and *Psidium* spp. (guava) (CABI, n.d.). It has a limited distributional range, being endemic to Sri Lanka.

B. occipitalis attacks *Mangifera* spp. (mango), *Psidium* spp. (guava), *Spondias purpurea* (red mombin), *Averrhoa carambola* (carambola), *Citrus* spp. and *Manilkara zapota* (sapodilla) (CABI, n.d.). It has a relatively narrow range in southeast Asia (Drew and Romig, 2013).

B. pyrifoliae attacks *Psidium* spp. (guava) and *Prunus persica* (peach) (Allwood *et al.*, 1999). It is known from parts of southeast Asia (Drew and Romig, 2013).

2. Taxonomic Information

Name: *Bactrocera dorsalis* complex

Synonyms: None

Taxonomic position: Insecta, Diptera, Tephritidae, Dacinae, *Bactrocera*

The species included in the *Bactrocera dorsalis* complex are in the subgenus *Bactrocera* (*Bactrocera*). According to ICZN (1999), three species are treated as synonyms under *B. dorsalis s.l.*: *B. papayae*, *B. invadens* and *B. philippinensis*. Drew and Romig (2013) placed *B. philippinensis* as a synonym of *B. papayae*. Revision by Schutze *et al*. (2015a) placed *B. invadens* and *B. papayae* as junior synonyms of *B. dorsalis*. Drew and Romig (2016) provide an argument for treating these as separate species, but Schutze *et al.* (2017) published a rebuttal of this argument. Note that *B. invadens* was placed in the *Bactrocera dorsalis* complex by Drew *et al*. (2008) but then removed from the complex by Drew and Romig (2013). Based on Schutze *et al*. (2015a), *B. invadens* is considered a sibling species, or synonym, of *Bactrocera dorsalis*. The current protocol treats these names (*B. papayae*, *B. invadens* and *B. philippinensis*) as part of *B. dorsalis s.l.*

Table 1. Common names and synonyms of six species in the *Bactrocera dorsalis* complex included in the protocol

***Bactrocera* species**	**Common name**	**Synonyms**
Bactrocera (*Bactrocera*) *carambolae* Drew and Hancock, 1994	Carambola fruit fly	None
Bactrocera (*Bactrocera*) *caryeae* (Kapoor, 1971)		*Chaetodacus ferrugineus incises* Bezzi, 1916
		Dacus (*Strumeta*) *caryeae* Kapoor, 1971
Bactrocera (*Bactrocera*) *dorsalis s.l.* (Hendel, 1912)	Oriental fruit fly	*Bactrocera conformis* Doleschall, 1858
		Dacus dorsalis Hendel, 1912
		Chaetodacus ferrugineus var. *okinawanus* Shiraki*, 1933*
		Dacus (*Bactrocera*) *semifemoralis* Tseng, Chen & Chu, 1992
		Dacus (*Bactrocera*) *yilanensis* Tseng, Chen & Chu, 1992
		Bactrocera papayae Drew and Hancock, 1994
		Bactrocera philippinensis Drew and Hancock, 1994
		Bactrocera invadens Drew *et al.*, 2005
Bactrocera (*Bactrocera*) *kandiensis* Drew and Hancock, 1994		None
Bactrocera (*Bactrocera*) *occipitalis* (Bezzi, 1919)		*Chaetodacus ferrugineus* var. *occipitalis* Bezzi, 1919; Drew and Hancock, 1994
		Dacus (*Strumeta*) *dorsalis* var. *occipitalis* (Bezzi, 1919); Hardy and Adachi, 1954; Hardy, 1969
		Dacus (*Strumeta*) *occipitalis* (Bezzi, 1919); Hardy, 1974
Bactrocera (*Bactrocera*) *pyrifoliae* Drew and Hancock, 1994		None

3. Detection

Fruit flies of the genus *Bactrocera* are detected mainly by male lure trap or in fruits. Only male adult fruit flies are captured by male lure trapping, while all immature stages such as eggs (Figure 2(a)), early to final instar larvae (Figures 2(b) to (d)), puparia and pupae (Figures 2(e) and (f)) can be found during inspection of fruits.

3.1 Trapping

Guidance on trapping *Bactrocera* fruit flies is given in Appendix 1 of ISPM 26 (*Establishment of pest free areas for fruit flies (Tephritidae)*). Additional information on trapping methods is provided by Drew (1982), Drew and Romig (2010), and FAO and IAEA (2018). The *Bactrocera dorsalis* complex includes species that respond to different male lures. When the lure responsiveness information is available, it can be used as supporting information for species identification. Five of the target species in this diagnostic protocol are methyl eugenol responding species. The only exception is *B. pyrifoliae*, which has been reported to respond to an alternative lure: cue lure (Drew and Romig, 2013).

Additional information on attractants for trapping, such as synthetic food attractants and hydrolysed protein substances, are available in Appendix 1 of ISPM 26.

3.2 Inspection of fruits

Fruits with soft areas, dark stains, dark pin spots, rot, orifices or injuries that might have originated from female oviposition or larval feeding activities should be targeted for inspection. In order to detect punctures made by female flies during oviposition, fruits should be examined under a microscope by an expert. If larval exit holes are observed, the fruit containers should be inspected for pupae. Second and third instar larvae and pupae are not likely to occur when unripe fruits are collected and packed; however, these fruits might host eggs and first instar larvae, which are more difficult to detect. Potentially infested fruits that show typical punctures made by ovipositioning female flies (Figure 3) should be cut open to search for eggs or larvae inside. The success of detection depends on careful sampling and examination of fruits.

Once detected, immature larvae can be reared to adults for identification (section 3.3). Rearing of adults is required to accurately identify a fly to species level or as part of the *Bactrocera dorsalis* complex. The incubation of infested fruits is a common practice to obtain adult flies, which is necessary to identify species in this protocol. Even if there are no signs of fruit fly infestation, an incubation could be conducted as an oviposition mark is often difficult to recognize.

3.3 Rearing larvae to obtain adults

Larvae can be reared to adults by placing infested fruits in cages containing a pupation medium (e.g. damp vermiculite, sand or sawdust) at the bottom. The cages are covered with cloth or fine mesh. Once the larvae emerge from the fruit, they will move to the pupation medium. Each sample should be observed and pupae gathered daily. The pupae are placed in containers with the pupation medium, and the containers are covered with a tight lid that enables proper ventilation. Once the adults emerge, they must be kept alive for several days to ensure that the tegument and wings acquire the rigidity and characteristic coloration of the species. Flies can be fed with honey (sugar) and water. The adults are then killed by freezing, or by exposure to ethyl acetate or other killing agents appropriate for morphological examination, and then mounted on pins. Prior to mounting (before they harden), it is useful to gently squeeze the apical part of the preabdomen with forceps, then squeeze the base and apex of the oviscape to expose the aculeus tip for females, and to pull out the aedeagus for males. Alternatively, this will need to be dissected later in flies.

4. Identification

Identification at the level of the species or the *Bactrocera dorsalis* complex requires morphological examination of adult flies. It is generally difficult and not reliable to morphologically identify eggs, larvae or pupae to the species level. It is not possible to identify a fly to the *Bactrocera dorsalis* complex using immature life stages.

Molecular methods of *Bactrocera* species identification have been reported and provide additional information to support morphological identifications of specimens. DNA sequencing of the *cytochrome oxidase I* DNA barcode does not provide adequate resolution to identify many species in the *B. dorsalis* complex (details in section 4.4). Other molecular methods lack the specificity data needed to demonstrate that a test is accurate for species identification. For example, the molecular profiles of all six pest species targeted in this protocol are not known using ribosomal DNA analysis (section 4.4). DNA can be used to distinguish *B. carambolae* from *B. dorsalis s.l.* and a method for doing this is provided in this protocol (section 4.3.2). The use of a fly leg for DNA extraction is recommended when molecular data are to be collected. For guidance on preparing a specimen for molecular study, see section 4.3.1.

4.1 Preparation of adults for identification

Proper preparation of specimens is essential for accurate morphological identification. General instructions on preparation of adult fruit fly specimens are given by Drew (1991) and White and Elson-Harris (1992).

Every attempt should be made to preserve all characters on at least one side of the centre line, regardless of the mounting method (Foote *et al.*, 1993).

Characters on the head, wing, leg, thorax and abdomen of a fly can be examined from pinned specimens under magnification using a stereomicroscope at ≥20×. This magnification level is appropriate for observation of spot and colour patterns and wing morphology (Figure 1). Microscopic examination is required to measure characters on the genitalia that are described in section 4.1.1.

Structures of the ovipositor such as the oviscape, eversible membrane and aculeus have been used as important taxonomic characters at species level (Hardy, 1949, 1969; Hardy and Adachi, 1954; Drew and Hancock, 1994). Since the review by Drew and Hancock (1994), aculeus length has been used in particular for distinguishing some of the fruit fly species within the *Bactrocera dorsalis* complex, and male aedeagus length, which is highly correlated with aculeus length, has also been used because only males are trapped in lure trapping surveys. Care must be taken when interpreting genitalic morphometric information for species diagnostics, as some members of the *B. dorsalis* complex exhibit a wide range of aedeagus lengths over their geographical distribution (Krosch *et al.*, 2013; Schutze *et al.*, 2015a). Preparation methods for male genitalia are included in section 4.1.1.

To assist in identification of characters under a stereomicroscope, the following can be applied:

- Examination of the costal band below the R_{2+3} vein will be made easier by putting white paper underneath the wing or by using transmitted light.
- When black markings on abdominal tergites 3–5 are difficult to observe due to damage such as colour change, observation may be made easier by wetting with a paintbrush dipped in 70% ethanol or clearing with 10% potassium hydroxide (KOH).
- When the inner yellow membrane in lateral vittae (Figure 4) is partially removed, which makes the boundary of the lateral vittae difficult to see, an alternative is to measure the width of the translucent window in the scutum (Figure 5(b)).
- In measuring the width of lateral vittae (example of measuring indicated in Figure 5(b)), adjustment of the angle to give the widest value of the vittae is important.

4.1.1 Preparation of adults for microscopic examination of genitalia

The procedures for dissection of the genitalia are mainly based on White and Elson-Harris (1992), White and Hancock (1997), and Foote *et al*. (1993). When measuring the length of genitalia, it is recommended that the relative length to body size also be calculated. The length of the CuA_1 vein along the discal medial cell of the wing has been used as an index of body size in prior studies (Ebina and Ohto, 2006).

Preparation of the abdomen for dissection and examination of genitalia can be accomplished by first removing the abdomen from the specimen and soaking it in a 10% solution of KOH at 95 °C for 10 to 20 minutes depending on the condition of the specimen. Once the KOH soak is complete, the digested abdomen can be transferred to a spot of glycerol.

For aculeus examination, the dissection should be carried out in a drop of glycerol with two fine forceps (or dissection needles). The oviscape should be broken from the rest of the abdomen and then it is possible to telescope the aculeus out of the oviscape by gently squeezing the oviscape with one pin (Figure 6(b)). It is necessary to finish removal of the aculeus by holding the oviscape with one pin and pulling the aculeus out with the other (for more details, see Foote *et al*., 1993). If the telescoping method fails, the oviscape will need to be torn open to remove the aculeus.

For aedeagus examination, it is recommended that the epandrium–surstylus assemblage (Figure 7(c)) be pulled from the rest of the abdomen. Using two pins, it is possible to straighten the aedeagus (Figure 8). It is then recommended that a small coverslip be placed over the aedeagus, leaving the epandrium, hypandrium and aedeagus base outside of the coverslip. The coverslip is carefully moved away from the epandrium so as to stretch the aedeagus out into a straight line. It is then measured from the base of the basiphallus (enclosed by the hypandrium) to just before the aedeagal glans (Figure 8(d)). In general, the aedeagus should be preserved in glycerol. However, if the specimens are to be used only for measurement, it is sufficient to glue onto a paper stage.

4.2 Morphological identification of adults

Members of the *Bactrocera dorsalis* complex are identified using a combination of morphological characters. The diagnostic characters required to complete an identification to the six species covered by this protocol and to the *Bactrocera dorsalis* complex as a whole are provided below. Additional resources on general characters for tephritid fruit fly identification are provided in White and Elson-Harris (1992).

4.2.1 Characters to identify the subgenus *Bactrocera* (*Bactrocera*)

Methods to identify fly specimens to the genus *Bactrocera* are not within the scope of the current protocol. However, proper screening of specimens is important to ensure that flies being diagnosed are within the subgenus *Bactrocera* (*Bactrocera*). The work of White and Elson-Harris (1992) provides a useful resource for those general identifications. Characters used to identify fruit flies to the tribe Dacini, including the genus *Bactrocera*, are useful in the identification of flies to the subgenus *Bactrocera* (*Bactrocera*). These flies have reduced chaetotaxies on the head, with ocellar (Figure 9(c)) and postocellar (Figure 9(c)) bristles absent (atrophied); the first flagellomere (Figure 9(a)) is at least three times as long as broad; and wing cell cup extension is very long (Figure 10, top). In addition to these characteristics, fruit flies of the genus *Bactrocera* have separate abdominal tergites (Figure 7(a)) (except for first and second tergites). In addition to the above characteristics of the genus *Bactrocera*, the subgenus *Bactrocera* also has the characteristics listed below.

The presence of diagnostic characters of other *Bactrocera* subgenera is useful in diagnosing flies, via exclusion, as not being members of the *Bactrocera dorsalis* complex. For example, flies in the subgenus *Bactrocera* (*Afrodacus*) lack anterior supra-alar bristles (Figure 11) and flies in the subgenus *Bactrocera* (*Gymnodacus*) lack pectens on tergite 3 (Figure 7(a)). The characters listed below are used for defining the subgenus *Bactrocera*. In starting identification, it is important to confirm that the fruit flies in question meet the definition. At this stage of identification, superficially similar species in other subgenera such as *Afrodacus* or *Gymnodacus* that could be intercepted during plant inspection can be excluded.

List of diagnostic characters of subgenus *Bactrocera* (*Bactrocera*):

- posterior lobe of male surstylus short (Figure 7(c))
- abdominal sternite 5 of male deeply concave on posterior margin (Figure 7(b))
- abdominal tergite 3 of male with pecten (Figure 7(a))
- postpronotal bristles absent (Figure 11)
- anterior supra-alar (a. sa.) bristles present (Figure 11)
- prescutellar acrostichal (prsc.) bristles usually present (Figure 11)
- one pair of apical scutellar (sc.) bristles present (Figure 11).

4.2.2 Characters to identify the *Bactrocera dorsalis* complex

Characters useful for the identification of adult flies following the terminology of Drew and Romig (2013) are listed in Table 2. The set of characters used to identify the *Bactrocera dorsalis* complex in this protocol follows Drew and Romig (2013) except for scutum colour. Scutum colour in Drew and Romig (2013) is black, but herein black and red–brown are included in the description of the complex.

A specimen must have characters that match the descriptions provided in Table 2 for the fly to be confidently identified as a *B. dorsalis* complex species.

Table 2. A combination of characters to diagnose the *Bactrocera dorsalis* complex

Structure	Description
Head	Face yellow with distinct facial spots present (Figures 9(a), 9(b), 12)
Scutum	Colour mostly black to mostly red–brown (inter-regionally variable) (Figure 13)
	Lateral vittae present (Figure 11) and yellowish (Figures 13 and 14)
	Medial vittae absent (Figure 11)
Scutellum	Yellowish colour (Figures 1 and 13)
	With a dark basal band (Figures 11 and 13)
	Never with other dark patterns (Figure 13)
Femora	Entirely or mostly fulvous (reddish-yellow or tawny) colour but may possess dark patterns particularly on and around apices (Figure 15)
Wing	Cells bc and c hyaline (colourless) or, at most, with an extremely pale tint (Figures 10 and 16)
	Without dense microtrichia covering cells bc and c (Figure 10)
	Costal band narrow (never confluent with R_{4+5}) (Figure 10)
	Narrow anal streak present (diagonal marking that is above anal lobe) (Figures 10 and 16)
Abdomen	With a "T" pattern on tergites 3–5 (Figures 7(a) and 17)

4.2.3 Morphological identification of six economically important species belonging to the *Bactrocera dorsalis* complex

Morphological identification of species in the *Bactrocera dorsalis* complex is difficult in part because of a high level of character variability within species and overlap in characters between species. Ranges of variations in each diagnostic character shown in Table 3 are compiled from various sources including Drew and Hancock (1994), Drew and Romig (2013, 2016), and Schutze *et al*. (2015a, b). In Table 3, some character descriptions are recorded with indications of being "inter-regionally" or "intra-regionally" variable because some of the regional populations seem to have clearly unique variations in qualitative or quantitative characters.

Identification at species level is generally difficult when specimens lack a combination of characteristics typical for one of the species. This is particularly evident in diagnosis of *B. dorsalis s.l.* and *B. carambolae* when genitalia lengths can match either species. As mentioned, hybrids are possible between these species but cannot be diagnosed with confidence using morphology.

An identification to one of the six species in the protocol requires the adult specimen to be examined for the characters provided in Table 3. This can be accomplished using the key in section 4.2.4 to screen specimens and then identification can be confirmed by comparing fly morphology to information in Table 3. If one or more characters are inconsistent between the specimen and the descriptions provided in Table 3, then the specimen cannot be diagnosed as one of these species. Morphometric examination of genitalia does not always provide a clear diagnosis because of overlap in the range of aedeagus and aculeus sizes between *B. dorsalis s.l.* and *B. carambolae* (Table 3). These characters are included because they can be informative in distinguishing some specimens of *B. dorsalis s.l.* from *B. carambolae*. When specimens match both *B. dorsalis s.l.* and *B. carambolae* based on morphology, then a molecular test (section 4.3) should be run to distinguish between these species.

Table 3. Diagnostic morphological characters of adult fruit flies of six economically important species of the *Bactrocera dorsalis* complex

Structure	**Species**					
	Bactrocera carambolae	*Bactrocera caryeae*	*Bactrocera dorsalis s.l.*	*Bactrocera kandiensis*	*Bactrocera occipitalis*	*Bactrocera pyrifoliae*
Head						
Facial spots (Figures 9(a), 9(b), 12)	Medium-sized, oval (Figure 12(a))	Large, elongate oval (Figure 12(b))	Medium to large, circular to oval (inter-regionally variable) (Figure 12(c))	Large, oval (Figure 12(d))	Large, oval (Figure 12(e))	Medium-sized, circular (Figure 12(f))
Abdomen						
Tergites 3–5 (Figures 7(a), 17, 18)	With medium-width medial longitudinal black stripe (Figures 17(a) and 18(a))	With narrow medial longitudinal black stripe (Figures 17(b) and 18(b))	With narrow to medium-width medial longitudinal black stripe (Figures 17(c) and 18(c))	With very narrow medial longitudinal black stripe (Figures 17(d) and 18(d))	With very broad medial longitudinal black stripe (Figures 17(e) and 18(e))	With narrow to medium-width medial longitudinal black stripe (Figures 17(f) and 18(f))
Tergite 3	With a narrow transverse black band across anterior margin (constituting a “T” pattern) widening to cover lateral margins	With a broad transverse black band across anterior third to half	Exhibits variations from transverse black band across anterior margin (constituting a “T” pattern) to broad lateral bands	With a narrow transverse black band across anterior margin (constituting a “T” pattern)	With a narrow transverse black band across anterior margin widening to cover lateral margins	With a narrow to medium-width transverse black band widening to cover outer third of lateral margins
Tergite 4	With rectangular anterolateral (occasionally triangular) black markings	With broad lateral black bands	Without any markings or with anterolateral black markings (occasionally rectangular in shape)	With very narrow anterolateral black markings	Exhibits variations from anterolateral black markings to broad lateral bands	With a narrow to medium-width transverse black band widening to cover outer third of lateral margins
Tergite 5	With anterolateral black markings	With broad lateral black bands	Without any markings or with anterolateral black markings	With very narrow anterolateral black markings	With broad lateral black bands that cover lateral margins	With broad lateral black bands that cover lateral margins

(Table 3 continued on next page)

(Table 3 continued)

Structure	Species					
	Bactrocera carambolae	*Bactrocera caryeae*	*Bactrocera dorsalis s.l.*	*Bactrocera kandiensis*	*Bactrocera occipitalis*	*Bactrocera pyrifoliae*
Thorax						
Scutum colour (Figure 13)	Dull black (Figure 13(a))	Entirely black (Figure 13(b))	Black to red–brown (inter or intra-regionally variable) (Figure 13(c))	Black (Figure 13(d))	Black (Figure 13(e))	Entirely black (Figure 13(f))
Postpronotal lobe (Figures 5, 11, 13, 19)	Entirely yellow (Figure 19(a))	Yellow with dark anteromedial corner (Figure 19(b))	Entirely yellow (Figure 19(c))	Yellow with dark anteromedial corner (Figure 19(d))	Entirely yellow (Figure 19(e))	Entirely yellow (Figure 19(f))
Anterior margin of anepisternal stripe (Figures 5(a) and 14)	Reaching midway between anterior margin of notopleuron and anterior npl. bristle; convex (anterior margin) (Figure 14(a))	Reaching midway between anterior margin of notopleuron and anterior npl. bristle; straight (anterior margin) (Figure 14(b))	Reaching midway between anterior margin of notopleuron and anterior npl. bristle; straight to convex (anterior margin) (Figure 14(c))	Slightly wider than notopleuron, equal in width to notopleuron; straight (anterior margin) (Figure 14(d))	Reaching midway between anterior margin of notopleuron and anterior npl. bristle; convex (anterior margin) (Figure 14(e))	Equal in width to notopleuron; convex (anterior margin) (Figure 14(f))
Basal band of scutellum (Figures 11 and 13)	Narrow (Figure 13(a))	Moderately broad (Figure 13(b))	Narrow (Figure 13(c))	Narrow (Figure 13(d))	Narrow (Figure 13(e))	Narrow (Figure 13(f))
Lateral vittae (Figures 4, 5, 11)	Broad, parallel-sided, ending at or behind ia. bristles (Figure 4(a))	Very narrow; either entirely parallel-sided or narrowing posteriorly; ending at or just before ia. bristles (Figure 4(b))	Narrow to broad (inter-regionally variable), parallel-sided, ending at or just behind ia. bristles (Figure 4(c))	Narrow, parallel-sided, ending at ia. bristles (Figure 4(d))	Broad, parallel- or subparallel-sided; either ending at ia. bristles or (in some specimens) ending behind ia. bristles (Figure 4(e))	Narrow; either subparallel-sided and ending before ia. bristles or (in some specimens) parallel-sided and ending at ia. bristles (Figure 4(f))

(Table 3 continued on next page)

(Table 3 continued)

Structure	Species					
	Bactrocera carambolae	*Bactrocera caryeae*	*Bactrocera dorsalis s.l.*	*Bactrocera kandiensis*	*Bactrocera occipitalis*	*Bactrocera pyrifoliae*
Wing						
Costal band (Figures 10 and 16)	Narrow, slightly overlapping R_{2+3}, moderately broad around apex of wing (Figure 16(a))	Very narrow, confluent with R_{2+3}, very narrow around apex of wing (Figure 16(b))	Narrow, generally confluent with R_{2+3} (inter- or intra-regionally variable), narrow to moderately broad around apex of wing (Figure 16(c))	Narrow, confluent with R_{2+3}, narrow around margin of wing (Figure 16(d))	Narrow, distinctly overlapping R_{2+3}, broad around apex of wing extending to mid-point between R_{2+3} and R_{4+5} (Figure 16(e))	Narrow, confluent with R_{2+3}, narrow but slightly expanding around apex of wing (Figure 16(f))
Legs						
Femora (Figure 15)	Fulvous, generally with a large elongate oval black marking on outer surface of fore femora (Figure 15(a))	Fulvous with large dark fuscous markings on all femora (Figure 15(b))	Generally fulvous, occasionally with a small dark marking on outer surface of fore femora (inter-regionally variable) (Figure 15(c))	Fulvous with large dark markings on all femora (Figure 15(d))	Generally fulvous, occasionally with a small preapical dark spot on outer surface of fore femora (Figure 15(e))	Fulvous with a small apical marking on fore femora and dark fuscous around apices of mid and hind femora (Figure 15(f))
Genitalia						
Aculeus length (mm) (Figure 6)	1.3–1.6	n/a	1.4–2.2 (inter- or intra-regionally variable)	n/a	n/a	n/a
Ratio (CuA_1/Acul.)	1.4–1.6	n/a	1.0–1.8	n/a	n/a	n/a
Aedeagus length (mm) (Figure 8(d))	2.0–2.7	n/a	2.3–3.5 (inter- or intra-regionally variable)	n/a	n/a	n/a
Ratio (Aed./CuA_1)	1.2–1.3	n/a	1.2–1.4	n/a	n/a	n/a

Acul., aculeus length; Aed., aedeagus length; CuA_1, first anterior branch of cubitus vein (see Figure 10); ia., intra-alar; n/a, not available; npl., notopleural; R_{2+3}, R_{4+5}, posterior branches of radial vein (see Figure 10).

4.2.4 Diagnostic key to six economically important species belonging to the *Bactrocera dorsalis* complex (adult)

1. Postpronotal lobe yellow with dark anteromedial corner (Figures 19(b) and (d)) **2**

– Postpronotal lobe entirely yellow (Figures 19(a), (c), (e), (f)) **3**

2. Scutum entirely black (Figure 13(b)), abdominal tergites 3–5 with broad black dorsolateral markings (Figures 17(b) and 18(b)); lateral vittae very narrow (Figure 4(b)) ..... ***B. caryeae***

– Scutum mostly black (Figure 13(d)), abdominal tergites 3–5 with "T" pattern and tergites 4–5 with very narrow anterolateral black markings (Figures 17(d) and 18(d)); lateral vittae narrow (Figure 4(d)) ..... ***B. kandiensis***

3. Costal band distinctly overlapping R_{2+3} and expanding broadly around apex of wing reaching mid-point between R_{2+3} and R_{4+5} (Figure 16(e)) ..... ***B. occipitalis***

– Costal band widening slightly (Figure 16(c)) to moderately (Figure 16(a)) around apex of wing **4**

4. Abdominal tergites 3–5 with broad black dorsolateral markings (Figures 17(f) and 18(f)) ..... ***B. pyrifoliae***

– Abdominal tergites 3–5 without broad black dorsolateral markings **5**

5. Costal band slightly overlapping R_{2+3}, moderately broad around apex of wing (Figure 16(a)); abdominal tergite 3 with a narrow transverse black band across anterior margin (constituting a "T" pattern), widening to cover lateral margins; tergite 4 with rectangular (occasionally triangular) anterolateral or narrow lateral black markings; tergites 3–5 with medium-width medial longitudinal black stripe (Figures 17(a) and 18(a)) ..... ***B. carambolae***

– Costal band confluent with R_{2+3}, narrow to moderately broad around apex of wing (Figure 16(c)); abdominal tergite 3 exhibits variations from black band across anterior margin (constituting a "T" pattern) to broad lateral bands, tergite 4 without markings or with anterolateral or narrow lateral black margins (occasionally rectangular), tergite 5 without markings or with anterolateral black markings (Figures 17(c) and 18(c)) ..... ***B. dorsalis s.l.***

4.3 Molecular identification of *Bactrocera carambolae*

Molecular identification of the six target species has been confounded by their very close genetic relationships and uncertain taxonomy (Boykin *et al*., 2014; Hendrichs *et al*., 2015). Molecular tests alone are not recommended for identification of the six species. However, molecular methods can provide useful information to support morphological identifications when new records are reported from the morphological diagnosis. When identifying *B. carambolae* and *B. dorsalis s.l.* specimens using this protocol, a molecular test is necessary for accurate identification whenever adult morphology alone cannot distinguish between the two species.

DNA sequencing of either the internal transcribed spacer 1 (ITS1) or 2 (ITS2) nuclear DNA regions has been proposed as a reliable way to distinguish between the species *B. carambolae* and *B. dorsalis s.l.* (Boykin *et al*., 2014; Schutze *et al*., 2015a). The ITS1 method as described by Boykin *et al*. (2014) for distinguishing between the two species is included in the current protocol. This method is designed to diagnose a fly as *B. carambolae* based on the presence of a unique DNA insertion that is not present in *B. dorsalis s.l.* The ITS1 method has not been shown to distinguish *B. carambolae* from all other *Bactrocera dorsalis* complex species. Specificity of the method for *B. carambolae* has been examined using only four species in the *Bactrocera dorsalis* complex: *B. dorsalis s.l., B. occipitalis*, *B. opiliae* and *B. cacuminata.*

In this diagnostic protocol, methods (including references to brand names) are described as published, as these define the original level of sensitivity, specificity and reproducibility achieved. Laboratory

procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

4.3.1 DNA extraction for molecular tests

Boykin *et al.* (2014) and Ball and Armstrong (2008) provide protocols for DNA extraction using commercial kits that are useful because small amounts of starting material such as one fruit fly leg can give enough DNA yield and quality for PCR. The methods used to preserve fruit flies for morphological and molecular examination are not the same. Ethanol is a common preservative for fruit fly DNA. Although fruit fly specimens can be preserved in ≥95% ethanol at −20 °C or colder for long-term storage, ethanol can alter the colouring of adult specimens, which can hinder morphological identification. All identifications performed using this protocol require morphological examination. In cases where molecular methods are to be used, it is therefore recommended that a leg be removed and stored in ethanol for DNA extraction and that the remaining specimen be prepared for morphology work. Further examples of methods are provided by Plant Health Australia (2016).

4.3.2 ITS1 PCR and DNA sequencing to distinguish *B. carambolae* from *B. dorsalis s.l.*

The Boykin *et al*. (2014) study compared a large collection of ITS1 sequences from *B. dorsalis s.l.* and *B. carambolae* specimens. Although many primer sets for analysis of ITS1 have been reported in the scientific literature (e.g. Plant Health Australia, 2016), the ITS7/ITS6 primer set reported by Boykin *et al.* (2014) is reported here to simplify comparison with reference sequences from that study and stored in GenBank. Other primer sets that target the same region of ITS1 could also function adequately. None of the published primer sets for this target gene have been tested for reproducibility or sensitivity.

The ITS7 (forward) and ITS6 (reverse) primers are:

ITS7 (5′- GAA TTT CGC ATA CAT TGT AT-3′) (Boykin *et al.*, 2014)

ITS6 (5′- AGC CGA GTG ATC CAC CGC T-3′) (Armstrong and Cameron, 2000)

PCR can be carried out in 30 µl reactions according to Boykin *et al*. (2014), using the master mix and cycling parameters given in Table 4.

Sanger sequencing of PCR products should be carried out using each primer to generate two independent DNA sequence reads in alternate directions. These sequences should be aligned to identify conflicting information. Chromatograms should be edited to resolve conflicting signals. If multiple peaks at a nucleotide are observed in the sequences generated using both the forward and reverse primers then the site should be assigned as an ambiguous base (i.e. N = A, C, T or G). The final edited sequence should be at least 400 base pairs (bp) in length for data interpretation.

Table 4. Master mix composition, cycling parameters and amplicons for PCR to distinguish *Bactrocera carambolae* from *B. dorsalis s.l.*

Reagents	Final concentration
PCR grade water	–[†]
PCR buffer	1×
$MgCl_2$	2.0 mM
dNTPs	200 µM of each
Primer (forward)	0.2 µM
Primer (reverse)	0.2 µM
DNA polymerase	0.6 U
DNA sample	2 µl
Cycling parameters	
Initial denaturation	94 °C for 2 min
Number of cycles	35
- Denaturation	94 °C for 15 s
- Annealing	60 °C for 20 s
- Elongation	69 °C for 60 s
Final elongation	68 °C for 5 min
Expected amplicons	
Size	500–550 bp (the amplicon size varies for species and individuals)

[†] For a final reaction volume of 30 µl.

bp, base pairs; PCR, polymerase chain reaction.

4.3.3 Controls for molecular tests

For the test result to be considered reliable, appropriate controls should be considered for each series of nucleic acid extractions and PCR amplifications of the target pest. As a minimum, a positive nucleic acid control and a negative amplification control (no template control) should be used for the ITS1 PCR test.

Positive nucleic acid control. This control is used to monitor the efficiency of the test method (apart from the extraction). Pre-prepared (stored) genomic DNA may be used.

Negative amplification control (no template control). This control is necessary to rule out false positives due to contamination with other genetic material during the preparation of the reaction mixture. PCR grade water that was used to prepare the reaction mixture is added in place of template DNA.

Negative extraction control. This control is used to monitor contamination during nucleic acid extraction. This requires processing extraction blanks alongside the samples to be tested.

4.3.4 Interpretation of molecular test results

The size of ITS1 is different for *B. carambolae* and *B. dorsalis* because of a 44-bp insertion in *B. carambolae* located near the binding site of the ITS7 primer. The inserted DNA is identical in all *B. carambolae* studied. The sequence of the insertion is:

5´- GAA AAA TTA ATA AAA AGT TAA ATG ATC TTT TTA TAA AAA AT-3´

The ITS1 sequence is variable between conspecific specimens of these two species (Boykin *et al.*, 2014). Consequently, an identical match for sites outside of the insertion region is not expected.

However, the test sequence should be at least 99% similar to one of the reference sequences for the interpretation to proceed. It is possible to distinguish between *B. carambolae* and *B. dorsalis s.l.* after comparing the DNA sequence of the tested specimen with a representative sequence of each species: GenBank KC446737 for *B. carambolae* and KC446776 for *B. dorsalis s.l.* If the tested sequence is most similar to *B. carambolae* and has the 44-bp insertion region, then it can be diagnosed as *B. carambolae*. If the tested sequence is most similar to *B. dorsalis s.l.* and lacks the insertion region, then it is diagnosed as not *B. carambolae*. Several other species in the *B. dorsalis* complex lack the insertion and a match with *B. dorsalis s.l.* cannot exclude those as a possible identification.

4.4 Other molecular methods of identification

Plant Health Australia (2016) has compiled a resource for identification of *Bactrocera* species using DNA methods. That resource summarizes three molecular options for identification: conventional PCR and restriction fragment length polymorphism (RFLP) of the ITS1 region (Plant Health Australia, 2016), PCR-RFLP analysis of a segment of ribosomal DNA array including the ITS1 and 18S gene regions (Armstrong *et al*., 1997; Armstrong and Cameron, 2000), and DNA barcoding of the *cytochrome oxidase subunit I* (*COI*) gene (Armstrong and Ball, 2005) based on the Barcode of Life Data Systems resource (Ratnasingham and Hebert, 2007). Molecular profiles for the species *B. caryeae*, *B. kandiensis*, *B. occipitalis* and *B. pyrifoliae* are not available for either of the PCR-RFLP methods described in the Plant Health Australia resource, precluding the use of these methods as a diagnostic test for these pests.

DNA barcode records of the *COI* gene are not available for *B. pyrifoliae*, and cannot distinguish the other five species from each other (Armstrong and Ball, 2005). The work by Leblanc *et al*. (2015) demonstrates that this complex is not a monophyletic group and a molecular identification of the complex is not possible using *COI* sequence data.

5. Records

Records and evidence should be retained as described in section 2.5 of ISPM 27 (*Diagnostic protocols for regulated pests*).

In cases where other contracting parties may be adversely affected by the diagnosis, records and evidence (in particular, preserved or slide-mounted specimens, photographs of distinctive taxonomic structures, DNA extracts and photographs of gels, as appropriate) should be kept for at least one year in a manner that ensures traceability.

6. Contact Points for Further Information

Further information on this protocol can be obtained from:

Pest Identification and Diagnostics Section, Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan (Kenji Tsuruta; email: tsurutak@pps.maff.go.jp; tel.: +81-45-622-8940; fax: +81-45-621-7560).

Regional R&D Training Center for Insect Biotechnology (RCIB), Department of Biotechnology, Mahidol University, 272 Rama VI Road, Ratchathewee, Bangkok 10400, Thailand (Sujinda Thanaphum; email: sujinda.tha@mahidol.ac.th; tel.: +66814333963; fax: +6623547160).

William F. Barr Entomological Museum, Department of Plant, Soil and Entomological Sciences, University of Idaho, 875 Perimeter Drive MS 2339, Moscow, ID 83844-2339, United States of America (Luc Leblanc; email: leblancl@uidaho.edu; tel.: +1 208-885-6274; fax: +1 208-885-7760).

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), which will in turn forward it to the Technical Panel on Diagnostic Protocols (TPDP).

7. Acknowledgements

The first draft of this protocol was written by Kenji Tsuruta (Ministry of Agriculture, Forestry and Fisheries, Japan (see preceding section)), Sujinda Thanaphum (Mahidol University, Thailand (see preceding section)), Luc Leblanc (University of Idaho, United States of America (see preceding section)) and Norman Barr (United States Department of Agriculture, United States of America). The following experts provided comments on earlier versions that improved the quality of the protocol: Jane Royer (Queensland Department of Agriculture and Fisheries, Australia), Mark Schutze (Queensland University of Technology, Australia), Josephine Moraa Songa (Kenya Agricultural & Livestock Research Organization, Kenya), George Momanyi (Kenya Plant Health Inspectorate Service, Kenya), Sharon Reid (Fera Science Ltd., Sand Hutton, York, United Kingdom), Yuji Kitahara (Ministry of Agriculture, Forestry and Fisheries, Japan), Eddy Dijkstra (Plant Protection Service, Netherlands) and Ken Hong Tan (Tan Hak Heng, Penang, Malaysia).

8. References

The present annex may refer to ISPMs. ISPMs are available on the International Phytosanitary Portal (IPP) at https://www.ippc.int/core-activities/standards-setting/ispms.

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9. Figures

Figure 1. *Bactrocera dorsalis s.l.*, female (habitus).
Source: Photo courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

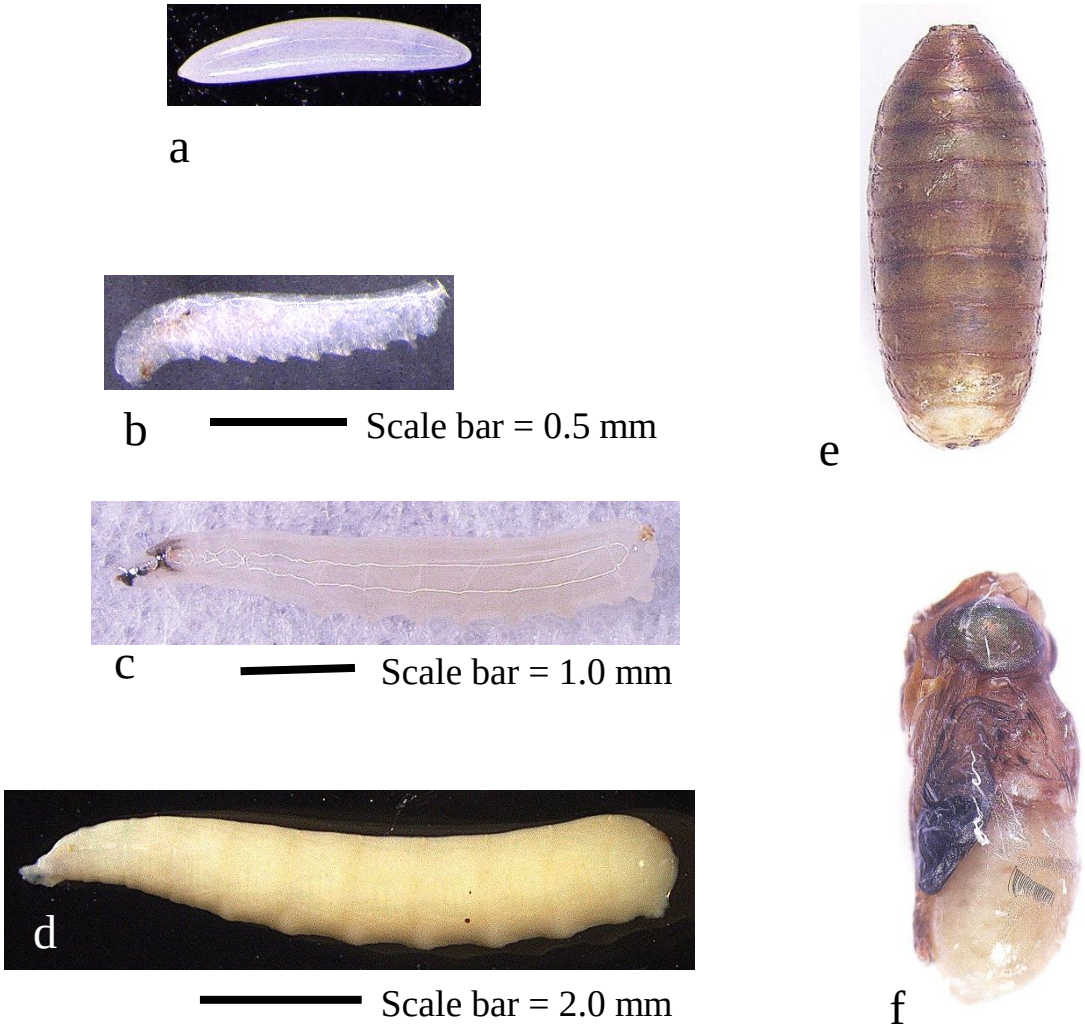


Figure 2. Immature stages of *Bactrocera dorsalis s.l.*: (a) egg; (b) first instar larva; (c) second instar larva; (d) third instar larva; (e) puparium; (f) pupa.
Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

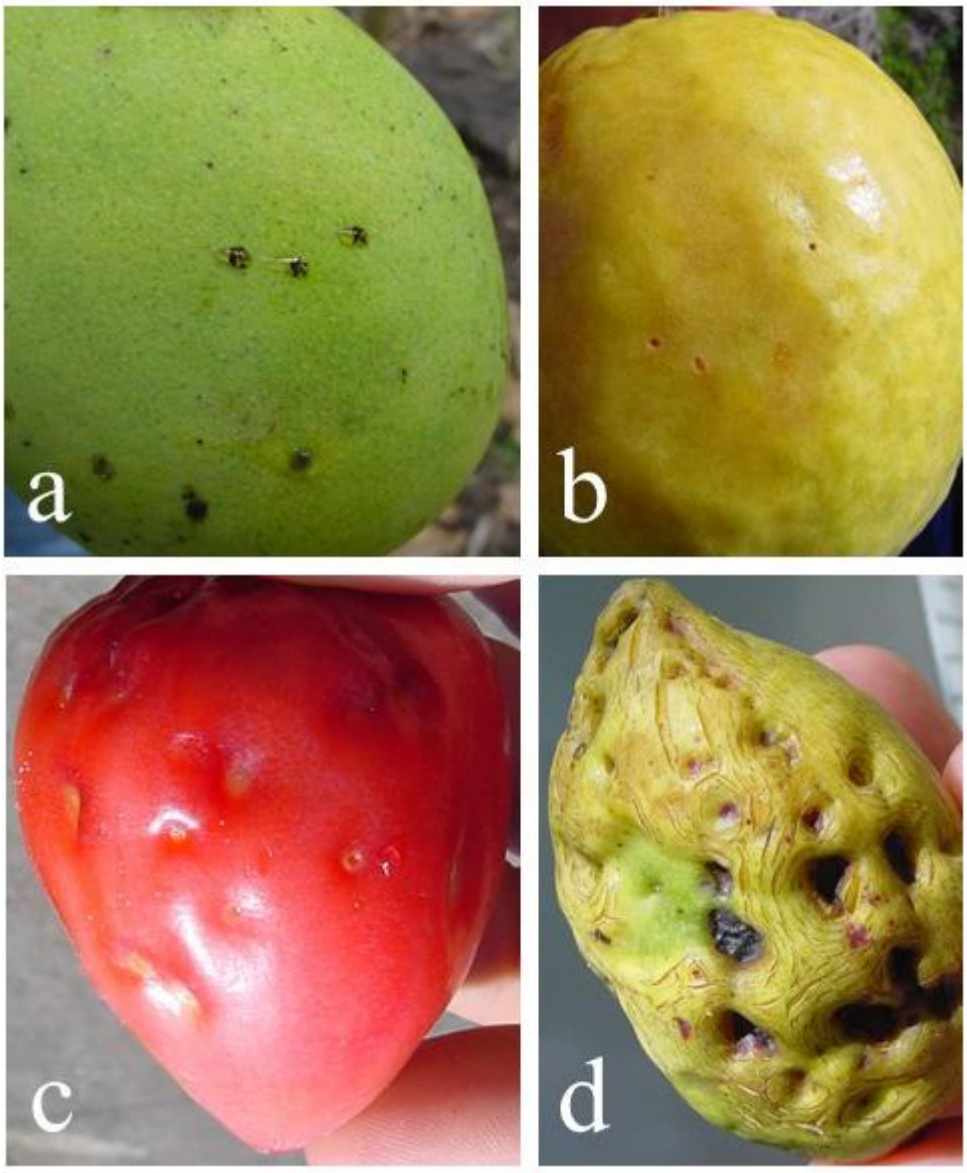


Figure 3. Oviposition punctures on (a) *Mangifera* spp. (mango), (b) *Psidium* spp. (guava), (c) *Syzygium samarangense* (java apple), (d) *Terminalia catappa* (tropical almond).
Source: Photos courtesy of Luc Leblanc, University of Idaho, United States of America.

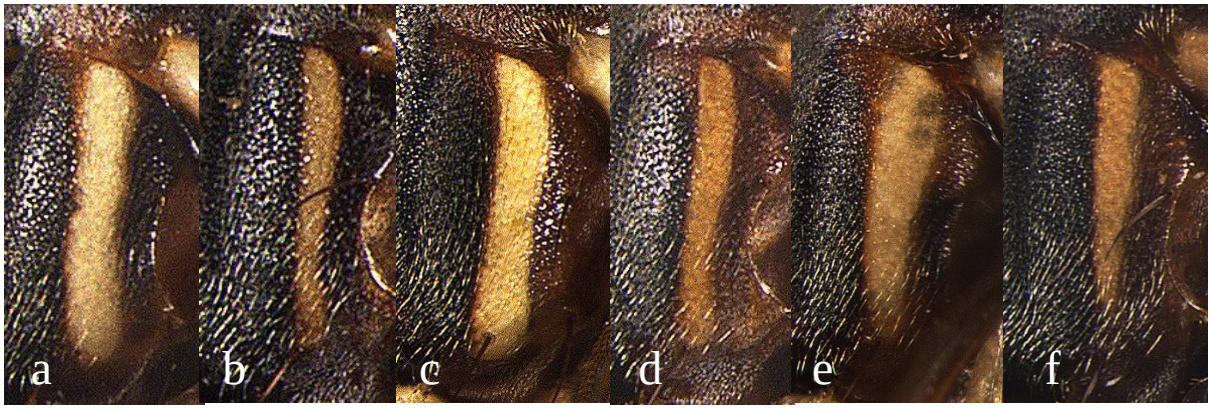


Figure 4. Lateral vittae: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis s.l.*; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*.
Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

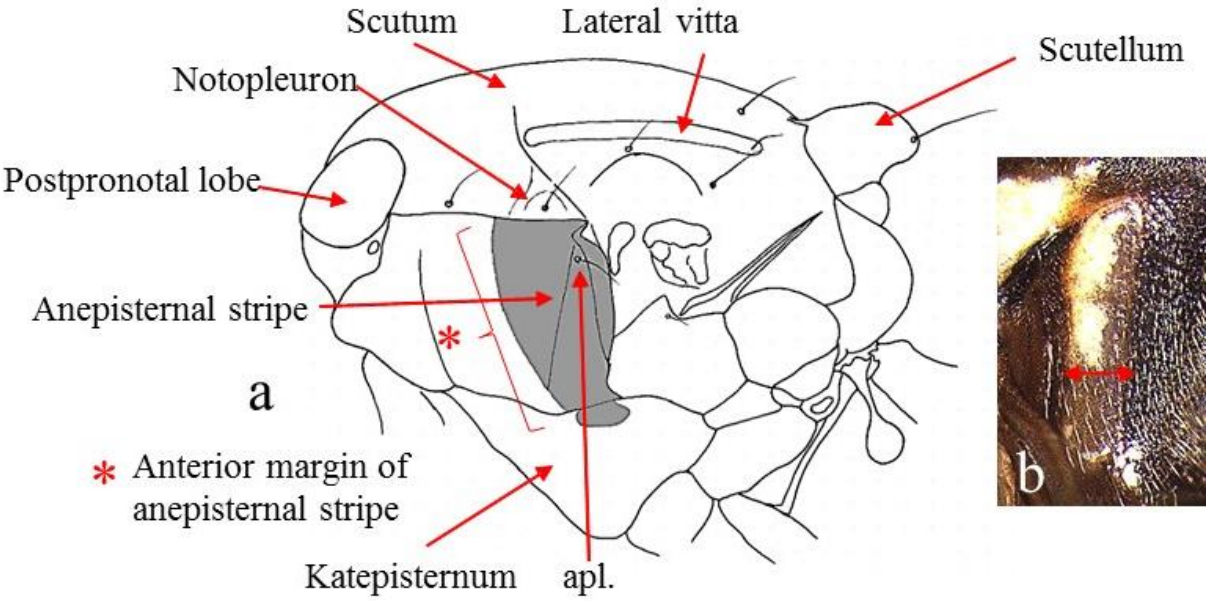


Figure 5. (a) Lateral view of Dacinae thorax. (b) Damaged lateral vitta, showing translucent window.
apl., anepisternal bristle.
Source: Photo and line drawing courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

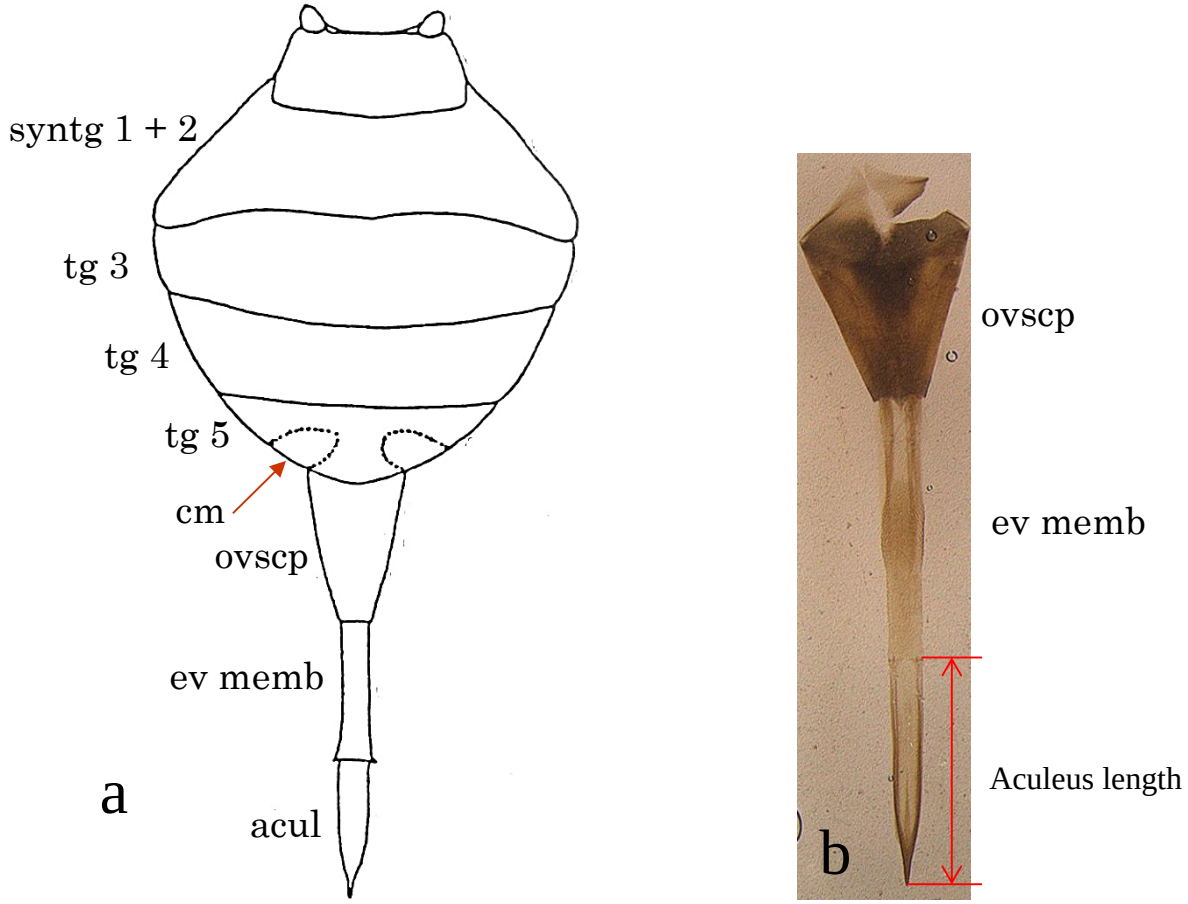


Figure 6. Dacinae abdomen: (a) female in dorsal view; (b) genitalia (fully extended). acul, aculeus; cm, ceromata; ev memb, eversible membrane; ovscp, oviscape; syntg 1 + 2, syntergites 1 + 2; tg3, tergite 3; tg4, tergite 4; tg5, tergite 5.
Source: Line drawing (a) adapted from Ito (1988) and photo (b) courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

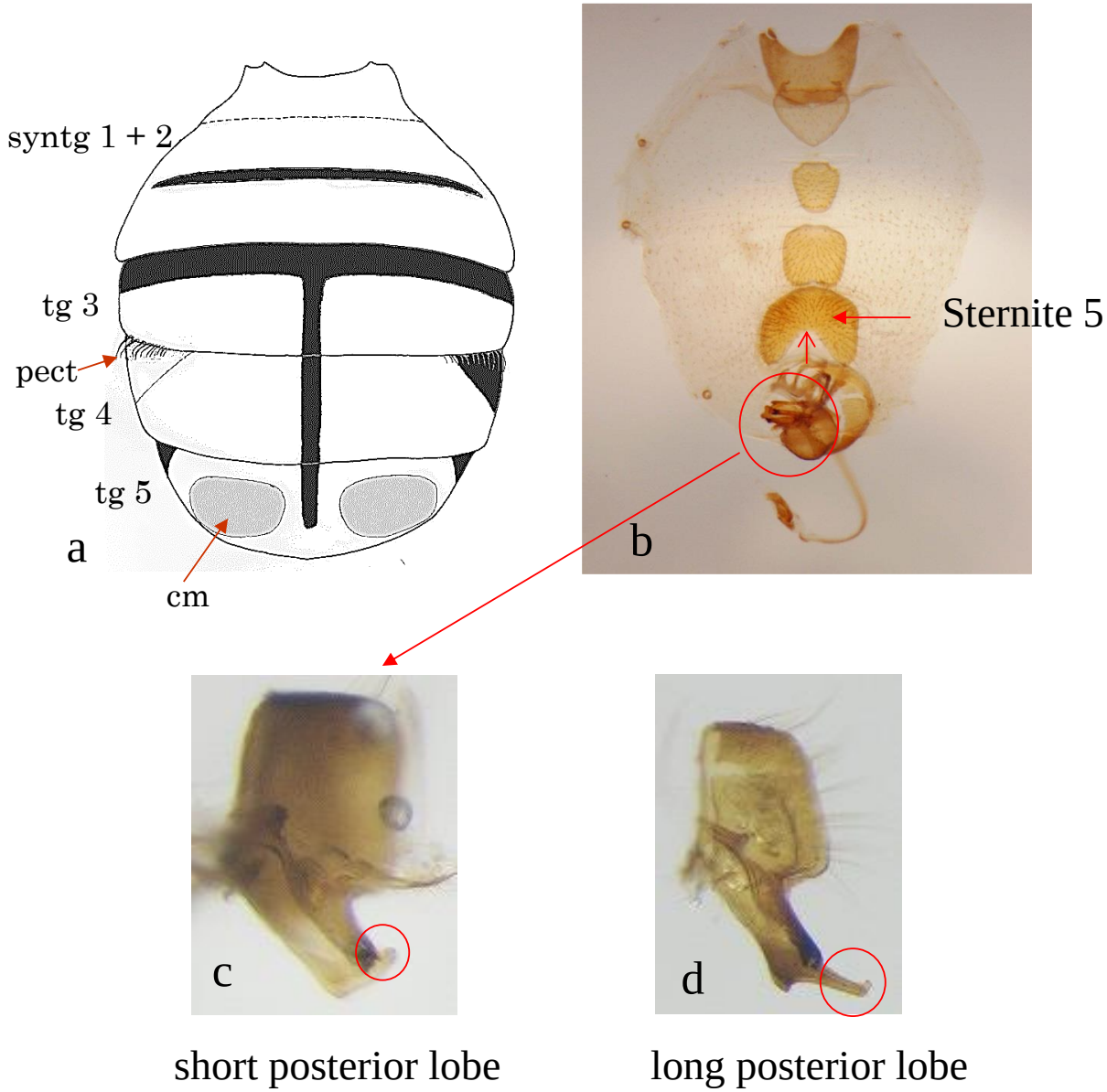


Figure 7. Dacinae abdomen: (a) male in dorsal view; (b) male in ventral view; (c) epandrium and lateral surstylus, showing short posterior lobe; (d) epandrium and lateral surstylus, showing long posterior lobe. cm, ceromata; pect, pecten; syntg 1 + 2, syntergites 1 + 2; tg3, tergite 3; tg4, tergite 4; tg5, tergite 5.
Source: Photos and line drawing courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

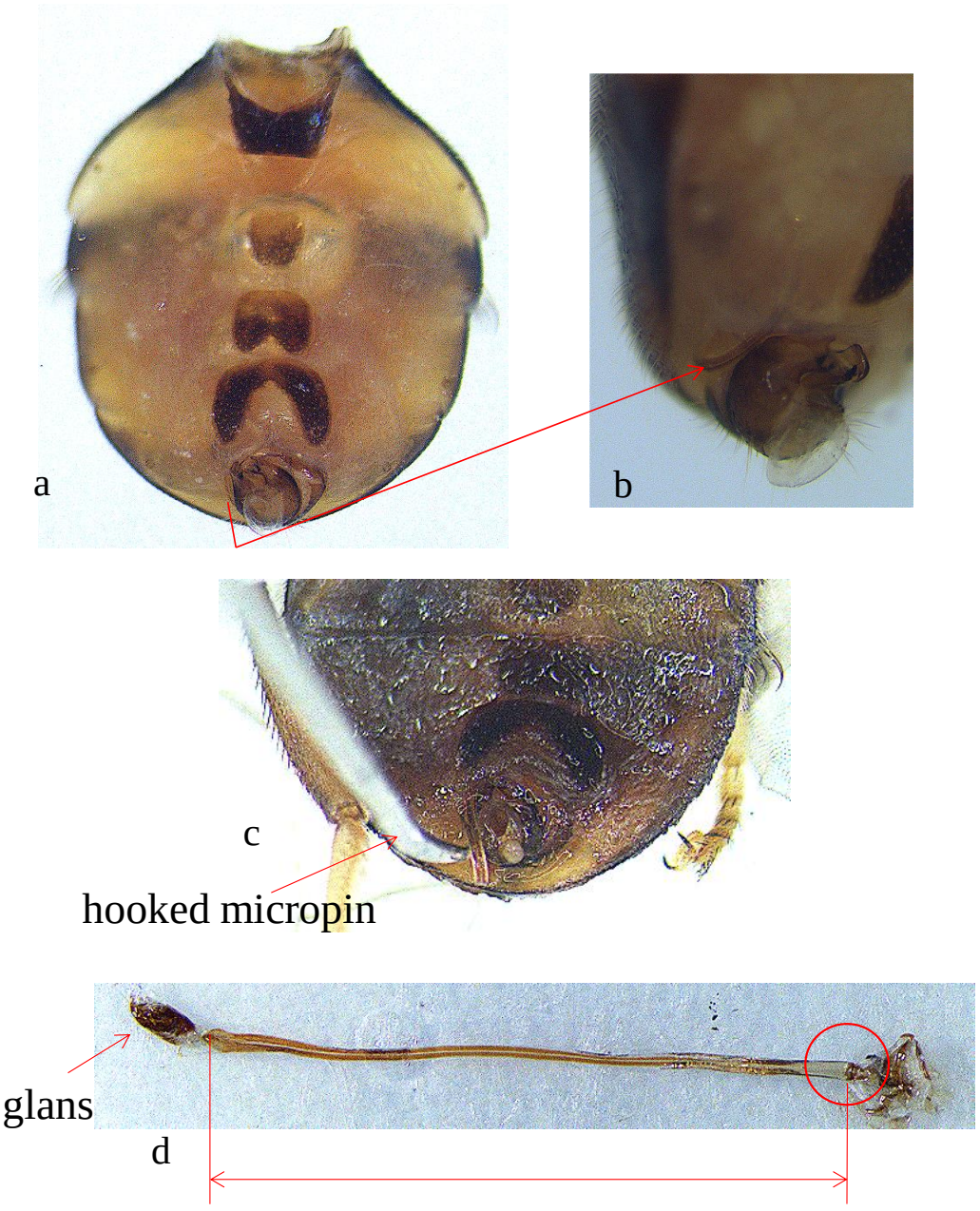
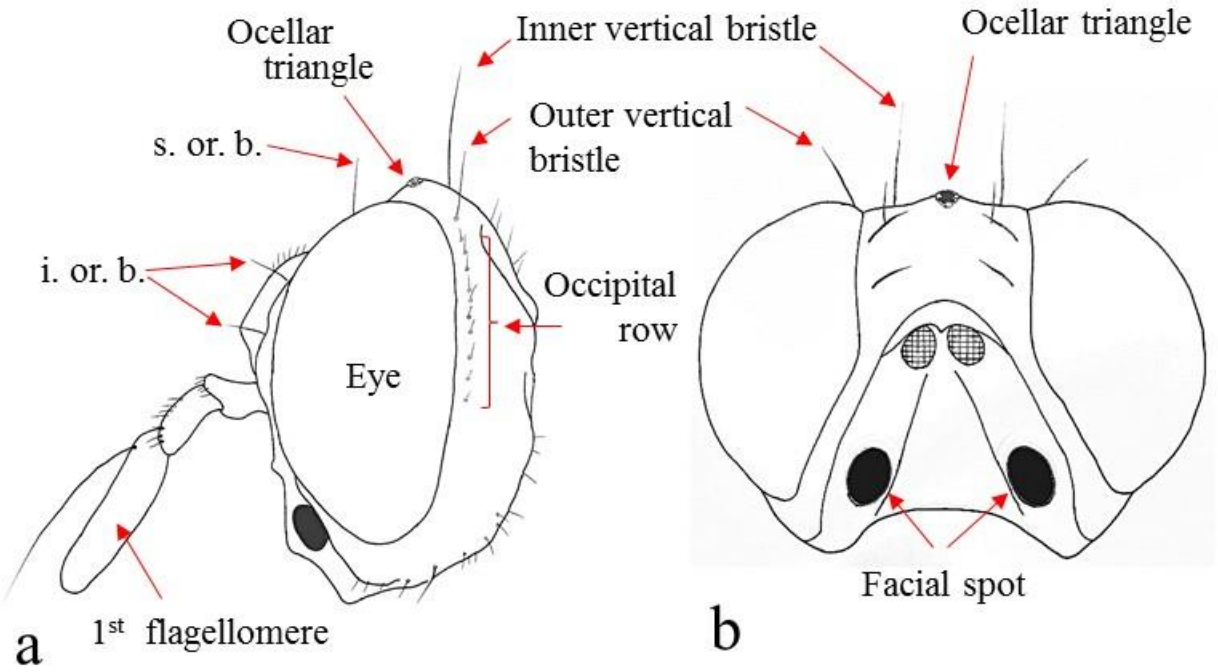


Figure 8. Male abdomen and aedeagus (*B. dorsalis s.l.*): (a) abdomen in ventral view (KOH treated); (b) part of aedeagus appearing rightside (when base of abdomen set upside-down and viewed from ventral side) of epandrium; (c) pulling out aedeagus using hooked micropin; (d) extended aedeagus, showing the part to be measured.
Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.



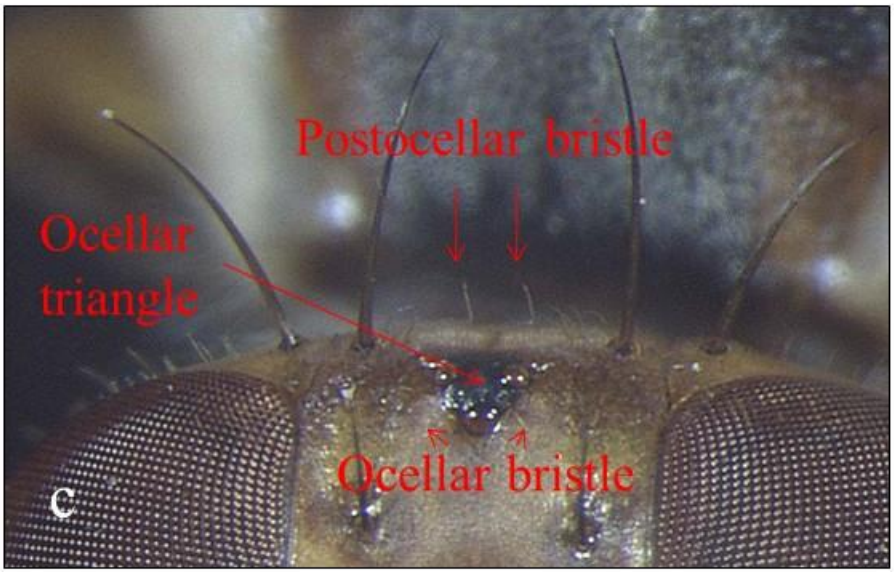


Figure 9. (a) Lateral view of Dacinae head. (b) Frontal view of Dacinae head. (c) Dorsal view of Dacinae head (vertex). i. or. b., inferior fronto-orbital bristles; s. or. b., superior fronto-orbital bristles.
Source: Photo and line drawings courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

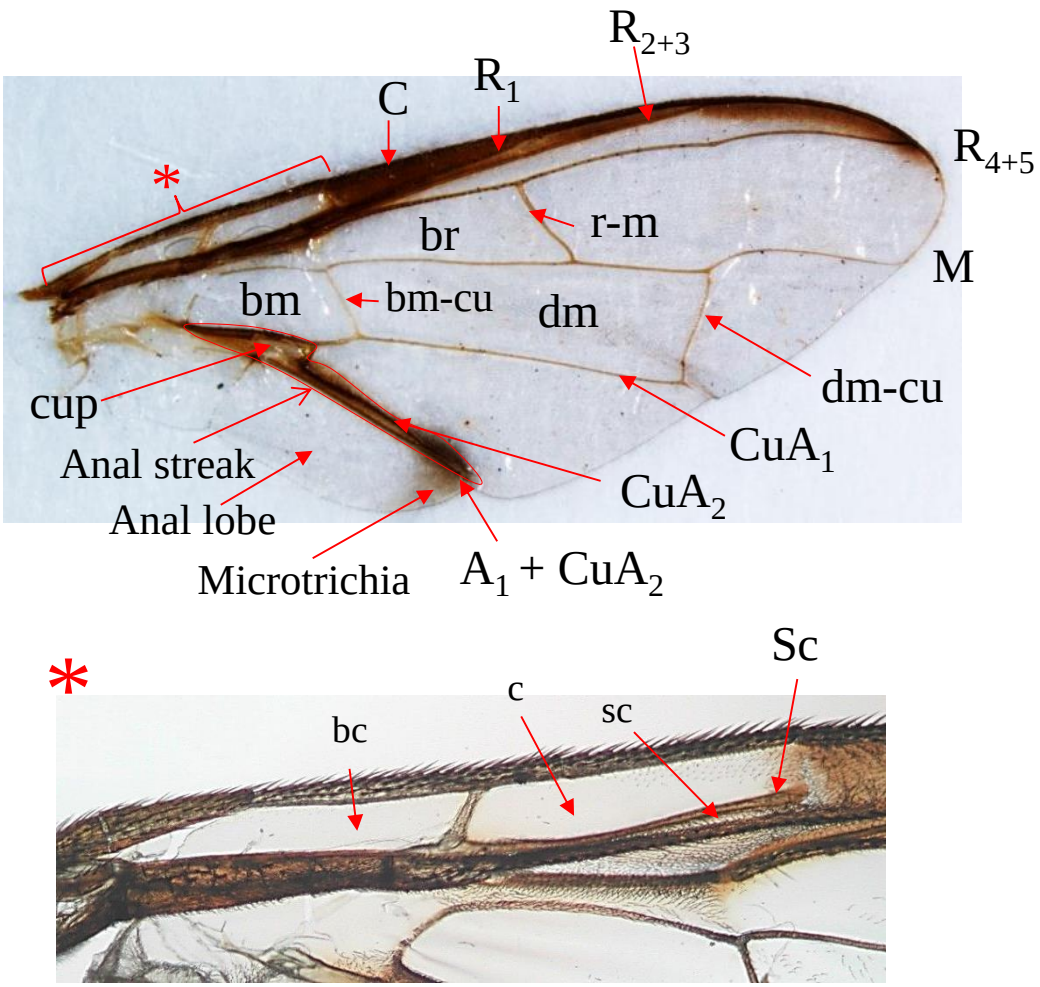


Figure 10. Wing of Dacinae (top) with a magnified view of c and bc cells marked by asterisk (bottom). Veins: A_1, branch of anal vein; bm-cu = basal medial-cubital crossvein; C, costa; CuA_1, CuA_2, anterior branches of cubitus; dm-cu, discal medial-cubital crossvein; M, media; R_1, anterior branch of radius; R_{2+3}, R_{4+5}, combined posterior branches of radius; r-m, radial-medial crossvein; Sc, subcosta. Cells: bc, basal costal; bm, basal medial; br, basal radial; c, costal; cup, posterior cubital; dm, discal medial; sc, subcostal. Anal streak, areas around cup and cup extension indicated by red outline.
Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

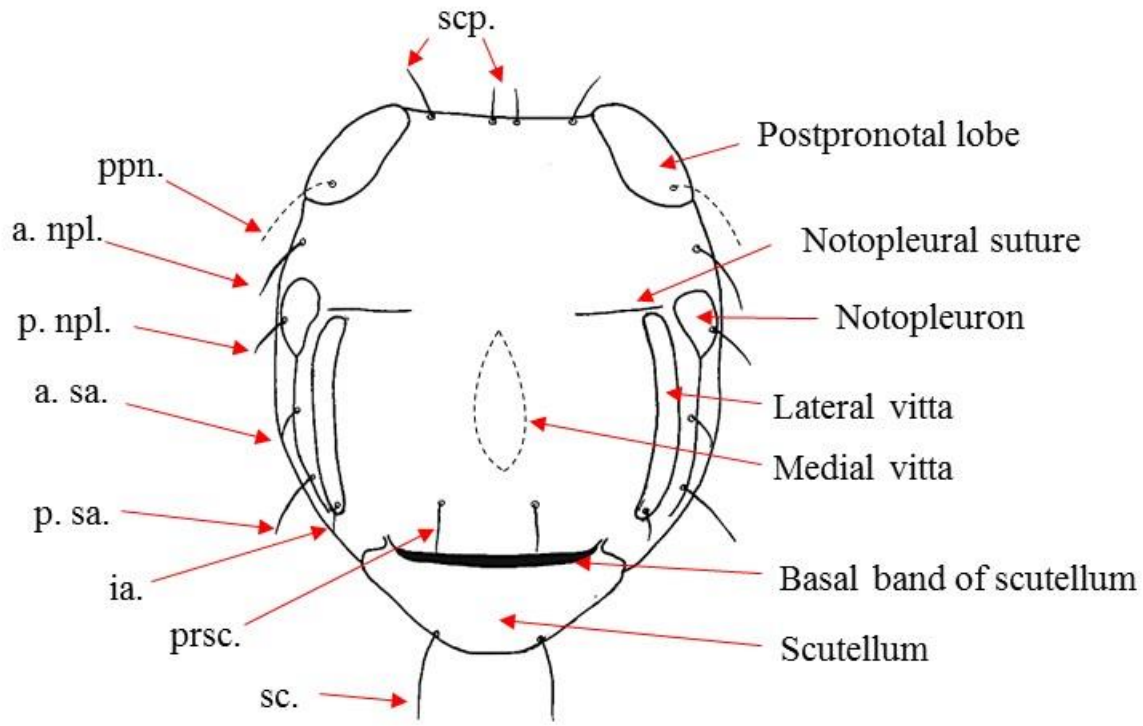


Figure 11. Dorsal view of Dacinae thorax. a. npl., anterior notopleural bristle; a. sa., anterior supra-alar bristle; ia., intra-alar bristle; p. npl., posterior notopleural bristle; ppn., postpronotal bristle; prsc., prescutellar bristle; p. sa., posterior supra-alar bristle; sc., scutellar bristle; scp., scapular bristle.
Source: Line drawing courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

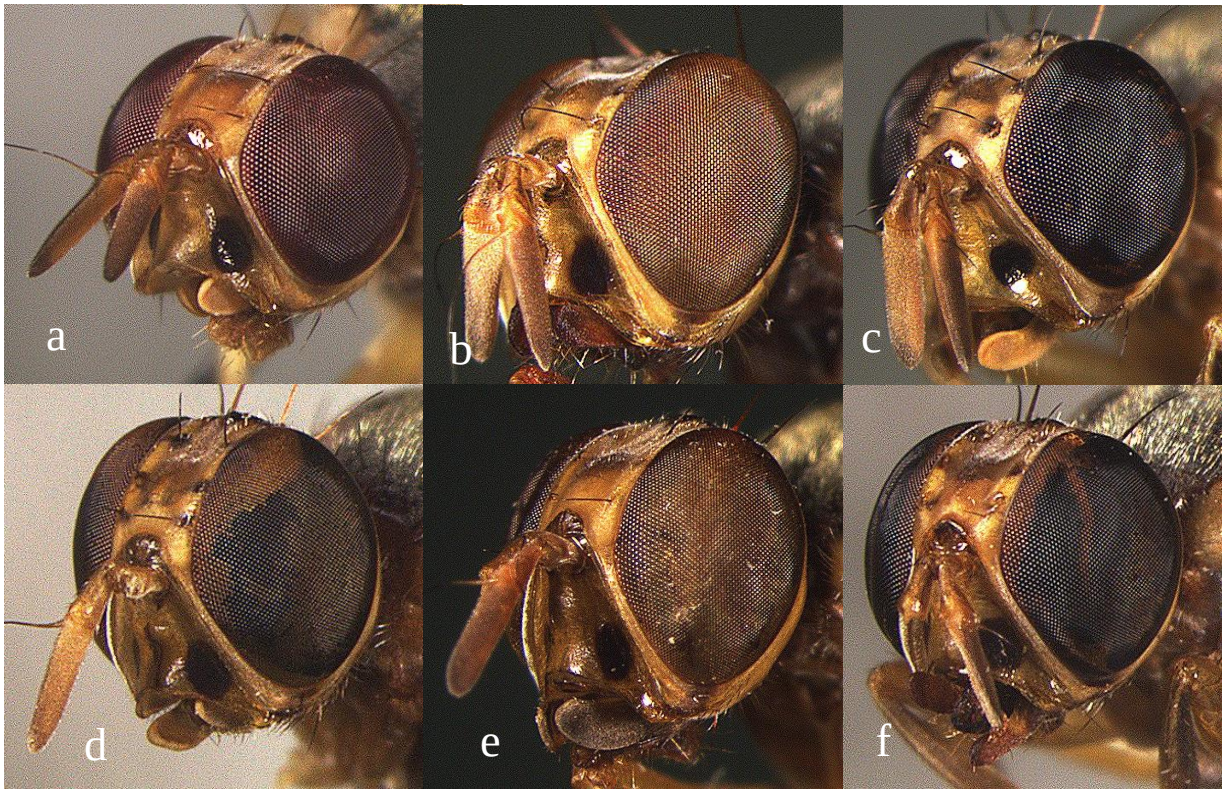


Figure 12. Head in anterolateral view: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis s.l.*; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*.
Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

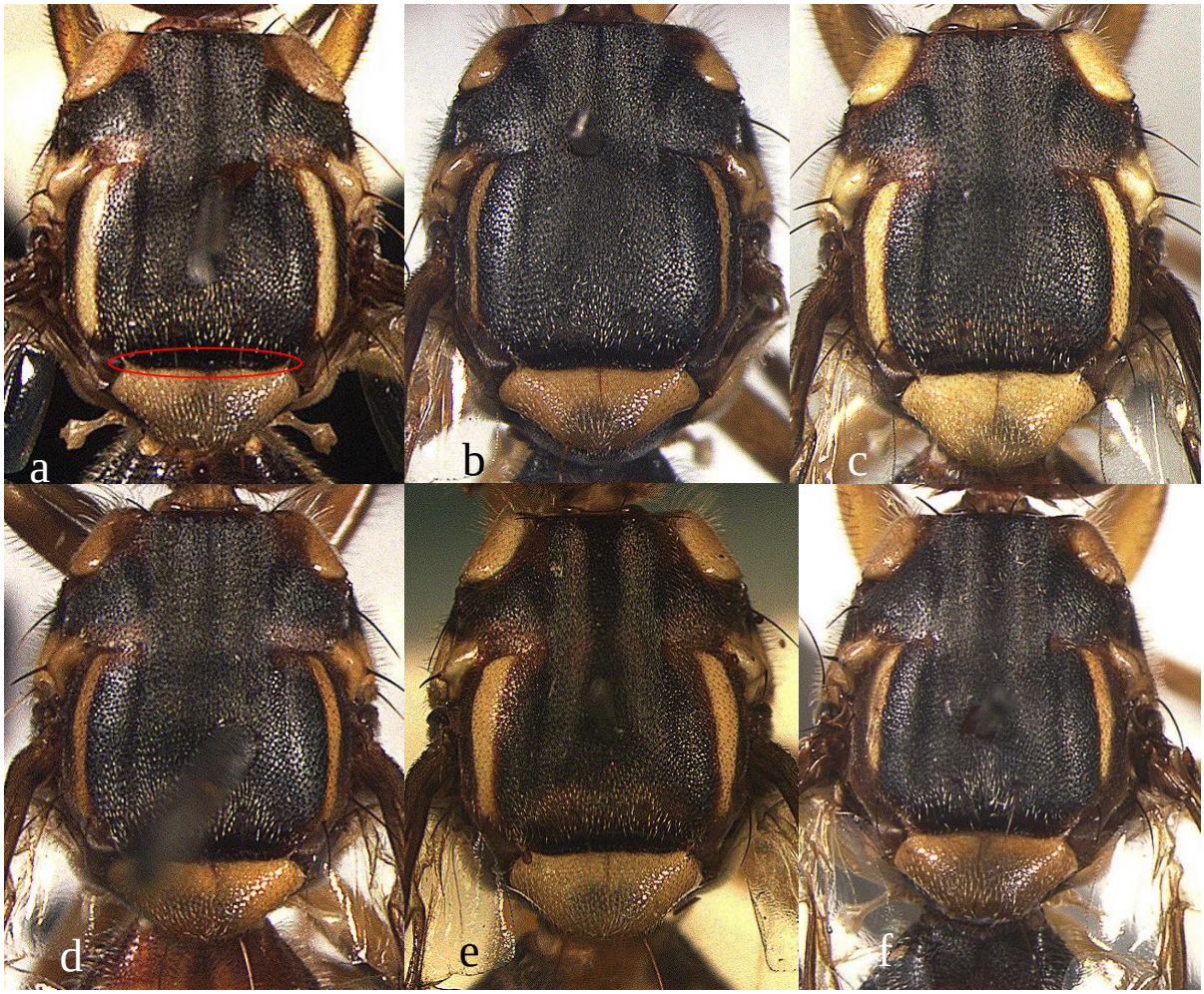


Figure 13. Thorax in dorsal view: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis s.l.*; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*. Basal band indicated by red circle in image (a).
Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

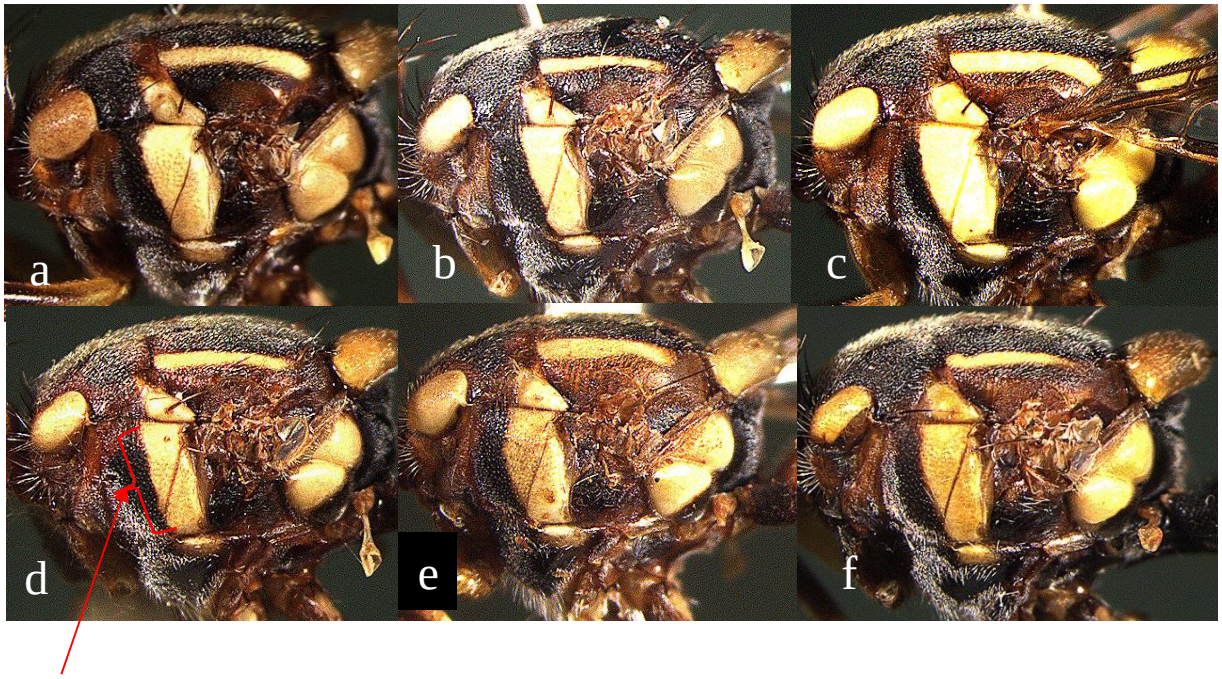


Figure 14. Thorax in lateral view: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis s.l.*; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*. The margin of the episternal stripe is marked in (d).
Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

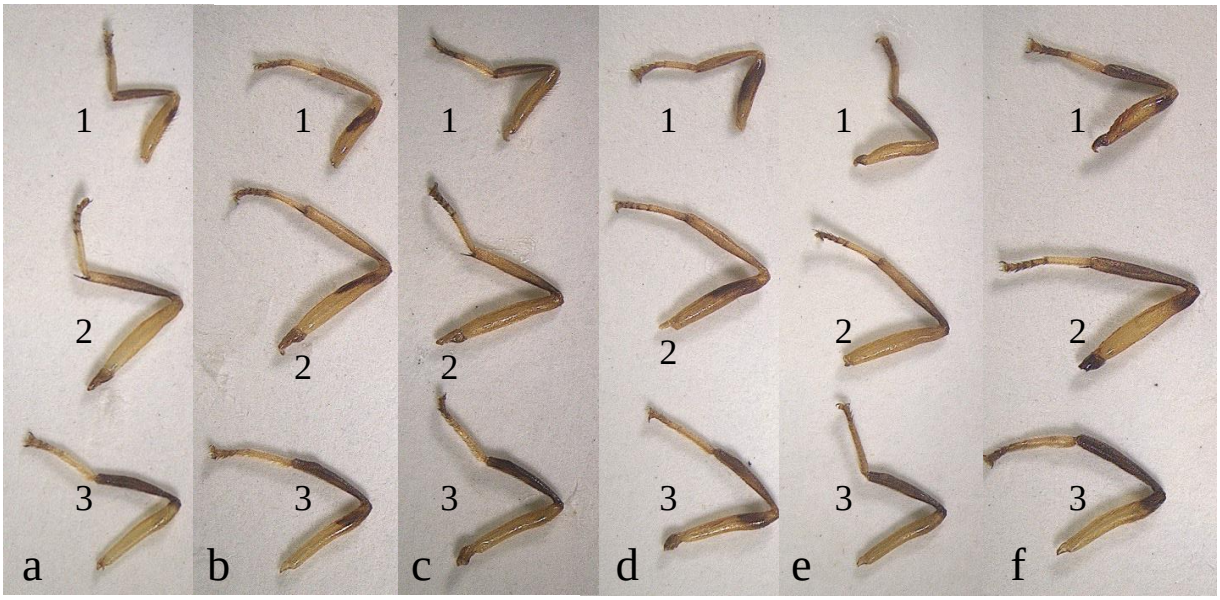


Figure 15. Legs: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis s.l.*; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*. 1, fore leg (outer surface); 2, mid leg; 3, hind leg (inner surface, when folded back alongside abdomen).
Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

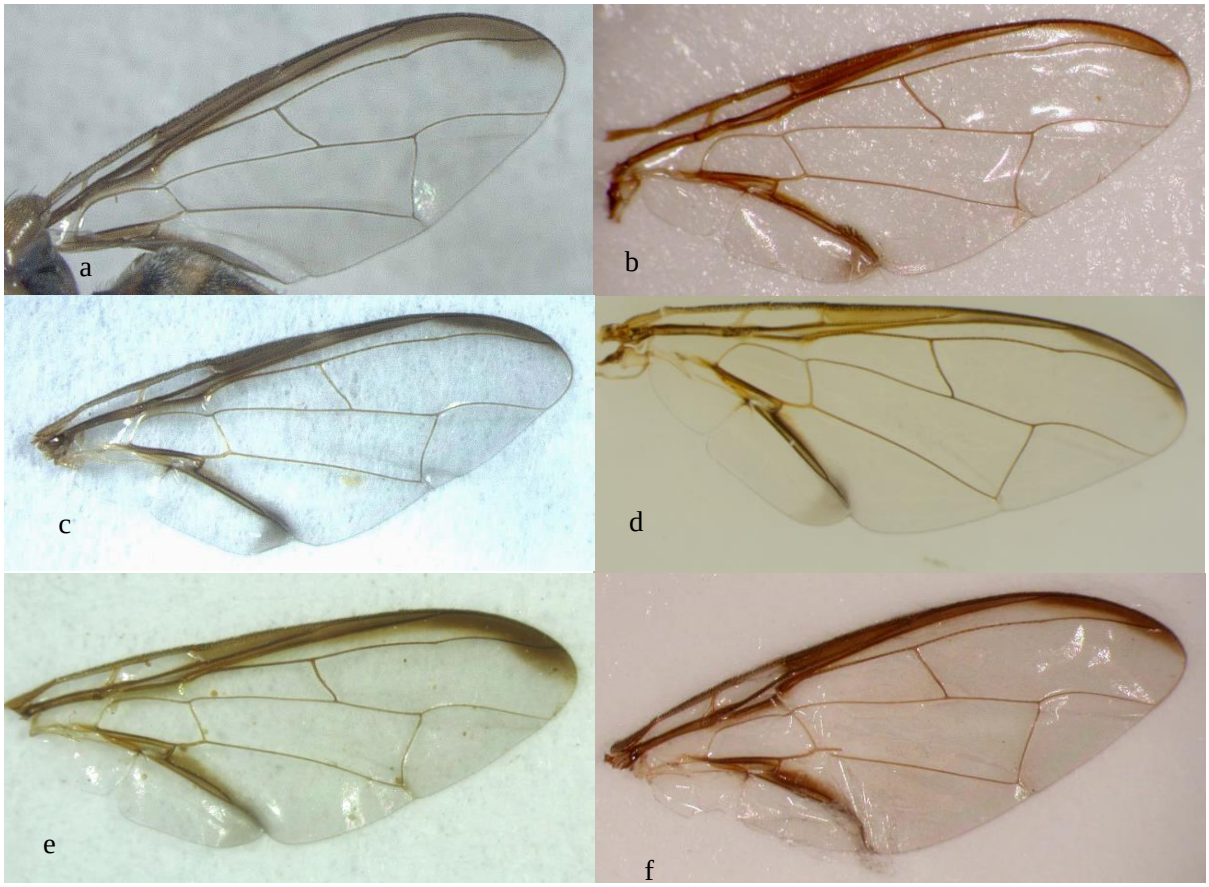


Figure 16. Wings: (a*) Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis s.l.*; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*.
Source: Photos (a–c, e–f) courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan; (d) courtesy of Luc Leblanc, University of Idaho, United States of America.

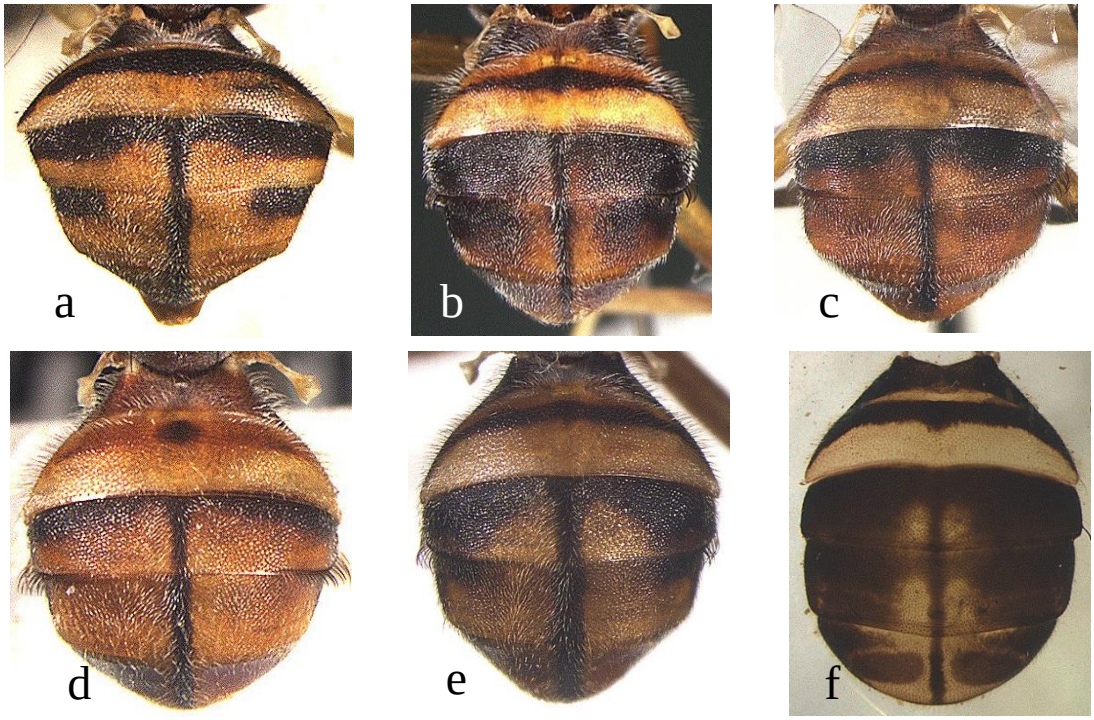


Figure 17. Abdomen in dorsal view: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis s.l.*; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*.
Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

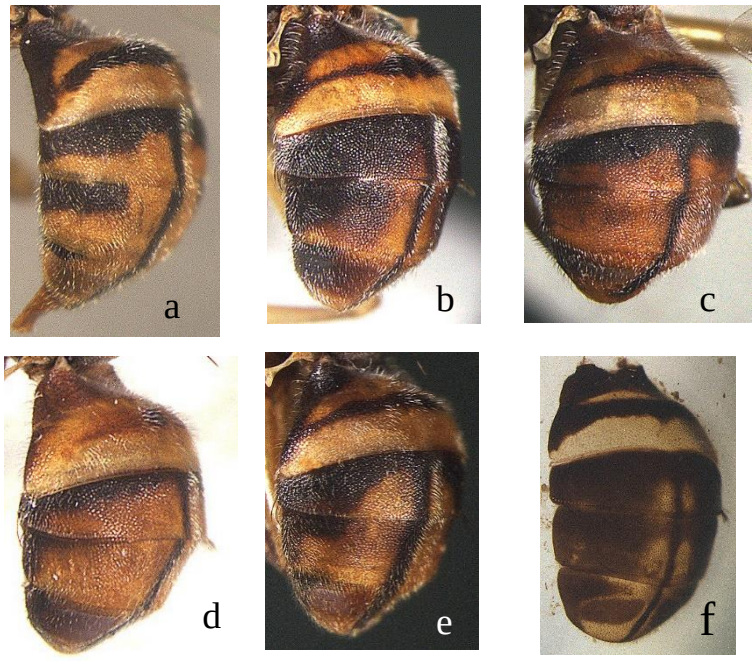


Figure 18. Abdomen in dorsolateral view: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis s.l.*; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*.
Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

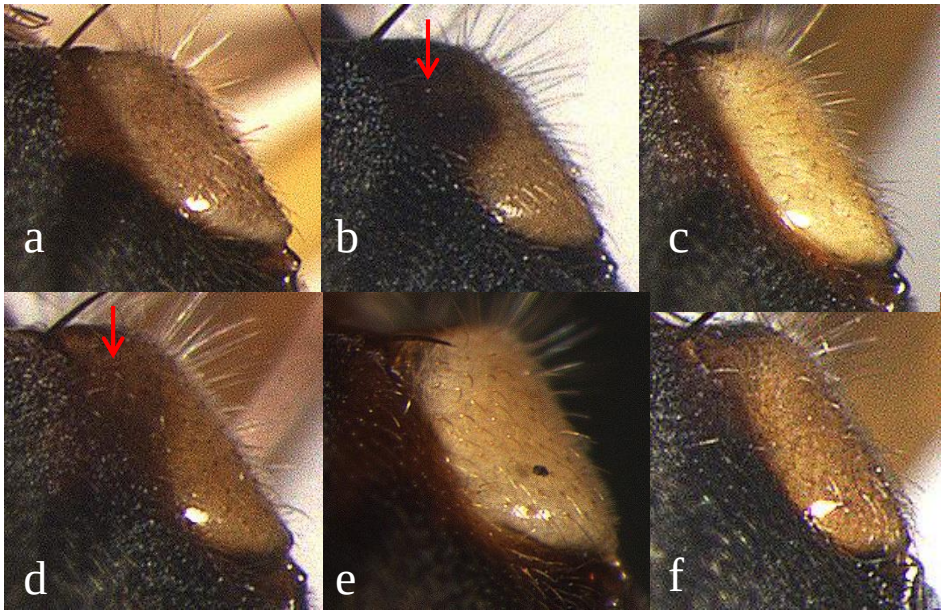


Figure 19. Postpronotal lobes in dorsal view: (a) *Bactrocera carambolae*; (b) *Bactrocera caryeae*; (c) *Bactrocera dorsalis*; (d) *Bactrocera kandiensis*; (e) *Bactrocera occipitalis*; (f) *Bactrocera pyrifoliae*.
Source: Photos courtesy of Yokohama Plant Protection Station, Ministry of Agriculture, Forestry and Fisheries, Japan.

Publication history
This is not an official part of the standard

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2017-07 First consultation.

2017-11 Reviewed by the Lead based on consultation comments.

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2018-03 SC approved draft to be submitted to the 45-day DP notification period (2018_eSC_May_06).

2018-07 DP notification period. Objection received.

2018-09 DP drafting group revised.

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ISPM 27. Annex 29. *Bactrocera dorsalis* (2019). Rome, IPPC, FAO.

Publication history last updated: 2019-03

CPM recommendation: High-throughput sequencing (HTS) technologies as a diagnostic tool for phytosanitary purposes

Status box

This is not an official part of the CPM Recommendation and it will be modified by the IPPC Secretariat after adoption.	
Date of this document	2018-12-06
Document category	Draft CPM recommendation
Current document stage	*To* CPM-14 (2019)
Major stages	2018-03 Topic Next Generation Sequencing technologies as a diagnostic tool for phytosanitary purposes proposed for inclusion in the IPPC work programme for a CPM Recommendation by Australia, EPPO and New Zealand. 2018-04 CPM-13 added topic to IPPC work programme for CPM Recommendation. 2018-05 Adjusted following CPM-13. 2018-05 Consultation (15 May – 15 August 2018). 2018-09 Revised following consultation period. 2018-10 CPM Bureau. 2018-12 CPM Bureau. 2019-04 CPM-14 adopted the CPM recommendation on Preparing to use high-throughput sequencing (HTS) technologies as a diagnostic tool for phytosanitary purposes (R-08).
Notes	This is a draft document and it was presented for consultation period in English only 2018-08 Title amended in response to Consultation (“Next generation sequencing” changed to “High-throughput sequencing (HTS)”) 2018-10 Edited 2018-12 Edited (in part)

BACKGROUND

The Commission on Phytosanitary Measures (CPM) recognizes that accurate and timely pest diagnosis underpins export certification, import inspections and the application of appropriate phytosanitary measures[1]. It is widely accepted that the ability to detect and identify a plant pest varies with the accuracy, reproducibility and specificity of the detection tools.

High-throughput sequencing (HTS) technologies, also known as next generation sequencing (NGS) or deep sequencing technologies, have the potential to provide an alternative to traditional diagnostic methods for the detection and identification of organisms (e.g. bacteria, fungi, phytoplasmas, viruses and viroids). However, the HTS-based diagnostic outcomes may not be associated with evidence of living pests or damage to the plant or plant products by these organisms. As such, caution should be applied when interpreting the results of these highly sensitive technologies, such as HTS, for the detection and identification of pests. In particular, due consideration should be given to the risks and consequences of applying HTS-diagnostic results when implementing phytosanitary measures. Furthermore, HTS technologies may not suit all national plant protection organizations (NPPOs) due to the high cost in platform and other operational costs. It should also be noted that each NPPO may use a different HTS platform.

More information on HTS technologies is provided in Appendix 1.

ADDRESSED TO

Contracting parties and regional plant protection organizations.

RECOMMENDATIONS

The Commission notes that there are existing challenges and further work is needed on HTS technologies for pest detection and identification as the basis for applying phytosanitary regulations. Findings based on HTS technologies of an unknown microorganism need to be further investigated to demonstrate the potential of that microorganism to be a pest that would qualify as a regulated pest.

Before a contracting party proposes to use HTS technologies and their results as the basis for appropriate phytosanitary regulations, the Commission *encourages* contracting parties to:

(a) *establish* guidelines on what phytosanitary actions, if necessary including pest risk analysis, should be taken after detection of an unknown organism (e.g. fungi, bacteria or virus) or detection of non-viable organisms in plant material

(b) *ensure* that appropriate infrastructure and investments in Information Technology and bioinformatics, and education and training on bioinformatics, are in place for the appropriate data storage and interpretation of test results, and that there is effective implementation of these technologies

(c) *standardise* and *apply* best-practice operational guidelines for HTS, including proper interpretation of results and quality control measures (e.g. procedure controls) that ensure HTS data outputs are robust and accurate, have biological significance in a phytosanitary context, and are implemented in a harmonized way

(d) *validate* the reliability and accuracy of HTS by conducting trials comparing HTS against other existing diagnostic platforms

(e) *communicate* information on the interpretation of HTS results, especially regarding conclusions about the phytosanitary risk of organisms detected, to the NPPO of the exporting country

[1] See also CPM Recommendation R-07: The importance of pest diagnosis (https://www.ippc.int/en/publications/84234/)

(f) *implement* HTS training programmes, including delivery of best laboratory practice courses online, and *coordinate* international proficiency testing to independently assess laboratory capability

(g) *publish* HTS protocols (developed for corresponding HTS platforms), and *share* guidelines and training material for transparency

(h) *publish* information on the unexpected biological associations of quarantine organisms in plants and plant products that are revealed by HTS.

RECOMMENDATION(S) SUPERSEDED BY THE ABOVE

None.

This appendix is for reference purposes only and is not a prescriptive part of the CPM recommendation.

APPENDIX 1

Background

In December 2017, the Bureau of the Commission on Phytosanitary Measures (CPM) considered a paper prepared by the Standards Committee (SC) which reflected discussions by the IPPC Technical Panel on Diagnostic Protocols (TPDP) on opportunities and challenges in relation to the use of high-throughput sequencing (HTS) technologies as a diagnostic tool for phytosanitary purposes. The Bureau was asked to agree that the background paper be presented to CPM-13 with a request that the CPM note the challenges associated with the use of HTS technologies and that further work is needed on HTS technologies for pest detection and identification.

The SC prepared a paper on the use of HTS technologies as a diagnostic tool for phytosanitary purposes based on IPPC TPDP discussion on HTS opportunities and challenges.

In December 2017, the CPM Bureau was asked to agree that the background paper be presented to CPM-13 with a request to CPM to note the challenges associated with the use of HTS technologies and that further work is needed on HTS technologies for pest detection and identification.

The CPM Bureau agreed that since this was an emerging issue that would be of interest to contracting parties, a CPM recommendation should be drafted to provide policy advice and guidance to contracting parties and regional plant protection organizations (RPPOs) on the use of HTS technologies as a diagnostic tool for phytosanitary purposes.

Australia, New Zealand and the European and Mediterranean Plant Protection Organization (EPPO) presented a draft CPM recommendation during CPM-13 and it was agreed to include the topic in the IPPC work programme for a CPM recommendation on "High Throughput Sequencing technologies as a diagnostic tool for phytosanitary purposes".

What is HTS and how is it different to other testing methods?

High-throughput sequencing (HTS) technologies, also known as next generation sequencing (NGS) or deep sequencing technologies, allow the sequencing of the whole genome and can be used for all types of organisms being of particular interest for non-culturable organisms (e.g. viruses and viroids, and some bacteria, oomycetes and fungi). HTS technologies can be used for targeted detection of regulated pests and also allow the detection of unknown organisms (i.e. without a priori knowledge). HTS technologies allow the sequencing of the genetic material, which can be used to identify the genome of microorganisms of phytosanitary interest that currently, with traditional technologies, have not been identified. Applying these technologies has recently resulted in the discovery of previously undetected microorganisms, such as fungi, bacteria, phytoplasmas and particularly viruses where the use of the technology is more advanced than for other pathogens (examples provided in this document are for viruses and viroids). Researchers and diagnosticians using HTS technologies will continue to identify and describe new taxa from among the large volume of as yet undiscovered organisms for which challenging and quick decisions will have to be taken by national plant protection organizations (NPPOs) on the basis of very limited information and imprecisely evaluated potential phytosanitary risks (Olmos *et al.*, 2018). These technologies, therefore, enable a new and comprehensive approach to the detection and characterization of potential pests in a biological sample.

Phytosanitary testing for viruses and viroids in plants and plant products currently relies on a combination of specific (molecular and serological) and generic (visual inspection, electron microscopy and biological indicators or bioassays) approaches. While these methods are currently the best available and widely used in plant pest diagnostic laboratories they have some inherent weaknesses. The specific tests usually require a priori knowledge of the viral pathogens and each test needs to be developed and validated (including validation of the test for different pest-host combinations), making resource

demands on NPPOs. Moreover, such specific tests can also detect nucleic acid or protein traces of disintegrated pathogen particles, resulting in an overestimation of actual pathogen presence. The host range of many pathogens is not well defined and exotic viruses and viroids may not be detected in new pest-host combinations. While bioassays have traditionally been used to detect unknown viruses, further molecular or serological testing is usually required to confirm the identity of the causal agent when disease symptoms are observed. Bioassays are heavily reliant on environmental conditions for symptom expression and often produce ambiguous results as false positives and false negatives.

The time taken for bioassays means that plants spend extended periods of time in post-entry quarantine stations, significantly adding to costs and delays for importers. A further drawback with bioassays is that strains may not be detected if they are asymptomatic on the indicator host. Studies conducted so far have demonstrated HTS to be equivalent to or better than biological indexing assays in detecting viruses and viroids of agronomic significance (Al Rwahnih *et al.*, 2015; Barrero *et al*., 2017; Mackie *et al*., 2017; Rott *et al.*, 2017). Most importantly, the studies demonstrated that HTS is able to produce results significantly quicker than bioassays. Nevertheless, HTS technologies are used alongside other existing tests and do not replace the need to confirm the biological significance of the detected organism.

Owing to the limitations of traditional diagnostic methods, new robust, reliable and cost-effective methods are required to rapidly and reliably screen plants and plant products for viruses and viroids but also for other non-culturable or fastidious pests, and HTS technologies open up such possibilities. Metabarcoding or HTS technologies applied to polymerase chain reaction (PCR) amplicons of a DNA barcode region also have diagnostic applications for phytosanitary purposes. Other than viruses, the aforementioned diagnostic applications for phytosanitary purposes could be the most likely diagnostic applications of the HTS approach.

In routine diagnostics, some opportunities and possibilities for the use of HTS technologies are for (1) understanding the pest status in a region through surveillance programmes, (2) certification of nuclear stock and plant propagation material, (3) (post-entry) quarantine testing, and (4) monitoring of imported commodities for new potential pest risks. HTS offers a wide range of benefits for all of these applications (Al Rwahnih *et al*., 2015; Hadidi *et al.*, 2016; Rott *et al.*, 2017). Nevertheless, challenges are also associated with the implementation of these technologies, such as the requirements for laboratory infrastructure, bioinformatics, data sharing and validation of the data (Olmos *et al.*, 2018).

Regulatory and scientific challenges

As new technologies become available, there are inherent challenges associated with them. HTS technologies have similar challenges to other molecular detection or sequence based detection technologies. However, research findings based on HTS technologies have significant implications within a phytosanitary framework. For example, there is a risk that the movement of plant material may be restricted due to the perceived presence of a (previously unknown) microorganism that does not have the potential to be pathogenic to this plant material. Not all organisms associated with plants are pests and instead are part of the plant microbiome; some may be mutualists providing benefit to the host plant or may be commensal agents. Ensuring that regulatory decisions are made on pests, and not on mutualists or commensal agents, is a key criterion to the adoption of whole genome sequencing as a diagnostic method. There is also the issue, as with other indirect methods, that HTS technologies may detect non-viable organisms.

Correctly identifying or predicting pests from whole genome sequences are two separate but important challenges using these technologies. The correct interpretation of results is another major challenge in using HTS technologies. Very large and well curated databases of the whole genomes or barcodes of known pests and microorganisms are required as the reference for comparison with HTS generated sequence data. Because of the increased rate of new microorganism discovery, NPPOs will face the challenge of making decisions about the biological significance of a finding, for example the ability of a microorganism to infest plants or plant products, on the basis of nucleic acid data analysis without

complete information (or even having no information). This decision-making process, of determining if the organism in question is a pest, distances the diagnostic outcome from any analysis of pathogenicity and poses questions in deciding whether the data are linked to the actual presence of a viable and pathogenic biological entity that is a quarantine pest. However, this same challenge is present with molecular and first generation sequencing methods and particularly for viruses that are "new to science", so this is not a new problem. Other challenges in using HTS for regulatory purposes are noted by Martin *et al*. (2016), Massart *et al*. (2017) and Olmos *et al.* (2018).

To give NPPOs the confidence to adopt HTS technologies for pest diagnosis, internationally harmonized approaches are required, including the development of operational guidelines for reliably and repeatedly performing HTS including quality controls and validation data to interpret HTS outputs (Boonham *et al*., 2014). Validation of the technology against existing methods, which also takes into account the limits of current procedures, is also needed. HTS technologies need to be thoroughly validated for each target pest and matrix to demonstrate that they are "fit-for-purpose". Laboratory protocols would need to be available, along with a description of sample preparation, the process for data analysis and the databases to be used.

Global collaboration

There are a number of initiatives underway in different regions of the world that are exploring the use of HTS technologies as a diagnostic tool for phytosanitary purposes (for example in Australasia, Europe and North America). These include discussions on associated policies that may be developed. Coordination of outcomes from these initiatives is required to progress the timely development of internationally harmonized standards for the use of HTS in a regulatory setting.

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