REPORT

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4-8 April 2016

Eleventh Session of the Commission on Phytosanitary Measures

4-8 April, 2016

Food and Agriculture Organisation of the United Nations

CONTENTS

APPENDICES

1. Opening of the Session

1.1 FAO Opening

[1] FAO Deputy-Director-General for Operations Mr. Dan Gustafson welcomed delegates. He noted the relationship between high-level global initiatives such as the Paris Agreement on Climate Change, the 2015 UN Sustainable Development Goals, and the natural links to the normative work of IPPC in sustaining and improving plant health around the world. In reaffirming the importance of IPPC to FAO he also acknowledged work on new initiatives in the IPPC Secretariat for internal cohesion under "one IPPC". He reiterated the importance of strengthening partnerships with others and of the need to increase visibility and awareness in promoting the mission of IPPC, especially through the drive to secure a UN International Year of Plant Health in 2020.

1.2 IPPC towards 2020

[2] The Secretary of the IPPC presented the themes and goals, which will guide and inform the work of the Secretariat over the next five years[1].

2. Keynote Address on Plant Health and Food Security

[3] Dr. Rudy Rabbinge, University Professor Emeritus in Sustainable Development and Food Security at Wageningen University in the Netherlands addressed delegates[2].

3. Adoption of the Agenda

Provisional agenda

[4] The Chairperson detailed changes to the agenda and the order in which items would be addressed. The list of participants is presented in Appendix 03.

[5] The CPM:

(1) *Adopted* the Agenda and noted the Documents list. (See Appendix 01 and 02)

3.1 EU statement of competence

[6] The European Commission presented a paper outlining the Declaration of Competences and Voting Rights[3] submitted by the European Union (EU) and its 28 member states.

[7] The CPM:

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

4. Election of the Rapporteur

[8] The CPM:

(1) *Elected* Ms. Olga Lavrentjeva (Estonia) as rapporteur and Ms. Phyllis Githaiga (Kenya) as assistant.

[1] CPM 2016/INF/01

[2] Available on line https://www.ippc.int/static/media/files/publication/en/2016/05/Food_Security_RudyRabbingeFAO04042016v3RR.pdf

[3] CPM 2016/CRP/04**.** All CPM-11 (2016) documents are available at https://www.ippc.int/en/core-activities/governance/cpm/

5. Establishment of the Credentials Committee

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

[10] The Committee would be assisted by the FAO Legal Office in determining the validity of Contracting Parties' (CP) credentials.

[11] The CPM:

(1) *Elected* a Credentials Committee to conform to FAO rules.

(2) *Elected* Mr Ngatoko Ta Ngatoko (Cook Islands) as the Chairperson of the Credentials Committee. The Credentials Committee established one list containing 123 valid credentials in conformity with current rules established by the FAO governing bodies. The number to establish a quorum for the Commission was set at 91.

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

[12] The CPM chairperson, Ms Kyu-Ock Yim, presented her report[4].

[13] The CPM:

(1) *Noted* the report.

7. Report by the IPPC Secretariat

[14] The Secretary introduced the 2015 annual report[5].

[15] In response to a request from some CPs, the Secretariat agreed to make available, during CPM11, a summary report on the Workshop on Synergies among the Biodiversity-Related Conventions held in Geneva in February 2016.

[16] Some CPs highlighted the importance of reporting being forward looking and welcomed the linkage between activities and future direction, including liaison with partner organizations.

[17] The CPM:

(1) *Noted* the report.

8. Governance

8.1 Summary of the Strategic Planning Group report

[18] The Chairperson of the Strategic Planning Group (SPG), Ms. Lois Ransom, presented the report[6]. She noted the SPG had focused on the next five years leading up to the proposed International Year of Plant Health (IYPH) in 2020 under the theme of 'The IPPC towards 2020', and on planning the development of the strategic framework for the IPPC from 2020 to 2030 using 'the IPPC in 20 years' as a starting point. The concept of a commodity standard had also been discussed.

[19] The Chairperson emphasized the need for strategic planning and thinking, and stated that it was important for CPs to focus on their strategic needs for inclusion in the strategic framework. She encouraged CPs to submit discussion papers to the Secretariat to move the work of the SPG forward.

[20] Some CPs stated the importance of the SPG as a vital forum for analysis and discussion on strategic priorities and approaches for the IPPC. They expressed support for the SPG discussion paper by

[4] CPM 2016/29

[5] CPM 2016/35

[6] CPM 2016/25

Canada[7], supported by Australia, New Zealand and the United States, and their commitment to maintaining the SPG as a valuable forum to provide insight, feedback and guidance to assist the CPM, Bureau and Secretariat on strategic matters.

[21] One CP proposed that the next SPG meeting should focus on four strategic themes (1-4 below) and another CP proposed that that these topics be added to the next SPG agenda.

(1) Identify areas and activities, which would support the CPM's 2017 annual theme on "plant health and trade facilitation."

(2) Continue discussions on the International Year of Plant Health.

(3) Begin discussions about the content, elements, process and timetable for preparing and finalizing the new IPPC Strategic Framework by 2020.

(4) Discuss future funding directions and concepts over the next five years to better support the Secretary's resource mobilization efforts and to consider the strategic alignment of limited resources to priority activities.

[22] One CP added their support to the vital role of the SPG and noted that their in-kind contribution had been split between standard setting and strategic work and that they would be looking to renew this in-kind contribution to the SPG in 2016.

[23] The CPM:

(1) *Noted* the activities of the SPG as presented in the summary.

(2) *Agreed* the themes for the years leading to the IYPH would be:

a. 2016 – Plant Health and Food Security

b. 2017 – Plant Health and Trade Facilitation

c. 2018 – Plant Health and Environmental Protection

d. 2019 – Plant Health and Capacity Development

8.2 Framework for standards and implementation

[24] The Secretariat introduced the paper[8] on the Framework for Standards and Implementation which has been under discussion within the CPM, SPG, SC and CDC.

[25] Some CPs expressed concern about the process for amendment and the availability of the most current version of the Framework on the IPP and proposed, that for clarity and transparency, the Framework for Standards and Implementation be presented annually to CPM for endorsement. They further noted that the Standards Committee and future Implementation and Capacity Development Committee would therefore be required to review the Framework annually and to propose changes to the CPM.

[26] CPs also expressed the views: that the consideration of new topics should be made against the framework; discussions on topics should be held at the CPM; there should be better cooperation between the SC and the CDC in updating the framework; items should not be included when their inclusion had been discussed by CPM but had not been agreed by CPM.

[27] The Secretariat noted that the framework was a flexible document to be reviewed on an annual basis and where gaps and topics could also be added, removed and priorities changed by the CPM based on recommendations. The essential element was to present the overall work programme of the CPM to more easily see completed work, work under development and future planned work. This was the reason for the inclusion of both the items under the oversight of the SC and CDC. The CPM agreed that a

[7] https://www.ippc.int/static/media/files/publication/en/2015/10/06_SPG_2015_Oct_CFIA_ACIA_-Reflection_on_the_future.DOCX

[8] CPM 2016/20

framework was needed but identifying those gaps will come from the CPM's subsidiary bodies and endorsed by the CPM annually.

[28] The CPM:

(1) *Endorsed* the use of the Framework for Standards and Implementation to record the standards and other tools for implementation that support and enable the implementation of the Convention and ISPMs to facilitate harmonisation. This Framework would include standards and other tools that have been adopted/developed, are under development or are planned to be developed.

(2) *Adopted* the Framework for Standards and Implementation (Appendix 04) and *agreed* that it is a working document which will be periodically updated, and provides transparency of existing or proposed standards and tools for implementation and assists with the identification of gaps and it would be a means of capturing agreed priorities for standards and implementation facilitation tools that are separately approved by CPM.

(3) *Agreed* that the Framework for Standards and Implementation is updated and maintained by the Secretariat, with responsibility for review and amendment resting jointly with the SC and CDC (or the CDC replacement) and reviewed by SPG.

(4) *Agreed* that the updated framework is presented annually to the CPM for endorsement.

(5) *Agreed* that the most current version of the Framework for Standards and Implementation will be maintained and be fully accessible on the IPP.

8.3 Concept of a commodity standard

[29] The Chairperson of the working group Ms. Jane Chard introduced the papers[9].

[30] Some CPs stated the need to shift the focus of standard setting to include developing more commodity standards to the benefit of both importing and exporting countries. They proposed to develop as a pilot a fully-fledged commodity specific ISPM with a narrow scope that included options for specific requirements and pest management measures. The benefits and challenges of developing commodity specific standards would be obtained as a result of the process of development of such a pilot standard. They considered that there is a continuum of scopes from broad to very narrow commodity standards and that further analysis was not needed to define and apply layers for commodity standards in the Framework for Standards and Implementation.

[31] Some CPs acknowledged the complexity that CPs face in dealing with commodities through ISPMs and proposed to follow the regular process for topics as they could not see the urgency to add an extra process. They suggested developing a regional standard for a specific commodity of interest as a possible way forward. However other CPs pointed out that the development of a regional standard would not provide CPs with the experience in developing a global standard.

[32] CPs also made the following observations:

- a need to firstly identify gaps in the Framework for Standards and Implementation for a commodity standard
- consider resource implications
- having liaised with Codex Alimentarius Commission due to their specific experience with commodity standards was considered as a good approach
- focus more on defining requirements and guidelines for harmonization that are appropriate to the effective management for phytosanitary risk management
- monitor how the current commodity-type standards that are already on the *List of topics for IPPC standards* evolve (e.g. grain, wood, cut-flowers, plants for planting and wood handicrafts) and consider merits, challenges and limiting factors for commodity type standards

[9] CPM 2016/17, CPM 2016/INF/17, CPM 2016/CRP/02

- some CPs had concerns with the proposal to establish a mechanism for dealing with emerging issues as they felt that a new mechanism was not needed. It was clarified that this proposal was related to emerging issues that require global concerted action from all CPs and that "mechanism" was maybe not the most appropriate term
- in response to questions, the Secretariat confirmed that there had been 11 submissions for topics in answer to the 2015 call for topics for IPPC standards. The SC had recommended four topics to the CPM for addition to the *List of topics for IPPC standards* (refer to section 9.4), but had not recommended to the CPM any of the proposals that were made for commodity specific ISPMs with a narrow scope.

[33] The CPM:

(1) *Noted* the considerations by the WG, SPG and SC and the CDC in relation to the concept of commodity standards.

(2) *Agreed* that the development of commodity standards is no more relevant, feasible or higher priority than any other standards or implementation tools and that there is nothing in the current standard setting procedure that prevents CPs from proposing topics for standards that harmonize the management of phytosanitary risks on a particular commodity or group of commodities.

(3) *Agreed* that a standard need not be tagged as a particular type, such as a commodity standard, but rather focus on defining requirements or guidance for harmonization that are appropriate to the effective management of phytosanitary risks that the standard is intended to achieve and which is defined in its scope.

(4) *Agreed* that a combined call for topics for standards and tools for implementation should be made, which would be reviewed with input from the SC and CDC, or its successor.

(5) *Requested* the SC and CDC to review and adapt the current process to allow the call to be made, including any changes to the assessment criteria needed.

(6) *Agreed* that any submission in response to a call for topics and tools should clearly define the problem needing resolution in sufficient detail to determine how it fits into the Framework of Standards and Implementation and the cost/benefit of the development of the standard or tool.

(7) *Encouraged* CPs to provide phytosanitary resources relevant to the management of pests associated with commodities or groups of commodities for possible inclusion in the phytosanitary resources web page in response to specific calls for resources.

(8) *Requested* the Bureau, in consultation with SC and CDC, to urgently establish a means for dealing with emerging issues that require global action.

8.4 Capacity development and implementation oversight

8.4.1 Review of the Capacity Development Committee (CDC)

[34] The Secretariat introduced the review[10]. The Secretariat gave an overview of the Capacity Development Committee (CDC) evaluation process and provided the outcome of the discussions held related to the evaluation. The CPs thanked those who contributed to the review exercise to finalize the report. The Secretariat proposed that the CDC mandate be extended until the new oversight subsidiary body to the CPM was established and active.

[35] The CPM:

(1) *Discussed* the recommendations of the CDC Review.

(2) *Agreed* to the extension of the CDC until a new oversight committee is established and active.

(3) *Thanked* the members of the CDC for their continuous commitment and productive work in support of IPPC capacity development.

[10] CPM 2016/16

8.4.2 Proposal for a new implementation oversight body

[36] The Secretariat introduced the proposal[11].

[37] CPs expressed broad support for the creation of the new subsidiary body on implementation, but agreed that it was premature to create the new body with the proposed Terms of Reference (ToR) and Rules of Procedures (RoP) prepared by the Secretariat. Some contracting parties proposed that a small group be formed to develop the Terms of Reference for a Focus Group on Establishing an Implementation Committee.

[38] The small group reported back to CPM proposing the Focus Group Terms of Reference for adoption in CPM 2016/CRP/08 and informed the CPM that the European and Mediterranean Plant Protection Organization (EPPO) had offered to host this meeting from 18-22 July 2016.

[39] The CPM:

(1) *Agreed* to abolish the CDC and set up a new subsidiary body on the basis of Rule IX of the Rules of Procedure of the CPM. The CDC should be retained until the new subsidiary body is established.

(2) *Agreed* to establish a Focus Group to carefully consider and propose the purpose, scope and functions of the new subsidiary body, and propose governance, membership, and rules of procedure.

(3) *Agreed* the Focus Group would share the result of their work with the Strategic Planning Group (SPG) meeting in October 2016 for testing and refining prior to Bureau consideration.

(4) *Agreed* the Bureau should recommend to CPM-12 (2017), a Terms of Reference and Rules of Procedure for the new subsidiary body, ensuring the newly drafted documents provided clarity on the purpose, scope, functions, governance, membership, and rules of procedure.

(5) *Adopted* the Focus Group Terms of Reference as outlined in Appendix 05.

(6) *Agreed* that each region, through their Bureau member, should nominate a representative to participate in the Focus Group, by 15th May 2016.

9. Standard Setting

9.1 Report on the activities of the Standards Committee

[40] The Chairperson of the Standards Committee (SC) Mr. Bart Rossel presented the report[12]. He highlighted the activities of the SC in 2015 as well as providing an insight into future activities. He noted the high volume of work within standard setting and highlighted the significant input provided throughout the year by the SC, technical panels and expert working group members as well as the stewards of draft ISPMs. He also acknowledged the professionalism and dedication of the IPPC Secretariat's Standard Setting Unit notwithstanding significant workload and resource constraints.

[41] The CPM:

(1) *Noted* the report on the activities of the Standards Committee in 2015.

9.2 Adoption of International Standards for Phytosanitary Measures

[42] The Secretariat introduced the paper[13] and the drafts proposed by the Standards Committee (SC) for adoption as International Standards for Phytosanitary Measures (ISPMs) by the Commission on Phytosanitary Measures (CPM), noting that no formal objections were received. The Secretariat also

[11] CPM 2016/18; CPM 2016/INF13; CPM 2016/INF/17

[12] CPM 2016/19

[13] CPM 2016/05 rev1, CPM 2016/12

delivered a presentation[14], outlining the standards under development and in particular the high volume of work regarding Phytosanitary Treatments and Diagnostic Protocols.

[43] The CPM:

(1) *Adopted* the Amendments to ISPM 5 *Glossary of Phytosanitary Terms* (1994-001) (Appendix 19). The previous versions of ISPM 5 *Glossary of Phytosanitary Terms* are revoked and replaced by this newly adopted version.

(2) *Adopted* ISPM 37 on *Determination of host status of fruit to fruit fly (Tephritidae)* (2006-031) (Appendix 19).

(3) *Adopted* the PT 20 Irradiation Treatment for *Ostrinia nubilalis* (2012-009) as Annex 20 to ISPM 28 (*Phytosanitary treatments for regulated pests*) (Appendix 19).

(4) *Adopted* the PT 21 Vapour Heat Treatment for *Bactrocera melanotus* and *B. xanthodes* on *Carica Papaya* (2009-105) as Annex 21 to ISPM 28 (*Phytosanitary treatments for regulated pests*) (Appendix 19).

(5) *Noted* that the Standards Committee adopted on behalf of CPM the following five diagnostic protocols as Annexes to ISPM 27 (*Diagnostic protocols for regulated pests*):

- DP 08: *Ditylenchus dipsaci* and *Ditylenchus destructor* (2004-017)
- DP 09: Genus *Anastrepha* Schiner (2004-015)
- DP 10: *Bursaphelenchus xylophilus* (2004-016)
- DP 11: *Xiphinema americanum sensu lato* (2004-025)
- DP 12: *Phytoplasma* (2004-018)

(6) *Noted* the change in process for co-publishing agreements.

[44] The CPM also discussed ink amendments to standards as indicated in CPM 2016/12.

[45] The Secretariat informed the CPM of the work undertaken to translate and incorporate ink amendments, previously noted by CPM in English, into the other official language versions of ISPMs. This work was carried out in the context of the CPM-10 (2015) approved mechanism for revoking standards, and work had been concluded for French and Spanish. The translations had been reviewed respectively by the Language review group (LRG) for Spanish and the Technical Panel for the Glossary member for French. The Secretariat confirmed they would make efforts to undertake the same process for the remaining FAO official languages, but highlighted that extra-budgetary resources would need to be identified. Several members thanked the Standard Setting Unit for progressing with this essential work. This should help facilitate the implementation of updated revisions of ISPMs, particularly in non-English speaking countries.

[46] The Secretariat also reported that, in parallel, the language review groups for Spanish and Chinese reviewed ISPM 5 (Glossary of phytosanitary terms) in the two languages, and made a number of adjustments to correct translation issues, which were then reviewed by FAO Translation services. This would help ensure that the Glossary presents consistent and correct phytosanitary terminology in the FAO languages concerned.

[47] The CPM thanked the contracting parties for providing resources through the LRGs and the TPG to carry out this important work.

[48] The CPM:

(1) *Noted* the process for translating and incorporating ink amendments previously noted in English to the other official language versions of ISPMs.

[14] IPP link to the presentation

(2) *Noted* the ink amendments to the currently adopted phytosanitary treatments as presented in Appendix 06.

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

(4) *Invited* contracting parties to support the work of aligning language versions of ISPMs by making donations to the IPPC Trust fund for this purpose.

9.3 Noting translation adjustments to International Standards for Phytosanitary Measures adopted at CPM-10

[49] The Secretariat introduced the paper noting that Language Review Groups (LRGs) were active for Arabic, Chinese, French and Spanish[15].

[50] The CPM:

(1) *Noted* that the following have been reviewed by the Arabic, Chinese, French and Spanish LRGs and FAO translation services:

- Amendments to ISPM 5 (*Glossary of phytosanitary* terms) (2013).
- Annex 3 (*Phytosanitary procedures for fruit fly (Tephritidae) management*) of ISPM 26 (*Establishment of pest free areas for fruit flies (Tephritidae*)).
- PT 16 (Cold treatment for *Bactrocera tryoni* on *Citrus sinensis*) as annex to ISPM 28 (*Phytosanitary treatments for regulated pests*).
- PT 17 Cold treatment for *Bactrocera tryoni* on *Citrus reticulata x* C. sinensis as annex to ISPM 28 (*Phytosanitary treatments for regulated pests*).
- DP 5 (*Phyllosticta citricarpa* (McAlpine) Aa on fruit) as annex to ISPM 27 (*Diagnostic protocols for regulated pests*) and
- DP 6 (*Xanthomonas citri* subsp. *citri*) as annex to ISPM 27 (*Diagnostic protocols for regulated pests*).

(2) *Noted* that the following have been reviewed by the Arabic, French and Spanish LRGs and FAO translation services:

a) PT 18 (Cold treatment for *Bactrocera tryoni* on *Citrus limon*) as annex to ISPM 28 (*Phytosanitary treatments for regulated pests*) and

b) PT 19 (Irradiation treatment for *Dysmicoccus neobrevipes, Planococcus lilacinus* and *Planococcus minor*) as annex to ISPM 28 (*Phytosanitary treatments for regulated pests*).

(3) *Noted* that the LRG for Russian is currently not operational because no Coordinator has been nominated.

(4) *Encouraged* contracting parties to nominate a Coordinator for the Russian LRG.

(5) *Urged* its members who participate in LRGs to ensure that the deadlines for the CPM adopted LRG process are followed and due dates respected.

(6) *Agreed* that once the Secretariat has applied the changes as indicated in track changes in the Attachments 1 to 30 of CPM 2016/06, the previous versions of the ISPMs are revoked and replaced by the newly noted versions.

(7) *Thanked* contracting parties and regional plant protection organizations involved in the LRGs, as well as FAO translation services, for their efforts and hard work to improve the language versions of ISPMs.

[15] CPM 2016/06

9.4 Topics for IPPC standards - New topics and adjustments to the List of topics for IPPC standards

[51] The Secretariat introduced the paper[16] with proposed adjustments to the CPM adopted *List of topics for IPPC standards*[17] (LOT) which can be viewed on the International Phytosanitary Portal (IPP).

[52] Some CPs proposed the topic on *PRA for Commodities* should not be added to the list of topics as they felt that NPPOs needed practical knowledge and experience, which could be gained through a pilot project on one commodity standard with a narrow scope and not by working on a concept standard.

[53] Another CP supported the proposed addition of the topic as the approach was consistent with other approaches taken for previous standards. They noted that such a standard could provide a policy framework and ultimately create a link between ISPM 11 (*Pest risk analysis for quarantine pests*) and others similar to that currently done by both ISPM 27 *(Diagnostic protocols for regulated pests*) and ISPM 28 (*Phytosanitary treatments for regulated pests*).

[54] It was also proposed that, as commodity standards were considered of high priority, efforts should be made to select a topic for a pilot on a specific commodity standard by CPM in 2016, for example by selecting one of the topics proposed in the 2015 call for topics or by holding an extra call for commodity specific topics during 2016. But other CPs felt the regular standard setting procedure should be followed.

[55] Another CP noted that if the intention was a standard on *PRA for Commodities*, it would be better to consider the re-organization of the PRA conceptual framework (ISPM 2 and 11) as well as other topics in the work program like "pest risk management". In this way if a standard on PRA for specific commodities were to be approved in the future, it could be added as an annex of this revised conceptual framework.

[56] Several CPs expressed support not to remove the topic on *Safe handling and disposal of waste with potential pest risk generated during international voyages (2008-004, priority 2)* from the *List of topics for IPPC standards.* In their view, the matter required special attention to promote sound waste management and to protect plant health. In response, the Secretariat noted that so far an insufficient number of experts from CPM had come forward to work on such a standard.

[57] In response to a question raised on the reorganization of the IPPC fruit fly standards, the Secretariat confirmed that this work was intended to harmonize the fruit-fly standards and to adjust the text accordingly. The Secretariat also clarified that this work will be presented to and reviewed by the Standards Committee in May 2016.

[58] One CP reiterated the importance of providing the proposed new topics in line with the Framework for standards and implementation to allow CPs to consider the overall context of proposals and how the new topics would address the gaps.

[59] It was also suggested that topics linked to trade, which were of more benefit to CPs, be given higher priority.

[60] The CPM:

(1) *Added* the following topics, with the indicated priorities and IPPC Strategic Objectives, to the *List of topics for IPPC standards* :

i. Draft ISPM on *Audit in the phytosanitary context (*2015-003), with priority 2 and IPPC Strategic Objectives A, B and C.

[16] CPM 2016/10

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

ii. Draft Supplement on *Guidance on the concept of the likelihood of establishment component of a pest risk analysis for quarantine pests* (2015-010*)* to ISPM 11 (*Pest risk analysis for quarantine pests*), with priority 4 and IPPC Strategic Objective A, B and C.

iii. Revision of ISPM 12 on *Phytosanitary certificates (*2015-011), with priority 2 and IPPC strategic Objective C .

(2) *Requested* the SC to reconsider the topic on PRA for Commodities (2015-002) as well as other proposals for commodity standards which were made during the 2015 call for topics, with further input from the CP who submitted the topic.

(3) *Noted* the reorganization of the IPPC fruit fly standards and minor technical updates and *added* this work to the *List of topics for IPPC standards*, with priority 2 and IPPC strategic objectives A, B and C.

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

(5) *Requested* the Secretariat to incorporate these changes into the *List of topics for IPPC standards* and post on the IPP[18].

(6) *Agreed* not to remove the topic on *Safe handling and disposal of waste with potential pest risk generated during international voyages (2008-004)*, priority 2, from the *List of topics for IPPC standards.*

(7) *Urged* CPs to respond to a future call for experts on the topic on *Safe handling and disposal of waste with potential pest risk generated during international voyages (2008-004), with priority 2.*

9.5 Adjustments to the IPPC standard setting procedure

[61] The Secretariat introduced the paper[19]. A small group was convened to discuss this matter further and presented their findings to CPM[20].

[62] The CPM:

(1) *Adopted* the proposed changes to the IPPC Standard setting procedure, which forms Annex III of the Rules of Procedure of the Commission on Phytosanitary Measures (Appendix 07).

(2) *Agreed* that the SC regional input after the second consultation was not practical (as currently described in CPM-7 (2012) decision 2 on improving the IPPC Standard setting procedure) and should not be implemented.

(3) *Agreed* that the creation of an editorial team was not practical (as currently described in CPM-7 (2012) decision 20 on improving the IPPC Standard setting procedure) and should not be implemented.

(4) *Noted* the consequential changes for "Provisions for the availability of standard setting documents", namely that:

- Draft PTs and DPs presented to the SC are posted for the SC in the e-decision forum; discussions reported in the following SC report.

(5) *Amended* Rule 6 of the Rules of Procedure for the SC as following:

Rules of Procedure for the SC

Rule 6. Approval

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

[19] CPM 2016/11, CPM 2016/INF/17, CPM 2016/INF/20 and CPM 2016/CRP/02

[20] CPM 2016/CRP/12

Approvals relating to specifications or draft standards are sought by consensus. Final drafts of ISPMs which have been approved by the SC are submitted to the CPM without undue delay.

Situations where consensus is required but cannot be reached shall be described in the meeting reports detailing all positions maintained and presented to the CPM for discussion and appropriate action.

(1) *Asked* the Secretariat to review all related IPPC procedures and make consequential changes according to the revisions to the Standard setting procedure, once adopted.

(2) *Requested* the Secretariat to implement the changes with immediate effect.

10. Implementation and Facilitation

10.1 Report on CDC activities

[63] The Secretariat presented the report[21] which provided a record of the activities of the Capacity Development Committee (CDC) during 2015, including activities and meetings. The Secretariat also acknowledged the contribution of the Republic of Korea in providing resource materials to CPs at the CPM.

[64] The CPM acknowledged the work of the CDC and the CD team and the completion of the Standards and Trade Development Facility (STDF) project 350[22].

[65] The CPM:

(1) *Noted* the 2015 report of activities in capacity development.

(2) *Requested* contracting parties and other organizations to provide active contribution of technical resources for the Phytosanitary.info page.

(3) *Requested* support of the entire phytosanitary community to consider implementing the IPPC National Phytosanitary Capacity Development Strategy[23].

10.2 Implementation pilot on surveillance

[66] The Secretariat introduced the report[24]. The Secretariat recalled that CPM-10 had delegated management of the implementation pilot to the IPPC Secretariat, under the oversight of the Bureau and had urged contracting parties and regional plant protection organizations (RPPOs) to commit to an increased emphasis on plant pest surveillance and contribute resources and motivate others to contribute resources to support the implementation pilot where possible.

[67] One CP stated it was important to concentrate on activities with selected pests that currently have an impacton agriculture and trade. The CP proposed forming a working group to define a more focused and practical pest surveillance plan.

[68] One CP suggested an additional or alternative activity be added to the list of preparatory phase activities. The activity would be to develop simple guidance for contracting parties wanting to access donor funds for implementation of national level surveillance related programs. They gave the example that, now the IPPC has been recognised by the Global Environmental Facility, some simple guidance could help contracting parties to access funds through the appropriate agencies of their national governments. There may be other global funds that simple guidance could help contracting parties to access and RPPOs may also play an important role in this respect.

[21] CPM 2016/08 rev1

[22] http://www.standardsfacility.org/PG-350

[23] IPPC National Phytosanitary Capacity Development Strategy: https://www.ippc.int/en/publications/76/

[24] CPM 2016/15

[69] A small group met to discuss starting limited activities under the implementation pilot using three example pests. Information will be collated for the example pests and the Secretariat will issue a call for technical resources accordingly. A discussion, with experts, on the use of these resources will take place in June 2016.

[70] The CPM:

(1) *Acknowledged* the efforts of experts and their contracting parties who have collaborated with the IPPC Secretariat to identify and prioritize work activities for inclusion in the implementation pilot.

(2) *Noted* the work plan developed by the IPPC Secretariat and experts (Appendix 08).

(3) *Urged* contracting parties, RPPOs and other relevant organizations to contribute resources to allow the implementation pilot project on surveillance to formally commence and stand a success with expected impacts.

10.3 Report on the Implementation, Review and Support System (IRSS)

[71] The Secretariat introduced the report[25] describing the integrated work activities within both the implementation pilot project on surveillance and the work programme of the IPPC Secretariat.

[72] Some CPs called on the Secretariat and the Bureau to ensure that the cross-cutting function of the IRSS is maintained, whereby the IRSS provides for a liaison between the implementation and standard setting activities of the IPPC and hence also between the implementation and standard setting pillars of the Secretariat structure. They also suggested that the Secretariat and Bureau consider possible Triennial Review Group roles under the new implementation oversight body.

[73] The CPM:

(1) *Noted* the 2015 IRSS work activities that will contribute to the success of the implementation pilot project on surveillance and the IPPC work programme.

10.4 Report on the activities of the Subsidiary Body on Dispute Settlement (SBDS)

[74] The Chairperson of the SBDS presented the report[26]. She noted that emphasis in SBDS would continue to be on dispute avoidance and that it was felt there was a need for increased promotion of the IPPC Dispute Avoidance and Settlement Process (DASS), through regular and continuous communication by the IPPC contact points in their ministries.

[75] One CP noted that IPPC had produced an extremely important tool to help contracting parties resolve disagreements on phytosanitary related measures. However, they felt the emphasis was more on "Dispute Avoidance" rather than actual Dispute Settlement.

[76] The CP suggested the IPPC could play a key role in resolving disputes such as those at the Sanitary and Phytosanitary Measures (SPS) level (Geneva) by offering technical and science-based assistance with a pro-active approach. This would raise the profile of IPPC and encourage participation from other countries.

[77] One CP welcomed the focus on dispute avoidance and advocated putting activities for 2016 on hold until the focus group (agreed under agenda 8.4.2) had completed its work regarding the purpose, scope and functions of "oversight" and related activities that would include dispute avoidance/settlement.

[25] CPM 2016/14

[26] CPM 2016/33

[78] Another CP, in contrast, stated that putting the work of the SBDS on hold could affect their current on-going disputes and that it would also be difficult to delay already planned 2016 activities while waiting for the focus group outcome.

[79] In response to matters raised, the Secretariat noted that to put SBDS activities on hold would be problematic as they were currently providing technical guidance for two other FAO departments regarding disputes.

[80] The Secretariat acknowledged the valuable contribution Japan had made to the SBDS and the work of the outgoing Chair of SBDS.

[81] The CPM:

(1) *Noted* the activities of the Subsidiary Body on Dispute Settlement in 2015.

10.5 Report on the status of ISPM 15 Symbol Registration

[82] The representative of the FAO Legal Office presented the status report[27] with specific regard to achievements made in 2015 and work plan for 2016. The representative also stressed the importance of Members' cooperation in responding to FAO's requests for assistance during the registration process, in order to be able to complete the registration in each country in a timely and effective manner.

[83] In response to questions, the representative noted that the FAO Legal Office could not provide general guidance on the type of information requested by national trademark offices as this depended on national legislation in force and the type of specific objections made. The representative added that the issue was frequently a matter of recognition of privileges and immunities in FAO and that sometimes, additional technical information was required, including procedures for accrediting agencies authorized to use the mark; evidence of use of the symbol in a specific country etc. for which FAO would require assistance from the NPPOs. The representative encouraged CPs requiring further assistance to contact the FAO Legal Office through the IPPC Secretariat.

[84] The CPM:

(1) *Noted* the progress made in 2015 and the work plan for 2016 with regard to 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.

(3) *Encouraged* contracting parties to reimburse the IPPC Secretariat for registration and registration renewal costs as soon as practically possible.

10.6 Report on ePhyto

[85] The Chairperson of the steering group Mr. Nico Horn, presented the report[28]. He noted that following endorsement by CPM-10 (2015), with respect to moving forward with the establishment of an ePhyto hub to facilitate the exchange of electronic certificates, the Secretariat had submitted a proposal for the development of a hub and supporting infrastructure, to produce and receive certificates, to the Standards and Trade Development Facility (STDF). The proposal was fully approved at the STDF meeting of 12th and 13th October 2015 for funding of USD1 million.

[86] In response to matters raised by CPs, the Secretariat explained that the pilot would be very short term (3-6 months) and that therefore some essential criteria had to be met by countries in order to eligible to take part in a practical sense (e.g. legal basis in the country for accepting ePhyto – digital signatures).

[27] CPM 2016/07

[28] CPM 2016/23

He stated that phase one of the pilot would be testing the generic system and hub with those countries prepared to engage.

[87] The Project Manager for ePhyto, Mr Shane Sela, presented information on the proposed pilot project[29].

[88] He explained that the purpose of the pilot was to verify that the ePhyto Solution components, the hub and generic system meet the needs of contracting parties as well as to verify that the training and support tools required for contracting parties to commence participation in ePhyto were both useful and effective.

[89] He noted that in surveying possible participants, the focus and criteria for selection had been on countries currently ready (within a short timeframe 6-8 months) to participate. He also reported that the pilot is seeking to select a French speaking country to complete the selection of countries invited to participate. Countries which applied for the project, but were not quite ready to take part in the initial pilot, will be able to apply to participate in a second phase of the project, which will take place later in 2016. Assistance will be available to help some countries meet the criteria required to take part in the project. CPs expressed their thanks for the development and updates and also expressed expectations for transparency to be ensured in further developments.

[90] The CPM:

(1) *Noted* the work of the ePhyto Steering Group and the IPPC Secretariat in advancing development of ePhyto.

(2) *Supported* the continued work of the Secretariat and the ePhyto Steering Group under the oversight of the CPM Bureau.

(3) *Acknowledged* the support provided by the Republic of Korea in hosting the 2nd International Symposium on ePhyto.

(4) *Acknowledged* the support provided by Canada with the contribution for the Project Manager of the project.

(5) *Supported* the implementation of the STDF project to pilot the hub and the generic national system to promote the use of ePhyto by CPs worldwide including developing countries.

(6) *Requested* the Secretariat report back to CPM12 on progress in implementation of the ePhyto project.

(7) *Noted* the decision of the Bureau regarding ePhyto pilot program contracting party participants.

11. Integration and Support

11.1 Communication and Advocacy

11.1.1 Report on National Reporting Obligations

[91] The Secretariat presented the report[30] with proposed procedures for National Reporting Obligations (NROs) taking into account the IPPC provisions and previous CPM decisions related to NROs.

[92] A CP requested that the analysis of possible obstacles to meeting reporting obligations be reported on at a future CPM.

[93] The CPM:

(1) *Considered* the proposed IPPC NROs General and Specific Procedures (Appendix 09, tables a and b) and provided suggestions for improvement and revision, as appropriate.

(2) *Adopted* the IPPC NROs General and Specific procedures (presented in Appendix 09 tables a and b)

[29] CPM 2016/CRP/03

[30] CPM 2016/28

(3) *Agreed* that the International Phytosanitary Portal (IPP) remains the preferred mechanism through which IPPC contracting parties meet their national reporting obligations.

[94] The Secretariat presented the NRO Quality Control Guidelines[31].

[95] The CPM:

(1) *Agreed* to "the NRO Quality Control guidelines" presented in Appendix 10.

[96] The Secretariat presented the NRO Work Plan (2014-2023)[32].

[97] The CPM:

(1) *Considered* the proposed NRO Work Plan (see Appendix 11) and provided suggestions for its improvement and revision.

(2) *Agreed* to the NRO Work Plan (see Appendix 11) and established 3 high priorities for the next 2 years:

a. Monitoring, updating and maintaining the system of official contact points;

b. Continuing to invest in, support and improve the IPP as the CPM's primary tool for communicating with NPPOs and with the public;

c. Creating, posting and updating regulated pest lists and pest reports.

(3) *Agreed* that annual oversight and adjustments to a detailed work plan would be overseen by the NROAG and a progress update would be provided annually to CPM.

(4) *Encouraged* contracting parties to provide extra budgetary resources (financial and in-kind) as the full implementation of the IPPC NRO Work Plan would only be possible with the allocation of sustainable extra-budgetary resources.

11.1.2 Annual Communications Work Plan 2016

[98] The Secretariat introduced the paper[33], provided an update on IPPC Communication and Advocacy Activities and presented a proposed work plan for CPM consideration and approval.

[99] The Secretariat noted the changes already underway regarding communication and advocacy in terms of operational arrangements as well as the improved profile of the IPPC. New tools and opportunities were being utilized to increase the profile of the IPPC activities, such as regular IPPC Seminars and increased timeliness and focus of new articles. It was also noted that there would be greater integration of the IPPC communications activities into the FAO corporate guidelines and policies and this would be done in a way that maintained the IPPC profile and identity. In addition, the Secretariat noted that the changes needed to be undertaken without costs to the Secretariat, as this would negatively impact the approved CPM work programme.

[100] Some CPs noted strong concerns regarding this initiative, as they felt this would lead to reduced visibility and undermine attempts by the IPPC community to increase awareness of the importance of the IPPC activities.

[101] Some CPs proposed adjustments and improvements to be included in the 2016 Work Plan (Appendix 12).

[102] The CPM:

[31] CPM 2016/26
[32] CPM 2016/27
[33] CPM 2016/30

(1) *Agreed* to the IPPC Communication and Advocacy Work Plan for 2016-2020 with the following high priorities:

a. Building and improving the IPPC website.

b. Developing advocacy documents and other communication efforts which support the annual strategic themes and IYPH.

(2) *Requested* the IPPC Secretariat to work closely with FAO to maintain the authority and identity of the IPPC website.

11.1.3 Report on the activities relating to the International Year of Plant Health in 2020 (IYPH 2020)

[103] Mr Ralf Lopian introduced two papers: an Update on the Efforts to Proclaim an International Year of Plant Health in 2020[34]; and information on the Scope, Objectives and Structures for the International Year of Plant Health[35].

[104] The CPM:

(1) *Applauded* the valuable voluntary contributions.

(2) Thanked Mr Lopian and the government of Finland for providing leadership in progressing the IYPH initiative.

(3) *Thanked* Ireland for their generous financial contribution.

(4) *Encouraged* other volunteers to join the IYPH 2020 volunteer programme.

(5) *Encouraged* NPPOs to promote the concept and importance of the IYPH 2020 in their capitals and through their FAO permanent representatives.

(6) *Encouraged* other potential donors to support efforts to proclaim the year 2020 as the International Year of Plant Health.

[105] Following deliberations from an in-session working group, Mr Lopian presented the objective of the IYPH 2020 and draft Terms of Reference for a Steering Committee as described in Appendix 13.

[106] The CPM:

(1) *Considered and adopted* the defining scope of "Plant Health" in the context of the IYPH 2020.

(2) *Considered and adopted* the main objective of the IYPH 2020.

(3) *Considered and adopted* the specific objectives for the IYPH 2020.

(4) *Established* a Steering Committee for the IYPH 2020 and approved the Terms of reference (Appendix 13).

(5) *Agreed* that each region, through their Bureau member, should nominate a representative and an alternate to participate in the Steering Committee, by 15 May 2016.

(6) *Noted* the preliminary time and work schedule for the IYPH 2020.

(7) *Agreed* on the valuable support role RPPOs can and should play in the IYPH.

11.2 Partnerships and Liaison

[107] The Secretariat presented the information paper[36] on partnerships and liaison. The Secretariat noted it was developing new relationships and enhancing old ones.

[34] CPM 2016/36

[35] CPM 2016/34

[36] CPM 2016/INF/18

11.2.1 Report on IPPC Regional Workshops

[108] The Secretariat introduced the report[37] on IPPC regional workshops in 2015.

[109] CPs expressed their support for regional workshops. Several CPs urged the Secretariat to continue such initiatives and in particular for Africa in 2016. CPs encouraged a physical Secretariat presence in these workshops so that they could communicate challenges in the region to the Secretariat directly.

[110] The Secretary to the IPPC underlined how such initiatives were part of a three level approach for increasing IPPC impacts: 1) CPM at the global level; 2) TC-RPPOs at the regional level, and 3) the IPPC Regional Workshops at the national level. The Secretary also noted that IPPC regional workshops were an efficient and useful way to connect these levels and expressed the hope for active participation and for contributions from donors.

[111] The CPM:

(1) *Encouraged* donors, contracting parties and RPPOs to contribute funding to the IPPC Regional workshops.

(2) *Noted* that the IPPC regional workshops are a valuable and essential tool for developing phytosanitary capacity for contracting parties and that the change of content in the IPPC Regional Workshops has been a successful strategy to increase and align the knowledge on IPPC related issues in all regions.

11.2.2 Report on the Technical Consultation among Regional Plant Protection Organizations

[112] The Secretariat introduced the agenda item[38] and invited the Executive Director of the North American Plant Protection Organization (NAPPO), Ms. Stephanie Bloem, to report on the 27th Technical Consultation among Regional Plant Protection Organizations (TC-RPPOs).

[113] CPs acknowledged the important work of the RPPOs, and of the need for teamwork as emphasized in the presentation[39]. A CP acknowledged the work being undertaken on surveillance by the APPPC with the IPPC. Caribbean countries recognized the importance of the regional work, in particular in Capacity Development. Caribbean countries also raised the need to have FAO legal advice to establish an RPPO for the Caribbean, and would welcome expertise from other regions.

[114] A CP referred to the TC-RPPOs proposal for topics for the side programmes at next year's CPM-12 and suggested that the topic on private standards should be set aside until the WTO-SPS had a clear position on the matter as pertaining to plant health.

11.2.3 Oral reports from selected international organizations

[115] The following organizations presented their reports:

- Convention on Biological Diversity (CBD)[40]
- World Trade Organization: Sanitary and Phytosanitary (SPS) Committee and Standards and Trade Development Facility (STDF)[41]
- International Atomic Energy Agency (IAEA)[42]

[37] CPM 2016/09

[38] CPM 2016/INF/02

[39] https://www.ippc.int/static/media/files/publication/en/2016/05/S.Bloem_-_CPM_on_27th_TC_of_RPPO.pdf

[40] https://www.ippc.int/static/media/files/publication/en/2016/05/cbd-cpm11.pdf

[41] CPM 2016/INF/09

[42] CPM 2016/INF/11

11.2.4 Written reports from international organizations

[116] Written reports or statements were presented by the following international and regional organizations:

- Report from the Phytosanitary Measures Research Group Activities for 2015[43]
- Report from the International Forestry Quarantine Research Group (IFQRG)[44]
- Report from the International Advisory Group for Pest Risk Analysis[45]

11.3 Financial Report and Budget

11.3.1 IPPC Secretariat financial Report for 2015

[117] The Secretariat presented the report[46] containing financial statements for resources available in 2015 from FAO's Regular Programme (RP) budget and the Extra-Budgetary (EB) Trust Fund sources that were administered by the IPPC Secretariat during the reporting period.

[118] The CPM acknowledged the contribution of 135.000 USD from the Republic of Korea to the multi-donor trust fund in 2016. The CPM encouraged other CPs to establish sustainable funding for the IPPC in their own countries.

[119] The CPM:

(1) *Noted* the 2015 Financial Report of the International Plant Protection Convention Secretariat
(2) *Adopted* the 2015 Financial report for the Special trust fund of the IPPC (Multi-donor) (Table 3)
(3) *Encouraged* contracting parties to contribute to the Special trust fund of the IPPC (Multi-donor)
(4) *Thanked* contracting parties which contributed to the Secretariat of the International Plant Protection Convention's work programme in 2015.

11.3.2 IPPC Secretariat Work Plan and Budget for 2016

[120] The Secretariat presented the work plan and budget[47].

[121] The CPM:

(1) *Approved* the IPPC Secretariat Work Plan and IPPC Multi-donor Trust Fund budget for 2016 (Appendix 14)
(2) *Noted* the IPPC Secretariat Regular programme budget for 2016 (Appendix 14).

11.4 Resource mobilization

[122] The Secretariat presented the report[48] on resource mobilization.

[123] The Secretary also made a presentation[49] to CPM.

[124] CPs expressed the following views and suggestions:

[43] CPM 2016/INF/14
[44] CPM 2016/INF/10
[45] CPM 2016/INF/04
[46] CPM 2016/31
[47] CPM 2016/22
[48] CPM 2016/24
[49] https://www.ippc.int/static/media/files/publication/en/2016/05/Xia_Resource_Mobilization_of_the_IPPC_Secretarait_Updated_03-04-2016.pdf

- IPPC should harness FAO resources such as IT, Communications and Legal Services.
- Gradually moving to a new model of mandatory assisted contributions would provide sustainability.
- Increase the visibility of the IPPC and help stakeholders and a wider audience understand the work of IPPC.
- Engage the SPG to explore a possible voluntary contribution agreement and continue discussions around the topic of country hosted or sponsored events including expert groups and drafting groups.
- Adopt the successful approach used in the past for logo registration and write to governments underlining the nature of the current critical financial situation.
- To increase implementation activities, the Secretariat should asses the resources available and re-allocate accordingly.
- Any consideration for mandatory contributions would only be a long term option and not settle IPPC current financing difficulties.
- There is a need for a good balance between regular budget and extra-budgetary contributions to ensure operational activities go ahead even when there is a shortfall in extra-budgetary contributions.
- The CPM should clearly articulate the "value added" resulting from IPPC work as this could enhance the CPM's ability in generating additional contributions to the trust fund.

[125] Some CPs noted that a possible contribution agreement based on voluntary contributions would require careful preparation, which could be undertaken as part of the IYPH 2020. The proposal could then be adopted by CPM that year.

[126] France confirmed they would be enhancing their contribution to the IPPC Secretariat in 2016 by continuing to provide an expert for a fifth consecutive year. France would also be making an additional contribution of USD 25.000 towards technical activities for standard setting in 2016.

[127] The United States confirmed their contribution of USD 140.000 to the trust fund to support the IYPH and ePhyto development.

[128] The CPM:

(1) *Noted* the work on resource mobilization which has been done by the IPPC Secretariat in 2015 and planned for in 2016

(2) *Agreed* to initiate a strategic discussion on sustainable funding such as: sustained contributions; contributions from industry; and contributions generated by articulating the "added value" of IPPC at the SPG and Bureau meetings, and report back to CPM-12 in 2017.

11.5 Recognition of Important Contributions

11.5.1 Expert Contributions

11.5.2 Donors

[129] The Secretariat introduced the paper[50] and acknowledged the services and contributions received, implemented or finalized in 2015.

[130] The CPM:

(1) *Acknowledged* the contributions of following members of the following groups who left in 2015:

[50] CPM 2016/32

- Standards Committee (SC): Mr. DDK SHARMA, India and Ms. Alice NDIKONTAR, Cameroon.
- Subsidiary Body for Dispute Settlement (SBDS): Mr. Similo MVIMBELA, Swaziland and Mr. Chusak Wongwichakorn, Thailand.
- National Reporting Obligations Advisory Body (NROAG): Mr. Sam BISHOP, United Kingdom.

(2) *Acknowledged* the donors who provided financial, in-kind staff and hosting or contribution support for IPPC Activities as their contributions are essential to ensure the Secretariat is able to deliver the CPM work programme as presented in Appendix 15, Table 1.

(3) *Acknowledged* the contributions of technical panel members who left their respective panels in 2015 as well as contracting parties, regional plant protection organizations, organizations and, in particular, individual experts for their efforts in the development of the ISPMs adopted at CPM-11 (2016) as presented in Appendix 15, Table 2.

(4) *Acknowledged* the contributions of the Capacity Development Committee (CDC) members, contracting parties, regional plant protection organizations, organizations and, in particular, individual experts for their efforts in capacity development activities as presented in Appendix 15, Table 3.

(5) *Acknowledged* the contributions of National Reporting Obligations Advisory Group (NROAG) members for their efforts in NROAG activities as presented in Appendix 15, Table 4.

(6) *Acknowledged* the contributions of Subsidiary Body for Dispute Settlement members, contracting parties, and, in particular, an individual expert for his efforts in dispute settlement and avoidance activities as presented in Appendix 15, Table 5.

(7) *Acknowledged* the contributions of ePhyto Steering Group members and individual experts for their efforts in ePhyto activities as presented in Appendix 15, Table 6.

12. CPM Recommendations

[131] The Secretariat introduced the paper[51] on the proposed recommendation on the importance of pest diagnosis.

[132] Some CPs, whilst acknowledging the proposed recommendation, noted that some outstanding strategic issues still required further consideration.

[133] The CPM:

(1) *Adopted* the recommendation on the importance of pest diagnosis (Appendix 16).

(2) *Encouraged* advocacy for enhanced attention by contracting parties to the issue of pest diagnosis.

(3) *Encouraged* continued liaison with RPPOs and research and educational organizations on pest diagnosis issues.

(4) *Encouraged* the IPPC Secretariat to publicize national, regional and international developments in pest diagnosis and diagnostic protocols on the phytosanitary resources page.

13. Contracting Parties Reports of Successes and Challenges of Implementation

[134] Two presentations were made:

National Plant Protection Organization of Kenya – Successful Experiences/Challenges in Implementing IPPC[52]

[51] CPM 2016/21 rev1

[52] https://www.ippc.int/static/media/files/publication/en/2016/05/Kenya_CPM11_SUCCESS_OF_IMPLEMENTATION_OF_IPPC_2016-04-06.pdf

[135] The representative of the NPPO of Kenya spoke of achievements in launching the Center of Phytosanitary Excellence (COPE), a Plant Import and Quarantine Regulatory System (PIQRS), as well as the recognition of KEPHIS plant health Laboratory as a COMESA Regional Reference Laboratory for Plant Health. She also described the challenges of meeting reporting obligations due to bureaucracy in the government structure.

General Surveillance Framework supports pest status determination in Australia[53]

[136] The representative of the Australian Department of Agriculture and Water Resources, Mr. Kim Ritman, described the system used to verify the status of key plant pests in Australia. He outlined the framework and its fundamental components and explained that as many of these elements as possible needed to be supported with evidence to an agreed minimum standard so that a level of confidence in the system could be achieved.

[137] A CP described the challenges faced in trade due to the absence of guaranteed timeline/s in processing market access requests. The CP also pointed out that 1 new commodity provides significant benefits to the economy of a small island country. The CP felt that IPPC can effectively contribute to addressing this issue through the good science it upholds and therefore asked the Secretariat to progress this in the best way possible. The Chairperson reminded the CPM that next year's theme on Trade Facilitation would be a good occasion on which to bring forward this type of issue in a timely manner.

14. Special Topics Session: Sea Containers

[138] A special topics session was held on the issue of sea containers[54]. Presentations[55] were given by NPPOs, relevant international organizations and stakeholders involved in the movement of sea containers.

[139] Presentations outlined the complex logistics of the movement of sea containers and the potential risks of the spread of pests.

[140] There was extensive discussion on possible options: continuing the development of the standard; changing the status of the topic to "pending"; or deleting the topic from the work programme.

[141] Industry representatives proposed to review their guidance on the cleanliness of sea containers in order to update them with language to address this risk.

[142] Most CPs were in favour of moving the standard to pending as they felt more time was required to assess and address the potential pest risk with the tools available (e.g. CTU code , CPM Recommendation on Sea Containers (CPM 10/2015_01)).

[143] Other CPs felt that the CTU code was a useful tool but an ISPM would be complementary in providing NPPOs with guidance on monitoring.

[144] The CPM:

(1) *Recognized* the risk of pests and regulated articles, other than cargo, that can be moved with sea containers.

(2) *Agreed* that the harmonization of requirements through the development of a draft ISPM on minimizing pest movement by sea containers (2008-001) is considered as complex to achieve.

[53] https://www.ippc.int/static/media/files/publication/en/2016/05/AU_General_Surveillance_Framework_-_CPM_April_2016_002.pdf

[54] CPM 2016/13, CPM 2016/INF/05, CPM 2016/INF/06, CPM 2016/INF/07, CPM 2016/INF/12 Rev. 1, CPM 2016/CRP/06 and CPM 2016/CRP/07

[55] Available at: https://www.ippc.int/en/core-activities/governance/cpm/scientific-sessions-during-commission-phytosanitary-measures/2016-special-topic-session-sea-containers/

(3) *Recognized* that the implementation of the IMO/ILO/UNECE CTU Code, and of the Recommendation CPM 10/2015_01 on Sea Containers would help address the risks of sea containers being contaminated.

(4) *Agreed* that the status of the topic on Minimizing Pest Movement by Sea Containers (2008-001) should be changed to pending and reconsidered by the CPM in maximum five years, to allow for the implementation of the CTU Code and Recommendation CPM 10/2015_01 and an analysis of their impact on reducing pest movement by sea containers.

(5) *Agreed* that some coordinated actions should be considered for assessing and addressing the pest risks associated with sea containers.

(6) *Encouraged* NPPOs to gather information on the movement of pests via the sea containers to help clarify the risk.

(7) *Requested* the Bureau (at its June 2016 meeting) to consider the development of a "set of complimentary actions" which, combined, may offer some value in assessing and managing the pests threats associated with sea containers and to propose such a possible program of complimentary actions to CPM-12 (2017).

(8) *Encouraged* interested parties and CPs to submit discussion papers by 15 May 2016 to the IPPC Secretariat for the CPM Bureau to consider.

15. Confirmation of membership and potential replacements for CPM subsidiary bodies

15.1 CPM Bureau members and potential replacement members

[145] The CPM:

(1) *Elected* the Chairperson Ms Lois Ransom (Australia) for the CPM Bureau.

(2) *Elected* the Vice-Chairperson Mr Francisco Javier TRUJILLO ARRIAGA (Mexico) for the CPM Bureau.

(3) *Elected* members for the CPM Bureau from FAO regions not represented by the Chairperson and Vice-Chairperson (Appendix 17).

(4) *Elected* replacements for members of the CPM Bureau.

15.2 SC and SBDS members and potential replacement members

SC

[146] The CPM:

(1) *Noted* the current membership of the Standards Committee and the potential replacements for the Standards Committee.

(2) *Confirmed* new members and potential replacements, as appropriate (Appendix 18 table A1 and table A2).

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

SBDS

[147] The CPM:

(1) *Noted* the current membership of the Subsidiary Body on Dispute Settlement (Appendix 18 table B1 and table B2).

(2) *Confirmed* new members and potential replacements, as appropriate.

16. Any other business

New IPPC website

[148] The Secretariat presented the new home page of the IPPC website designed for the whole IPPC community, explained the key features of improved usability and broader coverage, the emphasis the IPPC Secretariat will be placing on involving the IPPC Community, and possibilities to network with the whole Convention and a wider audience. Some CPs expressed support for the re-design of the home page. Concerns were raised concerning visibility and accessibility for users less familiar with the IPPC who may not immediately see the value of IPPC, significant achievements and contributions.

[149] The Secretariat emphasized that guidance material would be made available in all FAO languages when the site is released in June 2016. In addition, it was noted that the new developments involved only the home page, whilst data entry and usability for NPPOs that log in would not be changed.

[150] The Secretary to the IPPC requested to NPPO and RPPOs to provide "brief news items" for the revised home page on a regular basis.

17. Date and venue of the next CPM Session

[151] CPM-12 (2017) was scheduled for 5-11 April 2017 in Incheon, Republic of Korea.

[152] CPM expressed its thanks to the Republic of Korea and discussed the challenges and opportunities that holding the CPM outside Rome would present. The Secretariat informed the CPM that it would make every endeavor to make CPM-12 (2017) a success and apply lessons learned for the future.

[153] The Secretariat stated that all efforts would be made to ensure support would be available to CPs to guarantee a quorum at CPM-12 (2017)

18. Adoption of the Report

[154] The report was adopted.

Appendix 01 – Detailed Agenda

1. Opening of the Session
    1.1 FAO opening
    1.2 IPPC towards 2020
2. Keynote Address on Plant Health and Food Security
3. Adoption of the Agenda
    3.1 EU Statement of Competence
4. Election of the Rapporteur
5. Establishment of the Credentials Committee
6. Report from the CPM Chairperson
7. Report from the IPPC Secretariat
8. Governance
    8.1 Summary of the Strategic Planning Group report
    8.2 Framework for standards and implementation
    8.3 Concept of a commodity standard
    8.4 Capacity development and implementation oversight
        8.4.1 Review of the Capacity Development Committee (CDC)
        8.4.2 Proposal for a new implementation oversight body
9. Standard Setting
    9.1 Report on the activities of the Standards Committee
    9.2 Adoption of International Standards for Phytosanitary Measures
    9.3 Noting translation adjustments to International Standards for Phytosanitary Measures adopted at CPM-10
    9.4 Topics for IPPC standards - New topics and adjustments to the List of topics for IPPC standards
    9.5 Adjustments to the IPPC standard setting procedure
10. Implementation and Facilitation
    10.1 Report on CDC activities
    10.2 Implementation pilot on surveillance
    10.3 Report on the Implementation, Review and Support System (IRSS)
    10.4 Report on the activities of the Subsidiary Body on Dispute Settlement (SBDS)
    10.5 Report on the status of ISPM 15 Symbol Registration

10.6 Report on ePhyto

11. Integration and Support

11.1 Communication and Advocacy

11.1.1 Report on National Reporting Obligations

11.1.2 Annual Communications Work Plan 2016

11.1.3 Report on the activities relating to the International Year of Plant Health in 2020 (IYPH 2020)

11.2 Partnerships and Liaison

11.2.1 Report on IPPC Regional Workshops

11.2.2 Report on the Technical Consultation among Regional Plant Protection Organizations

11.2.3 Oral reports from selected international organizations

11.2.4 Written reports from international organizations

11.3 Financial Report and Budget

11.3.1 2015 Financial Report

11.3.2 2016 Budget

11.4 Resource mobilization

11.5 Recognition of Important Contributions

12. CPM Recommendations

13. Contracting Parties Reports of Successes and Challenges of Implementation

14. Special Topics Session: Sea Containers

15. Confirmation of membership and potential replacements for CPM subsidiary bodies

15.1 CPM Bureau members and potential replacement members

15.2 SC and SBDS members and potential replacement members

16. Any other business

17. Date and venue of the next CPM Session

18. Adoption of the Report

Appendix 02 – List of Documents

Document number	Agenda item	Document Title	Available Languages
CPM 2016/02	03	Provisional Detailed Agenda	EN/FR/ES/RU/AR/ZH
CPM 2016/03	15.2	SC and SBDS Members and potential replacement members	EN/FR/ES/RU/AR/ZH
CPM 2016/04	15.1	CPM Bureau members and potential replacements members	EN/FR/ES/RU/AR/ZH
CPM 2016/05	09.2	Adoption of International Standards for Phytosanitary Measures	EN/FR/ES/RU/AR/ZH
CPM 2016/05_01	09.2	Adoption of International Standards for Phytosanitary Measures -The draft amendments to the Glossary (1994-001)	EN/FR/ES/RU/AR/ZH
CPM 2016/05_02	09.2	Adoption of International Standards for Phytosanitary Measures - The Draft ISPM on Determination of host status of fruit to fruit fly (2006-031)	EN/FR/ES/RU/AR/ZH
CPM 2016/05_03	09.2	Adoption of International Standards for Phytosanitary Measures - Phytosanitary Treatment - High temperature forced air treatment for *Bactrocera melanotus and B. xanthodes (Diptera: Tephritidae*) on *Carica papaya* (2009-105)	EN/FR/ES/RU/AR/ZH
CPM 2016/05_04	09.2	Adoption of International Standards for Phytosanitary Measures -Phytosanitary Treatment - Irradiation treatment for *Ostrinia nubilalis* (2012-009)	EN/FR/ES/RU/AR/ZH
CPM 2016/06	09.3	Noting translation adjustments to International Standards for Phytosanitary Measures adopted at CPM-10	EN/FR/ES/RU/AR/ZH (+ annexes in FR/ES/ AR/ZH)
CPM 2016/07	10.5	Report on the status of ISPM 15 Symbol Registration	EN/FR/ES/RU/AR/ZH
CPM 2016/08	10.1	Report on CDC Activities	EN/FR/ES/RU/AR/ZH
CPM 2016/09	11.2.1	Report on IPPC Regional Workshops	EN/FR/ES/RU/AR/ZH
CPM 2016/10	09.4	Topics for IPPC Standards - New topics and adjustments to the *List of topics for IPPC Standards*	EN/FR/ES/RU/AR/ZH
CPM 2016/11	09.5	Adjustments to the IPPC standard setting process	EN/FR/ES/RU/AR/ZH
CPM 2016/12	09.2	Adoption of International Standards for Phytosanitary Measures - Ink Amendments	EN/FR/ES/RU/AR/ZH
CPM 2016/13	14	Special Topics Session: Sea Containers	EN/FR/ES/RU/AR/ZH
CPM 2016/14	10.3	Report on the Implementation, Review and Support System (IRSS)	EN/FR/ES/RU/AR/ZH
CPM 2016/15	10.2	Implementation Pilot on Surveillance	EN/FR/ES/RU/AR/ZH
CPM 2016/16	08.4.1	Review of the Capacity Development Committee (CDC)	EN/FR/ES/RU/AR/ZH
CPM 2016/17	08.3	Concept of a commodity standard	EN/FR/ES/RU/AR/ZH
CPM 2016/18	08.4.2	Proposal for a new implementation oversight body	EN/FR/ES/RU/AR/ZH
CPM 2016/19	09.1	Report on activities of the Standards Committee	EN/FR/ES/RU/AR/ZH
CPM 2016/20	08.2	Framework for standards and implementation	EN/FR/ES/RU/AR/ZH
CPM2016/21Rev.01	12.1	CPM Recommendations - Proposed recommendation on the importance of pest diagnosis	EN/FR/ES/RU/AR/ZH

Document number	Agenda item	Document Title	Available Languages
CPM 2016/22	11.3.2	IPPC Secretariat Work Plan and Budget for 2016	EN/FR/ES/RU/AR/ZH
CPM 2016/23	10.6	Report on ePhyto – ePhyto Update	EN/FR/ES/RU/AR/ZH
CPM 2016/24	11.4	Resource mobilization	EN/FR/ES/RU/AR/ZH
CPM 2016/25	08.1	Summary of the Strategic Planning Group report	EN/FR/ES/RU/AR/ZH
CPM 2016/26	11.1.1	Report on National Reporting Obligations - NRO Quality Control Guidelines	EN/FR/ES/RU/AR/ZH
CPM 2016/27	11.1.1	Report on National Reporting Obligations - NRO Work Plan (2014 – 2023)	EN/FR/ES/RU/AR/ZH
CPM 2016/28	11.1.1	Report on National Reporting Obligations - IPPC National Reporting Obligations Procedures	EN/FR/ES/RU/AR/ZH
CPM 2016/29	06	Report from the CPM Chairperson	EN/FR/ES/RU/AR/ZH
CPM 2016/30	11.1.2	Annual Communications Work Plan 2016 - Communication and Advocacy Activities and IPPC Secretariat Work Plan (2016-2020)	EN/FR/ES/RU/AR/ZH
CPM 2016/31	11.3.1	2015 Financial Report - Financial Report and Resource mobilization	EN/FR/ES/RU/AR/ZH
CPM 2016/32	11.5	Recognition of Important Contributions	EN/FR/ES/RU/AR/ZH
CPM 2016/33	10.4	Report on the activities of the Subsidiary Body on Dispute Settlement (SBDS) in 2015	EN/FR/ES/RU/AR/ZH
CPM 2016/34	11.1.3	Report on the activities relating to the International Year of Plant Health in 2020 (IYPH 2020)- Scope, Objectives and Structures for the International Year of Plant Health	EN/FR/ES/RU/AR/ZH
CPM 2016/35	07	Report from the IPPC Secretariat	EN/FR/ES/RU/AR/ZH
CPM 2016/36	11.1.3	Report on the activities relating to the International Year of Plant Health in 2020 (IYPH 2020) - Update on the efforts to proclaim an International Year of Plant Health in 2020	EN/FR/ES/RU/AR/ZH

Information Papers (INF)

Document number	Agenda item	Document Title	Available Languages
CPM 2016/INF/01	01.2	IPPC towards 2020	EN/FR/ES/RU/AR/ZH
CPM 2016/INF/02	11.2.2	Summary Report of the Twenty-seventh Technical Consultation among Regional Plant Protection Organizations	ENGLISH ONLY
CPM 2016/INF/03	16	Any other business – Info regarding the Pre-CPM Training session and Side Sessions	ENGLISH ONLY
CPM 2016/INF/04	11.2.4	Written reports from international organizations - Report from the International Advisory Group for Pest Risk Analysis	ENGLISH ONLY
CPM 2016/INF/05	14	Special Topics Session: Sea Containers - Logistics of movement of sea containers and the IMO / ILO / UN ECE Code of practice for packing cargo transport units (CTU Code)	ENGLISH ONLY

Document number	Agenda item	Document Title	Available Languages
CPM 2016/INF/06	14	Special Topics Session: Logistics of Sea Containers	ENGLISH ONLY
CPM 2016/INF/07	14	Special Topics Session: Programme	ENGLISH ONLY
CPM 2016/INF/08	11.2.4	Written reports from international organizations – STDF Overview	ENGLISH ONLY
CPM 2016/INF/09	11.2.4	Written reports from international organizations – Activities of the SPS Committee and other relevant WTO activities in 2015	EN/FR/ES
CPM 2016/INF/10	11.2.4	Written reports from international organizations – IFQRG Report	ENGLISH ONLY
CPM 2016/INF/11	11.2.4	Written reports from international organizations – Report IAEA/FAO Division	ENGLISH ONLY
CPM 2016/INF/12	14	Special Topics Session: Role of sea containers in unintentional movement of invasive contaminating pests (so-called “hitchhikers”), and opportunities for mitigation measures	ENGLISH ONLY
CPM 2016/INF/13	08.4.2	Proposal for a new implementation oversight body - New Zealand's intervention	ENGLISH ONLY
CPM 2016/INF/14	11.2.4	Written reports from international organizations – Report from the Phytosanitary Measures Research Group Activities for 2015	ENGLISH ONLY
CPM 2016/INF/15	13	Contracting Parties Reports of Successes and Challenges of Implementation- General Surveillance Framework supports pest status determination in Australia	ENGLISH ONLY
CPM 2016/INF/16	13	Contracting Parties Reports of Successes and Challenges of Implementation National Plant Protection Organization of Kenya	ENGLISH ONLY
CPM 2016/INF/17	08.2; 08.3; 08.4.2; 09.5; 11.1.1	Statements from the European Union and its Member States regarding various CPM Agenda items	ENGLISH ONLY
CPM 2016/INF/18	11.2	Partnerships and Liaison	ENGLISH ONLY
CPM 2016/INF/19	10.2; 11.3.1; 17	Comments from the Republic of Korea regarding various CPM Agenda items	ENGLISH ONLY
CPM 2016/INF/20	09.5	Comments from Japan regarding various CPM Agenda items	ENGLISH ONLY

Appendix 03 – List of Participants

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Appendix 04 – Framework for Standards and Implementation

Framework for Standards and Implementation

2016-04-08

Adopted by CPM-11 (2016)

LEGEND

Red text: indicates gaps for new topics, new revisions to adopted ISPMs that are not already on the *List of topics for IPPC standards* or gaps for other guidance.

Underlined text: indicates topics on the *List of topics for IPPC standards* for revisions to adopted ISPMs (topic number in brackets)

Bolded text: indicates topics on the *List of topics for IPPC standards* for new ISPMs (topic number in brackets) or guidance being drafted

Adopted ISPMs are listed with title and ISPM number.

ISPMs or proposed gaps that cover or should cover both conceptual issues and implementation issues in one standard are centred.

IPPC Area: GENERAL IPPC Strategic Objectives (SOs): A3, A4, B1, B2, B3, D2, D4			
	Concept standards - "what"	**Implementation standards - "how"**	**Other guidance**
1.	Audits (Priority 1)	No gap.	
2.	No gap.	No gap.	Organization and provision of information on technical resources. Available guidance: Phytosanitary resource page (roster of experts, projects database, activities calendar, technical documents)
3.	No gap.	No gap.	Cooperation with other Organizations e.g. environmental. Available guidance: Memorandums of Understanding: Ozone Secretariat, CBD; Partnership paper (CPM 9/2014/21).
4.	No gap.	No gap.	Environmental protection and climate change e.g. surveillance of wild flora. Available guidance: Guide to implementation of phytosanitary standard in forestry; ICPM-7 decisions in relationship to Cooperation with the CBD: Threats to biodiversity by IAS)
5.	No gap.	No gap.	International cooperation between NPPOs, e.g. regional centres of expertise
6.	No gap.	No gap.	How standards are used in or relate to different areas (e.g. Market access, IAS, climate change)
7.	No gap.	No gap.	Advocacy for NPPO resource mobilisation

IPPC Area: GENERAL RIGHTS AND OBLIGATIONS IPPC SOs: A1, A2, B2, B3, B4, C3, D3, Y4			
	Concept standards - "what"	**Implementation standards - "how"**	**Other guidance**
8.	Elements of an effective NPPO e.g. training, engagement of stakeholders, competency (Priority 1)	No gap.	Elements of an effective NPPO e.g. training, engagement of stakeholders, competency. Available guidance: NPPO management (draft manual); PCE tool; Explanatory document (2005) on ISPM 20 *(Guidelines for a phytosanitary import regulatory system)* (includes appendix on rights, roles & responsibilities in relation to the IPPC, ISPMs and SPS))
9.	Revision: Pest reporting (ISPM 17) (Priority 2)		
10.	Revision: Guidelines on lists of regulated pests (ISPM 19) (Priority 2)		
11.	Guidelines for the notification of non-compliance and emergency action (ISPM 13)		
12.	National legislation requirements (Priority 4)	No gap.	
13.	No gap.	No gap.	International Cooperation between contracting parties. Available guidance **Stakeholder relations** (draft manual))
14.	No gap.	No gap.	Elements of an effective RPPO. Available guidance: Procedure for the recognition of new RPPOs; ICPM-4 (2002); Role and functions of the Technical Consultation among RPPOs ICPM-5 (2003)
15.	No gap.	No gap.	Information exchange. Available guidance: Recommendation information exchange (ICPM 2/1) ; Role of IPPC contact points (CPM 1/1)
16.	No gap.	No gap.	Pest reporting. Available guidance: Explanatory document (2005) on ISPM 17 *(Pest reporting)*. Regulated pest lists clarification of terminology and its use in ISPM 19.

IPPC Area: GENERAL RIGHTS AND OBLIGATIONS IPPC SOs: A1, A2, B2, B3, B4, C3, D3, Y4			
	Concept standards - "what"	**Implementation standards - "how"**	**Other guidance**
17.	No gap.	No gap.	Guidelines for the revision of national phytosanitary legislation – FAO Establishing an NPPO (manual), establishment of an NPPO (training kit)

IPPC Area: PRINCIPLES AND POLICIES (interpretation of the Convention) IPPC SOs: B2, B3, C3, D1, D3			
	Concept standards - "what"	**Implementation standards - "how"**	**Other guidance**
18.	Phytosanitary principles for the protection of plants and the application of phytosanitary measures in international trade (ISPM 1)	No gap.	1) Undue delay and prompt action, Operation of a NPPO manual, Operation of a NPPO (training kit)
19.	Glossary of phytosanitary terms (ISPM 5) Terminology of the Convention on Biological Diversity in relation to the Glossary of phytosanitary terms (ISPM 5 – Appendix 1)	No gap.	Available guidance: Annotated Glossary: Explanatory document (2013) on ISPM 5 *(The Glossary of phytosanitary terms)*
20.	Efficacy of measures (Priority 4)	No gap.	Efficacy of measures
21.	No gap.	Recognition of pest free areas and areas of low pest prevalence (ISPM 29)	Technical Justification including reliability of scientific information
22.	Guidelines for the determination and recognition of equivalence of phytosanitary measures (ISPM 24)		Available guidance: **Equivalence** (draft manual)
23.	**Authorization of entities other than national plant protection organizations to perform phytosanitary actions (2014-002)** (Priority 2 (from 3))	No gap.	
24.	No gap.	No gap.	Appropriate level of protection
25.	No gap.	No gap.	State of plant protection in the world

IPPC Area: PEST STATUS IPPC SOs: A1, A2, B1			
	Concept standards - "what"	**Implementation standards - "how"**	**Other guidance**
26.	Determination of pest status in an area (ISPM 8) (Priority 1)		
27.	Revision: Regulated non-quarantine pests: concept and application (ISPM 16), to broaden to pests and clarify the concepts related to quarantine pests, RNQP and pests of national concern (Priority 2) Guidelines on the interpretation and application of the concept of official control for regulated pests (ISPM 5 - Supplement 1)	No gap.	Available guidance: IPPC coverage of aquatic plants (CPM recommendation CPM-9/2014/01); GMOs, Biosafety and Invasive Species: ICPM 3 (2001) decision
28.	Host and non host status (Priority 3)	Determination of host status of fruit to fruit flies (Tephritidae) (ISPM 37) (Priority 1)	
29.	Guidelines for surveillance (ISPM 6) (Priority 1)		
30.	No gap.	Specific guidance on surveillance for a pest or a group of pests (Priority 3)	Guidance on surveillance for a pest or a group of pests. Available guidance: **Surveillance** (manual), Technical resources (manuals, standard operating procedures, public outreach materials, projects, etc.) on general and specific pest surveillance available on phytosanitary.info
31.	Requirements for the establishment of pests free areas (ISPM 4) (Priority 4 (from 2)) Establishment of pest free areas for fruit flies (Tephritidae) (ISPM 26)		
32.	Requirements for the establishment of pest free places of production and pest free production sites (ISPM 10)		
33.	Requirements for the establishment of areas of low pest prevalence (ISPM 22)		
34.	No gap.	Specific guidance on PFA, PFPP and ALPP for a pest or a group of pests (Priority 4)	

IPPC Area: PEST STATUS IPPC SOs: A1, A2, B1			
Concept standards - "what"		**Implementation standards - "how"**	**Other guidance**
		Establishment of areas of low pest prevalence for fruit flies (ISPM 30) Control measures for an outbreak within a fruit fly-pest free area (ISPM 26 - Annex 2)	

IPPC Area: PEST RISK ANALYSIS IPPC SOs: C2, C3, B2, B3, B4			
	Concept standards - "what"	**Implementation standards - "how"**	**Other guidance**
35.	Framework for pest risk analysis (ISPM 2)	Pest risk analysis for quarantine pests (ISPM 11) Pest risk analysis for regulated non-quarantine pests (ISPM 21) Categorization of commodities according to their pest risk (ISPM 32) Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms (ISPM 3) Guidance on climate change (supplement to ISPM 11) (Priority 3)	Commodity and host pest lists Available guidance: **PRA awareness toolkit** (proposed manual); PRA training (manual and eLearning)
36.	Revision and combination of PRA standards (including ISPM 2, 11 and 21) (priority 4)		Commodity and host pest lists
37.	**Guidance on pest risk management (2014-001)** (Priority 2 (from 1))	Specific guidance on pest risk management for pests or a group of pests (Priority 3)	
38.	Risk communication (Priority 3)		
39.	Guidelines on the understanding of potential economic importance and related terms including reference to environmental considerations (ISPM 5 - Supplement 2)	Economic analysis in PRA (Priority 2)	
40.	Diversion from intended use (Priority 2? to be determined) (concept standard or supplementary document)	No gap.	Diversion from intended use

IPPC Area: PEST MANAGEMENT IPPC SOs: A1, A2, B1, B2, B4, C2, D1			
	Concept standards - "what"	**Implementation standards - "how"**	**Other guidance**
41.	Management of regulated pests (Priority 4)	No gap.	**CDC document on Phytosanitary Treatments** (draft manual)
42.	No gap.	No gap.	Pest management options
43.	Contingency planning and emergency response (Priority 1)	No gap.	
44.	No gap.	**Criteria for treatments for wood packaging material in international trade (2006-010) (draft annex to ISPM 15)** (Priority 2) **Revision of annex 1 and 2 of ISPM 15 (Inclusion of the Phytosanitary treatment *Sulphuryl fluoride fumigation of wood packaging material* (2006-010A) and *Revision of dielectric heating section* (2006-010B).**	Available guidance: Replacement of MeBr (CPM 3/1)
45.	Phytosanitary treatments for regulated pest (ISPM 28 and annexes)	Non-commodity specific phytosanitary treatments for regulated pests (e.g. soil drench, sterilization) (Annexes to ISPM 28) (Priority 4)	Available guidance: Explanatory document (2006) on ISPM 18 *(Guidelines on the use of irradiation as a phytosanitary treatment)*
46.	Guidelines for the use of irradiation as a phytosanitary measure (ISPM 18) (2014-007) (Priority 3 (from 2))		
47.	No gap.	**Requirements for the use of fumigation as a phytosanitary measure** (2014-004) (Priority 1)	
48.	No gap.	**Requirements for the use of temperature treatments as a phytosanitary measure** (2014-005) (Priority 1)	
49.	No gap.	**Requirements for the use of modified atmosphere treatments as a phytosanitary measure** (2014-006) (Priority 2)	

IPPC Area: PEST MANAGEMENT IPPC SOs: A1, A2, B1, B2, B4, C2, D1			
	Concept standards - "what"	**Implementation standards - "how"**	**Other guidance**
50.	No gap.	**Requirements for the use of chemical treatments as a phytosanitary measure** (2014-003) (Priority 3)	
51.	Guidelines for pest eradication programmes (ISPM 9)		
52.	No gap.	**Phytosanitary procedures for fruit fly (Tephritidae) management** (2005-010)	
53.	Integrated measures plants for planting (ISPM 36)		
54.	Systems approach (ISPM 14) Clarification on the concepts of integrated measures and systems approach (Priority 4)	Pest free potato (Solanum spp.) micropropagative material and minitubers for international trade (ISPM 33) Systems approach for pest risk management of fruit flies (Tephritidae) (ISPM 35) Specific guidance on systems approaches for commodities or pests (Priority 4)	

IPPC Area: PHYTOSANITARY IMPORT & EXPORT REGULATORY SYSTEMS IPPC SOs: A3, B4, C1, C2, C3, D3			
	Concept standards - "what"	**Implementation standards - "how"**	**Other guidance**
55.	Phytosanitary certification system (ISPM 7)	Phytosanitary certificates (ISPM 12) Electronic phytosanitary certificates, information on standard XML schemes and exchange mechanisms (ISPM 12 - Appendix 1)	Available guidance: e-Phyto (proposed system), **Import verification manual, export verification manual**
56.	Consignments in transit (ISPM 25)		Available guidance: **Transit** (proposed manual)
57.	No gap.	Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms (ISPM 3) Phytosanitary treatments for regulated pests (ISPM 28)	Available guidance: **Phytosanitary treatments based on historical evidence** (Position paper-TPPT draft)
58.	Guidelines for a phytosanitary import regulatory system (ISPM 20)		Available guidance: Explanatory document (2005) on ISPM 20 (*Guidelines for a phytosanitary import regulatory system*)
59.		Guidelines for a phytosanitary import regulatory system (ISPM 20) **Use of specific import authorization (2008-006) (ISPM 20, new annex)** (Priority 4 (from 3))	
60.	No gap.	Guidelines for inspection (ISPM 23)	
61.	Methodologies for sampling of consignments (ISPM 31)		Available guidance: Explanatory document (2009) on ISPM 31 (*Methodologies for sampling of consignments*)
62.	No gap.	Design and operation of post-entry quarantine stations for plants (ISPM 34)	
63.	No gap.	No gap.	Dispute settlement manual
64.	**Phytosanitary pre-import clearance (2005-003)** (Priority 3)	No gap.	

IPPC Area: PHYTOSANITARY IMPORT & EXPORT REGULATORY SYSTEMS IPPC SOs: A3, B4, C1, C2, C3, D3			
	Concept standards - "what"	**Implementation standards - "how"**	**Other guidance**
65.	No gap.	No gap.	Traceability **Proposed Traceback Guidance;** Market access (manual)
66.	No gap.	No gap.	Pathways
67.	No gap.	**Minimizing pest movement by air containers and aircrafts (2008-002)** (Priority 3 (from 1))	
68.	No gap.	**International movement of cut flowers and foliage (2008-005)** (Priority 4)	
69.	No gap.	**Safe handling and disposal of waste with potential pest risk generated during international voyages (2008-004)** (Priority 2 (from 3))	
70.	No gap.	**International movement of growing media in association with plants for planting (2005-004)** (Priority 1)	
71.	No gap.	**Minimizing pest movement by sea containers (2008-001)** (Priority 1)	Available guidance: CPM Recommendation on sea containers (CPM-10/2015/1)
72.	No gap.	**International movement of grain (2008-007)** (Priority 1)	Available guidance: Internet trade (e-commerce) in plants and other regulated articles (CPM recommendation CPM-9/2014/2)
73.	No gap.	**Guidelines for regulating wood packaging material in international trade (ISPM 15)** (Revision to include fraudulent use) (Priority 2)	Available guidance: Explanatory document (2014) on ISPM 15 (*Guidelines for regulating wood packaging material in international trade*); **Dielectric heat treatment** (draft manual); Quick guide to Dielectric heating
74.	No gap.	**International movement of used vehicles, machinery and equipment (2006-004)** (Priority 3)	
75.	No gap.	**International movement of seeds (2009-003)** (Priority 1)	

IPPC Area: PHYTOSANITARY IMPORT & EXPORT REGULATORY SYSTEMS IPPC SOs: A3, B4, C1, C2, C3, D3			
	Concept standards - "what"	**Implementation standards - "how"**	**Other guidance**
76.	No gap.	**International movement of wood (2006-029)** (Priority 1)	
77.	No gap.	**International movement of wood products and handicrafts made from wood (2008-008)** (Priority 2 (from 1))	

IPPC Area: DIAGNOSTICS IPPC SOs: A1, B1, B4			
	Concept standards - "what"	**Implementation standards - "how"**	**Other guidance**
78.	Diagnostic protocols for regulated pests (ISPM 27)	Annexes to Diagnostic protocols for regulated pests (ISPM 27)	**Guide to delivering Phytosanitary Diagnostic Services (manual)**
79.	No gap.	Requirements for diagnostics (Priority 2)	
80.	No gap.	No gap.	International or regional cooperation for diagnostics (e.g. Regional centres of expertise)

Appendix 05 – Terms of Reference for a Focus Group on establishing an Implementation Committee

Background and Purpose

The Secretariat Evaluation report suggested "one advisory body should be created, the nature of which will have to be determined by the CPM, to support implementation and provide capacity development for CPs; this body would replace the current ad-hoc bodies on CDC, IRSS TRG and NROAG and assume their mandates".

The CDC review recommended the CPM abolish the current CDC and establish an oversight committee, named "Implementation Committee".

The Bureau, at its June 2015 meeting discussed the results of the CDC Review. Several Bureau members felt that a new committee should be formed only once the Implementation unit had been formed in the Secretariat so that it would be clear what activities should be considered by this committee.

The consideration of implementation should be done in the light of the papers on this subject presented at CPM 2014/20 Rev.1 and the report of the OEWG on implementation as noted in CPM 2015/23.

Based on the discussions on the formation of an implementation committee as presented in CPM 2016/18, CPM 11 has identified the need to have further and in depth discussions and analysis about the establishment of the new IPPC implementation committee, which could be undertaken by a focus group.

Tasks
The focus group will consider, discuss and report on the following matters:

a) The purpose and scope.
b) Functions of the new implementation committee.
c) The functions of subsidiary bodies and existing ad hoc groups such as the Subsidiary Body on Dispute Settlement (SBDS), the National Reporting Obligations Advisory Group (NROAG), the e-Phyto Steering Group (ESG), the Capacity Development Committee (CDC), the Implementation Review and Support System (IRSS) Triennial Review Group (TRG) and their tasks and any necessary transitional arrangements.
d) Governance, work planning and prioritization within CPM decisions.
e) Relationships with the CPM, the CPM Bureau, the IPPC Secretariat, the Standards Committee and the Strategic Planning Group (SPG).
f) Resource analysis for operation of the new implementation committee.
g) Any other issues relevant to the establishment of the new implementation committee.
h) Terms of reference.
i) Rules of procedure.
j) Name of the new implementation committee.

Membership
The focus group will include one representative from each FAO region and the following:

a) A CPM Bureau member
b) CDC Chair or representative
c) SBDS Chair or representative
d) SC Chair or representative

e) Regional Plant Protection Organization representative
f) FAO or another organization implementation body representative

The focus group should have a combined knowledge in CPM governance, implementation and capacity development activities.

Each FAO region will nominate their representative through their Bureau member.

The focus group will be supported by the IPPC Secretariat.

Date and venue
The focus group meeting should be held prior to the end of July to allow presentation of the findings and recommendations at the SPG meeting in October 2016.

Reporting
The report of the focus group should be considered by the IPPC Secretariat, the SPG and Bureau. The resultant amended recommendations on the ToR and RoP for the new implementation committee should be submitted to CPM-12.

Appendix 06 – Ink amendments to the adopted Annexes to ISPM 28 (*Phytosanitary treatments for regulated pests*)

PT#	PT Title	Changes in the treatment schedule	Rationale for ink amendment to reflect end-point
PT 1	**Irradiation treatment for *Anastrepha ludens***	Minimum absorbed dose of 70 Gy to prevent the emergence of adults of *Anastrepha ludens*. ~~Efficacy and confidence level of the treatment is $ED_{99.9968}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule prevents emergence of not less than 99.9968% of adults of *Anastrepha ludens*.	The confirmatory trials demonstrated that the stated dose prevented adult emergence from the fruit that were treated containing third instar larvae that were identified as the most tolerant life stage.
PT 2	**Irradiation treatment for *Anastrepha obliqua***	Minimum absorbed dose of 70 Gy to prevent the emergence of adults of *Anastrepha obliqua*. ~~Efficacy and confidence level of the treatment is $ED_{99.9968}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule prevents emergence of not less than 99.9968% of adults of *Anastrepha obliqua*.	The confirmatory trials demonstrated that the stated dose prevented adult emergence from the fruit that were treated containing third instar larvae that were identified as the most tolerant life stage.
PT 3	**Irradiation treatment for *Anastrepha serpentina***	Minimum absorbed dose of 100 Gy to prevent the emergence of adults of *Anastrepha serpentina*. ~~Efficacy and confidence level of the treatment is $ED_{99.9972}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule prevents emergence of not less than 99.9972% of adults of *Anastrepha serpentina*.	The confirmatory trials demonstrated that the stated dose prevented adult emergence from the fruit that were treated containing third instar larvae that were identified as the most tolerant life stage.

PT#	PT Title	Changes in the treatment schedule	Rationale for ink amendment to reflect end-point
PT 4	**Irradiation treatment for *Bactrocera jarvisi***	Minimum absorbed dose of 100 Gy to prevent the emergence of adults of *Bactrocera jarvisi*. ~~Efficacy and confidence level of the treatment is $ED_{99.9981}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule prevents emergence of not less than 99.9981% of adults of *Bactrocera jarvisi*.	The confirmatory trials demonstrated that the stated dose prevented adult emergence from the fruit that were treated containing 1-day old eggs and third instar larvae that were identified as the most tolerant life stages.
PT 5	**Irradiation treatment for *Bactrocera tryoni***	Minimum absorbed dose of 100 Gy to prevent the emergence of adults of *Bactrocera tryoni*. ~~Efficacy and confidence level of the treatment is $ED_{99.9978}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule prevents emergence of not less than 99.9978% of adults of *Bactrocera tryoni*.	The confirmatory trials demonstrated that the stated dose prevented adult emergence from the fruit that were treated containing 1-day old eggs and third instar larvae that were identified as the most tolerant life stages.
PT 6	**Irradiation treatment for *Cydia pomonella***	Minimum absorbed dose of 200 Gy to prevent the emergence of adults of *Cydia pomonella*. ~~Efficacy and confidence level of the treatment is $ED_{99.9978}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule prevents emergence of not less than 99.9978% of adults of *Cydia pomonella*.	The confirmatory trials demonstrated that the stated dose prevented adult emergence from the fruit that were treated containing fifth instar larvae that were identified as the most tolerant life stage.

PT#	PT Title	Changes in the treatment schedule	Rationale for ink amendment to reflect end-point
PT 7	**Irradiation treatment for fruit flies of the family Tephritidae (generic)**	Minimum absorbed dose of 150 Gy to prevent the emergence of adults of fruit flies. ~~Efficacy and confidence level of the treatment is $ED_{99.9968}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule prevents emergence of not less than 99.9968% of adult fruit flies.	The confirmatory trials demonstrated that the stated dose prevented adult emergence from the fruit that were treated containing the most tolerant life stage of a number of economically important species in the Tephritidae.
PT 8	**Irradiation treatment for *Rhagoletis pomonella***	Minimum absorbed dose of 60 Gy to prevent the development of phanerocephalic pupae of *Rhagoletis pomonella*. ~~Efficacy and confidence level of the treatment is $ED_{99.9921}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule prevents the development of not less than 99.9921% of phanerocephalic pupae of *Rhagoletis pomonella.*	The confirmatory trials demonstrated that the stated dose prevented the formation of the phanerocephalic pupa in fruit that were treated containing third instar larvae that were identified as the most tolerant life stage.
PT 9	**Irradiation treatment for *Conotrachelus nenuphar***	Minimum absorbed dose of 92 Gy to prevent the reproduction in adults of *Conotrachelus nenuphar*. ~~Efficacy and confidence level of the treatment is $ED_{99.9880}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule prevents the reproduction in not less than 99.9880% of adults of *Conotrachelus nenuphar.*	The confirmatory trials demonstrated that the stated dose prevented successful reproduction (development of F1 beyond the first instar) in treated adults that were identified as the most tolerant life stage.

PT#	PT Title	Changes in the treatment schedule	Rationale for ink amendment to reflect end-point
PT 10	**Irradiation treatment for *Grapholita molesta***	Minimum absorbed dose of 232 Gy to prevent the emergence of adults of *Grapholita molesta*. ~~Efficacy and confidence level of the treatment is $ED_{99.9949}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule prevents emergence of not less than 99.9949% of adults of *Grapholita molesta.*	The confirmatory trials demonstrated that the stated dose prevented adult emergence from the fruit that were treated containing fifth instar larvae that were identified as the most tolerant life stage.
PT 11	**Irradiation treatment for *Grapholita molesta* under hypoxia**	Minimum absorbed dose of 232 Gy to prevent oviposition of *Grapholita molesta*. ~~Efficacy and confidence level of the treatment is $ED_{99.9932}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule prevents oviposition of not less than 99.9932% of *Grapholita molesta.*	The confirmatory trials demonstrated that the stated dose prevented egg laying (oviposition) in adults that emerged from the fruit that were treated containing fifth instar larvae that were identified as the most tolerant life stage.
PT 12	**Irradiation treatment for *Cylas formicarius elegantulus***	Minimum absorbed dose of 165 Gy to prevent the development of F1 adults of *Cylas formicarius elegantulus*. ~~Efficacy and confidence level of the treatment is ED99.9952 at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule prevents the development of not less than 99.9952% of F1 adults of *Cylas formicarius elegantulus.*	The confirmatory trials demonstrated that the stated dose prevented F1 adult production from eggs laid by treated adults that were identified as the most tolerant life stage.

PT#	PT Title	Changes in the treatment schedule	Rationale for ink amendment to reflect end-point
PT 13	**Irradiation treatment for *Euscepes postfasciatus***	Minimum absorbed dose of 150 Gy to prevent the development of F1 adults of *Euscepes postfasciatus*. ~~Efficacy and confidence level of the treatment is $ED_{99.9950}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule prevents the development of not less than 99.9950% of F1 adults of *Euscepes postfasciatus.*	The confirmatory trials demonstrated that the stated dose prevented F1 adult production from eggs laid by treated adults that were identified as the most tolerant life stage.
PT 14	**Irradiation treatment for *Ceratitis capitata***	Minimum absorbed dose of 100 Gy to prevent the emergence of adults of *Ceratitis capitata.* ~~Efficacy and confidence level of the treatment is $ED_{99.9970}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule prevents emergence of not less than 99.9970% of adults of *Ceratitis capitata.*	The confirmatory trials demonstrated that the stated dose prevented adult emergence from the fruit that were treated containing third instar larvae that were identified as the most tolerant life stage.
PT 15	**Vapour heat treatment for *Bactrocera cucurbitae* on *Cucumis melo* var. *reticulatus***	**[Scope of the treatment** This treatment comprises the vapour heat treatment of *Cucumis melo* var. *reticulatus* (netted melon) fruit to result in the mortality of eggs and larvae of melon fly (*Bactrocera cucurbitae*) at the stated efficacy.] **Treatment schedule** ~~The efficacy and confidence level of the treatment is effective dose $(ED)_{99.9889}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule kills not less than 99.9889% of eggs and larvae of *Bactrocera cucurbitae*.	The confirmatory trials demonstrated that the stated dose killed the treated eggs and third instar larvae that were identified as the most tolerant life stages.

PT#	PT Title	Changes in the treatment schedule	Rationale for ink amendment to reflect end-point
PT 16	**Cold treatment for *Bactrocera tryoni* on *Citrus sinensis***	**[Scope of the treatment** This treatment comprises the cold treatment of fruit of *Citrus sinensis* (orange) to result in the mortality of eggs and larvae of *Bactrocera tryoni* (Queensland fruit fly) at the stated efficacy.] **Treatment schedule** ~~For cultivar "Navel" the efficacy is effective dose (ED)$_{99.9981}$ at the 95% confidence level.~~ ~~For cultivar "Valencia" the efficacy is ED$_{99.9973}$ at the 95% confidence level.~~ For cultivar "Navel", there is 95% confidence that the treatment according to this schedule kills not less than 99.9981% of eggs and larvae of *Bactrocera tryoni*. For cultivar "Valencia", there is 95% confidence that the treatment according to this schedule kills not less than 99.9973% of eggs and larvae of *Bactrocera tryoni*.	The confirmatory trials demonstrated that the stated dose killed the treated first instar larvae that were identified as the most tolerant life stage.

PT#	PT Title	Changes in the treatment schedule	Rationale for ink amendment to reflect end-point
PT 17	**Cold treatment for *Bactrocera tryoni* on *Citrus reticulata* × *Citrus sinensis***	**[Scope of the treatment** This treatment comprises the cold treatment of fruit of *Citrus reticulata* × *Citrus sinensis* (tangor) to result in the mortality of eggs and larvae of *Bactrocera tryoni* (Queensland fruit fly) at the stated efficacy.] **Treatment schedule** ~~The efficacy is effective dose (ED)$_{99.9986}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule kills not less than 99.9986% of eggs and larvae of *Bactrocera tryoni*.	The confirmatory trials demonstrated that the stated dose killed the treated first instar larvae that were identified as the most tolerant life stage.

PT#	PT Title	Changes in the treatment schedule	Rationale for ink amendment to reflect end-point
PT 18	**Cold treatment for *Bactrocera tryoni* on *Citrus limon***	**[Scope of the treatment** This treatment applies to the cold treatment of fruit of *Citrus limon* (lemon) to result in the mortality of eggs and larvae of *Bactrocera tryoni* (Queensland fruit fly) at the stated efficacy.] **Treatment schedule** **Schedule 1: 2 °C or below for 14 continuous days** ~~The efficacy is effective dose (ED)$_{99.99}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule kills not less than 99.99% of eggs and larvae of *Bactrocera tryoni*. **Schedule 2: 3 °C or below for 14 continuous days** ~~The efficacy is ED$_{99.9872}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule kills not less than 99.9872% of eggs and larvae of *Bactrocera tryoni*.	The confirmatory trials demonstrated that the stated dose killed the treated first instar larvae that were identified as the most tolerant life stage.

PT#	PT Title	Changes in the treatment schedule	Rationale for ink amendment to reflect end-point
PT 19	**Irradiation treatment for *Dysmicoccus neobrevipes*, *Planococcus lilacinus* and *Planococcus minor***	Minimum absorbed dose of 231 Gy to prevent the reproduction of adult females of *Dysmicoccus neobrevipes*, *Planococcus lilacinus* and *Planococcus minor*. ~~Efficacy and confidence level of the treatment is $ED_{99.99023}$ at the 95% confidence level.~~ There is 95% confidence that the treatment according to this schedule prevents the reproduction of not less than 99.99023% of adult females of *Dysmicoccus neobrevipes*, *Planococcus lilacinus* and *Planococcus minor*.	The confirmatory trials demonstrated that the stated dose prevented F1 larval development from eggs laid by treated female adults that were identified as the most tolerant life stage.

Appendix 07 – IPPC Standard Setting Procedure as adopted by CPM-11 (2016)

INTERNATIONAL PLANT PROTECTION CONVENTION
STANDARD SETTING PROCEDURE
(ANNEX 3 OF THE RULES OF PROCEDURE OF THE COMMISSION ON PHYTOSANITARY MEASURES)

The process for the development of International Standards for Phytosanitary Measures (ISPMs) is divided into four stages:

- Stage 1: Developing the *List of topics for IPPC standards*
- Stage 2: Drafting
- Stage 3: Consultation for draft ISPMs
- Stage 4: Adoption and publication.

Relevant Interim Commission on Phytosanitary Measures (ICPM) / Commission on Phytosanitary Measures (CPM) decisions on many aspects of the Standard setting procedure have been compiled in the IPPC Procedure manual for standard setting, which is available on the International Phytosanitary Portal (IPP, www.ippc.int).

STAGE 1: Developing the *List of topics for IPPC standards*

Step 1: Call for topics

The IPPC Secretariat makes a call for topics[56] every two years. Contracting parties (CPs) and regional plant protection organizations (RPPOs) submit detailed proposals for new topics or for the revision of existing ISPMs to the IPPC Secretariat. Submissions should be accompanied with a draft specification (except for Diagnostic protocols (DPs)), a literature review and justification that the proposed topic meets the CPM-approved criteria for topics (available in the IPPC Procedure manual for standard setting). To indicate a global need for the proposed topic, submitters are encouraged to gain support from CPs and RPPOs in other regions.

A separate call for submissions for Phytosanitary treatments (PTs) is made.

The Standards Committee (SC), taking into account the IPPC Strategic Framework and the *Criteria for justification and prioritization of proposed topics*, reviews the submissions. The SC reviews the *List of topics for IPPC standards* (including subjects), adding topics and giving each topic a recommended priority. This list is recommended to the CPM.

The CPM reviews, changes and adopts the *List of topics for IPPC standards*, including assigning a priority for each topic.

A revised *List of topics for IPPC standards* is made available.

Step 2: Annual review of the *List of topics for IPPC standards*

Annually the SC reviews the *List of topics for IPPC standards* and recommends changes (including deletions, or changes in priority) to the CPM. In exceptional circumstances, in response to a specific need, the SC may recommend an addition to the *List of topics for IPPC standards*.

The CPM reviews the *List of topics for IPPC standards* recommended by the SC. The CPM changes and adopts the *List of topics for IPPC standards*, including assigning a priority for each topic. A revised *List of topics for IPPC standards* is made available.

[56] This is a call for "technical area", "topic", "Diagnostic Protocol (DP)", see the *Hierarchy of terms for standards* in the IPPC Procedure manual for standard setting.

In any year, when a situation arises in which an ISPM or a revision to an ISPM is required urgently, the CPM may add such a topic into the *List of topics for IPPC standards*.

Stage 2: Drafting

Step 3: Development of a specification

The SC should be encouraged to assign a lead steward and assistant(s) for each topic. These assistants could be from outside the SC, such as potential SC replacement members, former SC members, technical panel (TP) members or expert working group members.

The SC reviews the draft specification. The SC should endeavour to approve draft specifications for consultation at the SC meeting following the CPM session when new topics have been added to the *List of topics for IPPC standards*.

Once the SC approves the draft specification for consultation, the IPPC Secretariat makes it publicly available. The IPPC Secretariat solicits comments through the IPPC Online Comment System (OCS) from CPs, RPPOs, relevant international organizations, and other entities as decided by the SC. The length of the consultation for draft specifications is 60 days. The IPPC contact point or information point submits comments to the IPPC Secretariat using the OCS.

The IPPC Secretariat compiles the comments received, makes them publicly available and submits them to the steward and the SC for consideration. The specification is revised and approved by the SC, and made publicly available.

Step 4: Preparation of a draft ISPM[57]

An expert drafting group (EDG) (i.e. expert working group (EWG) or TP) drafts or revises the draft ISPM in accordance with the relevant specification. The SC may request the IPPC Secretariat to solicit comments from scientists around the world to ensure the scientific quality of draft DPs. The resulting draft ISPM is recommended to the SC.

The SC or the SC working group established by the SC (SC-7) reviews the draft ISPM at a meeting (for a DP or PT, the SC reviews it electronically) and decides whether to approve it for consultation, to return it to the steward or an EDG or to put it on hold. When the SC-7 meets, comments from any SC members should be taken into account.

STAGE 3: Consultation and review

Draft ISPMs are submitted to two consultation periods except for draft DPs which are submitted to one consultation period unless decided otherwise by the SC.

Step 5: First consultation

Once the SC approves the draft ISPM for the first consultation, the IPPC Secretariat makes it publicly available. The IPPC Secretariat solicits comments through the OCS from CPs, RPPOs, relevant international organizations, national plant protection services of non-CPs, and other entities as decided by the SC. The length of the First consultation for draft ISPMs is 90 days. The IPPC contact point or information point submits comments to the IPPC Secretariat using the OCS. The IPPC Secretariat compiles the comments received, makes them publicly available and submits them to the steward for consideration.

The steward reviews the comments, prepares responses to the comments, revises the draft ISPM and submits them to the IPPC Secretariat. These are made available to the SC. Taking the comments into

[57] This procedure refers to "draft ISPMs" and "standards" to simplify wording, but also applies to any part of an ISPM, including annexes, appendices or supplements.

account, the SC-7 or TP (for draft DPs or draft PTs) revises the draft ISPM and recommends it to the SC.

For draft ISPMs other than draft DPs and draft PTs, responses to the major issues raised in the comments are recorded in the report of the SC-7 meeting. Once the SC-7 recommends the draft ISPM to the SC, the IPPC Secretariat makes it publicly available.

For draft PTs or draft DPs, once the SC has approved them and the responses to comments, the drafts and responses to comments are made publicly available. A summary of the major issues discussed by the SC for the draft DP or draft PT is recorded in the report of the following SC meeting.

Alternatively to approving the draft ISPM, the SC may for example return it to the steward or an EDG, submit it for another round of consultation or put it on hold.

Step 6: Second consultation

Once the SC or SC-7 approves the draft ISPM for the second consultation, the IPPC Secretariat solicits comments through the OCS from CPs, RPPOs, relevant international organizations, national plant protection services of non-CPs, and other entities as decided by the SC. The length of the Second consultation is 90 days. The IPPC contact point or information point submits the comments to the IPPC Secretariat using the OCS. The IPPC Secretariat compiles the comments received, makes them publicly available and submits them to the steward for consideration.

The steward reviews the comments, prepares responses to the comments, revises the draft ISPM and submits the revised draft ISPM to the IPPC Secretariat. These are made available to the SC and the revised draft ISPM, other than draft PTs, is made available to CPs and RPPOs.

The SC reviews the comments, the steward's responses to the comments and the revised draft ISPM. For draft ISPMs other than draft PTs, the SC provides a summary of the major issues discussed by the SC. These summaries are recorded in the report of the SC meeting.

For draft PTs, once the SC has approved them and the responses to comments, the drafts and responses to comments are made publicly available. A summary of the major issues discussed by the SC for the draft PT is recorded in the report of the following SC meeting.

Alternatively to recommending the draft ISPM to the CPM, the SC may for example return it to the steward or an EDG, submit it for another round of consultation, or put it on hold.

STAGE 4: Adoption and publication

Step 7: Adoption

- For draft ISPMs other than draft DPs:

Following recommendation by the SC, the draft ISPM is included on the agenda of the CPM session. The IPPC Secretariat should make the draft ISPM presented to the CPM for adoption available in the languages of the Organization as soon as possible and at least six weeks before the opening of the CPM session.

If all CPs support the adoption of the draft ISPM, the CPM should adopt the ISPM without discussion.

If a CP does not support the adoption of the draft ISPM, the CP may submit an objection[58]. An objection must be accompanied by technical justification and suggestions for improvement of the draft ISPM which are likely to be acceptable to other CPs and be submitted to the IPPC Secretariat no later than 3

[58] An objection should be a technically supported objection to the adoption of the draft standard in its current form and sent through the official IPPC contact point (Refer to the *Criteria to help determine whether a formal objection is technically justified* as approved by CPM-8 (2013), recorded in the IPPC Procedure manual for standard setting).

weeks before the CPM session. Concerned CPs should make every effort to seek agreement before the CPM session. The objection will be added to the CPM agenda and the CPM will decide on a way forward.

When the need for a minor technical update to an adopted ISPM is identified by a TP or the SC, the SC can recommend the update for adoption by the CPM. The IPPC Secretariat should make the update to the adopted ISPM available in the languages of the organization as soon as possible and at least six weeks prior to the opening of the CPM meeting. Minor technical updates to adopted ISPMs presented to the CPM are subject to the objection process as described above.

- For draft DPs:

The CPM has delegated its authority to the SC to adopt DPs on its behalf. Once the SC approves the DP, the IPPC Secretariat makes it available on defined dates twice a year and CPs are notified[59]. CPs have 45 days to review the approved DP and submit an objection, if any, along with the technical justification and suggestions for improvement of the approved DP. If no objection is received, the DP is considered adopted. DPs adopted through this process are noted by the CPM and attached to the report of the CPM meeting. If a CP has an objection, the draft DP should be returned to the SC.

When a technical revision[60] is required for an adopted DP, the SC can adopt the updates to adopted DPs via electronic means. The revised DPs shall be made publicly available as soon as the SC adopts them. DPs revised through this process are noted by the CPM and attached to the report of the CPM meeting.

Step 8: Publication

The adopted ISPM is made publicly available.

CPs and RPPOs may form a Language Review Group (LRG) and, following the CPM-agreed LRG process[61], may propose modifications to translations of adopted ISPMs.

[59] For translation of DPs, contracting parties would follow the mechanism for requesting the translation for DPs into FAO languages posted on the IPP (https://www.ippc.int/en/core-activities/standards-setting/member-consultation-draft-ispms/mechanism-translate-diagnostic-protocols-languages/).

[60] A technical revision for DPs has been defined by the SC and is recorded in the IPPC Procedure manual for standard setting.

[61] https://www.ippc.int/en/core-activities/governance/standards-setting/ispms/language-review-groups/

Appendix 08 – Implementation Pilot on Surveillance Work Plan

A. Preparatory phase (2015-2017):
Using existing resources and, where possible, extra-budgetary contributions, a preparatory work phase would set the foundation and strategy for work plan activities. This phase will build on past studies of challenges and successes of surveillance-related activities to provide further strategic direction for the next phases of the pilot project.

This would include:

- Aggregation and analysis of baseline studies, existing tools, guidance and projects that can be leveraged and built upon.
- Aggregation and analysis of case studies of past successes and challenges in surveillance, to be able to provide specific examples of development of surveillance activities in different contexts.
- Revision of ISPM 6 and other surveillance-related ISPMs (Revision of ISPM 8. *Determination of pest status in an area* (2009-005) and Revision of ISPM 4. *Requirements for the establishment of pest free areas* (2009-002)), taking into account the outcomes of IRSS studies, as a component of the pilot project for updated guidance on surveillance to contracting parties.
- Identification of stakeholders (including the civil society), degree of involvement and roles at various levels (sub-national, national, regional, international, etc.).
- Development of indicators to measure the success of the pilot project and broader implementation programme.
- Exploration of options to encourage national and regional-level participation and to take ownership for actions and results in the pilot project, for long term sustainability.
- Establishment of a monitoring and evaluation framework to allow for responsiveness and continuous improvement in the pilot project as well as the IPPC implementation programme.
- Development of feedback mechanisms of the pilot project, through the IRSS project, National Reporting Obligations (NROs), Standard-Setting and Capacity Development programmes.
- Conducting a budget, timeline and work plan revision for the pilot project.

B. Project implementation phase (2017-2020):
This phase has two initial components of activities consisting of the design and development of technical resources and their implementation. A third component on feedback mechanisms is cross-cutting and concurrent with the other two components.

1. Design and development of relevant technical resources
Technical resources (e.g. guidance and tools) would be designed and developed, or where they exist contributed resources would be adapted, to satisfy the global needs identified in the preparatory phase through an ongoing analysis of materials. The areas in which technical resources would be elaborated are the following:

- Development of technical resources as needed on surveillance-related activities and of training material, including on:
  - Guidance on common understanding of general surveillance,
  - Guidance on collection and validation of information at country level,
  - Guidance on specific surveillance including delimitation and trace-back and cross-border cooperative surveillance programmes,
  - Guidance on utilizing this information to meet NROs and inform other national phytosanitary processes, e.g. PRA or establishment of a list of regulated pests,
  - Guidance on Decision-Support-Systems on surveillance.
- Support of regional and national data collection, management and information exchange initiatives:

- Facilitate the establishment and/or improvement of systems and tools,
- Promote stakeholders (including the civil society) involvement through existing information exchange mechanisms.

- Development of technical resources to set and/or update national policies and legislations on surveillance activities, to meet NROs, and to support NPPOs to engage with relevant resources, e.g. resource mobilization, appropriate technical competencies.
- Development of awareness raising and communication material, tools and campaigns to facilitate the involvement of stakeholders (including the civil society), and to convey the message of surveillance and NRO related-activities. This work would contribute to the International Year of Plant Health (IYPH).

2. Coordinated implementation and support activities

Developed and/or contributed resources (manuals, e-learning resources, etc.) would be disseminated for implementation through coordinated national and regional activities to ensure their long-term use.

The steps for long-term implementation would be the following:

- Development or adaptation of training material to support the technical resources when necessary (e-learning, workshops activities, etc.).
- Facilitation and training opportunities to use the training materials through existing fora as well as through training mechanisms including workshops, mentoring programmes, dissemination of e-learning tools, manuals, videos, etc.
- Facilitation and training opportunities to use the data systems and the functioning of data management.
- Development of training mechanisms for setting and maintaining surveillance-related activities, e.g. project and programme design and management, human resource management, resource mobilization for long term planning and advocacy.
- Facilitation and training to ensure that technical information generated through a national surveillance programme is appropriately utilized to meet surveillance-related NROs and used in other phytosanitary processes.
- Development of implementation plans for surveillance-related ISPMs.
- Promotion and encouragement of establishing and sustaining partnerships and other types of collaborative mechanisms to leverage existing resources to establish and maintain functional surveillance programmes and associated activities.

C. Feedback mechanisms (concurrent phase)

Active feedback would be encouraged during the duration of the pilot project to inform future work activities and future implementation programme streams. Feedback mechanisms would translate into the following activities:

- Review of surveillance-related ISPMs and technical resources, incorporating broad feedback from contracting parties and other stakeholders (including the civil society), using the existing mechanisms and programmes: IRSS, NROs.
- Establishing reporting and feedback mechanisms for ongoing activities, and for determining implementation priorities.
- Assessment and improvement of the methods used to encourage national and regional-level participation, actions and results in the pilot project as well as sharing successes and challenges of implementation.
- Preparation of materials on the state of implementation of surveillance-related activities.

Appendix 09 – General and Specific IPPC National Reporting Obligations (NROs) procedures

1a - General IPPC National Reporting Obligations (NROs) procedures

The following general NROs procedures are established as per Article VIII, paragraph 1(a) of the IPPC.

	Subject	NROs Procedures	Remarks
1.	The use of **electronic media**	The primary, and preferred, modality of communicating NROs is electronically, where applicable, as this is more efficient than paper communications and substantially reduces resources needed by the Secretariat to process paper. For the purposes of the IPPC, the phrases "make available to", "reported to", "submit to", "transmit to" and "communicate to" the Secretary means that the Secretary to the IPPC must be notified directly and the preferred mechanism of undertaking this is by publishing on the IPP by contracting parties (apart from the nomination of the IPPC Official Contact Point, which is published on the IPP by the Secretariat).	CPM-1 (2006) agreed to the use, wherever possible, of electronic communications between official contact points and the Secretariat (Report of CPM-1 (2006), paragraph 152).
2.	The use of the **International Phytosanitary Portal** (**IPP**)	1) To make optimal use of the Secretariat resources and ensure fast and effective communication, posting NRO information on the IPP is considered by CPM to be meeting contracting parties' (CPs) national reporting obligations, which includes those which specifically need to be sent to the Secretary, other CPs, national plant protection organizations (NPPOs), regional plant protection organizations (RPPOs) or any combination thereof. 2) The IPP is the preferred IPPC information exchange mechanism for NPPOs, CPs, the Secretariat and RPPOs to meet NROs. 3) Any NRO that needs to be reported to the Secretary, is reported by CPs on the IPP and hence public (apart from the nomination of the IPPC Official Contact Point which is published on the IPP by the Secretariat). 4) Official Contact Points can nominate editors to assist the CP meeting their NROs, but this needs to be done by formally notifying the Secretary of such. 5) Once a report is posted on the IPP by a CP it should be regularly checked, by Official Contact Points or their editors, and updated to reflect the most recent legislation in force and the status quo of the present time. 6) The IPP makes allowance for either the direct uploading of NRO information onto the IPP, or can be done by making links available to their CP website/s where the NRO data is maintained. 7) The Secretariat is available to provide guidance to CPs in meeting their NROs, but should not upload NRO information on behalf of CPs.	ICPM-3 (2001) adopted the proposal for an IPP (Report of ICPM-3 (2001), paragraph 53). CPM-6 (2011) agreed to the Secretariat's recommendations to improve IPPC reporting, particularly through the IPP, as outlined in Appendix 6 to the Report of CPM-6 (2011), paragraph 90. The form to nominate an IPP editor by an Official Contact Point is available on the IPP (https://www.ippc.int/en/publications/ippc-official-contact-point-notification-form/).

3.	Pest reporting through **Regional Plant Protection Organizations**	In accordance with Article VIII1(a) of the IPPC, CPs cooperate in the exchange of information on plant pests. CPs may also undertake their pest reporting through their RPPOs. CPs need, however, to liaise with their RPPO first to ensure that they have a mechanism to allow pest reporting in this way. Should a CP wish to provide pest reports through its RPPO, the CP needs to provide the Secretariat with a signed form notifying that they are using this possibility/option for reporting. The CP can withdraw from pest reporting through RPPO and continue reporting directly to the Secretariat. The Secretariat needs to be informed about this change.	CPM-4 (2009) endorsed reporting through an RPPO (Report of CPM-4 (2009) paragraph 135). The form for contracting parties to provide legal authority to RPPOs to undertake pest reporting on their behalf is available on the IPP (https://www.ippc.int/publications/national-pest-reporting-through-regional-plant-protection-organizations).
4.	Reporting **information other** than NROs by countries	CPs can post other information on the IPP that they deem beneficial to other contracting parties, but meeting NROs should be given priority.	This option was envisaged in the Report of the Working Group on Information Exchange, as adopted by ICPM-3 (Report of CPM-3 (2001), paragraph 53 and Appendix XV).
5.	**Non-contracting parties**	Countries that are not CPs are encouraged to use the IPP. They can appoint "IPPC information points" and post IPPC-related information on the IPP.	At its 1st Session in 2001 the CPM decided that "countries that were not a contracting party should be allowed to post information on the IPP" (Report of CPM-1 (2006), paragraph 152).

1b - Specific IPPC National Reporting Obligations (NROs) procedures

Background:
In 2001, ICPM-3 adopted the interpretations of the IPPC on information exchange enclosed in the Report of the Working Group on Information Exchange (Report of the ICPM-3 (2001), paragraph 53 and Appendix XV). Since then, apart from the role of the IPPC Contact Points adopted by the CPM-1 (2006) (Appendix XVIII), no additional advice was adopted by the CPM on other National Reporting Obligations. The procedures below were drafted based on guidance provided by the NROAG in 2014 and 2015.

All obligations mentioned in the table constitute national reporting obligations of all Contracting Parties to the IPPC. The following procedures are agreed as per Article VIII 1(a) of the IPPC as presently in force. **Articles IV** (General provisions relating to the organizational arrangements for national plant protection), **VII** (Requirements in relation to import), **VIII** (International Cooperation), **XII** (Secretariat) and **XIX** (Languages) of the IPPC constitute the legal basis for the obligations set out in this table. Three types of reporting obligations were identified: basic (an obligation regardless of circumstances), event-driven (triggered by a specific event) and on request (triggered by a request); while there are two reporting methods: public or bilateral.

Article of the IPPC	Type	Method	Responsible Entity	Receiving entity: in accordance with the IPPC text	Languages (Art. XIX of the IPPC)	Reason	Remarks
VIII.2	**Designate an Official Contact Point (OCP) for the exchange of information**						
	Basic	Public	Contracting Party	Not specified	In accordance with Article XIX, paragraph 3 (e and f), "requests for information from contact points as well as replies to such requests, but not including any attached documents" and "any document made available by contracting parties for meetings of the Commission" shall be in at least one of the official languages of FAO.	1. Official Contact Points are central to the NRO programme and the broader IPPC programme. 2. It is important to facilitate the exchange of information on implementation of the IPPC as a whole, e.g. standard setting.	1. It takes a lot of time to manage changes to contact point. 2. There is a need to rely on many sources to ensure maintenance of the Official Contact Point system. 3. There is a need to create increased awareness and priority that NPPOs and CPs give to this task.

The Role of IPPC Contact Points (adopted in the report of the CPM-1 (2006), paragraph 152 and Appendix XVIII):

1. The IPPC contact points are used for all information exchanged under the IPPC among contracting parties, between the Secretariat and contracting parties and, in some cases, between contracting parties and Regional Plant Protection Organizations (RPPOs).

2. The IPPC contact point should:

- have the necessary authority to communicate on phytosanitary issues on behalf of the contracting party, i.e. as the contracting party's single IPPC enquiry point;
- ensure the information exchange obligations under the IPPC are implemented in a timely manner;
- provide coordination for all official phytosanitary communication between contracting parties related to the effective functioning of the IPPC;
- redirect phytosanitary information received from other contracting parties and from the IPPC Secretariat to appropriate official(s);
- redirect requests for phytosanitary information from contracting parties and the IPPC Secretariat to the appropriate official(s);
- keep track of the status of appropriate responses to information requests that have been made to the contact point.

3. *The role of the IPPC contact point is central to the effective functioning of the IPPC*, and it is important that the IPPC contact point has adequate resources and sufficient authority to ensure that requests for information are dealt with appropriately and in a timely manner.

4. Article VIII.2 requires contracting parties to designate a contact point, and therefore it is the contracting party which is responsible for making, and informing the Secretariat of, the nomination. There can be only one contact point per contracting party. The contracting party, by making the nomination, agrees that the nominee has the necessary authority to fulfil the functions of the contact point as determined within the framework of the IPPC. Individual persons cannot appoint themselves as contact points.

The following should be additionally respected by contracting parties while making an IPPC Official Contact Point (OCP) nomination[4] :

OCP nominations of contracting parties should be sent to the Secretary to the IPPC, preferably using the nomination form prepared for this purpose and available on the IPP.

An OCP should be a specific individual (with first name/s and family name/s) and not an entity or specific office.

The nomination of a new OCP must be signed by the person supervising and/or responsible for the new OCP. No self-nomination will be accepted.

Nominations should be submitted in a timely manner so that there is no gap in any official correspondence with the national OCP.

It is preferable for the OCP to be in the NPPO as the NPPO is responsible for the implementation of most of the IPPC actions.

Outgoing OCP should not nominate the new (incoming) OCP but arrange for the nomination to reach the Secretariat in a timely manner.

RPPOs and FAO representatives may facilitate the nomination of an OCP.

Should a CP unofficially indicate a Contact Point, the Secretariat will invite the relevant CP to submit a formal nomination in accordance with the procedures set forth in this document. Confirmation of the informal contact point as OCP or the designation of a new OCP should be notified to the Secretariat within 3 months from receiving the Secretariat's invitation.

Once the IPPC Secretary has made the OCP public on the IPP, the OCP is then responsible for keeping their contact information up-to-date.

Editors are nominated by OCPs to assist in delivery of the NROs, including the physical uploading of data on the IPP.

11. Countries which are not contracting parties to the IPPC can appoint an '*Information point'* for the purpose of the exchange of phytosanitary information.

[4] Recommendations and guidance provided by the NROAG.

IV.4 XII.4(d)	**Submit a description of the NPPO and its changes**						
	Basic	Public	Contracting parties	Secretary	Art. XIX.3 (a) sets out that information provided according to Article IV.4 shall be in at least one of the official languages of FAO.	1. Availability of information on NPPOs and their internal organization enhances their reliability and accessibility. 2. It ensures a degree of transparency and access to information on the internal organization of NPPOs.	
	1. A description of the NPPO should have the form of an organogram. Ideally, a description of its organizational arrangements should be included in that organogram (i.e. who is responsible for which area and what are the connections between different parts of the NPPO). That would implement both obligations set forth in Article IV.4 of the IPPC, i.e. a description of the NPPO and a description of its organizational arrangements for plant protection. [4] 2. A description of the NPPO should also identify the organizations that act under the authority of the NPPO as provided in Article IV.2 (a-g).[4]						
VII.2(b) XII.4(d)	**Publish and transmit phytosanitary requirements, restrictions and prohibitions**						
	Basic	Public	Contracting party	Any contracting party or parties that CPs believe may be directly affected by such measures.	1. Art XIX 3 (b) sets out that cover notes giving bibliographical data on documents transmitted according to Article VII paragraph 2 (b) shall be in at least one of the official	To facilitate safe and efficient international movement of plant, plant products and other regulated articles. To result in minimum impediment of international movement of plants, plant products and other regulated articles.	1. This was initially understood by the IPP Support Group to mean "all legislation and regulations". 2. In accordance with Article VII.2(b) of the IPPC, "*Contracting parties shall, immediately upon their adoption, publish and transmit phytosanitary requirements, restrictions and prohibitions to any contracting party or parties that CPs believe may be directly affected by such measures*". In accordance with Article XII 4 (d) of the IPPC, "*the Secretary shall disseminate information received from contracting parties on phytosanitary requirements,*

					languages of FAO. 2. Art XIX 3 (c) sets forth that information provided according to Article VII 2 (b) shall be in at least one of the official languages of FAO.		*restrictions and prohibitions referred to Art. VII 2(b)*". Article VII.2 (b) does not explicitly set out any obligation for CPs to notify phytosanitary requirements, restrictions and prohibitions to the IPPC Secretariat. Article XI.4 should be therefore interpreted as implying the duty of the Secretariat to publish phytosanitary requirements, restrictions and prohibitions only where such information is received from the relevant Contracting Parties.
1. Article XII.4 (d) sets forth the duty of the Secretary to disseminate information received from CPs on phytosanitary requirements, restrictions and prohibitions referred to Art. VII 2(b). The ICPM-3 adopted the recommendation that "all information on restrictions, requirements, and prohibitions be available through national or RPPO websites and/or national webpages within the IPPC website linked through IPP" (Report of the ICPM-3, Appendix XV, paragraph 18). CPs are encouraged to make phytosanitary requirements more widely available than in the past through their inclusion in the IPP (available to all countries whether affected or not by such measures).[4] 2. Phytosanitary requirements, restrictions and prohibitions can also be posted by CP on their own websites or RPPOs websites. In such cases information should be linked through the IPP.[4]							
VII.2(d) **XII.4(b)**	**Publish specified points of entry for plants or plant products**						
	Basic	Public	Contracting party	Secretary, RPPOs of which the contracting party is a member, all contracting parties which the contracting party believes to be directly affected, other contracting parties upon request.	Art XIX 3 (c) sets forth that information provided according to Article VII paragraph 2 (d) shall be in at least one of the official languages of FAO.	To facilitate safe and efficient international movement of plant and plant products. To result in minimum impediment of international movement of plants, plant products and other regulated articles.	Specified points of entry should be selected by a CP if a CP requires consignments of particular plants or plant products to be imported only through these points of entry.

[4] Recommendations and guidance provided by the NROAG.

1. Information on points of entry could be reported together with phytosanitary requirements, restrictions and prohibitions. [4] 2. In cases where there are no restrictions concerning entry points for consignments of plants and plant products into a country, no report is needed. However, it is recommended that the information about the lack of restrictions should be posted on the IPP. [4]							
VII.2(i) **XII.4(c)**	**Establish and update lists of regulated pests**						
	Basic	Public	Contracting party	Secretary, RPPOs of which they are members, other contracting parties on request.	Art XIX 3 (c) sets out that information provided according to Article VII paragraph 2(i) shall be in at least one of the official languages of FAO.	To allow trading partners access to information on pests regulated by importing country and for which they will need to meet established national measures.	1. A "list of pests (occurring within a country)" is not synonymous with a "list of regulated pests". 2. National surveillance systems need to be strengthened to establish and update lists of regulated pests. 3. Extensive Capacity Development, including pest identification, surveillance and pest risk assessment, is needed in a number of CPs before they can meet this NRO.
1. Regulated pest lists should be made available on IPP and therefore public to ensure that all provisions of the IPPC are met.[4]							
IV.2(b) **VIII.1(a)**	**Reporting of the occurrence, outbreak or spread of pests, and of controlling those pests** **International cooperation: Exchange of information on plant pests, particularly the reporting of the occurrence, outbreak or spread of pests that may be of immediate or potential danger**						
	Event driven	Public	NPPO and		Art XIX 3 (d) sets forth that notes giving bibliographical	1. It forms a basis for	1. A large number of CPs do not have the capacity to undertake

			Contracting party		data and a short summary of relevant documents on information provided according to Article VIII paragraph 1(a) shall be in at least one of the official languages of FAO.	cooperation among CPs. 2. It contributes towards the identification of phytosanitary risks. 3. As mentioned in the preamble of IPPC, avoidance of spread and introduction of pests of plants.	pest reporting in a sustainable manner. 2. Political commitment to pest reporting is required. The awareness on this issue should be increased to achieve this goal. 3. National surveillance systems need to be strengthened. Capacity development for surveillance and pest identification is needed for some CPs.
	1. Art. VIII 1(a) sets out that the reporting of pests will be undertaken "… *in accordance with such procedures as may be established by the Commission* …". The responsibilities of and requirements for CPs in reporting the occurrence, outbreak and spread of pests in areas for which they are responsible are set out in ISPM No.17, as adopted by ICPM-4 in 2002. 2. All reporting requirements established under ISPM 17 are fully met when pest reports are published through the IPP. [4] 3. Pest reports can also be made through existing RPPOs on condition that a CP signs an appropriate form to satisfy the legality of that action and the technical mechanism exist for the exchange of such data. [4] 4. A pest report should contain important information that allows CPs to adjust as necessary their phytosanitary import requirements and to take actions taking into account any changes in pest risk.[4] 5. When in doubt as to the qualification of a pest as a "pest of immediate or potential danger" and therefore its reporting, the reporting of any pest is desirable.[4]						
IV.4	**Provide a description of organizational arrangements for plant protection**						
	On request	Bilateral communication only but public posting on the IPP is	Contracting party	Other contracting parties upon request.	Art XIX.3 (a) sets forth that information provided according to Article IV paragraph 4 shall be in at least one of the official languages of FAO.	CPs may obtain clarifications as to the operation of the NPPO.	Not all CPs have developed such information, or kept existing data up-to-date.

[4] Recommendations and guidance provided by the NROAG.

		encouraged					
	1. This obligation is considered to be transmitted as a bilateral obligation. [4] 2. This requirement does not relate to the general structure of an NPPO (mentioned in the first sentence of Art IV.4), but to organizational arrangements described in Article IV.2 & 3. [4] 3. The report should contain a description of functions and responsibilities in relation to plant protection. It can be combined in one report with a NRO regarding a description of a NPPO and made public on the IPP as a single report. [4]						
VII.2(c)	**Make available the rationale for phytosanitary requirements, restrictions and prohibitions**						
	On request	Bilateral communication only but public posting on the IPP is encouraged	Contracting party	On request, to any contracting party	Article XIX.3 (e) sets out that requests for information from contact points as well as replies to such requests, but not including any attached, documents, shall be in at least one of FAO Languages.	1. To ensure CPs can safely trade with minimal negative impact on trade and research. 2. To ensure that unjustified measures are not in place. 3. To result in minimum impediment of international movement of plants, plant products and other regulated articles.	1. There is a global lack of PRAs on "old" regulated pests, on pathways and commodities. 2. There is also a lack of technical capacity within NPPOs.
	1. When requested to provide the rationale for certain phytosanitary requirements, restrictions and prohibitions, CPs should provide information as to the compliance of such measures with the requirements set out in Article VI.1 (a) and (b) for quarantine and regulated non-quarantine pests. [4] 2. It is suggested that one of FAO languages is to be used in reporting to facilitate transparency and communication. [4]						
VII.2 (f)	**Inform of significant instances of non-compliance with phytosanitary certification**						
	Event driven	Bilateral communication only	Importing contracting party	Exporting or re-exporting contracting party	Article XIX.3 (e) sets forth that requests for information from contact points as well as replies to such requests, but not including any attached, documents, shall be in at least one of FAO Languages.	To notify the exporting or re-exporting country of significant problems, e.g. interceptions of quarantine nature.	1. If needed a mechanism could be established to allow CPs to exchange this information on a bilateral basis, limited to parties concerned only. 2. Most CPs already have bilateral mechanisms in place to report non-compliance.

1. This obligation is considered to apply only to contracting parties concerned. [4] 2. If needed, a mechanism could be established to facilitate CPs to exchange information through the IPP but on a bilateral basis, restricted to parties concerned only. This has been requested by some CPs through IPPC capacity development activities. [4] 3. ISPM No. 13 sets out guidelines for the notification of on non-compliance. [4] 4. It is suggested that one of FAO languages is to be used in reporting to facilitate transparency and communication. [4]							
VII.2 (f)	**Report the result of its investigation regarding significant instances of non-compliance with phytosanitary certification**						
	Event driven	Bilateral communication	Exporting or re-exporting contracting party	On request by an importing contracting party	Article XIX.3 (e) sets out that requests for information from contact points as well as replies to such requests, but not including any attached, documents, shall be in at least one of FAO Languages.	To allow exporting or re-exporting country to justify and improve CP phytosanitary procedures.	A frequent lack of response to non-compliance communications is noted by many CPs.
	1. ISPM No. 13 sets out guidelines for the notification of on non-compliance.[4] 2. It is suggested that one of FAO languages is to be used in reporting to facilitate transparency and communication. [4]						
VII.2(j)	**Develop and maintain adequate information on pest status and make such information available**						
	On request	Bilateral communication but public posting on the IPP is encouraged	Contracting party, to best of its ability	Pest status information should be made available on request by CPs.	Art XIX 3 (c) sets out that information provided according to Article VII 2 (j) shall be in at least one of the official languages of FAO.	To enable categorization of pests, and for use in the development of appropriate phytosanitary measures	National surveillance systems need to be strengthened to carry out this task.
	1. ISPM No. 8 provides additional guidance on this reporting obligation, including the definition of the term 'pest status'. [4] 2. The term 'Categorization' is understood to refer to the differentiation of regulated and non-regulated pests. [4] 3. ISPM No. 6 provides guidance on what is meant by 'adequate' information. [4]						
VII.6	**Immediately report emergency action**						
	Event driven	Public	Contracting party	Contracting parties concerned, Secretary, RPPOs of which the contracting party is a member.	Article XIX.3 (e) sets out that requests for information from contact points as well as replies to such requests, but not including any attached, documents, shall be in at least one of FAO Languages.	Report new phytosanitary challenges that may affect the national phytosanitary status and those of partner / neighbouring countries.	1. Based on the Glossary of Phytosanitary Terms, "emergency action" is "a prompt phytosanitary action undertaken in a new or unexpected phytosanitary situation". The Glossary defines "phytosanitary action" as "an official operation, such as inspection, testing, surveillance or

[4] Recommendations and guidance provided by the NROAG.

							treatment, undertaken to implement phytosanitary regulations or procedures". 2. Information on emergency actions are often included in pest reports.
1. ISPM No. 13 contains partial (connected only to non-compliance of imported consignments) guidelines for the notification of emergency actions. [4] 2. When fulfilling the reporting obligation set out in Article VII.6, both emergency measures and emergency actions should be addressed. [4] 3. It is suggested that one of FAO languages is to be used in reporting to facilitate transparency and communication. [4]							
VIII.1(c)	**Cooperate in providing the technical and biological information necessary for pest risk analysis**						
	On request	Bilateral communication but public posting on the IPP is encouraged	Contracting party, to the extent practicable	Other contracting parties.	Article XIX.3 (e) sets out that requests for information from contact points as well as replies to such requests, but not including any attached, documents, shall be in at least one of FAO Languages.	To support the pest risk analysis (PRA) process	Timeliness in providing that information is desirable.
1. This obligation is considered as a bilateral obligation. CPs are nevertheless encouraged to disseminate technical and biological information necessary for pest risk analysis through the IPP. [4] 2. It is suggested that one of FAO languages is to be used in reporting to facilitate transparency and communication. [4]							

Appendix 10 – NRO Quality Control Guidelines

The National Reporting Obligations Advisory Group (NROAG) noted that the quality of National Reporting Obligations (NROs) information is variable and could be improved substantially by providing further guidance on each NRO and ensuring more consistent quality by the Secretariat of the reports uploaded. However, the NROAG and the Secretariat unanimously agreed that such quality control needs to be undertaken in a way that does not make any quality judgement of the technical content of such reports.

The purpose of quality control is to provide administrative support to Contracting Parties in ensuring that the reports they upload are easily located by the IPP users, correctly found when the IPP search tool is used and are easily understood from the title as to their content.

The following points are produced in consultation with the NROAG and are to provide guidance to the IPPC Secretariat on what can be communicated to contracting parties to improve the quality of the NROs on the IPP:

(1) Information has been misplaced on the IPP, e.g. a description of an NPPO has been reported as a pest report.
(2) Clarity can be improved regarding the title of the document, e.g. key information may be missing that would improve search results or understanding.
(3) Missing or corrupt files (which do not open) were identified.
(4) Missing or dead links (which do not open) were identified.
(5) Information was misplaced erroneously within the reporting form which causes confusion and makes files or links non-functional.
(6) New reports were added instead of updating the old (existing) report.
(7) Generic links were identified that do not provide the relevant information.
(8) Non-functional e-mail addresses were provided.
(9) Duplicate of reports, or text in a report, are identified.
(10) Typos, punctuation and spelling mistakes affecting searches, summaries or usability of data were identified.
(11) The selection of relevant keywords to make the information easily located.

Although the Secretariat will communicate the above points or information to the Official Contact Points (OCPs), with copies to the IPP country's editor/s, it remains the responsibility of the NPPO/OCP/editors to undertake the corrections or provide adequate updates if deemed necessary by them. Only on the request from the OCPs, and with their written permission, will the Secretariat physically undertake any of the above corrections.

The Secretariat will provide a feedback system on the IPP that will allow IPPC users to submit comments on perceived NRO data quality issues which will be transmitted to the relevant IPPC Contact Points.

[4] Recommendations and guidance provided by the NROAG.

Appendix 11 – NRO Work Plan (2014 – 2023)

No.	Task	Progress	Deadline	Performance indicators	Estimated budget (USD)*	Lead entities supported by
1.	Maintenance of **Official Contact Points** data	completed/ongoing	2015 March/+	Database with the up-to-date contact details of OCPs available	Existing P2 (NRO Officer) on IPPC TF*	Secretariat
2.	**NRO work programme**					
2.1	develop /revise NRO work plan	Submitted to CPM-11	2016 March	Revised NRO work programme established and endorsed	Existing P4 on RP and P2 (NRO Officer) on IPPC TF*	CPM; Secretariat; NROAG
2.1.1	adjustment of the NRO work programme after mid-term review	to be completed	2019+	An updated NRO Work Plan agreed by CPM in 2019	Existing P4 on RP and P2 (NRO Officer) on IPPC TF*	Secretariat; NROAG
2.1.2	review work of NROAG and make recommendations on its future role, composition and functions	to be completed	2018	Paper submitted on the future role, composition and functions of NROAG submitted to CPM in 2018.	Existing P4 on RP and P2 (NRO Officer) on IPPC TF*	Secretariat; NROAG
2.2	Establishment of a monitoring and evaluation framework to allow for responsiveness and continuous improvement of NRO	to be completed/ongoing	2016 May/+	Clear goals that should be reached and evaluation steps that shows success or problems within overall NRO and can serve for NRO improvement also in specific aspects if needed	Existing P2 (NRO Officer) on IPPC TF*	CPM; Secretariat; NROAG
2.3	Development of indicators to measure the success of overall implementation.	to be completed	2016 May	Evaluation and feedback to each CPM about overall results of NRO implementation system	Existing P2 (NRO Officer) on IPPC TF*	Secretariat
3.	**Terminology**					

3.1	request SC (TPG) to provide clarity on terminology used in the Convention to describe NROs	completed	2015 March	Request passed to the SC in 2015.	0	Secretariat
3.2	agree on terminology to use in classifying the various NROs.	completed	2016 April	NRO Procedures submitted to CPM-11 in 2016	0	Secretariat
4.	**NROs interpretations**					
4.1	Develop paper/s for CPM on reporting procedures, hosting on IPP and in particular pest reporting. Consolidate all CPM decisions relating to IE and NROs, in particular inconsistencies.	completed	2016 April	NRO Procedures submitted to CPM-11 in 2016	Existing P4 on RP and P2 (NRO Officer) on IPPC TF*	Secretariat
5.	**Manuals**			Manuals available in languages		
5.1	Revise the NRO manual	Completed in February 2015, but will be updated as and when necessary. Available in 5 FAO languages	2015 March	The editors' guide was made available in February 2015.	Existing P2 (NRO Officer) on IPPC TF*	Secretariat
6.	**Training**			Training materials and courses available.		
6.1	training: develop training tools and materials, including e-learning	Completed/ongoing (NROs UPDATE newsletter has been issued monthly since October 2014; pre-CPM-10 training was prepared; an NRO exercise was prepared for the 2016 IPPC regional workshops	2016 April	NROs UPDATE issues monthly with NRO news and capacity development material.	Existing P2 (NRO Officer) on IPPC TF*	Secretariat; NROAG
6.2	finalize NRO training: training tools and materials	to be completed	2018	Development of a NRO eLearning module through the Southern Africa Solidarity Trust Fund.	$40,000	Secretariat; NROAG; FAO Africa regional and sub-regional office.
7.	**Reminders**					

7.1	reminder for ALL NROs.	completed (letters sent out and put on IPP in September 2014)	2015 March	Reminder letter sent to all CPs	Existing P2 (NRO Officer) on IPPC TF*	Secretariat
7.2	description of NPPO – reminder of the NRO	completed (letters sent out and put on IPP in May 2015)	2016 April	Reminder letter sent to all CPs	Existing P2 (NRO Officer) on IPPC TF*	Secretariat
7.3	develop an automated IPP reminder system	Completed	2016 April	Reminders system established and functioning	Existing P2 (NRO Officer) on IPPC TF*	Secretariat
7.4	optimize the NRO reminder system	to be completed	2018	System with updates functional.	Existing P2 (IT) on IPPC TF*	Secretariat
8.	**The NRO website**					
8.1	develop user requirements for the NRO website	Completed/ongoing (new IPP website was launched in February 2015 which will be upgraded continuously)	2016 December	Specifications and work plan for the revision of the NRO web pages.	Existing P2 (NRO Officer) on IPPC TF; Existing P2 (IT) on IPPC TF*	Secretariat; NROAG
8.2	re-development of the NRO website	to be completed	2018	NRO website functioning	Existing P2 (IT) on IPPC TF	Secretariat
8.3	optimization of new NRO website	to be completed	2019+	NRO website revision functional	Existing P2 (NRO Officer) on IPPC TF*	Secretariat; NROAG
8.4	develop new tools for NROs	to be completed	2019+	Release of new tools if and when requested by CPs and Secretariat.	Existing P2 (NRO Officer) on IPPC TF; Existing P2 (IT) on IPPC RP*	Secretariat; NROAG
9.	**Concept notes for project funding**			Concept notes prepared and funding obtained		
9.1	prepare concept notes for project funding e.g. staffing support, training (virtual and face-to-face), new tools, new software, Apps, on-line training.	completed/ongoing	2015 August	NRO project proposals available	Existing P4 on RP*	Secretariat

9.2	submit selected project proposals for funding	ongoing	2016 April	Proposal submitted or include in other project proposal submissions	Existing P4 on RP*	Secretariat
10.	**Awareness raising & coordination**					
10.1	transmit strong need for surveillance, PRA and diagnostics capacity development in support of NRO programme, particularly regulated pest lists and pest reporting to CDC and Implementation programme meeting.	completed	2015 August	Awareness raising through the monthly NROs UPDATE; relevant input into the CDC; included in IPPC Regional Workshops.	Existing P4 on RP and P2 (NRO Officer) on IPPC TF*	Secretariat
10.2	take advantage of existing synergies with other projects for implementation	ongoing	2016 April	Included into Implementation Pilot Project on Surveillance	Existing P4 on RP*	Secretariat
11.	**Pest reporting**					
11.1	complete existing pest reporting through RPPO tool	ongoing pilot project with EPPO – system established in the IPP and waiting for automatic feed of pest reports from EPPO	2016 March	Increased pest reports provided through EPPO	Existing P2 (IT) on IPPC TF	Secretariat
11.2	expand use of reporting through RPPO tool	working with PPPO/SPC to align their pest reporting system to allow the automatic uploading of pest reports waiting for alignment of NAPPO pest reporting system	2016 April-2018	Pest reporting for PPPO members through PPPO Pest reporting through NAPPO	Existing P2 (NRO Officer) on IPPC TF; Existing P2 (IT) on IPPC TF*	Secretariat
11.3	change format of reporting and retrieving data (NROAG to test before broader testing & release)	Completed / ongoing; Simplification of data entry forms already taken place, other changes once NRO tool developed.	2016 April-2018	A functional NRO tool for reporting	Existing P4 on RP; Existing P2 (NRO Officer) on IPPC TF; Existing P2 (IT) on IPPC TF	Secretariat

11.4	Explore possible synergies with environment sector	ongoing	2017 May	Identified and started practical cooperation	Existing P2 (NRO Officer) on IPPC TF*	CPM; Secretariat; NROAG
12.	**Regulated pest lists**			More pest lists made available, with updates, by CPs and reports provided		
12.1	request revision of the ISPM 19 – call for topics & SC; include in standard framework review (representative of NROAG chair to attend)	completed	2015 March	E-mail to SC via the Secretariat	Existing P4 on RP	Secretariat
12.2	improve reporting of regulated pest lists	to be completed	2018	Greater number of countries reporting regulated pest lists through the IPP	Existing P4 on RP; Existing P2 (NRO Officer) on IPPC TF	Secretariat
12.3	reporting by all CPs of regulated pest lists – monitoring and evaluation process	to be completed	2019+	Statistics and survey on increased regulated pest list reporting through the IPP	Existing P2 (NRO Officer) on IPPC TF	Secretariat
13.	**Emergency actions**					
13.1	emergency actions: request a study for IRSS to explore constraints to meet emergency action reporting, including towards a possible revision of ISPM 13 as might be requested by IPPC Contracting Party or RPPO	ongoing (change in approach by Bureau: the study is to be prepared by the Secretariat and NROAG)	2016 July	Development and completion of an emergency action survey by CPs	Existing P4 on RP; Existing P2 (NRO Officer) on IPPC TF	Secretariat; NROAG
13.2	emergency actions: address constraints to reporting emergency actions identified by the study	to be completed	2018	Increased emergency action reporting through the IPP	Existing P4 on RP; Existing P2 (NRO Officer) on IPPC TF	Secretariat; NROAG

*Three IPPC Secretariat staff will be involved in the Work Plan: Existing P4 (Information Officer) paid by the Regular Programme, and:
Existing P2 (NRO Officer) paid by the Trust Fund whose work on NROs per year is estimated to cost 76 406 USD (equivalent of 60% of working time dedicated to NROs), and
Existing P2 (IT – Web developer) paid by the Trust Fund whose work on NROs per year is estimated to cost 31 836 USD (equivalent of 25% of working time dedicated to NROs)

Appendix 12 – Communications and Advocacy Work Plan (2016 – 2020)

No.	Task	Deadline	Performance indicators	Lead entities	Supported by	Target audience
1.	**Improve the IPPC website**	2018 March	Improved usability and increased traffic visiting the IPPC website	Secretariat	Not applicable	Contracting parties, NPPOs, RPPOs, Academia, Researchers, Industry and general public
	1.1 Re-design and launch of a new IPP home page	2016 May	New home page in 6 FAO languages with improved usability	Secretariat	Not applicable	Contracting parties, NPPOs, RPPOs, other relevant stakeholders
	1.2 Revised IPPC website pages – approximately 60 pages in 6 languages)	2017 March	Revised pages in 6 FAO languages	Secretariat	Not applicable	Contracting parties, NPPOs, RPPOs, Academia, Researchers, Industry and general public
	1.3 Migration of www.ippc.int web pages to www.fao.org/ippc	2018 March	IPPC web presence on www.fao.org without loss of functionality or services	Secretariat, FAO	FAO	FAO members
	1.4 Re-development of IPPC data entry and work management tools of the IPP on www.ippc.int e.g. PCE, NRO data entry, OCS and on line registration.	2018 December	New tools for data entry and work programme management	Secretariat	Not applicable	Contracting parties, NPPOs, RPPOs
	1.5 Maintain and continue the development of the www.phytosanitary.info	2020 December	Updated information and new resources available	Secretariat: Implementation and Facilitation Unit and Integration and Support Team	Not applicable	Contracting parties, NPPOs, RPPOs
2.	**Advocacy**					
	2.1 Revision of brochures, pamphlets and factsheets	2017 March	Revised 2015 brochures, pamphlets and factsheets	Secretariat	NPPOs, RPPOs	Contracting parties, NPPOs, RPPOs

No.	Task	Deadline	Performance indicators	Lead entities	Supported by	Target audience
	2.2 Development of new advocacy material (at least 4 new publications per year) e.g. e-Phyto, surveillance, food security, and NROs	2020 January	At least four new publications per annum	Secretariat	NPPOs, RPPOs	Contracting parties, NPPOs, RPPOs, Government decision makers, Academia, Researchers, Industry and general public
	2.3 Development of promotional videos – at least 2 per annum and 1 at least on annual theme (see 3.1 for details)	2020 January	At least two new videos per annum, one on the annual theme.	Secretariat	NPPOs, RPPOs	Contracting parties, NPPOs, RPPOs, Government decision makers, Academia, Researchers, Industry and general public
	2.4 Production and distributions of the Annual Report of the IPPC Secretariat	1 March annually	An annual report made available at CPM each year.	Secretariat	Not applicable	Contracting parties, NPPOs, RPPOs, Government decision makers, Academia, Researchers, Industry and general public
	2.5 Attendance at international technical meetings to increase awareness of IPPC activities, achievements and needs – at least 2 per annum.		Attend two international technical meetings per annum	Secretariat	Not applicable	NPPOs, Researchers, Academia
3.	**Communications**					
	3.1 Development and implementation of Annual work plans for the following themes: 2016 - Food Security, 2017 - Trade Facilitation, 2018 - Environmental Protection, 2019 - Capacity Building and 2020 the International Year of Plant Health.	January of each year	Promotion of annual theme through at least one seminar per theme, development of at least one specific pamphlet or brochure on the annual theme, and ensure there is at least one press	Secretariat	NPPOs, RPPOs	Contracting parties, NPPOs, RPPOs, Government decision makers, Academia, Researchers, Industry and general public

No.	Task	Deadline	Performance indicators	Lead entities	Supported by	Target audience
			release per theme per year.			
	3.2 IPPC Seminar Series	December of each year	Minimum of 3 per annum (at least one on annual theme)	Secretariat	FAO	Contracting parties, NPPOs, RPPOs
	3.3 Utilizing FAO communication system, including press releases	Related to key events and opportunities	Number of IPPC press releases through FAO, level of engagement of FAO social media	Secretariat	FAO	Contracting parties, Government decision makers, Academia, Researchers, Industry and general public
	3.4 Providing support (2016-2019) leading up to the IYPH in 2020	An activity at least every 4 months	Promotion of NPPOs and RPPOs activities in support of IYPH	Secretariat	NPPOs, RPPOs, FAO, other international organizations	Contracting parties, NPPOs, RPPOs, Government decision makers, Academia, Researchers, Industry and general public
	3.5 Development and implementation of the work plan for IYPH 2020	2020	See IYPH paper	Secretariat	NPPOs, RPPOs, FAO, International Organizations	Contracting parties, NPPOs, RPPOs
	3.6 News items	Throughout year	At least 70 IPPC headline news items per annum and improved reader statistics. A monthly IPPC newsletter and news from RPPOs and NPPOs more widely available.	Secretariat	NPPOs, RPPOs	Contracting parties, NPPOs, RPPOs, Government decision makers, Academia, Researchers, Industry
	3.7 Technical publications, including those produced through IPPC projects – at least 3 per annum.	Annual	Three key publications per annum.	Secretariat	Not applicable	Contracting parties, NPPOs, RPPOs, Government decision makers, Academia, Researchers, Industry
	3.8 Utilization of social media to improve awareness of IPPC achievements and activities, including through FAO social media systems.		At least three new social media items documented per month	Secretariat	FAO	NPPOs, Academia, Researchers,

No.	Task	Deadline	Performance indicators	Lead entities	Supported by	Target audience
						Industry and general public
	3.9 Annual competitions e.g. photos, videos, apps, logo	Annual	At least 1 competitions per annum	Secretariat	Not applicable	Contracting parties, NPPOs, RPPOs
4	**Monitoring and Evaluation**					
	4.1 Measure the effectiveness of the communications and advocacy activities	Annual	Annual survey conducted of selected stakeholders and user statistics / engagement.	Secretariat	FAO	NPPOs, RPPOs
	4.2 Evaluation of effectiveness and usability of the IPPC websites	Annual	Provision of user feedback and statistical analysis, and adjustments to improve usability and effectiveness.	Secretariat	FAO	NPPOs, RPPOs
	4.3 Adjustments to the communications and advocacy programme to improve effectiveness and efficacy as needed.	Annual	Annual improvements in communications and advocacy programme.	Secretariat	FAO	NPPOs, RPPOs

Appendix 13 – Terms of Reference of the International Year of Plant Health Steering Committee

Background

The Tenth Session of the Commission of Phytosanitary Measures (CPM-10) strongly supported a proposal by Finland for an International Year of Plant Health (IYPH) in 2020. The 39th FAO Conference in 2015 was informed of the proposal and the support it had received at CPM-10. This gave rise to further positive responses from many Conference delegations and an official request to the Director-General of the FAO has been made for the subject of declaring an IYPH in 2020 to be included on agendas of relevant FAO meetings with a view to receiving a supportive FAO resolution at the 40th FAO Conference in 2017. This would allow the proposal to progress to the General Assembly of the United Nations in 2018 for decision.

IYPH objective

The main objective of the IYPH is *to raise awareness of the importance and impacts of plant health in addressing issues of global importance, including hunger, poverty, threats to the environment and economic development.*

Plant health in this context is usually considered the discipline that uses a range of measures to control and prevent pests, weeds and disease causing organisms to spread into new areas, especially through human interaction such as international trade

However, while this is largely the domain of the IPPC and national governments, the scope of the IYPH cannot be limited to these activities.

The IYPH Steering Committee

Purpose

To achieve the main objective of the IYPH, a Steering Committee is required to plan and oversee the implementation of IYPH activities over the next six years. This Committee will undertake a number of tasks to mid-2022 that will span actions to secure agreement by the United Nations General Assembly to the IYPH, the development of materials in support of this, as well as planning and oversight of the 2020 agenda, monitoring its impact and evaluating its success.

Role and Responsibility

The Committee will ensure that contracting parties, RPPOs, representatives of other FAO departments and other international organisations and major donors actively contribute to the planning and implementation of the IYPH. It will do this through the development of a detailed work plan that identifies actions, communication and engagement, responsibilities, resources, costs and funding for planning and implementing the IYPH. It will identify, solicit and secure revenue sources to fund both the planning and implementation of the IYPH that are separate from funds that support the core business of the IPPC, as outlined in the IPPC strategic objectives and annual work plan and approved by the CPM.

The Steering Committee will focus on the specific work related to the planning and implementation of the IYPH to ensure that IPPC objectives are achieved. It will do this in collaboration with any official FAO systems and structures that are responsible for achieving FAO outcomes for the IYPH, and will assist the IPPC Secretariat to develop any resource materials, as may be required, to assist the successful progress of the proposal for the IYPH through FAO decision processes.

A number of specific outcomes will contribute to achieving the main objective and lead to stronger public and political support to plant health, and better coordination within the plant health community. The key areas of focus for the Steering Committee include:

- Raising the awareness of the public and political decision makers at the global, regional and national levels about plant health
- Promoting and strengthening of national, regional and global plant health efforts and their resources in light of increasing trade and new pest risks caused by climate change
- Educating the public and increasing its knowledge about plant health
- Enhancing dialogue and stakeholder involvement in plant health

- Increasing information about the state of plant protection in the world
- Facilitating the establishment of plant health partnerships on national, regional and global levels.
- The Committee will use Strategic Planning Group (SPG) analysis of IPPC annual themes and the advocacy resources developed for each theme to assist its planning for the IYPH.

Tasks

The key tasks of the Steering Committee from 2016 to 2022 are:

(1) Securing the proclamation of 2020 as the International Year of Plant Health.

(2) Developing advocacy materials in support of the IYPH and a tool kit for contracting parties and RPPOs.

(3) Visualising the 2020 agenda and translating this into a program of varied activities.

(4) Define and allocate roles and responsibilities in the IYPH.

(5) Monitor implementation of the IYPH agenda and evaluate outcomes against objectives and outputs.

(6) Identify, solicit and secure identify revenue sources to fund both the planning and implementation of the IYPH.

Governance

The Steering Committee is convened under the authority of the CPM and guidance of the Bureau to plan and implement activities in the IYPH 2020 that successfully achieve the main objective of the IYPH.

IPPC Secretariat function

The IPPC Secretariat will facilitate meetings of the Steering Committee.
In case of the practical implementation of the IYPH, the IPPC Secretariat will provide facilitation and/or coordination assistance to the Steering Committee depending on the availability of extra-budgetary resources. Funds from extra-budgetary resources must be allocated by the Bureau on behalf of the CPM.

Funding

The Steering Committee must be self-funded through donations of cash through the IPPC trust fund mechanism, or in-kind contributions of members and their organisation.

Reporting

The Steering Committee will provide written progress reports on its activities to each meeting of the CPM and SPG. Reports will address planning, stakeholder engagement, performance against indicators and IYPH outcomes/objectives, risks and their mitigation, resources and budget.

Chair

The Chair of the Steering Committee will be elected annually by the members from amongst its membership. An independent Chair may be appointed with unanimous agreement of the members, and subject to available funds that may be required to support their participation. Should the Steering Committee members consider it necessary, a Vice Chair may be elected from the membership in the same manner. There is no provision for an independent Vice Chair.

Membership

The Steering Committee should have sufficient members to tackle all subjects related to the IYPH 2020 through the four years of planning in the lead up to the IYPH in 2020; oversight of implementation in 2020; and post IYPH reviews through 2022.

It should ideally be composed of:

- 1 member and 1 alternate member from contracting parties in each of the seven FAO regions (7 members and 7 alternates)
- 5 - 7 members from collaborating International Organisations and Regional Plant Protection Organisations. These may include CBD, WCO and WTO/SPS Committee as relevant partners with a strong interest in the main objective of the IYPH

- 1 member each of the IPPC Standards Committee, Capacity Development Committee and Subsidiary Body for Dispute Settlement
- 1 member from the IPPC Secretariat, who will also represent wider FAO interests
- A maximum of 3 representatives from major donors and contributors to the IYPH 2020

For continuity, members or alternates may be drawn from Rome-based permanent representatives to the FAO. Three RPPO representatives from different regions, which may rotate across all regions over the planning and implementation cycle, will coordinate RPPO input into planning and implementation through the annual and intercessional actions of the RPPO technical consultation forum, in which IYPH will be a standing item.

Expectation of Steering Committee members

Members of the Steering Committee are expected to allocate sufficient time and the resources to fully participate in the Committee's activities. These commitments are expected to increase significantly in the lead up to critical delivery points including FAO and UN decisions and throughout 2020. The Chair and Vice Chair are expected to provide strong leadership and actively champion the IYPH throughout its planning and implementation. RPPOs are expected to act as a focal point for planning and coordinating IYPH activities within their region.

Meetings

The Steering Committee will meet as often as required per year. At least two meetings per year should be held in Rome, with one of these face to face meetings adjacent to the annual CPM meeting. All efforts should be undertaken to convene meetings by tele or video-conferencing, with intercessional activities enabled by electronic tools such as email or community sites eg. Sharepoint.

Review

The Steering Committee will be dissolved on 30 June 2022. Any residual actions will be assessed by the Bureau, which will allocate them to a relevant body(s) to finalise.
The ToRs of the IYPH Steering Committee will be regularly reviewed by the CPM Bureau and updated as necessary.

Appendix 14 – IPPC Secretariat Work Plan and Budget for 2016

IPPC Secretariat Work Plan and Budget for 2016

(in thousand USD)

IPPC Mission - Protecting the world's plant resources from pests Activity	Deliverables (Products and output)	Source of funding (in 000 USD)		
		FAO RP	IPPC Multi-donor TF	311/EC - EU TRAVEL TF
GOVERNANCE/MANAGAMENT/STRATEGY				
STAFF COSTS		493	127	-
OPERATIONAL COSTS (INCLUDING CONSULTANTS)		569	122	297
Commission on Phytosanitary Measures (CPM) - 11th Session				
Translation	CPM documents translated	80	-	-
Presenting ISPMs for adoption and noting	4 Draft ISPMs presented to CPM translated in 3 languages and revised in two languages; min. 2 DPs translated after adoption Language Review Group (LRG) process organized for 4 languages for adopted ISPMs	70	-	-
Interpretation	Interpretation conducted well during CPM	70	-	-
Participants from developing countries - travel	Participants travel organized according to EU rules	-	-	100
Report writer	CPM report drafted	8	-	-
Printing, messengers, security officers, catering, other	All services completed	20	-	-
Bureau/Financial committee				
Travel	Travel organized well and on time	-	-	20
Standards Committee (SC)				
Oversee the Standards Committee (SC) work and organize meetings to ensure a consensus-based review of draft standards (SC and SC-7 meetings, SC e-decisions)	2 SC meetings and 1 SC-7 meeting successfully organized and outcomes processed and published Approximately 25 SC e-forums and 15 SC e-polls opened, and the equivalent of SC e-decisions processed	120	-	40
Capacity Development Committee (CDC)		40	-	-
Subsidiary Body on Dispute Settlement (SBDS)				
Travel of participants from developing countries	Travel organized well and on time	8	-	-
Strategic Planning Group (SPG)				
Travel of participants from developing countries	Travel organized well and on time	-	-	20
National Reporting Obligations Advisory Group (NROAG)				
Participants from developing countries - travel	Travel organized well and on time	10	-	-
Communication and Advocacy				
Improve IT Tools (OCS, IPP) to better fit user needs	New OCS developed and launched, training material developed and trainings organized and delivered IPP improved (participants database) Virtual meetings tools used	-	44	-
Start re-design of the IPPC website and phytosanitary.info	Improved home page, usability and functionality.	5	-	-
Management Secretariat wide information technology needs	Service both web and IT tool needs of the Secretariat by ensuring appropriate prioritization, consistency of standards and quality, and technical support.	-	-	-
Contribute to the implementation of the 2016 communication work plan and to the development of the 2017 communication work plan (standard setting activities)	2016 communication work plan implemented and 2017 communication work plan developed (standard setting activities)	-	13	-

(Continued)

IPPC Mission - Protecting the world's plant resources from pests Activity	Deliverables (Products and output)	Source of funding (in 000 USD) FAO RP	IPPC Multi-donor TF	311/EC - EU TRAVEL TF
Awareness through news dissemination	Monthly newsletter distribution, maintain IPPC news feeds and social media communications systems.	-	10	-
International Year of Plant Health (IYPH) promotion	Development of support and tools associated with IYPH	-	5	-
Translation of communications and advocacy material		-	20	-
Coordination and integration of Secretariat advocacy needs and activities.	Increased availability of advocacy material on a wide range of IPPC activities and subjects e.g. resource mobilization, IPPC web and printed needs.	-	-	-
Development and dissemination of publications to support Secretariat activities	New advocacy material for donors	5	5	-
	Key publications such as Annual Report	8	5	-
Partnership and Liaison				
Regional workshops		40	-	117
Technical Consultation among Regional Plant Protection Organizations (TC-RPPO)	Travel organized well and on time	10	-	-
Provide coordination and integration of partnerships and liaison programme.	Work with Secretariat staff to ensure new partnership with CABI, WCO and re-new CBD partnership. Provided support for liaison activities for other Secretariat members. Travel for 5 missions	-	20	-
Organize and conduct side sessions, workshops and trainings	External workshops relevant for IPPC : CBD, SPS, WTO, STDF, RPPOs, NPPOs, FAO Reg PPOs, FAO Units (EST, AGP, EMPRES, AGDF, etc.)	10	-	-
Staff development and trainings	Adequate trainings and development opportunities organized and proposed to staff	5	-	-
Resource Mobilization		-	-	-
Secretariat staff travel	Travel organized well and on time	10		
Other				
ISPM 15 Symbol registration	2nd round of new registration	40	-	-
Scientific Advisory Group		10	-	-
Subtotal Governance/Management/Strategy		**1,062**	**249**	**297**

(Continued)

IPPC Mission - Protecting the world's plant resources from pests Activity	Deliverables (Products and output)	Source of funding (in 000 USD) FAO RP	IPPC Multi-donor TF	311/EC - EU TRAVEL TF
STANDARD SETTING UNIT (SSU)				
STAFF COSTS		677	233	-
OPERATIONAL COSTS (INCLUDING CONSULTANTS)		239	74	40
Work Programming				
Implement the newly adopted standard setting procedure to streamline the process	Newly adopted standard setting procedure implemented; documentation, procedures, tools and systems updated	-	-	-
Update standard setting information	List of topics (LOT) updated in 6 languages twice a year Procedure manual for standard setting and style guide updated Standard setting pages on IPP checked twice a year and updated as needed Standard operating procedures updated Pdf searchable database updated twice a year and publically shared	3	-	-
Expert Input				
Organize 1 call for experts (EWG members for the revision of ISPM 8 (Priority 1), and Pest Risk Management (Priority 2) and for TP members) and 1 call for DP authors	Submissions reviewed and experts / authors selected	2	-	-
Oversee EWGs work, ensure experts feel engaged and satisfied. Organize 2 EWG meetings: Grain (priority 1) and Sea containers (priority 1) or Waste (priority 2)	2 EWGs meetings successfully organized and outcomes processed and published as appropriate	30	45	20
Oversee TPs work, ensure experts feel engaged and satisfied, and organize 4 face-to-face meetings : TPDP (8 drafts), TPPT (13 drafts), TPG, TPFQ (4 drafts)	4 face to face TP meetings successfully organized and outcomes processed and published as appropriate Intersessional TP work plan carried out (including virtual meetings)	56	29	20
Develop and update training materials for CPs and SC members to increase the effectiveness of their participation in the standard setting process, deliver trainings as needed	Training material for CPs participation in the standard setting process and for SC members updated as needed Mentoring programme for new SC members implemented	33	-	-
Consultation				
Organize consultation processes on draft specifications and draft standards to ensure all views are collected	2 member consultations on draft specifications organized through the OCS in 3 languages (incl. translations) 2 member consultations on 15 draft ISPMs, organized through the OCS in 3 languages 1 substantial concerns commenting period on 5 draft ISPMs organized through the OCS 2 DP notification periods organized on 6 draft DPs 4 draft ISPMs presented to CPM in 6 languages with possibility of formal objections 4 DP expert consultations organized for 6 draft	87	-	-
Adoption				
Ensure translation and publication of specifications and standards	Approved specifications are revised in 3 languages and published; Adopted ISPMs published in 6 languages (including after LRG review) All adopted ISPMs are published in 6 languages (except DPs) 7 co-publishing agreements managed according to the procedure Explanatory document on ISPM 5 updated Revoking of standards All ISPMs in LRG process are republished	28	-	-
Subtotal SSU		**916**	**307**	**40**

IPPC Mission - Protecting the world's plant resources from pests Activity	Deliverables (Products and output)	Source of funding (in 000 USD) FAO RP	IPPC Multi-donor TF	311/EC - EU TRAVEL TF
IMPLEMENTATION FACILITATION UNIT (IFU)				
STAFF COSTS		872	360	-
OPERATIONAL COSTS (INCLUDING CONSULTANTS)		100	38	-
Capacity Development				
Production of resources: technical manuals, guidelines, e-learning, etc.	IPPC Risk Communication technical resource	-	-	-
	Pest Free Area (PFA) manual	-	-	-
	350 project outputs	70	-	-
	Soils and plant health paper for IYS	-	-	-
	Legal and policy frameworks of plant protection paper	-	-	-
	Climate change and plant health paper	-	-	-
	Manual on grain	-	33	-
Promotion of resources through websites	Resources made available on the phytosanitary.info webpage	-	-	-
Organize and conduct side sessions, workshops and trainings	Internal workshops at CPM and through IPPC projects	5	-	-
Project formulation and development	Training for trainers project	-	-	-
	IYPH project	-	-	-
	Diagnostic initiative development	-	-	-
Project management	FAO projects covering around 31 countries	-	-	-
Implementation Review and Support System-IRSS				
Proposals of IPPC recommendations	Identify issues that could be addressed as IPPC recommendations	-	-	-
Production of desk studies	IRSS study on diversion from intended use	-	-	-
	IRSS study on Xylella fastidiosa position	-	-	-
Evaluation and feed back on desk studies and technical resources	Set and implement procedures to follow up on the use of desk studies, technical resources and related recommendations	-	-	-
Consultant	Consultant (COF.REG.INT)	-	-	-
National Reporting Obligations (NRO)				
Develop capacity in CPs e.g. surveillance to all CPs to meet their reporting obligations	CPs increased ability to deliver national surveillance systems to collate, verify and where appropriate meet NROs	-	-	-
Improved NRO delivery	Greater CPs participation (especially pest reporting through RPPOs, listing of regulated pests, and emergency action) and introduction NRO quality advice system; editor training	-	-	-
Awareness raising for NROs	Focus on awareness raising for NROs	-	5	-
Maintenance of Official Contact Points database	Up to date database of OCPs available	-	-	-
ISU training tools and materials, including e-learning; manuals and guidance	Training tools and material available on general IPPC activities, NROs, IPP, dispute avoidance; training workshops on NROs	-	-	-
Dispute Avoidance		-	-	-
ISU training tools and materials, including e-learning; manuals and guidance	Training tools and material available on general IPPC activities, NROs, IPP, dispute avoidance; training workshops on NROs	-	-	-
In country liaison and training	Travel	5	-	-
New Technology and Tools (PCE, ePhyto)				
Project management	Training of PCE Facilitators	-	-	-
	Country application of PCE	-	-	-
Tools development	Development of the PCE environmental module	20	-	-
	Development of the IPPC implementation indicators	-	-	-
	Development of the Monitoring and evaluation framework	-	-	-
Subtotal IFU		**972**	**398**	**-**

Total (thousand USD)		**2,950**	**954**	**337**

Appendix 15 – List of Donors and Contributors Supporting IPPC Activities

Table 1

Financial

The Secretariat would like to recognize the donors who continue to provide financial support for IPPC Activities as their contributions are essential to ensure the Secretariat is able to deliver the CPM work programme. Further details of these contributions can be found in the 2015 Financial Report and the 2016 Budget.

Country / Organization	Category	Activity
Australia	Contributions from previous years that were used in 2015	
European Union	Contributions from previous years that were used in 2015	
Ireland	New contribution in 2015	IYPH
Japan	New contribution in 2015	
Korea, Republic of	New contribution in 2015	
New Zealand	New contribution in 2015	
South Africa	New contribution in 2015	
Sweden	Contributions from previous years that were used in 2015	
Switzerland	Contributions from previous years that were used in 2015	
United Kingdom	Contributions from previous years that were used in 2015	

In-kind Staffing, Hosting or Technical Contributions

The Secretariat would like to recognize the donors who continue to provide in-kind staff support for IPPC Activities and their contributions are also essential to ensure the Secretariat is able to deliver the CPM work programme.

A number of countries continue to host key IPPC meetings, plus those countries that made technical contributions to the standard setting process, which positively contribute to the delivery of the CPM work programme. These include those countries that hosted meeting that contributed to the adoption of ISPMs or Annexes to ISPMs in 2015.

Country / Organization	Activity
Brazil	Hosted the 2011 TPFF meeting
Canada	In-kind staffing 50% FTE[62] (standard setting)
European Plant Protection Organisation (EPPO)	Hosted the 2012, 2013 and 2014 TPDP meetings
FAO/IAEA Joint Division	Hosted the 2010 TPFF
	Organized the 2011 TPFF
	In-kind staffing 5% FTE (standard setting)
FAO Near East	IPPC Regional Workshop in North Africa and Near East, Jordan.
France	100% FTE (standard setting)
Germany	Hosted the 2008 TPDP meeting
Indonesia	Hosted June TPDP 2014 meeting
IICA	IPPC Regional Workshop for Latin America and the Caribbean 6th CDC meeting in Costa Rica
Japan	Hosted the July 2010, December 2012 and July 2013 TPPT meetings

[62] Full time equivalent

	In-kind staffing 100% FTE 6 months (dispute settlement)
	In-kind staffing 100% FTE 2 years (capacity development)
Korea, Republic of	IPPC Regional Workshop for Asia
	Hosted November 2015 Second IPPC global Symposium on ePhyto.
New Zealand	Submitted treatment: 4. PT Vapour Heat treatment for Bactrocera melanotus and B. xanthodes (Diptera: Tephritidae) on Carica papaya (2009-105)
	In-kind staffing 10% FTE (standard setting)
United States of America	Hosted the workshop the elaboration of the manuals on surveillance and diagnostic in May 2015
	Hosted the 2010 TPDP meeting
	Submitted treatment: PT Irradiation for Ostrinia nubilalis (2012-009)
	In-kind staffing 5% FTE (standard setting)

Table 2

Recognition related to Standard Setting activities

Gratitude is expressed for contributions of the following members of the Technical Panels who have left their respective panels since CPM-10 (2015) for phytosanitary treatments and diagnostic protocols: Ms. Ana Lia Terra, Uruguay, TPDP, Mr. Patrick Gomes, United States, TPPT and Mr. Aldo Malavasi, Brazil TPFQ.

Gratitude is expressed to the experts of the drafting groups and organizers or hosts for their active contribution in the development of the following ISPMs, or Annexes to ISPMs, adopted in 2015:

A. ISPMs developed by the Technical Panel on Pest Free Areas and Systems Approaches for Fruit Flies (2004-003):

1. ISPM on *Determination of host status of fruit to fruit fly (Tephritidae) (2006-031)*

Country / Organization	Expert	Role
Australia	Mr Robert DUTHIE	TPFF member
Brazil	Mr Aldo MALAVASI	TPFF member
	Mr Odilson RIBEIRO E SILVA	TPFF Steward
Chile	Mr Jaime Gonzalez	TPFF member
FAO/IAEA	Mr Rui CARDOSO-PEREIRA	Steward
Japan	Mr Kenji TSURUTA	TPFF member
Jordan	Ms Mary BAHDOUSHEH	TPFF member
Malaysia	Mr Keng Hong TAN	TPFF member
Mexico	Ms Ana Lilia MONTEALEGRE LARA	TPFF Steward
	Mr Martin Aluja	Invited expert to 2010 TPFF meeting
	Mr José Luis ZAVALA LÓPEZ	TPFF member
North American Plant Protection Organization (NAPPO)	Mr Walther ENKERLIN	Steward
South Africa	Mr Jan Hendrik VENTER	TPFF member
Suriname	Ms Alies VAN SAUERS-MULLER	TPFF member
United States of America	Ms Julie ALIAGA	TPFF Steward, TPFF Assistant Steward
	Mr Kevin M. HOFFMAN	Invited expert to 2011 TPFF meeting

2. ISPM 5 *Glossary of Phytosanitary Terms (1994-001)*

Country / Organization	Expert	Role
China	Ms Hong NING	TPG member
Denmark	Mr Ebbe NORDBO	TPG Assistant steward
Egypt	Mr Shaza Roushdy OMAR	TPG member
European Plant Protection Organization (EPPO)	Mr Andrei ORLINSKI	TPG member
	Mr Ian SMITH	Invited Expert

France	Ms Laurence BOUHOT-DELDUC	TPG member
New Zealand	Mr John HEDLEY	TPG Steward, TPG member
United States of America	Ms Stephanie BLOEM	TPG member
Uruguay	Ms Beatriz MELCHO	TPG member

B. ISPMs developed by the Technical Panel on Phytosanitary treatments as annexes to ISPM 28

3. PT Irradiation for *Ostrinia nubilalis* (2012-009)

Country / Organization	Expert	Role
Argentina	Mr Eduardo WILLINK	TPPT member
	Mr Ezequiel FERRO	TPPT member
	Mr Andrew PARKER	Invited expert Invited expert, International Atomic Energy Agency (IAEA)
Australia	Mr Matthew SMYTH	TPPT member, treatment lead
	Mr Jan Bart ROSSEL	Steward
	Mr Andrew JESSUP	TPPT member, treatment lead
	Mr David REES	TPPT Member
China	Mr Yuejin WANG	TPPT member
	Mr Daojian YU	TPPT member
Indonesia	Mr Antario DIKIN	Steward
Japan	Mr Toshiyuki DOHINO	TPPT member
New Zealand	Mr. Michael ORMSBY	TPPT member
Republic of Korea	Mr Min-Goo PARK	TPPT member
USA	Mr Patrick GOMES	TPPT member
	Mr Guy HALLMAN	TPPT member
	Mr Scott MYERS	TPPT member

4. PT Vapour Heat treatment for *Bactrocera melanotus* and B. *xanthodes* (Diptera: Tephritidae) on Carica papaya (2009-105)

Country / Organization	Expert	Role
Argentina	Mr Eduardo WILLINK	TPPT member
	Mr Ezequiel FERRO	TPPT member, assistant steward
	Mr Andrew PARKER	Invited expert, International Atomic Energy Agency (IAEA)
Australia	Mr Andrew JESSUP	TPPT member
	Mr Jan Bart ROSSEL	Steward
	Mr Matthew SMYTH	TPPT member
	Mr Glenn John BOWMAN	TPPT member
China	Mr Wang YUEJIN	TPPT member
	Mr Daojian YU	TPPT member
Germany	Mr Thomas SCHRÖDER	Invited expert
Indonesia	Mr Antarjo DIKIN	Steward
Japan	Mr Mitsusada MIZOBUCHI	TPPT member
	Mr Toshiyuki DOHINO	TPPT member
	Mr Motoi SAKAMURA	Host country representative
	Mr Hisashi SAKATA	Host organization representative
Jordan	Mr Mohammad Katbeh BADER	TPPT member
Republic of Korea	Mr Min-Goo PARK	TPPT member
South Africa	Ms Alice BAXTER	TPPT member
New Zealand	Mr Michael ORMSBY	TPPT member
	Mr Ray CANNON	TPPT member
USA	Mr Scott WOOD	TPPT member
	Mr Patrick GOMES	TPPT member
	Mr Guy HALLMAN	TPPT member
	Mr Larry ZETTLER	Scientific contribution

C. ISPMs developed by the Technical Panel on Diagnostic protocols as annexes to ISPM 27

5. DP 8 *Ditylenchus dipsaci* and *Ditylenchus destructor*

Country / Organization	Expert	Role
Argentina	Mr Eliseo Jorge CHAVES	Co-author
	Ms Maria Elena MANNA	Co-author
Australia	Mr Brendan Rodoni	TPDP member
Brazil	Ms Renata C.V. TENENTE	Scientific contribution
Canada	Mr Harvinder BENNYPAUL	Scientific contribution
	Mr Delano JAMES	Referee and TPDP member
China	Ms Liping Yin	TPDP member
France	Ms Géraldine ANTHOINE	Discipline Lead and TPDP member
Germany	Mr Johannes HALLMANN	Scientific contribution
	Mr Jens Unger	TPDP Steward
Jamaica	Ms Juliet Goldsmith	TPDP member
Netherlands	Mr Johannes de Gruyter	TPDP member
New Zealand	Mr Robert Taylor	TPDP member
Russia	Mr Mikhail PRIDANNIKOV	Scientific contribution
South Africa	Ms Antoinette SWART	Lead author
Spain	Mr P. CASTILLO	Scientific contribution
United Kingdom	Mr Thomas PRIOR	Scientific contribution
	Ms Jane Chard	Steward
United States of America	Mr Norman B Barr	TPDP member
	Mr Sergei SUBBOTIN	Scientific contribution

6. DP 9: Genus *Anastrepha Schiner* (2004-015)

Country / Organization	Expert	Role
Argentina	Ms Norma Christina VACCARO	Co-author
	Ms Alicia Leonor BASSO	Co-author
Australia	Mr Malik MALIPATIL	Referee and TPDP member
	Mr Brendan Rodoni	TPDP member
Brazil	Mr Roberto A. Zucchi	Scientific contribution
Chile	Ms Daniel Frías	Scientific contribution
France	Ms Valerie. Balmès	Scientific contribution
	Ms Géraldine ANTHOINE	Discipline Lead and TPDP member
Germany	Mr Jens Unger	TPDP (TPDP steward)
Jamaica	Ms Juliet Goldsmith	TPDP member
Mexico	Mr Vicente HERNÁNDEZ-ORTIZ	Lead author
New Zealand	Mr Robert Taylor	TPDP member
Netherlands	Mr Johannes de Gruyter	TPDP member
United Kingdom	Ms Jane Chard	TPDP steward
Uruguay	Ms Ana Lía TERRA	Discipline Lead and TPDP member
United States of America	Mr Norman Barr	TPDP member
	Mr Gary Steck	Scientific contribution
	Mr Allen L. Norrbom	Scientific contribution

7. DP: *Bursaphelenchus xylophilus* (2004-016)[63]

Country / Organization	Expert	Role
Australia	Mr Brendan Rodoni	TPDP member
Canada	Ms Isabel LEAL	Co-author
	Mr Sun FENCHENG	Co-author
China	Mr Jeff GU	Co-author
	Ms Liping Yin	TPDP member

[63] The following draft protocols are currently under the notification period process from 15 December 2015 to 30 January 2016 and may be adopted

France	Mr Philippe Castagnone	Scientific Contribution
	Ms Corinne Sarniguet	Scientific Contribution
	Ms Géraldine ANTHOINE	Discipline lead and TPDP member
Germany	Mr Martin Brandstetter	Scientific Contribution
	Ms Helen Braasch	Scientific Contribution
	Mr Thomas Schröder	Lead author
	Mr Jen Unger	TPDP Steward
Jamaica	Ms Juliet Goldsmith	TPDP member
Japan	Mr Yasuharu Mamiya	Scientific Contribution
Malta	Mr Clifford Borg	Scientific Contribution
Netherlands	Mr Johannes de Gruyter	TPDP member
New Zealand	Mr Robert Taylor	TPDP member
Portugal	Mr Manuel Mota	Scientific Contribution
Poland	Mr Witold Karnkowski	Scientific Contribution
Russia	Mr Alexander Ryss	Scientific Contribution
Spain	Ms Adela Abelleira Argibay	Scientific Contribution
United Kingdom	Ms Jane CHARD	TPDP Steward
	Mr Thomas Prior	Scientific Contribution
	Ms Sue Hockland	Scientific Contribution
United States	Mr Weiming Ye	Scientific Contribution
	Mr Norman Barr	TPDP member

8. DP: *Xiphinema americanum sensu lato* (2004-025)[64]

Country / Organization	Expert	Role
Argentina	Mr Eliseo Jorge Chaves	Co-author
Australia	Mr Brendan RODONI	TPDP member
Canada	Mr Delano James	TPDP member
China	Ms Liping Yin	TPDP member
France	Ms Géraldine ANTHOINE	Discipline lead and TPDP member
	Mr Alain Buisson	Scientific Contribution
Germany	Mr Jen Unger	TPDP Steward
Jamaica	Ms Juliet Goldsmith	TPDP member
Netherlands	Mr Johannes de Gruyter	TPDP member
New Zealand	Mr Robert Taylor	TPDP member
South Africa	Ms Antoinette Swart	Co-author
Spain	Ms Adela Abelleira Argibay	Scientific Contribution
Switzerland	Mr Sebastian Kiewnick	Scientific Contribution
Slovenia	Ms Sasa Širca	Co-author
United Kingdom	Ms Jane CHARD	TPDP Steward
	Ms Sue Hockland	Co-author
	Mr Thomas Prior	Lead author
United States of America	Mr Norman B. Barr	TPDP member

9. DP: *Phytoplasmas* (2004-018) [65]

Country / Organization	Expert	Role
Australia	Ms Fiona CONSTABLE	Scientific Contribution
Canada	Mr Brendan RODONI	Discipline Lead and TPDP member
	Mr Delano James	Discipline Lead and TPDP member
China	Ms Liping Yin	TPDP member
France	Ms Géraldine ANTHOINE	TPDP member
Germany	Mr Wilhelm JELKMANN	Scientific Contribution
	Mr Jen Unger	TPDP Steward

[64] The following draft protocols are currently under the notification period process from 15 December 2015 to 30 January 2016 and may be adopted

[65] The following draft protocols are currently under the notification period process from 15 December 2015 to 30 January 2016 and may be adopted

Jamaica	Ms Juliet Goldsmith	TPDP member
Netherlands	Dr Jos. VERHOEVEN	Scientific Contribution
	Mr Johannes de Gruyter	TPDP member
New Zealand	Ms Lia W. LIEFTING	Scientific Contribution
	Mr Robert Taylor	TPDP member
Spain	Ms Ester TORRES	Scientific Contribution
United Kingdom	Mr P. JONES	Scientific Contribution
	Ms Jane CHARD	TPDP Steward
United States of America	Mr Norman B. Barr	TPDP member

Table 3

Recognition related to Implementation Facilitation activities

Deep gratitude is expressed to Capacity Development Committee (CDC) members who have provided in-kind contributions for reviewing technical resources, which is an essential activity to manage the Phytosanitary.info webpage:

Expert
Ms. Magda González ARROYO
Mr. Sam BISHOP
Mr. Haw Leng HO
Mr. Marc GILKEY
Ms. Sally JENNINGS
Ms. Stella Nonyem ORAKA

Japan is thanked for its in-kind contribution which was extremely appreciated, as Mr. Yuji KITAHARA worked for 2 years for capacity development and terminated his mission in October 2015. All contributors to the CDC review are acknowledged:

Expert
Ms. Renata CLARKE
Mr. Masato FUKUSHIMA
Mr. Francesco GUTIERREZ
Mr. Ralf LOPIAN
Ms. Parul PATEL
Mr. Sankung SAGNIA

Contributors to the CPM-10 (2015) side sessions are deeply thanked for their inputs to make these events a success:

Expert
Mr. Shoki AL DOBAI
Mr. Khalid ALHUDAIB
Ms. Magda González ARROYO
Ms. Ellie BARHAM
Mr. Neil BOONHAM
Mr. Mark BURGMAN
Mr. Lava KUMAR
Ms. Kenza LE MENTEC
Mr. Edoardo PETRUCCO TOFFOLO
Ms. Françoise PETTER
Mr. Davide RASSATI
Ms. Shiroma SATHYAPALA
Mr. Moulay Hassan SEDRA
Mr. Ron SEQUERIA
Ms. Suzanne SHARROCK
Mr. Roberto VALENTI

Participants who attended the workshop on the elaboration of the manuals on surveillance and diagnostic held in San Juan, Puerto Rico on 19-29 May 2015 and provided in-kind expertise for the elaboration of the manuals on surveillance and diagnostic are thanked:

Expert
Mr. Ringolds ARNITIS
Ms. Magda González ARROYO
Mr. Pablo CORTESE
Mr. Christopher DALE
Mr. Robert FAVRIN
Ms. Amanda HODGES
Mr. Lalith KUMARASINGHE
Ms. Olga LAVRENTJEVA
Mr. Bouabid LBIDA
Ms. Hyok-In LEE
Mr. George MOMANYI
Mr. Mohammed Amal RAHEL
Mr. Julian SMITH
Mr. Paul STEVENS
Ms. Carol THOMAS
Ms. Rebecca WEEKES
Mr. Leroy WHILLBY
Mr. Hernan ZETINA

Ms. Leyinska WISCOVITCH and Mr. Norberto GABRIEL are thanked for the support they provided in organizing this meeting held in San Juan, Puerto Rico on 19-29 May 2015.

Ms. Anna Maria D'ONGHIA and Mr. Ralf LOPIAN are thanked for their in kind contribution in providing comments to the pilot project on surveillance.

Mr. Cosimo LACIRIGNOLA and CIHEAM-IAM Bari staff, in particular Ms. Anna Maria D'ONGHIA and Mr. Khaled DJELOUAH, are warmly thanked for organizing a week of phytosanitary training for Master students and NPPO staff.

This list is not exhaustive and does not cover all in-kind contributions coming from persons and organizations.

Table 4

Recognition related to National Reporting Obligations Advisory Group (NROAG) activities

Gratitude is expressed to the members of the NROAG for their active contribution in reviewing National Reporting Obligations (NRO) papers and resources between sessions of the NROAG:

Country	Expert	Status
Argentina	Mr. Ezequiel Ferro	NROAG member
United Kingdom	Mr. Samuel Bishop	NROAG member
Thailand	Ms. Tasanee Pradyabumrung	NROAG member
Gabon	Mr. Séraphine MINKO	NROAG member
Italy	Mr. Federico Sòrgoni	NROAG member

Table 5

Recognition related to Subsidiary Body for Dispute Settlement (SDBS) activities

Gratitude is expressed to the members of the SBDS for their active contribution in reviewing dispute settlement and avoidance resources between sessions of the SBDS:

Country	Expert	Status
Gabon	Ms. Seraphine MINKO	SBDS Member
Bangladesh	Mr. Mohamed AHSAN ULLAH	SBDS Member
Netherlands	Ms. Mennie GERRITSEN-WIELARD	SBDS Member
Panama	Mr. Luis BENAVIDES	SBDS Member
Canada	Mr. Steve CÔTÉ	SBDS Member
Samoa	Ms. Talei FIDOW	SBDS Member

Japan is thanked for its in-kind contribution which was extremely appreciated, as Mr. Shinya NEGORO worked for 6 months for dispute avoidance and settlement, and terminated his mission on 31 July 2015.

Table 6

Recognition related to ePhyto Steering Group (ESG) activities

Gratitude is expressed to the members of the ESG for their active contributions between sessions of the ESG:

Country	Expert	Status
Netherlands	Mr. Nico Horn	ESG Member
Australia	Mr. Peter Neimanis	ESG Member
USA	Mr. Christian Dellis	ESG Member
Argentina	Mr. Walter Alessandrini	ESG Member
China	Ms. Maoyu Chen	ESG Member
Kenya	Mr. Josiah Syanda	ESG Member
Argentina	Mr. Diego Quiroga	ESG Member
Australia	Chinthaka Karunaratne	ESG resource person
Canada	Ms. Marie-Pierre Mignault	ESG resource person

Appendix 16 – CPM Recommendation on the importance of pest diagnosis

Background

Pest diagnosis is a cross-cutting issue that underpins most International Plant Protection Convention (IPPC) activities. In order to take action against a pest, it must be accurately identified. To enable safe trade, pest diagnosis must further be completed quickly and to a high level of confidence. Contracting parties regularly undertake pest diagnoses, for example to support export certification, import inspections and remedial actions where a quarantine pest is found, pest surveillance and eradication programmes. The diagnosis of some pests is particularly challenging because the modernization of taxonomic concepts and the diagnostic opportunities provided by new technologies are not available universally.

The results of the general survey of implementation of the Convention and its standards by the Implementation Review and Support System (IRSS) and other IRSS surveys indicate access to diagnostic support needs to be improved. This would assist some countries to undertake surveillance, determine pest status, undertake pest risk analysis etc. This is a fundamental issue that contracting parties and the CPM should address.

In addition to problems within contracting parties, many regions have identified a general trend in reduced expertise in core scientific disciplines, such as the taxonomy of pests, and classical diagnostic skills.

Recommendation addressed to Contracting Parties, Regional Plant Protection Organizations and the IPPC Secretariat:

The CPM recognizes that accurate and rapid pest diagnosis underpins export certification, import inspections and the application of appropriate phytosanitary treatments, enables effective pest surveillance and supports successful eradication programs. To increase the capacity and capability of contracting parties to diagnose pests:

The CPM encourages Contracting Parties to:

- ensure there are adequate laboratory facilities and expertise to support pest diagnostic and taxonomic activities underpinning phytosanitary activities through sufficient allocation of resources
- share knowledge and expertise with other countries where possible, for example by making places available on training programs, opening access to proficiency testing or publicizing examples of best laboratory practice, encourage diagnostic and taxonomic publications relating to phytosanitary activities in appropriate peer reviewed journals, in particular in open access formats
- share diagnostic protocols used by NPPOs on the IPPC phytosanitary resources page via links to NPPO resource pages
- encourage and support experts to contribute to the IPPC standard setting processes for Diagnostic Protocols
- consider strategic needs for expertise in taxonomy of pests and classical diagnostic skills, and, when necessary, pool resources with other NPPOs to ensure sufficient diagnostic capacity and capability is in place to meet future demand
-

The CPM encourages RPPOs to:

- support the development of diagnostic protocols and other resources relevant to their region and share them on the IPPC phytosanitary resources pages

- support the development of guidance on laboratory requirements for pest detection and on the overall management and technical aspects for a diagnostic laboratory
- Undertake knowledge exchange and training on diagnostic methods and laboratory capability
- Work to enhance expertise and capacity within the region, such as the identification of regional experts.
- Identify and encourage the development of centres of expertise that NPPOs within the region can access.

Appendix 17 – CPM Bureau Memberships and CPM Bureau replacement memberships

Updated 2016-04-08 after it was approved by the CPM
This refers to the document CPM 2016/04 Rev.1– CPM 2016/CRP/09

Table 01 - Current CPM Bureau replacement membership

Region	Country	Name	Nominated/ Re-nominated	Current term/duration	End of current term
Africa	Cote D'Ivoire	Mr Lucien KOUAME KONAN	CPM-7 (2012) CPM-9 (2014) CPM – 11 (2016)	3rd Term/2 years	2018
Asia	Republic of Korea	Ms Kyu-Ock YIM	CPM-5 (2010) CPM-7 (2012) CPM-9 (2014) CPM-11 (2016)	4th term / 2 years	2018
Europe	Netherlands	Mr Cornelis Antonius Maria VAN ALPHEN	CPM-9 (2014) CPM-11 (2016)	2nd term / 2 years	2018
Latin America and Caribbean (Vice-Chairperson)	Mexico	Mr Francisco Javier TRUJILLO ARRIAGA	CPM-11 (2016)	1st term/ 2 years	2018
Near East	Sudan	Mr Kamal El Din Abdelmahmoud Amein BAKR	CPM-11 (2016)	1st term/ 2 years	2018
North America	Canada	Ms Marie-Claude FOREST	CPM-11 (2016)	1st term / 2 years	2018
Southwest Pacific (Chairperson)	Australia	Ms Lois RANSOM	CPM-7 (2012) CPM-11 (2016)	2nd term / 2 year	2018

Table 2 - CPM Bureau replacement memberships (as of 2016-04-08)

Region	Country	Name	Nominated/ Re-nominated	Current term/duration	End of current term
Africa	Cameroon	Mr Francis LEKU AZENAKU	CPM-11 (2016)	1st term/ 2 years	2018
Asia	1 China	Mr Wang FUXIANG	CPM-11 (2016)	1st term/ 2 years	2018
	2 Indonesia	Mr Antarjo DIKIN	CPM-11 (2016)	1st term/ 2 years	2018
Europe	France	Ms Emmanuelle SOUBEYRAN	CPM-10 (2015)	1st term/ 2 years	2017
Latin America and Caribbean	Argentina	Mr Diego QUIROGA	CPM-11(2016)	1st term/ 2 years	2018
Near East	Egypt	Mr Ibrahim Imbaby EL SHOBAKI	CPM-11 (2016)	1st term/ 2 years	2018
North America	USA	Mr John GREIFER	CPM-11 (2016)	1st term/ 2 years	2018
Southwest Pacific	Australia	Mr Kim RITMAN	CPM-10 (2015)	1st term/ 2 years	2017

Appendix 18 – Standards Committee and Subsidiary Body on Dispute Settlement Membership Lists and Potential Replacements

Table A1 - Standards Committee Membership

FAO region	Country	Name	Nominated/ Re-nominated	Current term/duration	End of current term
Africa	Malawi	Mr David KAMANGIRA	CPM-11 (2016)	1st term/ 3 years	2019
	Algeria	Ms Nadia HADJERES	CPM-10 (2015)	1st term / 3 years	2018
	Kenya	Ms Esther KIMANI	CPM-9 (2014)	1st term / 3 years	2017
	Nigeria	Mr Moses Adegboyega ADEWUMI	Replacement member for Alice Ntoboh Sibon NDIKONTAR	Replacement	2018
Asia	China	Mr Lifeng WU	CPM-10 (2015)	1st term / 3 years	2018
	Indonesia	Mr HERMAWAN	CPM-11 (2016)	1st term /3 years	2019
	Kingdom of Thailand	Ms Walaikorn RATTANADECHAKUL	CPM-10 (2015)	1st term / 3 years	2018
	Vietnam	Ms Thanh Huong HA	CPM-7(2012) CPM-10 (2015)	2nd term/3 years	2018
Europe	France	Ms Laurence BOUHOT-DELDUC	CPM-10 (2015)	1st term / 3 years	2018
	Netherlands	Mr Nicolaas Maria HORN	CPM-9 (2014)	1st term / 3 years	2017
	Norway	Ms Hilde Kristin PAULSEN	CPM-7(2012) CPM-10 (2015)	2nd term/3 years	2018
	Poland	Mr Piotr WLODARCZYK	CPM-7(2012) CPM-10 (2015)	2nd term/3 years	2018
Latin America and Caribbean	Chile	Mr Álvaro SEPÚLVEDA LUQUE	CPM-10 (2015)	1st term / 3 years	2018
	Mexico	Ms Ana Lilia MONTEALEGRE LARA	CPM-7(2012) CPM-10 (2015)	2nd term/3 years	2018

FAO region	Country	Name	Nominated/ Re-nominated	Current term/duration	End of current term
	Argentina	Mr Ezequiel FERRO	CPM-8 (2013) CPM-11 (2016)	2nd term / 3 years	2019
	Brazil	Mr Jesulindo DE SOUZA	CPM-11 (2016)	1st term/ 3 years	2019
Near East	Egypt	Ms Shaza OMAR	CPM-11 (2016)	1st term / 3 years	2019
	Oman	Mr Suleiman Mahfoudh AL TOUBI	CPM-11 (2016)	1st term / 3 years	2019
	Iran	Ms Maryam JALILI MOGHADAM	CPM-10 (2015)	1st term / 3 years	2018
	Lebanon	Mr Youssef Al MASRI	CPM-11 (2016)	1st term / 3 years	2019
North America	Canada	Mr Rajesh RAMARATHNAM	CPM-11 (2016)	1st term / 3 years	2019
	USA	Ms Marina ZLOTINA	CPM-10 (2015)	1st term / 3 years	2018
Southwest Pacific	Australia	Mr Jan Bart ROSSEL	CPM-6 (2011) CPM-9 (2014)	2nd term/3 years	2017
	Papua New Guinea	Mr Pere KOKOA	CPM-10 (2015)	1st term / 3 years	2018
	New Zealand	Mr John HEDLEY	CPM-1 (2006) CPM-4 (2009) CPM-7 (2012) CPM-11 (2016)	4th term / 3 years	2019

Table A2-Standards Committee Potential Replacements

FAO region	Order	Country	Name	Nominated / Re-nominated	Current term/duration	End of current term
Africa	1	Congo	Ms Alphonsine LOUHOUARI TOKOZABA	CPM-11 (2016)	1st term / 3 years	2019
	2	Burundi	Mr Eliakim SAKAYOYA	CPM-11 (2016)	1st term / 3 years	2019
Asia	1	Japan	Mr Masahiro SAI	CPM-10 (2015)	1st term / 3 years	2018
	2	Philippines	Ms Merle Bautista PALACPAC	CPM-11 (2016)	1st term / 3 years	2019
Europe	1	United Kingdom	Mr Samuel BISHOP	CPM-10 (2015)	1st term/3 years	2018
	2	Turkey	Mr Nevzat BIRISIK	CPM-11 (2016)	1st term/3 years	2019
Latin America and Caribbean	1	Panama	Ms Judith Ivette VARGAS AZCÁRRAGA	CPM-9 (2014)	1st term / 3 years	2017
	2	Dominica	Nelson LAVILLE	CPM-11 (2016)	1st term / 3 years	2019
Near East	1	Libya	Mr Ali Amin KAFU	CPM-11 (2016)	1st term / 3 years	2019
	2	Jordan	Mr Nazir Al-BDUDOR	CPM-11 (2016)	1st term / 3 years	2019
North America	To replace Canada	Canada	Ms Marie-Claude FOREST	CPM-11 (2016)	1st term/ 3 years	2019
	To replace USA	USA	Ms Stephanie DUBON	CPM-11 (2016)	1st term / 3 years	2019
Southwest Pacific	1	New Zealand	Mr Stephen BUTCHER	CPM-4 (2009) CPM-7 (2012) CPM-11 (2016)	3rd term / 3 years	2019
	2	Australia	Mr Bruce HANCOCK	CPM-11 (2016)	1st term/ 3 years	2019

Table B1 - Subsidiary Body on Dispute Settlement Membership

FAO region	Country	Name	Nominated / Re-nominated	Current term / Duration	End of current term
Africa	Gabon	Ms Seraphine MINKO	CPM-10 (2015)	1st term / 2 years	2017
Asia	Bangladesh	Mr Mohamed AHSAN ULLAH	CPM-10 (2015)	1st term / 2 years	2017
Europe	Estonia	Ms Olga LAVRENTIEVA	CPM-11 (2016)	1st term / 2 years	2018
Latin America and Caribbean	Panama	Mr Luis BENAVIDES	CPM-8 (2013) CPM-10 (2015)	2nd term / 2 years	2017
Near East	Yemen	Mr Abdullah H. AL SAYANI	CPM-9 (2014) CPM-11 (2016)	2nd term / 2 years	2018
North America	Canada	Mr Steve CÔTÉ	CPM-7 (2012) CPM-9 (2014) CPM-11 (2016)	3rd term/ 2 years	2018
Southwest Pacific	Samoa	Ms Anoano SEUMALII	CPM-11 (2016)	1st term / 2 years	2018

Table B2 - Subsidiary Body on Dispute Settlement Potential Replacements

FAO region	Country	Name	Nominated / Re-nominated	Current term / Duration	End of current term
Africa	Mozambique	Ms Antonia VAZ TAMBOLANE	CPM-10 (2015)	1st term / 2 years	2017
Asia		VACANT			
Europe	France	Ms Clara PACHECO	CPM-11 (2016)	1st term / 2 years	2018
Latin America and Caribbean	Argentina	Ms María Julia PALACIN	CPM-10 (2015)	1st term / 2 years	2017
Near East	Lebanon	Ms Sylvana GERGES	CPM-11 (2016)	1st term / 2 years	2018
North America	USA	Mr John GREIFER	CPM-10 (2015)	1st term / 2 years	2017
Southwest Pacific	New Zealand	Mr Peter THOMSON	CPM-8 (2013) CPM-10 (2015)	2nd term / 2 years	2017

Appendix 19 – ISPMs adopted and noted by CPM-11

- Amendments to ISPM 5 *Glossary of Phytosanitary Terms* (1994-001).
- ISPM 37 on *Determination of host status of fruit to fruit fly (Tephritidae)* (2006-031).
- PT 20 Irradiation Treatment for *Ostrinia nubilalis* (2012-009) as Annex 20 to ISPM 28 (*Phytosanitary treatments for regulated pests*).
- PT 21 Vapour Heat Treatment for *Bactrocera melanotus* and *B. xanthodes* on *Carica Papaya* (2009-105) as Annex 21 to ISPM 28 (*Phytosanitary treatments for regulated pests*).

The following five diagnostic protocols as Annexes to ISPM 27 (*Diagnostic protocols for regulated pests*) adopted by Standards Committee on behalf of CPM:

- DP 08: *Ditylenchus dipsaci* and *Ditylenchus destructor* (2004-017)
- DP 09: *Genus Anastrepha Schiner* (2004-015)
- DP 10: *Bursaphelenchus xylophilus* (2004-016)
- DP 11: *Xiphinema americanum sensu lato* (2004-025)
- DP 12: *Phytoplasma* (2004-018)

[1]

DRAFT AMENDMENTS TO ISPM 5 (2014):
***GLOSSARY OF PHYTOSANITARY TERMS* (1994-001)**

[2]

Date of this document	2015-11-25
Document category	*Amendments to ISPM 5* (*Glossary of phytosanitary terms*) 2014 (1994-001)
Current document stage	*from* SC November 2015 *to* CPM
Major stages	CEPM (1994) added topic: 1994-001, Amendments to ISPM 5: Glossary of phytosanitary terms 2006-05 Standards Committee (SC) approved specification TP5 2012-10 Technical Panel for the Glossary of phytosanitary terms (TPG) revised specification 2012-11 SC revised and approved revised specification, revoking Specification 1 2014-02 TPG reviewed draft amendments to ISPM 5 (2014) 2014-05 SC reviewed and approved for member consultation 2014-7/11 member consultation 2014-12 TPG revised amendments and responded to member comments 2015-5 SC-7 approved for Substantial concerns commenting period (SCCP) 2015-06/09 SCCP 2015-10 TPG reviewed SCCP comments; there were no changes to the draft amendments incorporated 2015-11 SC withdrew "mark" (2013-007) and approved the draft 2014 Amendments to ISPM 5 to be submitted for adoption
Notes	2014-05 SC withdrew: identity (of a consignment) (2011-001), phytosanitary security (of a consignment) (2013-008), integrity (of a consignment), kiln-drying (2013-006), 2014-05-19 edited by Secretariat 2015-05 SC-7 withdrew: bark (2013-005) and visual examination (2013-010) 2015-05-25 Steward reviewed. 2015-11-16 Secretariat updated the draft Amendments to reflect the fact that CPM-10 (2015) noted ink amendments in relation to the expression "a commodity class" **NOTE: The explanations for each proposal are presented only in the version of the draft Amendments presented to member consultation and to the SC. For CPM, only the proposals will be presented.**

[4] **1. REVISIONS**

[5] **1.1 *additional declaration* (2010-006)**

[10] *Original definition*

[11]

additional declaration	A statement that is required by an importing country to be entered on a **phytosanitary certificate** and which provides specific additional information on a **consignment** in relation to **regulated pests** [FAO, 1990; revised ICPM, 2005]

[12] *Proposed revision*

[13]

additional declaration	A statement that is required by an importing country to be entered on a **phytosanitary certificate** and which provides specific additional information on a **consignment** in relation to **regulated pests** or **regulated articles** [FAO, 1990; revised ICPM, 2005]

[14] **1.2 *grain* (2013-018), *seeds***

[24] *Original definitions*

[25]

grain (as a **commodity class**)	**Seeds** intended for processing or consumption and not for **planting** (see **seeds**) [FAO, 1990; revised ICPM, 2001; revised CPM, 2015]
seeds (as a **commodity class**)	Seeds for **planting** or intended for planting and not for consumption or processing (see **grain**) [FAO, 1990; revised ICPM, 2001; revised CPM, 2015]

[26] *Proposed revision*

[27]

grain (as a **commodity class**)	Seeds (in the botanical sense) ~~intended~~ for processing or consumption, but ~~and~~ not for **planting** ~~(see **seeds**)~~ [FAO, 1990; revised ICPM, 2001; revised CPM, 2015]
seeds (as a **commodity class)**	Seeds (in the botanical sense) for **planting** ~~or intended for planting, and not for consumption or processing~~ ~~(see **grain**)~~ [FAO, 1990; revised ICPM, 2001; revised CPM, 2015]

[38] **1.3 *wood* (2013-011)**

[46] ***Original definition***

[47]

wood (as a **commodity class**)	**Round wood**, **sawn wood**, wood chips or **dunnage**, with or without **bark** [FAO, 1990; revised ICPM, 2001; revised CPM, 2015]

[48] *Proposed revision*

[49]

wood (as a **commodity class**)	**Commodities** such as **round wood**, **sawn wood**, wood chips ~~or dunnage~~ and wood residue, with or without **bark**, excluding **wood packaging material, processed wood material** and bamboo products [FAO, 1990; revised ICPM, 2001; revised CPM, 2015]

INTERNATIONAL STANDARDS FOR
PHYTOSANITARY MEASURES

ISPM 37

Determination of host status of fruit to fruit flies (Tephritidae)

Produced by the Secretariat of the
International Plant Protection Convention
Adopted 2016; published 2016

Publication history

This is not an official part of the standard

2006-11 SC added the topic *Determination of host susceptibility for fruit flies (Tephritidae)* (2006-031)

2009-05 SC approved draft specification for member consultation

2010-02 Member consultation

2010-04 SC approved Specification 50

2010-10 TPFF drafted ISPM

2011-05 SC reviewed and returned draft ISPM to TPFF

2011-08 TPFF revised draft ISPM

2012-04 SC approved draft ISPM for member consultation

2012-07 Member consultation

2013-05 SC-7 approved for SCCP

2013-07 SCCP

2013-11 SC approved draft ISPM to be submitted to CPM-9 for adoption

2014-04 Formal objections received 14 days prior to CPM-9

2014-04 Steward revised draft ISPM to respond to the formal objections

2014-05 SC reviewed and asked the TPFF to review

2014-05 TPFF reviewed

2014-11 SC approved draft ISPM to be submitted to CPM-10 for adoption

2015-03 Concerns raised at CPM-10 (2015), draft returned to SC

2015-04 Steward revised draft ISPM (following concerns discussed among interested parties)

2015-05: SC approved for SCCP

2015-10 TPFF revised draft ISPM

2015-11 SC reviewed and approved for submission to CPM-11 for adoption

2016-04 CPM-11 adopted the standard

ISPM 37. 2016. *Determination of host status of fruit to fruit flies (Tephritidae)*. Rome, IPPC, FAO.

Publication history last updated: 2016-04

CONTENTS

Adoption

This standard was adopted by the Eleventh Session of the Commission on Phytosanitary Measures in April 2016.

INTRODUCTION

Scope

This standard provides guidelines for the determination of host status of fruit to fruit flies (Tephritidae) and describes three categories of host status of fruit to fruit flies.

Fruit as referred to in this standard covers fruit in the botanical sense, including such fruits that are sometimes called vegetables (e.g. tomato and melon).

This standard includes methodologies for surveillance under natural conditions and field trials under semi-natural conditions that should be used to determine the host status of undamaged fruit to fruit flies for cases where host status is uncertain. This standard does not address requirements to protect plants against the introduction and spread of fruit flies.

References

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

Appendix 1 and Appendix 2 of ISPM 26 *(Establishment of pest free areas for fruit flies (Tephritidae))* also apply to this standard.

Definitions

Definitions of phytosanitary terms used in this standard can be found in ISPM 5 (*Glossary of phytosanitary terms*). In addition to the definitions in ISPM 5, in this standard the following definitions apply:

host status (of fruit to a fruit fly)	Classification of a plant species or cultivar as being a natural host, conditional host or non-host for a fruit fly species
natural host (of fruit to a fruit fly)	A plant species or cultivar that has been scientifically found to be infested by the target fruit fly species under natural conditions and able to sustain its development to viable adults
conditional host (of fruit to a fruit fly)	A plant species or cultivar that is not a natural host but has been scientifically demonstrated to be infested by the target fruit fly species and able to sustain its development to viable adults as concluded from the semi-natural field conditions set out in this standard
non-host (of fruit to a fruit fly)	A plant species or cultivar that has not been found to be infested by the target fruit fly species or is not able to sustain its development to viable adults under natural conditions or under the semi-natural field conditions set out in this standard

Outline of Requirements

This standard describes requirements for determining the host status of a particular fruit to a particular fruit fly species and designates three categories of host status: natural host, conditional host and non-host.

Requirements for determining host status include:

- accurate identification of the fruit fly species, test fruit and, for field trials, control fruit from a known natural host
- specification of parameters for adult and larval fruit fly surveillance and experimental design under semi-natural field conditions (i.e. field cages, greenhouses or bagged fruit-bearing branches) to determine host status and describe the conditions of the fruit (including physiological) to be evaluated
- observation of fruit fly survival at each stage of its development
- establishment of procedures for holding and handling the fruit for host status determination
- evaluation of experimental data and interpretation of results.

BACKGROUND

Fruit flies are economically important pests and the application of phytosanitary measures is often required to allow movement of their host fruit in trade (ISPM 26; ISPM 30 *(Establishment of areas of low pest prevalence for fruit flies (Tephritidae))*; ISPM 35 *(Systems approach for pest risk management of fruit flies (Tephritidae)))*. The host status of fruit is an important element of pest risk analysis (PRA) (ISPM 2 (*Framework for pest risk analysis*); ISPM 11 (*Pest risk analysis for quarantine pests*)). Categories of and procedures for determining host status should therefore be harmonized.

It is important to note that host status may change over time because of changes in biological conditions.

When host status is uncertain there is a particular need to provide harmonized guidance to national plant protection organizations (NPPOs) for determining the host status of fruit to fruit flies. Historical evidence, pest interception records and scientific literature generally may provide sufficient information on host status, without the need for additional larval field surveillance or field trials. However, historical records and published reports may sometimes be unreliable, for example:

- Fruit fly species and plant species or cultivars may have been incorrectly identified and reference specimens may not be available for verification.
- Collection records may be incorrect or dubious (e.g. host status based on (1) the catch from a trap placed on a fruit plant; (2) damaged fruit; (3) simply finding larvae inside fruit; or (4) cross-contamination of samples).
- Important details may have been omitted (e.g. cultivar, stage of maturity, physical condition of fruit at the time of collection, sanitary condition of the orchard).
- Development of larvae to viable adults may not have been verified.

Protocols and comprehensive trials to determine fruit fly host status have been documented in the scientific literature. However, inconsistencies in terminology and methodology contribute to variations in the determination of fruit fly host status. Harmonization of terminology, protocols and evaluation criteria for the determination of fruit fly host status will promote consistency among countries and scientific communities.

Surveillance by fruit sampling is the most reliable method to determine natural host status. Surveillance of natural infestation by fruit sampling does not interfere with the natural behaviour of fruit flies and takes into account high levels of variability in the fruit, fruit fly behaviour and periods of activity. Fruit sampling includes the collection of fruit and the rearing of fruit flies on it to

determine if the fruit is a host to the fruit fly (i.e. if the fruit can sustain fruit fly development to viable adults).

Field trials under semi-natural conditions allow fruit flies to exhibit natural oviposition behaviour, and because the fruit remains attached to the plant it does not degrade rapidly during the trials. However, field trials under semi-natural conditions can be resource-intensive and may be compromised by environmental variables.

Results of field trials carried out in a certain area may be extrapolated to comparable areas if the target fruit fly species and the physiological condition of the fruit are similar, so that fruit fly host status determined in one area does not need to be repeated in a separate but similar area.

GENERAL REQUIREMENTS

Determining to which of the three categories of host status (natural host, conditional host and non-host) a fruit belongs can be done through the following steps, as is outlined in the flow chart (Figure 1):

A. When existing biological or historical information provides sufficient evidence that the fruit does not support infestation[1] and development to viable adults, no further surveys or field trials should be required and the plant should be categorized as a non-host.

B. When existing biological and historical information provides sufficient evidence that the fruit supports infestation and development to viable adults, no further surveys or field trials should be required and the plant should be categorized as a natural host.

C. When existing biological and historical information is inconclusive, appropriate field surveillance by fruit sampling or field trials should be used to determine host status. Surveillance and trials may lead to one of the following results:

C1. If infestation with development to viable adults is found after field surveillance by fruit sampling, the plant should be categorized as a natural host.

C2. If no infestation is found after field surveillance by fruit sampling, and no further information indicates that the fruit has the potential to become infested, taking into consideration the conditions in which the commodity is known to be traded, such as physiological condition, cultivar and stage of maturity, the plant may be categorized as a non-host.

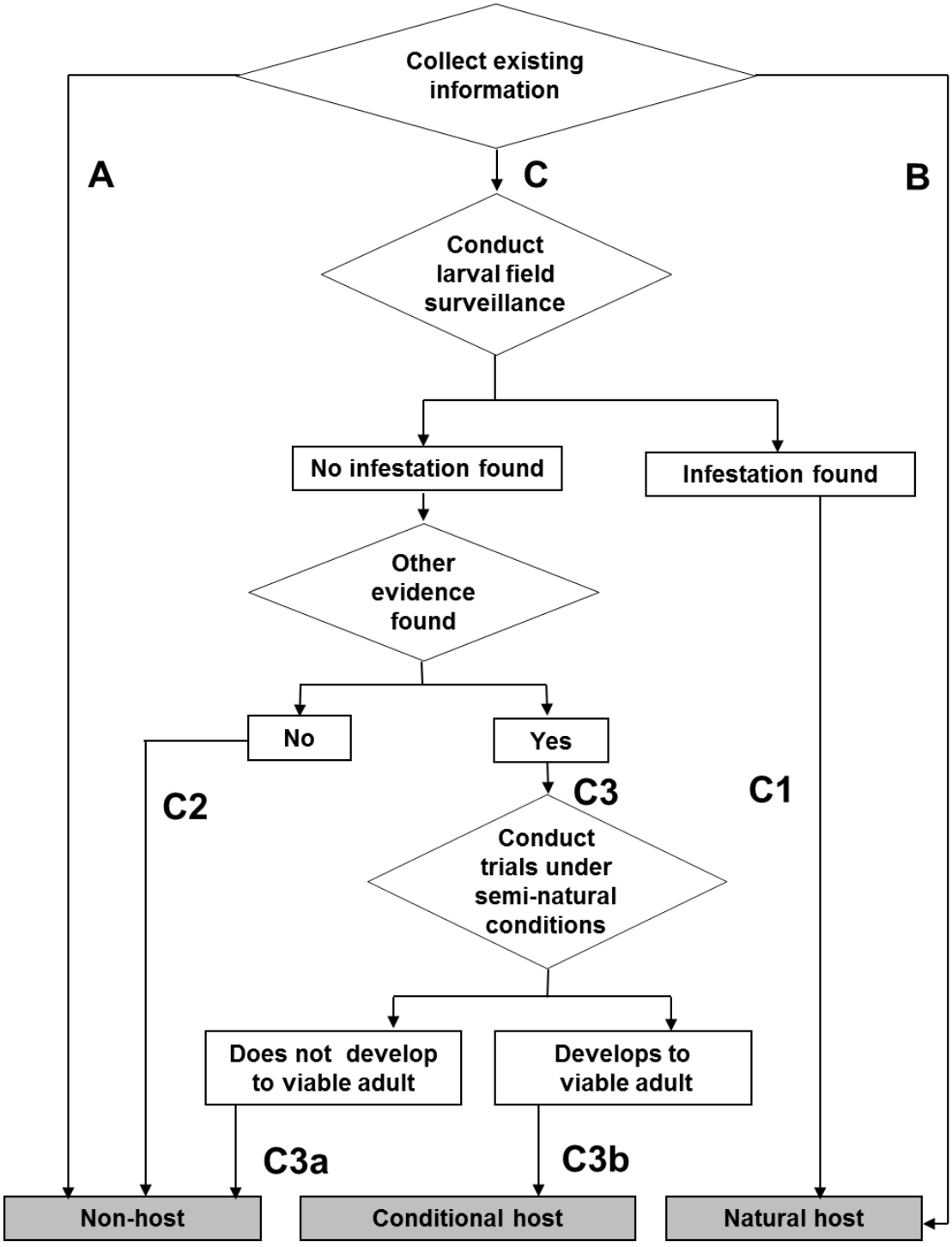
C3. If no infestation is found after field surveillance by fruit sampling, but available biological or historical information indicates that the fruit has the potential to become infested, additional field trials under semi-natural conditions may be needed to assess whether the target fruit fly can develop to viable adults on the particular fruit species or cultivar.

C3a. If the target fruit fly species does not develop to viable adults, the plant should be categorized as a non-host.

C3b. If the target fruit fly species develops to viable adults, the plant should be categorized as a conditional host.

[1] Hereinafter, “infestation” refers to infestation of a fruit by a target fruit fly species.

Figure 1. Steps for the determination of host status of fruit to fruit flies.



SPECIFIC REQUIREMENTS

Host status may be determined from historical production records or from trade or interception data indicating natural infestations. Where historical data do not provide clear determination of host status, surveillance by fruit sampling should be conducted to gather evidence of natural infestations and development to viable adults, or field trials under semi-natural conditions may be required. In cases where host status has not been scientifically determined by surveillance, or when there is a particular need to determine if a fruit is a conditional host or a non-host, trials conducted under semi-natural field conditions may be required.

Artificial conditions are inherent in laboratory tests in which fruit flies are presented with harvested fruit that undergoes rapid physiological changes and thereby may become more susceptible to infestation. The detection of infestation in laboratory tests for the determination of host status may therefore be misleading. In addition, it has been widely documented that under artificial conditions, females of polyphagous species will lay eggs in almost any fruit presented to them and, in most cases, the larvae will develop into viable adults. Therefore, laboratory tests may be sufficient for demonstrating non-host status, but are inappropriate for demonstrating natural or conditional host status.

The following elements are important considerations in planning field trials:

- the identity of the plant species (including cultivars where appropriate) and the target fruit fly species
- the physical and physiological variability of the fruit in the production area
- past chemical usage in the fruit production area
- target fruit fly incidence over the entire production area, and relevant harvest and export periods
- relevant information, including literature and records, regarding host status of the fruit and fruit fly species, and a critical review of such information
- the origin and rearing status of the fruit fly colony to be used
- known natural host species and cultivars to be used as controls
- separate field trials, where appropriate, for each fruit fly species for which determination of host status is required
- separate field trials for each cultivar of the fruit if cultivar differences are the purported source of host variability to infestation
- the placing of field trials in the fruit production areas
- compliance with sound statistical practice.

1. Natural Host Status Determination Using Surveillance by Fruit Sampling

Fruit sampling is the most reliable method to determine natural host status. The status of a natural host can be determined on the basis of confirmation of natural infestation and development to viable adults by sampling fruit during the harvest period.

Fruit samples should be representative of the range of production areas and environmental conditions, as well as of physiological and physical stages.

2. Host Status Determination Using Field Trials under Semi-natural Conditions

The objective of field trials is to determine host status under specified conditions of a fruit that has been determined not to be a natural host. Trials may include the use of field cages, greenhouses (including glass, plastic and screen houses) and bagged fruit-bearing branches.

The emergence of a viable adult in any one replicate of a field trial under semi-natural conditions indicates that the fruit is a conditional host.

The following subsections outline elements that should be taken into account when designing field trials.

2.1 Fruit sampling

The following requirements apply to fruit sampling in field trials:

- Where possible, sampling should target fruit suspected of being infested. Otherwise, sampling protocols should be based on the principles of randomness and replication and be appropriate for any statistical analysis performed.

- Period of time, the number of repetitions per growing season and the number of replicates should account for the variability of target fruit flies and fruit over time and over the production area. They should also account for early and late harvest conditions and be representative of the proposed area from where the fruit will be moved. The number and weight of the fruit required and replicates per trial to determine effectiveness, and appropriate confidence level, should be specified.

2.2 Fruit flies

The following requirements apply to operational procedures pertaining to the fruit flies used in field trials:

- Taxonomic identification of the fruit flies used for the field trials should be performed and voucher specimens be preserved.
- Basic information on target fruit fly species, including normal period of development and known hosts in the specific production area, should be compiled.
- The use of wild populations for the field trials is desirable. If wild flies cannot be obtained in sufficient numbers, the colony used should not be older than five generations at the initiation of the trials, whenever possible. The fruit fly population may be maintained on substrate, but the generation to be used in the trials should be reared on the natural host to ensure normal oviposition behaviour. Flies used in experimental replicates should all come from the same population and generation (i.e. cohort).
- The fruit fly colony should originate from the same area as the target fruit whenever possible.
- Pre-oviposition, oviposition and mating periods should be determined before the field trials so that mated female flies are exposed to the fruit at the peak of their reproductive potential.
- The age of the adult female and male flies should be recorded on the mating date and at the beginning of the field trials.
- The number of mated female flies required per fruit should be determined according to fruit size, female fecundity and field trial conditions. The number of fruit flies per replicate trial should be determined according to fruit fly biology, amount of fruit to be exposed and other field trial conditions.
- The exposure time of the fruit to the target fruit fly species should be based on fruit fly oviposition behaviour.
- An individual female fly should be used only once.
- The number of adults dying during the field trials should be recorded and dead fruit flies should be replaced with live adults of the same population and generation (i.e. cohort). High adult mortality may indicate unfavourable conditions (e.g. excessive temperature) or contamination of field trial fruit (e.g. residual pesticides). In such cases, the trials should be repeated under more favourable conditions.

In repeated field trials, fruit flies should be of a similar physiological age and have been reared under the same conditions.

2.3 Fruit

The following requirements apply to the fruit used in field trials. The fruit should be:

- of the same species and cultivar as the fruit to be moved
- from the same production area, or an area representative of it, as the fruit to be moved
- practically free from pesticides deleterious to fruit flies and from baits, dirt, other fruit flies and pests
- free from any mechanical or natural damage
- of a specified commercial grade regarding colour, size and physiological condition
- at an appropriate, specified stage of maturity (e.g. dry weight or sugar content).

2.4 Controls

Fruit of known natural hosts at known stage of maturity are required as controls for all field trials. These may be of different species or genera from the target fruit species. Fruit should be free of prior infestation (e.g. by bagging or from a pest free area). Fruit flies used in controls and experimental replicates (including control) should all come from the same population and generation (i.e. cohort).

Controls are used to:

- verify that female flies are sexually mature, mated and exhibiting normal oviposition behaviour
- indicate the level of infestation that may occur in a natural host
- indicate the time frame for development to the adult stage under the field trial conditions in a natural host
- confirm that environmental conditions for infestation are appropriate.

2.5 Field trial design

For this standard, field trials use field cages, greenhouses or bagged fruit-bearing branches. Trials should be appropriate for evaluating how the physical and physiological condition of the fruit may affect host status.

Fruit flies are released into large mesh field cages that enclose whole fruit-bearing plants or mesh bags that enclose the parts of plants with the fruit. Alternatively, fruit-bearing plants may be placed in greenhouses into which flies are released. The fruit-bearing plants can be grown in the enclosures or be introduced as potted plants for the trials. It is important to note that because female fruit flies are artificially confined within the specific enclosure under observation, they may be forced to lay eggs in the fruit of a conditional host.

Field trials should be conducted under conditions appropriate for fruit fly activity, especially oviposition, as follows:

- Field cages and greenhouses should be of an appropriate size and a design to ensure confinement of the adult flies and trial plants, allow adequate airflow and allow conditions that facilitate natural oviposition behaviour.
- Adults should be provided with satisfactory and sufficient food and water.
- Environmental conditions should be optimal and be recorded during the period of the field trials.
- Male flies may be kept in cages or greenhouses with the female flies if it is beneficial for encouraging oviposition.
- Natural enemies to the target fruit fly species should be removed from the cages before initiating the trials and re-entry should be prevented.
- Cages should be secured from other consumers of fruits (e.g. birds and monkeys).
- For controls, fruit from known natural hosts can be hung on branches of plants (not on the branches with test fruit). Controls must be separated from test fruits (in separate field cages, greenhouses or bagged fruit-bearing branches) to ensure the trial is not a choice test.
- The test fruit should remain naturally attached to plants and may be exposed to the fruit flies in field cages, bags or greenhouses.
- The plants should be grown under conditions that exclude as far as possible any interference from chemicals deleterious to fruit flies.
- A replicate should be a bag or cage, preferably on one plant at the experimental unit.
- Fruit fly mortality should be monitored and recorded and dead flies immediately replaced with live flies from the same population and generation (i.e. cohort) to maintain the same fruit fly incidence.

- The fruit should be grown under commercial conditions or in containers of a size that allows normal plant and fruit development.
- After the designated exposure period for oviposition, the fruit should be removed from the plant and weighed and the number and weight of fruit recorded.

The sample size to be used to achieve the confidence level required should be pre-determined using scientific references.

3. Fruit Handling for Fruit Fly Development and Emergence

Fruit collected under natural conditions (surveillance by fruit sampling) and semi-natural conditions (field trials), as well as control fruit, should be kept until larval development is complete. This period may vary with temperature and host status. Fruit handling and holding conditions should maximize fruit fly survival and be specified in the sampling protocol or experimental design of the field trial.

Fruit should be kept in an insect-proof facility or container under conditions that ensure pupal survival, including:

- appropriate temperature and relative humidity
- suitable pupation medium.

Furthermore, conditions should facilitate accurate collection of larvae and pupae, and viable adults emerging from the fruit.

Data to be recorded include:

- daily physical conditions (e.g. temperature, relative humidity) in the fruit holding facility
- dates and numbers of larvae and pupae collected from the test fruit and the control fruit, noting that:
  - the medium may be sieved at the end of the holding period
  - at the end of the holding period, the fruit should be dissected before being discarded, to determine the presence of live and dead larvae or pupae; depending on the stage of fruit decay, it may be necessary to transfer the larvae to an adequate pupation medium
  - all or a subsample of pupae should be weighed and abnormalities recorded
- emergence dates and numbers of all adults by species, including any abnormal adult flies.

4. Data Analysis

Data from larval surveillance and field trials may be analysed quantitatively to determine, for example:

- levels of infestation (e.g. number of larvae per fruit, number of larvae per kilogram of fruit, percentage of infested fruit) at a specific confidence level
- development time of larvae and pupae, and number of viable adults
- percentage of adult emergence.

5. Record-Keeping and Publication

The NPPO should keep appropriate records of larval field surveillance and field trials to determine host status, including:

- scientific name of the target fruit fly
- scientific name of the plant species or name of the cultivar
- location of the production area of the fruit (including geographic coordinates)
- location of voucher specimens of the target fruit fly (to be kept in an official collection)
- origin and rearing of the fruit fly colony used for the field trials
- physical and physiological condition of the fruit tested for infestation by fruit flies

- experimental design, trials conducted, dates, locations
- raw data, statistical calculations and interpretation of results
- key scientific references used
- additional information, including photographs, that may be specific to the fruit fly, the fruit or host status.

Records should be made available to the NPPO of the importing country upon request.

Research should, as far as possible, be peer reviewed and published in a scientific journal or otherwise made available.

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

APPENDIX 1: Bibliography

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This phytosanitary treatment was adopted by the Eleventh Session of the Commission on Phytosanitary Measures in 2016.
The annex is a prescriptive part of ISPM 28.

ISPM 28
Phytosanitary treatments for regulated pests

PT 20: Irradiation treatment for *Ostrinia nubilalis*

Scope of the treatment

This treatment comprises the irradiation of fruits and vegetables at a minimum absorbed dose of 289 Gy to prevent F_1 development past fifth instar, or a minimum adsorbed dose of 343 Gy to prevent F_1 egg hatching from irradiated parent pupae (the most tolerant life stage) of *Ostrinia nubilalis* (European corn borer)[1].

Treatment description

Name of treatment	Irradiation treatment for *Ostrinia nubilalis*
Active ingredient	N/A
Treatment type	Irradiation
Target pest	*Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae)
Target regulated articles	All fruits and vegetables that are hosts of *Ostrinia nubilalis*

Treatment schedules

Minimum absorbed dose of 289 Gy to prevent F_1 development past fifth instar in eggs through late pupae of *O. nubilalis.*

There is 95% confidence that the treatment according to this schedule prevents F_1 development past fifth instar of not less than 99.987% of late pupae of *O. nubilalis*.

Minimum absorbed dose of 343 Gy to prevent F_1 egg hatching in eggs through late pupae of *O. nubilalis.*

There is 95% confidence that the treatment according to this schedule prevents F_1 egg hatching in eggs of not less than 99.9914% of late pupae of *O. nubilalis*.

This treatment should be applied in accordance with the requirements of ISPM 18 (*Guidelines for the use of irradiation as a phytosanitary measure*).

These irradiation schedules should not be applied to fruits and vegetables stored in modified atmospheres because they may affect the treatment efficacy.

Other relevant information

Because irradiation may not result in outright mortality, inspectors may encounter live but non-viable *O. nubilalis* (larvae, pupae or adults) during the inspection process. This does not imply a failure of the treatment.

[1] The scope of phytosanitary treatments does not include issues related to pesticide registration or other domestic requirements for contracting parties' approval of treatments. Treatments adopted by the Commission on Phytosanitary Measures may not provide information on specific effects on human health or food safety, which should be addressed using domestic procedures before contracting parties approve a treatment. In addition, potential effects of treatments on product quality are considered for some host commodities before their international adoption. However, evaluation of any effects of a treatment on the quality of commodities may require additional consideration. There is no obligation for a contracting party to approve, register or adopt the treatments for use in its territory.

In evaluating this treatment the Technical Panel on Phytosanitary Treatments (TPPT) considered issues associated with the possible survival of sterile adults. If sufficient numbers of these were to escape from irradiated infested fruits and vegetables and fly into pest monitoring traps, a quarantine response could be triggered, possibly resulting in economic loss and trade restrictions. The TPPT considered that, based on the work described in Hallman and Hellmich (2009) and Hallman *et al.* (2010), the numbers of fit survivors would be sufficiently low to make this an unlikely event.

References

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

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Publication history

This is not an official part of the standard

2012 Treatment submitted (2012-009)

2012-12 TPPT reviewed treatment and requested additional information

2013-02 TPPT sent letter to Submitter through Secretariat

2013-05 Submitter responded

2013-07 TPPT recommended to SC for MC

2013-09 TPPT approved treatment schedule (virtual meeting)

2013-09 TPPT started drafting paper on adult emergence after irradiation

2014-02 TPPT approved paper on adult emergence after irradiation and submitted to Secretariat

2014-02 SC e-decision for approval for MC

2014-03 Secretariat applied changes suggested by forum and opened poll

2014-03 SC approved draft treatment for MC via poll (2014_eSC_May_06)

2015-02 Member consultation comments under review by TPPT

2015-05 TPPT May virtual meeting reviewed

2015-09 TPPT September meeting reviewed

2015-10 SC approved PT to be submitted for adoption by CPM (2015_eSC_Nov_06)

2016-04 CPM-11 adopted the PT

ISPM 28. Annex 20. *Irradiation treatment for* Ostrinia nubilalis*.* Rome, IPPC, FAO.

Publication history last updated: 2016-04

This phytosanitary treatment was adopted by the Eleventh Session of the Commission on Phytosanitary Measures in 2016.
The annex is a prescriptive part of ISPM 28.

ISPM 28
Phytosanitary treatments for regulated pests

PT 21: Vapour heat treatment for *Bactrocera melanotus* and *Bactrocera xanthodes* on *Carica papaya*

Scope of the treatment

This treatment comprises the treatment of fruit of *Carica papaya* in a high temperature forced air chamber to result in the mortality of eggs and larvae of *Bactrocera melanotus* and *Bactrocera xanthodes* (Pacific fruit fly) at the stated efficacy[1].

Treatment description

Name of treatment	Vapour heat treatment for *Bactrocera melanotus* and *Bactrocera xanthodes* on *Carica papaya*
Active ingredient	N/A
Treatment type	Physical (vapour heat)
Target pests	*Bactrocera melanotus* (Coquillett) (Diptera: Tephritidae) and *Bactrocera xanthodes* (Broun) (Diptera: Tephritidae)
Target regulated articles	Fruit of *Carica papaya* L.

Treatment schedule

Exposure in a forced air chamber:

- at a minimum of 60% relative humidity
- with air temperature increasing over a minimum of 3.5 hours from room temperature to 48.5 °C or above
- with air temperature held at 48 °C or above for a minimum of 3.5 hours
- with all fruit within the chamber maintaining a core temperature of 47.5 °C or above for a minimum of 20 minutes.

Once the treatment is complete, the fruit may be cooled (e.g. by hydro-cooling) to a core temperature of 30 °C in a period of time of no less than 70 minutes.

There is 95% confidence that the treatment according to this schedule kills not less than 99.9914% of eggs and larvae of *B. melanotus* and *B. xanthodes*.

[1] The scope of phytosanitary treatments does not include issues related to pesticide registration or other domestic requirements for contracting parties' approval of treatments. Treatments adopted by the Commission on Phytosanitary Measures may not provide information on specific effects on human health or food safety, which should be addressed using domestic procedures before contracting parties approve a treatment. In addition, potential effects of treatments on product quality are considered for some host commodities before their international adoption. However, evaluation of any effects of a treatment on the quality of commodities may require additional consideration. There is no obligation for a contracting party to approve, register or adopt the treatments for use in its territory.

Other relevant information

In evaluating this treatment the Technical Panel on Phytosanitary Treatments (TPPT) considered the technical justification for including other pest tephritid fruit flies (*Anastrepha ludens* (Loew), *Anastrepha suspensa* (Loew), *Bactrocera cucurbitae* (Coquillett), *Bactrocera dorsalis* (Hendel), *Bactrocera facialis* (Coquillett), *Bactrocera kirki* (Froggatt), *Bactrocera passiflorae* (Froggatt), *Bactrocera psidii* (Froggatt), *Bactrocera tryoni* (Froggatt) and *Ceratitis capitata* (Wiedemann)) and other fruit crops (all fruit hosts of tephritid fruit flies) in the treatment description as originally submitted. The TPPT recommended, however, including only two pest tephritid fruit flies, *B. melanotus* and *B. xanthodes,* for only one fruit crop, *C. papaya*, based on Waddell *et al.* (1993).

The fruit crop used to develop the schedule was *C. papaya* Waimanalo Solo.

References

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

Waddell, B.C., Clare, G.K., Maindonald, J.H. & Petry, R.J. 1993. *Postharvest disinfestations of* Bactrocera melanotus *and* B. xanthodes *in the Cook Islands.* Report 3. Wellington, New Zealand Ministry of Agriculture and Fisheries – Regulatory Authority. 44 pp.

Publication history

This is not an official part of the standard

2009 *High temperature forced air treatment for selected fruit fly species (Diptera: Tephritidae) on fruit* (2009-105) submitted.

2010-07 TPPT reviewed treatment and requested additional information.

2012-12 TPPT requested additional time for evaluation.

2013-07 TPPT changed the title to *High temperature forced air treatment for* Bactrocera melanotus *and* B. xanthodes *on* Carica papaya (2009-105) and recommended to SC for member consultation.

2013-09 TPPT approved treatment schedule (virtual meeting).

2014-02 SC e-decision for approval for member consultation.

2014-03 Secretariat applied changes suggested by forum and opened poll.

2014-03 SC approved draft treatment for member consultation via poll (2014_eSC_May_02).

2015-02 Member consultation comments under review by TPPT.

2015-05 TPPT May virtual meeting.

2015-09 TPPT September meeting.

2015-10 SC approved draft treatment for adoption by CPM (2015_eSC_Nov_07).

2016-04 CPM-11 adopted the phytosanitary treatment.

ISPM 28. Annex 21. *Vapour heat treatment for* Bactrocera melanotus *and* Bactrocera xanthodes *on* Carica papaya*.* Rome, IPPC, FAO.

Publication history last updated: 2016-04

This diagnostic protocol was adopted by the Standards Committee on behalf of the Commission on Phytosanitary Measures in August 2015.
The annex is a prescriptive part of ISPM 27.

ISPM 27
Diagnostic protocols for regulated pests

DP 8: *Ditylenchus dipsaci* and *Ditylenchus destructor*

Adopted 2015; published 2016

CONTENTS

1. Pest Information

Species within the large genus *Ditylenchus* Filipjev, 1936 are distributed worldwide, and most species are mycetophagous. However, the genus contains a few species that are of great importance as pests of higher plants (Sturhan and Brzeski, 1991). It is worth mentioning that though there are certain plants (e.g. beets, lucerne, clover) that are affected by both *Ditylenchus dipsaci* and *Ditylenchus destructor*, the two species rarely occur together in the same plant (Andrássy and Farkas, 1988).

1.1 *Ditylenchus dipsaci*

D. dipsaci sensu lato (*s.l.*), or stem nematode, attacks more than 1 200 species of wild and cultivated plants. Many weeds and grasses are hosts for the nematode and may play an important role in its survival in the absence of cultivated plants. Morphological, biochemical, molecular and karyological analyses of different populations and races of *D. dipsaci s.l.* have suggested that it is a complex of at least 30 host races, with limited host ranges. Jeszke *et al*. (2013) divided this complex into two groups, the first containing diploid populations characterized by their "normal" size and named *D. dipsaci sensu stricto* (*s.s*.). This group comprises most of the populations recorded so far. The second group is polyploidal and currently comprises *Ditylenchus gigas* Vovlas *et al*., 2011 (the "giant race" of *D. dipsaci* parasitizing *Vicia faba* (broad bean)); *D. weischeri* Chizhov *et al*., 2010 (parasitizing *Cirsium arvense* (creeping thistle)); and three undescribed *Ditylenchus* spp. called D, E and F, which are associated with plant species of the Fabaceae, Asteraceae and Plantaginaceae, respectively (Jeszke *et al.*, 2013). Of all these species only *D. dipsaci s.s.* and its morphologically larger variant *D. gigas* are plant pests of economic importance. This protocol includes information to distinguish between *D. dipsaci s.s.* and *D. gigas.*

D. dipsaci lives mostly as an endoparasite in aerial parts of plants (stems, leaves and flowers), but also attacks bulbs, tubers and rhizomes. This nematode is seed-borne in *V. faba*, *Medicago sativa* (lucerne/alfafa), *Allium cepa* (onion), *Trifolium* spp. (clovers), *Dipsacus* spp. (teasel) and *Cucumis melo* (melon) (Sousa *et al*., 2003; Sikora *et al*., 2005). Of great importance is the fact that the fourth stage juvenile can withstand desiccation for a long time, sometimes 20 years or more (Barker and Lucas, 1984). These nematodes clump together in a cryptobiotic state to form "nematode wool" when the plant tissue begins to dry (Figure 1). The wool can often be observed on the seeds in heavily infested pods and in dry plant debris (e.g. that which remains in the field after harvest). The presence of the infective fourth stage juveniles in seed and dry plant material is important in the passive dissemination of the nematode over long distances. The nematode in its desiccated state can survive passage through pigs and cattle on or in infected seed (Palmisano *et al.*, 1971).

Although *D. dipsaci* is seen as a pest of higher plants, Viglierchio (1971) reported that a Californian population of *D. dipsaci* from *Allium sativum* (garlic) could reproduce on soil fungi (*Verticilium* and *Cladosporium*) under laboratory conditions.

D. dipsaci is known to vector bacterial plant pathogens externally (i.e. *Clavibacter michiganensis* subsp. *insidiosus* (syn. *Clavibacter michiganensis* subsp. *insidiosum*, *Corynebacterium insidiosum*), causing alfalfa wilt).

According to EPPO (2013a), *D. dipsaci* is present in the following regions (interceptions excluded): Europe, Asia, Africa, North America, Central America and the Caribbean, South America and Oceania.

1.2 *Ditylenchus destructor*

D. destructor, or potato rot nematode, attacks almost exclusively the subterranean parts of plants (e.g. tubers, rhizomes and stem-like underground parts). It is a near-cosmopolitan species, common in temperate regions and responsible for severe losses in potato and hop production (EPPO, 2013a). The host range of the nematode is extensive, comprising more than 90 plant species, which include ornamental plants, crop plants and weeds. *Solanum tuberosum* (potato) is the principal host, the tubers developing wet or dry rot that will spread to other tubers in storage. Under certain conditions, wet rot

organisms may damage the tubers extensively, but will also kill the nematodes. *D. destructor* can survive only when dry rot organisms invade the tuber. Rojankovski and Ciurea (1986) found 55 species of bacteria and fungi associated with *D. destructor* in *S. tuberosum* tubers, with *Fusarium* spp. being the most common.

Other common hosts are *Ipomoea batatas* (sweet potato), bulbous iris (hybrids and selections derived from *Iris xiphium* and *Iris xiphioides*), *Taraxacum officinale* (dandelion), *Humulus lupulus* (hop), *Tulipa* spp. (tulip), *Leopoldia comosa* (grape hyacinth), *Hyacinthus orientalis* (hyacinth), *Gladiolus* spp. (gladiolus), *Dahlia* spp. (dahlia), *Coronilla varia* and *Anthyllis vulneraria* (vetch), *Beta vulgaris* (sugar beet, fodder beet and beetroot), *Calendula officinalis* (marigold), *Daucus carota* (carrot), *Petroselinum crispum* (parsley) and *Trifolium* spp. (red, white and alsike clover) (Sturhan and Brzeski, 1991). In the absence of higher plants, *D. destructor* reproduces readily on the mycelia of about 70 species of fungi and it is known to destroy the hyphae of cultivated mushroom (Sturhan and Brzeski, 1991). The species is able to survive desiccation and low temperatures, but does not form nematode wool as does *D. dipsaci* (Kühn, 1857) Filipjev, 1936. This species, however, overwinters as eggs, which makes eggs more vital in *D. destructor* than in *D. dipsaci*. *D. destructor* in seed potatoes and flower bulbs is a regulated pest in many countries (Sturhan and Brzeski, 1991). *D. destructor* was reported on *Arachis hypogaea* (groundnut/peanut) in South Africa, but these records are now considered to be a separate species, *Ditylenchus africanus* Wendt, Swart, Vrain and Webster, 1995, which is morphologically and morphometrically close to *D. destructor*.

According to EPPO (2013a), *D. destructor* is present in the following regions (interceptions excluded): Europe, Asia, Southern Africa, North America, South America and Oceania.

2. Taxonomic Information

Name: *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936

Synonyms: Synonyms of the type species *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 are listed in Siddiqi (2000)

Taxonomic position: Nematoda, Secernentea, Diplogasteria, Tylenchida, Tylenchina, Tylenchoidea, Anguinidae

Common names: Stem nematode, stem and bulb eelworm (English) (Sturhan and Brzeski, 1991)

Note: *D. dipsaci* is now considered as a species complex composed of a great number of biological races and populations differing mainly in host preference. Consequently a total of 13 nominal species have been synonymized with *D. dipsaci* and up to 30 biological races have been differentiated, mainly distinguished by host range and generally named after their principal host plant.

Name: *Ditylenchus destructor* Thorne, 1945

Synonyms: None

Taxonomic position: Nematoda, Secernentea, Diplogasteria, Tylenchida, Tylenchina, Tylenchoidea, Anguinidae

Common names: Tuber-rot eelworm, potato rot nematode (English) (Sturhan and Brzeski,1991)

De Ley and Blaxter (2003) have constructed the most recent classification system, combining morphological observations, molecular findings and cladistic analysis.

3. Detection

D. dipsaci and *D. destructor* both have the following common symptoms that allow their detection: swelling, distortion, discoloration and stunting of the above-ground plant parts and necrosis or rotting of the bulbs and tubers (Thorne, 1945).

Ditylenchus dipsaci

D. dipsaci shows parasitic adaptation in its ability to invade solid parenchyma tissue following enzymatic lysis of the pectic or middle lamella layer between adjacent cell walls, leading to separation and rounding of the cells. This causes the typical glistening appearance or mealy texture of infested tissues, reminiscent of the flesh of an over-ripe apple (Southey, 1993).

According to Vovlas *et al.* (2011), *D. gigas* (giant stem and bulb nematode) infestation of *V. faba* causes swelling and deformation of stem tissue or lesions, which turn reddish brown then black. In severe infestations the seeds appear dark, distorted and smaller in size than uninfested seeds, and they have speckle-like spots on the surface. Hosts other than *V. faba* are *Lamium purpureum, Lamium album, Lamium amplexicaule, Ranunculus arvensis, Convolvulus arvensis* and *Avena sterilis.*

Ditylenchus destructor

D. destructor commonly infects the underground parts of plants (tubers and stolons of potato, rhizomes of mint, and roots of hop and lilac), causing discoloration and rotting of plant tissue. The above-ground parts are sometimes also infected, causing dwarfing, thickening and branching of the stem and dwarfing, curling and discoloration of the leaves (e.g. in potato) (Sturhan and Brzeski, 1991). More often, however, no symptoms of infection are found in the above-ground parts of plants.

3.1 Hosts and symptoms

3.1.1 *Ditylenchus dipsaci*

According to Sturhan and Brzeski (1991), the principal hosts of *D. dipsaci* are Gramineae: *Avena sativa* (oat), *Secale cereale* (rye), *Zea mays* (maize), *Triticum aestivum* (wheat); Liliaceae: *A. cepa, A. sativum, Tulipa* spp.; Leguminosae: *M. sativa, Vicia* spp., *Pisum sativum, Trifolium* spp.; Solanaceae: *S. tuberosum, Nicotiana* spp.; Cruciferae: *Brassica campestris*; and Amarilidaceae: *Narcissus* spp. Other hosts include *D. carota, Fragaria* spp. (strawberry), *B. vulgaris, H. orientalis, Allium ampeloprasum* (leek), *Phlox drummondii, Phlox paniculata, Dianthus* spp. (carnation), *Apium graveolens* (celery), *Hydrangea* spp., *Lens culinaris* (lentil), *Brassica napus* (rape), *Petroselinum crispum* and *Helianthus annuus* (sunflower).

Various generations of *D. dipsaci* may be present in a host plant during a season, following each other. If affected parts of the plant die due to injuries by the pest, nematodes leave the host before it dies completely. When lacking host plants, the nematodes can enter non-host plants and feed there for a certain time, though they are unable to reproduce in non-host plants (Andrássy and Farkas, 1988). The most common symptoms of *D. dipsaci* infestation are stunted, chlorotic plants; thickened, stunted, gall-containing and distorted stems, petioles and flowers; and necrotic lesions in and rotting of bulbs and rhizomes, often appearing as brown rings when bulbs are sliced. *D. dipsaci* may also infest seeds, from, for example, *Phaseolus vulgaris* (snap bean, string bean or green bean), *V. faba, Allium* spp. and *M. sativa*. Small seeds generally show no visible symptoms of infestation but larger seeds may have a shrunken skin with discoloured spots.

3.1.1.1 Symptoms specific to Gramineae

Avena sativa* and *Secale cereale (McDonald and Nicol, 2005). Leaves become distorted, stems thicken, an abnormal number of tillers are produced, and the plant is short, bushy and stunted. In *S. cereale* cultivation, *D. dipsaci* occurs mainly in light soils poor in humus and naturally in areas where rye is regularly grown. The first signs of infestation can be observed in late autumn, but they are most conspicuous in spring. Several spots on plants with retarded growth in the rye field indicate damage by the pest. As infested *A. sativa* plants grow more slowly, they are conspicuous in the yellowing crop with their green colour. Affected *T. aestivum* has the same symptoms as other cereals and is attacked by *D. dipsaci* only in central and eastern Europe (Rivoal and Cook, 1993).

Zea mays is a poor host for *D. dipsaci* but invasion of the stem tissues of young plants produces necrosis in those tissues and causes the maize plants to die or fall over before harvest (Rivoal and

Cook, 1993). The leaves of the infested plants are crisp, and twisted like a corkscrew. Internodes are shortened and the bottom of the stem becomes hollow, while bigger plants break and lodge.

3.1.1.2 Symptoms specific to Liliaceae

***Allium cepa*, *Allium sativum* and *Allium cepa* var. *aggregatum* (shallot).** It is characteristic in most *Allium* spp. that leaves and bulbs become deformed on infestation with *D. dipsaci* (Figures 2, 3 and 4). The base of young plants becomes swollen and leaves become distorted. Older infected bulbs show swelling (bloat) of scales with open cracks often occurring at the root disc of the bulbs (Potter and Olthof, 1993). *A. cepa* attacked by *D. dipsaci* have a frosted appearance caused by the dissolution of cells that results from nematode feeding (Ferris and Ferris, 1998). Infested bulbs tend to rot readily in storage (Bridge and Hunt, 1986). The inner scales of the bulb are usually more severely attacked than the outer scales. As the season advances the bulbs become soft and when cut open show browning of the scales in concentric circles. Conversely, *D. dipsaci* does not induce deformation of leaves or swelling in *A. sativum*, but does cause leaf yellowing and death (Netscher and Sikora, 1990). Mollov *et al.* (2012) reported *D. dipsaci* for the first time from *A. sativum* in Minnesota, United States. The symptoms of the above-ground plant were stunting and chlorosis, while the symptoms of the bulbs were necrosis, underdevelopment and distortion. *Allium* spp. may have foliar spickels (i.e. blister-like swellings on the leaves). No symptoms of infestation are observed on infested *Allium* seeds.

***Tulipa* spp.** (Southey, 1993). Symptoms of *D. dipsaci* attack on tulip, both on growing plants and on bulbs, are quite different from those on *Narcissus* spp. In the field, infestation is best detected at flowering. The first sign is a pale or purplish lesion on one side of the stem immediately below the flower, which bends in the direction of the lesion. The lesion increases in size, the epidermis splits – revealing typical loose tissue beneath – and the damage spreads downwards and often upwards on to the petals. In more severe attacks, similar lesions extend down stems from leaf axils and growth may become distorted. Infestations start at the base of new bulbs, which arise as lateral offset buds from the base of the previous stems. The infection can be seen and felt on removal of the outer brown scales, as grey or brown soft patches on the outer fleshy scales. Infected bulbs do not show brown rings as they do in narcissus and hyacinth.

3.1.1.3 Symptoms specific to Leguminosae

Medicago sativa. *D. dipsaci* is the most important nematode pest of *M. sativa.* Infestation occurs readily in heavier soils and during times of high rainfall or in sprinkler-irrigated areas. "White flagging" associated with loss of leaf chlorophyll is often a feature of infested crops under conditions of moisture stress (Griffin, 1985). Infested fields often show irregular areas of sparse growth. Typical symptoms of nematode attack include basal swelling, dwarfing and twisting of stalks and leaves, shortening of internodes, and the formation of many axillary buds, producing an abnormal number of tillers to give the plant a bushy appearance (McDonald and Nicol, 2005). Infested plants sometimes do not grow tall enough for hay (Ferris and Ferris, 1998), and they often fail to produce flower spikes (McDonald and Nicol, 2005). *D. dipsaci* predisposes lucerne to *Phytophtora megasperma*. Damage by *D. dipsaci* is increased by the occurrence of other, saprophagous nematodes (*Rhabditis*, *Cephalobus* and *Panagrolaimus* species) on the diseased, broken plants, which also hasten the death of the plants (Andrássy and Farkas 1988). No symptoms of infestation are observed in infested *Medicago* seeds.

***Trifolium* spp.** (Cook and Yeates, 1993). Symptoms are quite similar to those described for *M. sativa*, except on red and white clovers. The pest invades red clover in particular in cool, rainy weather. Large, round areas of diseased plants appear in the field; plants are more diseased towards the inside of the area, frequently wilting in its centre. The bases of the plants are swollen like bulbs, and the leaves are crisp, shrivelled and with conspicuously thick veins. Flower initiations are swollen like galls, and a single flower gall may contain 5 000 nematodes (Courtney, 1962). Stems of white clover infected by *D. dipsaci* are short and swollen, buds are tufty, and the infested parts become brown in summer or autumn. Leaves are narrower than usual; however, their petioles are thicker and shorter. Flower buds are swollen at their bases (Andrássy and Farkas, 1988).

3.1.1.4 Symptoms specific to Solanaceae

Solanum tuberosum. *D. dipsaci* produces a funnel-shaped rot, which extends further into the tuber than the superficial rot caused by *D. destructor*. Stems and leaves are invaded by the nematode and this results in the typical stunting of the plant, accompanied by severe distortion of stems and petioles (Evans and Trudgill, 1992).

***Nicotiana* spp.** (Johnson, 1998). The infectious juveniles (fourth stage) enter the leaves and stems of tobacco seedlings during wet weather and induce small, yellow swellings (galls) that may extend 40 cm or more above the soil. As the number of galls increases, plant tissue begins to die prematurely. Lower leaves may fall off and upper leaves may turn yellow. Galls eventually rot, stopping growth of infected plants. Eventually, and especially in cool, damp weather and in heavy soils, the infected stems break and the plants fall over.

3.1.1.5 Symptoms specific to Cruciferae

Severe crown rot may develop in mature *B. campestris* infected with *D. dipsaci*.

3.1.1.6 Symptoms specific to Amarilidaceae

***Narcissus* spp.** (Southey, 1993). Typical symptoms are the presence of pale yellowish, blister-like swellings on the leaves (spickels) and concentric brown rings that can be seen when the bulbs are cut transversely (Figures 5 and 6). When bulbs are cut lengthwise, the necrosis is seen to have started at the neck, spreading downwards. Swellings are best seen before flowering when leaves are growing actively. In mild attacks, the swellings can be better felt between the finger and thumb than seen. *D. dipsaci* infection can be detected in dry bulbs with minimal bulb damage by cutting just below the neck. Careful examination in the early stages of infestation reveals glistening, spongy areas where cells have been separated. This is rapidly followed by brown necrosis.

3.1.1.7 Symptoms specific to other hosts

***Fragaria* spp.** *D. dipsaci* is the only species of *Ditylenchus* regarded as a pathogen of strawberry (Brown *et al.*, 1993). Damage is seen as small, distorted leaves, and short, thick and twisted petioles.

Family Asparagacae, subfamily Sciloideae (hyacinths) and other bulbs (Southey, 1993). Bulb symptoms are the same as in *Narcissus* spp., but distinct swellings are not usually seen on the plant leaves. The foliage may show pale yellow streaks, distortion and often slight swelling. Other liliaceous bulbs generally show the same symptoms as hyacinths. Symptoms of infestation in Amarylliaceae are similar to those in *Narcissus* spp.; for example, *Galanthus* spp. and *Nerine* spp. show swellings on their leaves and concentric, brown rings in bulbs.

Beta vulgaris* and *Daucus carota (Cooke, 1993). *D. dipsaci* feeding results in the death of the growing point in seedlings (leading to the formation of multiple crowns); cotyledons and leaves may become twisted, swollen and distorted; and galls may develop on leaves or petioles of slightly older plants. Later in the season, feeding on the crown may cause a rot known as crown canker, crown rot or collar rot. This is first visible as raised, greyish pustules, usually among the leaf scars. Rotting then develops outwards and downwards, expanding across the shoulder of the plant, allowing the crown to become detached when pulled. In *D. carota,* additional symptoms may include straddled leaves and discoloration of the head of the main root. Symptoms mainly occur on the root and stem of the plant 2–4 cm below and above ground level. Severe infestation causes leaf death and crown rot, especially in autumn (Figure 7).

***Phlox paniculata* and other ornamental plants** (Southey, 1993). On phlox, infested shoots show typical thickening and brittleness of stems and shortening of internodes that have a tendency to split. Characteristic and unique to this host is the crinkling and reduction of laminae of the upper leaves, the uppermost of which may be reduced to attenuated filaments. Examples of plants recorded as hosts, with malformed growth, swelling and so forth, are species and cultivars of *Anemone, Calceolaria, Cheiranthus, Gypsophila, Helenium, Heuchera, Lychnis, Lysimachia* and *Penstemon* (Roberts, 1981).

Edwards (1937) reported stunting, leaf malformation, rotting and failure to flower in *Primula* spp. Woody plants are not often attacked, but *Hydrangea* may be infested with *D. dipsaci*, causing distortion of non-woody shoots, swelling of petioles and main veins, and pronounced crinkling of leaf laminae. The crinkled leaves are usually the first sign of infection. Another woody plant, *Yucca smaliana*, shows leaf distortion and blister-like swellings.

3.1.2 *Ditylenchus destructor*

According to Sturhan and Brzeski (1991), *D. destructor* parasitizes mainly tubers (e.g. potato and dahlia), bulbs (e.g. bulbous iris, tulips and gladioli) and root crops (e.g. sugar beet and carrot). It is able to destroy the hyphae of *Agaricus hortensis* (cultivated mushroom). Other hosts include *I. batatas*, *A. sativum*, *P. vulgaris*, *Angelica sinensis* ("dong quai" or "female ginseng"), *Panax ginseng (*ginseng), *Taraxacum officinale*, *Begonia* spp. and bulbs of *Erytronium denscanis* (dog's tooth violet or doftooth violet).

***Solanum tuberosum* and *Dahlia* spp.** No symptoms are visible during the growth period. The nematodes enter potato tubers usually via the stolons. Most of the nematodes are located at the edge of the browning and undamaged parts. If a small sample from this part of the tuber is taken and placed in water, the mass of small nematodes is conspicuous even with a simple magnifying glass. The earliest symptoms of *D. destructor* infection are small, white, chalky or light-coloured spots that can be seen just below the skin of the tuber (Brodie, 1998). These spots later become larger and gradually darker (through grey, dark brown and black), and acquire a spongy texture (Figure 8). This is mostly a result of secondary invasion by bacteria, fungi and saprophytic nematodes (Brodie, 1998). On severely affected tubers there are typically slightly sunken areas with cracked, wrinkled, papery skin. The skin is not attacked but becomes thin and cracks as underlying infected tissues dry and shrink (Brodie, 1998). Finally, mummification of whole tubers may occur. Such fully damaged tubers float in water (Figure 9). In contrast, the skin of *S. tuberosum* infested with *D. dipsaci* is usually not cracked. The nematodes continue to reproduce inside the tubers after harvest and may build up to large numbers. Symptoms may be more visible after storage. Secondary infections of fungi, bacteria and free-living nematodes occur in general on infested tubers.

Beta vulgaris. Infestation results in dark, necrotic lesions on roots and rhizomes. Dallimore and Thorne (1951) reported symptoms similar to crown canker. In sugar beet, in addition to yield loss, sugar content will also be reduced.

Daucus carota. Infestation results in transverse cracks in the skin of the carrot with white patches in the cortical tissue. Secondary infections in these areas by fungi and bacteria may also result in decay. This damage is easily seen in a cross-section of the carrot. The nematode continues its destructive activity during winter storage and carrots become unsuitable for consumption.

***Iris* spp. and *Tulipa* spp.** (Southey, 1993). Infestation results in greyish linear marks that extend upwards from the basal plate on the outer fleshy scales. As infestation progresses, the damage spreads over and through the tissue of the bulb and leads to a secondary dry, fibrous rotting that results in collapse of the bulb. Ring-like brown spots are conspicuous when a cross-section is made of an infested bulb. Yellowing and dieback of the foliage are secondary symptoms caused by the damage to the bulb and eventual cessation of root functioning.

D. destructor infestation of ornamental *Liatris spicata* corms ("Gayflower", "Blazing Star" or "Button Snakeroot") in cold storage in South Africa showed a blackish rot with living nematodes at different stages in the tissue adjacent to the decaying areas (Van der Vegte and Daiber, 1983).

3.2 Nematode extraction

3.2.1 Extraction from bulbs and garlic

To extract the nematodes, the affected scales of bulbs (inner scales mainly) or garlic cloves are cut into small pieces and put in a container (e.g. Petri dish) with tap water at room temperature. To obtain a clear suspension the pieces may be placed on a sieve of 200–250 μm aperture covered with filter

paper, as a support (Oostenbrink dish technique). After 1 h or more the nematodes can be observed with a stereomicroscope (at least 40× magnification).

3.2.2 Extraction from soil and plant material

The Baermann funnel method is a reference technique for the extraction of nematodes from soil and plant material (bulbs, roots, potato peelings and seeds). A funnel has a piece of rubber tubing attached to its stem that is closed by a spring or screw clip. The funnel is placed in a support and almost filled with tap water**.** Soil or plant tissue cut into small pieces is placed in a muslin or in tissue paper, which is folded to enclose the material and is gently submerged in the water in the funnel. Active nematodes pass through the cloth and sink to the bottom of the funnel stem. After some hours, or overnight, a small quantity of water containing the nematodes is run off and observed under a microscope (Flegg and Hooper, 1970).

In a variation of the technique the funnel is replaced by a dish. Lumps of soil are broken up and stones and plant debris removed. Soil (50 ml) is spread evenly on a circle of single-ply paper towel supported on a coarse-meshed plastic screen standing in a plastic container. Water is added to the container until the soil is thoroughly wet but not immersed. The container is covered with a large Petri dish top to reduce evaporation of water. This set-up is left for at least 24 h after which the soil is discarded and the nematode suspension is poured from the container into a dish for examination with the aid of a dissection microscope. The soil can be replaced by finely chopped plant tissue (Kleynhans, 1997).

The Seinhorst mistifier technique for bulbs and roots differs from the Baermann funnel method in that plant sap and toxic decomposition products are washed away. It should be used in preference to the Baermann funnel method for plants such as *Narcissus* spp. In this method a Baermann funnel or Oostenbrink dish is placed in a mist or fog of water to avoid the depletion of oxygen. The mist is produced by nozzles spraying water over the plant material or by nozzles spraying water upwards so that droplets fall softly back onto the plant material. Live nematodes leave the plant tissue and are washed into the funnel or dish where they sediment. The nematodes are collected every 24 to 48 h in a glass beaker by opening the screw clip on the funnel stem or by collecting the specimens on a 20–25 µm sieve. Extraction can be continued for up to four weeks. This technique is described by Hooper (1986).

Another method to extract *Ditylenchus* spp. from plant material was adapted from a description by Oliveira *et al.* (2013). Plant material is cut in 1 cm pieces and they are placed in 500 ml jars filled with tap water. Two holes are punched into the lids of these jars, one providing access to the tube of an aquarium pump and one acting as an outlet for air. The material is kept for 72 h under continuous aeration from the pump. The resulting suspension is poured through a 1 000 µm sieve to remove plant debris and then through a 38 µm sieve to extract the nematodes from the suspension. This method of aerating the suspension prevents the rotting of the plant material so there is a minimal increase of bacterial and fungal feeders and many of the nematodes stay alive. The agitation through the aeration of the suspension containing the plant material results in more nematodes being dislodged from the root tissue and therefore in a much more accurate estimate of the infestation of the plant material.

Nematodes can also be extracted from plant material by the method of Coolen and D'Herde (1972). The plant material is washed, cut into pieces of about 0.5 cm, and 5 g portions are macerated in 50 ml tap water in a domestic blender at the lowest mixing speed for 1 min. The disadvantage of this method is that large nematode specimens, such as *D. dipsaci* adults, can be cut to pieces in the blender. The suspension of nematodes and tissue fragments are washed through a 750 µm sieve placed on top of a 45 µm sieve. The residue on the 45 µm sieve is collected and poured into two 50 ml centrifuge tubes. About 1 ml kaolin is added to each tube, the mixture is thoroughly stirred and then it is centrifuged at 3 000 r.p.m. for 5 min. The supernatant is decanted and sucrose solution (density 1.13 g/cm^3) is added to the tubes. The mixture is thoroughly stirred and centrifuged at 1 750 r.p.m. for 1 min. The supernatant is washed through a 45 µm sieve, the residue is collected and the nematodes are studied.

The testing of dried legumes and other pulse crops for the presence of *D. dipsaci* is a two-step procedure involving (1) soaking of a quantity of seed in aerated water overnight, and (2) extracting a portion of the soaked seed under mist for three days. The presence of nematodes in the soaking water and mist extract are determined by sieving aqueous fractions from each of the two steps followed by microscopic observation for identification. The process takes about seven days, but can be shortened to three days by eliminating step (2) (i.e. extraction under mist). The modified procedure consists of soaking the pulses overnight in aerated water, followed by sieving and microscopic observation for identification.

For extraction of nematodes from soil, the following method (after Kleynhans, 1997) can be used. Soil (250 ml) is washed through a coarse-meshed (2 mm) sieve into a 5 litre bucket. Tap water is added to make a volume of 5 litres. The suspension is stirred, then allowed to settle for 30 s before being poured through a 45 µm sieve. This procedure is repeated with the soil in the bucket two times, but shortening the setting times to 20 s and then 10 s. The residue is transferred from the 45 µm sieve to 50 ml centrifuge tubes. If the solution in the tubes is very sandy, 5 ml kaolin can be added to the tubes (and thoroughly mixed) to assist in the settling of the nematodes. The tubes are centrifuged at 1 750 r.p.m. for 7 min. The supernatant is decanted from each tube and discarded. A sugar solution (450 g/litre water) is added to the tubes and this sugar and soil mixture is thoroughly shaken before centrifuging again at 1 750 r.p.m. for 3 min. The supernatant is poured through a 45 µm sieve and the residue, with nematodes in it, is collected in a beaker for examination. This is a basic technique and depending on the skill of the technician and type of soil, up to 40% of the nematodes may be lost. Other methods that may be used for the extraction of nematodes from soil include the Flegg-modified Cobb technique and the Oostenbrink elutriator method (EPPO, 2013c). Hooper *et al*. (2005) describes different extraction methods adapted to take advantage of size, density and motility of nematodes.

4. Identification

Identification of *Ditylenchus* spp. by morphological means is restricted to adult specimens and preferably both male and female nematodes of a species are examined under a high-power microscope. Good-quality slide preparations should allow adult *D. dipsaci* and *D. destructor* to be identified with certainty by morphological examination alone. The morphological identification of *Ditylenchus* juveniles in a sample should be used only to confirm the presence of the species in the sample. As mycophagous *Ditylenchus* spp. frequently contaminate decaying plant material, care must be taken in the identification of specimens in both plant and soil samples.

4.1 Morphological identification

The identification of *D. dipsaci* and *D. destructor* should preferably be based on morphological methods. Molecular methods developed for identifying these species can be used for low infestation levels or when only juveniles are present. Molecular methods can be applied to damaged and atypical adults, and all life stages, including the juvenile stages, for which morphological identification to species is not possible.

4.1.1 Preparation of specimens

Temporary preparations for quick identification or study of features best seen in unfixed specimens are prepared as follows (Kleynhans, 1997):

- Live specimens are transferred to a small drop of water on a glass slide.
- The slide is briefly heated over a spirit flame, checking frequently for nematode movement. Heating should be stopped as soon as the specimens stop twitching.
- A coverslip is applied and sealed around the edge with nail varnish. When the varnish has dried, the slide with specimens is ready for study.

For light microscopy, live nematodes are extracted from soil or plant material, killed by gentle heat (65–70 °C), fixed in FAA (35% distilled water, 10% of 40% formalin, 5% glacial acetic acid, 50% of 95% alcohol) (Andrássy, 1984), transferred into glycerol (Hooper *et al*., 2005) and mounted in anhydrous glycerine between coverslip slides as described by Seinhorst (1959) and Goodey (1963).

For light microscopy identification work, magnification of 500× to 1 000× (oil immersion lens) in combination with differential interference contrast microscopy is recommended.

4.1.2 Morphological diagnostic characters

Keys for diagnosis for *Ditylenchus* species can be found in Viscardi and Brzeski (1993) and Brzeski (1998). A key to distinguish *Ditylenchus* spp. from other tylenchid and aphelenchid genera is presented in Table 1 below.

Table 1. Key to distinguish *Ditylenchus* spp. from other tylenchid and aphelenchid genera

1	Outlet of dorsal pharynx gland near base of stylet; median bulb roundish, ovoid or absent	Tylenchida – 2
	Outlet of dorsal pharynx gland in median bulb; median bulb a prominent feature, usually oblong	Aphelenchida
2	Anterior part of oesophagus (procorpus) and median bulb not united into single unit; stylet never exceptionally long	3
	Procorpus gradually widened and fused with median bulb; stylet very long, its base often located in anterior part of median bulb	Other genera
3	Adult female vermiform	4
	Adult female saccate or pyriform sessile parasite on roots	Other genera
4	Valvular median bulb	5
	Median bulb without valve[1]	Other genera
5	Pharynx glands contained within basal bulb, not overlapping or slightly overlapping intestine; cephalic framework rarely conspicuous; stylet weak to moderately strong	6
	Pharynx glands lobe-like, overlapping intestine; cephalic framework strong; stylet massive	Other genera
6	Single prodelphic ovary; vulva posterior	7
	Ovaries two, amphidelphic; vulva slightly post-equatorial	Other genera
7	Female not swollen; crustaformeria in female in form of quadricollumella with four rows of four cells each; bursa in males enveloping one-third or more of tail	*Ditylenchus*
	Female swollen; crustaformeria with more than 20 cells	Other genera

Source: Adapted from Heyns (1971) and Siddiqi (2000).

[1] A few non-plant-parasitic species of *Ditylenchus* do not have a valvular median bulb.

D. africanus, *D. destructor*, *D. dipsaci*, *D. gigas* and *D. myceliophagus* are morphologically and morphometrically similar, but can be differentiated from each other by the following (Table 2), providing both male and female specimens can be measured and studied.

4.1.2.1 Description of Ditylenchus dipsaci

After Sturhan and Brzeski (1991), Wendt *et al.* (1995) and Brzeski (1998). Details and views are provided in Figure 10.

Measurements (criteria described in EPPO (2013b)). (*Ex* Oat, *Avena sativa* L., after Blake, 1962, in Hooper, 1972.) (*n* = 48♀♀): L = 1.3 mm ± 0.009; a = 62 ± 5.6; b = 15 ± 1.4; c = 14 ± 2.1; V = 80 ± 1.5. (*n* = 23♂♂): L = 1.3 mm ± 0.017; a = 63 ± 11.3; b = 15 ± 1.7; c = 14 ± 2.1; T = 72.

General morphology. Body straight or almost so when relaxed. Lateral field with four incisures. Head continuous with adjacent body (Figure 10B). Stylet 10–13 µm long in females, 10–12 µm in males. Stylet cone about half of stylet length, knobs rounded and well developed. Median bulb muscular, with thickenings of lumen walls 4–5 µm long (Figure 10A). Basal bulb offset or overlapping intestine for a few micrometres. Excretory pore opposite posterior part of isthmus or glandular bulb. Postvulval part of uterine sac occupying about half to slightly more of vulva–anus distance (Figure 10D). Bursa envelops three-quarters of the tail in males. Spicules 23–28 µm long. Tails of both sexes conical with a pointed tip.

Morphological diagnostic characters. The number of lateral incisures (four) (Figure 10F), the comparatively long stylet, the length of the postvulval sac and the pointed tail (Figure 10D) are the distinguishing characters for this species (Andrássy, 2007). *D. dipsaci* can be distinguished from *D. gigas* by the shorter body of females (1.0–1.7 vs 1.6–2.2 mm) and the longer vulva–anus distance (202–266 vs 132–188 µm) (Vovlas *et al*., 2011). When observed in the lateral view, the spicule is more arched in *D. dipsaci* than in *D. destructor* (Figure 10C). See Karssen and Willemsen (2010) for more information on the spiculum and its use in the identification of *D. dipsaci* and *D. destructor*. It must be noted that the seed of *V. faba* contains mainly larvae of the fourth stage.

4.1.2.2 Description of Ditylenchus destructor

After Sturhan and Brzeski (1991) and Brzeski (1998). Details and views are provided in Figure 11.

Measurements (after Goodey, 1952, from various higher plant hosts). (*n* = 237♀♀): L = 1.07 (0.69–1.89) mm; a = 32 (18–49); b = 7 (4–12); c = 17 (9–30); V = 80 (73–90). (*n* = 231♂♂): L = 0.96 (0.76–1.35) mm; a = 35 (24–50); b = 7 (4–11); c = 14 (11–21); T = 65 (40–84).

General morphology. Adults of *D. destructor* are minute, worm-like animals, 0.8–1.4 mm long, 23–47 µm wide and slightly ventrally arcuate. Considerable morphometric variation occurs in adults according to their host and age. Males and females are similar in general appearance. Lateral field with six incisures (Figure 11F), reduced to two on the neck and tail regions. Cuticular and head annulation fine, head often narrower than adjacent body, about four head annules discerned by scanning electron microscopy (Wendt *et al*., 1995). Stylet 10–12 µm long, specimens with stylets of 14 µm have been described occasionally. Stylet cone 45–50% of stylet length, knobs distinct, rounded and sloping backwards. Median bulb muscular, with thickenings of lumen walls (or valve) about 3 µm long. Posterior bulb overlaps intestine for a short distance on the dorsal body side, although specimens with an offset glandular bulb are seen occasionally (Figure 11A). Excretory pore opposite oesophageal glands. Postvulval sac extending about three-quarters of the vulva–anus distance (Figure 11E). Eggs twice as long as wide (Andrássy, 2007). Lips of vulva thick, elevated (Figure 11B). Anterior ovary outstretched, sometimes reaching the oesophageal region. Postvulval part of uterine sac 40–98% of vulva–anus distance, not functioning as a spermatheca (Figure 11E). Male bursa surrounds 50–90% of the tail length. Spicules 24–27 µm long. The spiculum shape of *D. dipsaci* differs from *D. destructor* in having a ventral tumulus in the calomus area (Figure 12) (Karssen and Willemsen, 2010). Testis outstretched approaching the base of oesophagus. Tail of both sexes conical, three to five anal body widths long, usually ventrally curved, terminus rounded.

Morphological diagnostic characters. D. destructor is similar to *D. dipsaci*, but differs from that species by the lateral field showing six incisures (Figure 11F), the longer postvulval sac and the finely rounded tail terminus (Figure 11D). Morphologically *D. destructor* differs from *D. africanus* mainly in

the stylet length, which may overlap slightly, and the spicule length, which implies that males must be present in the population. As polymerase chain reaction (PCR) technology is sufficiently sensitive to resolve differences between closely related genera, Wendt *et al*. (1995) used restriction fragment length polymorphisms (RFLPs) to separate *D. destructor* from *D. africanus*. When observed in the lateral view, the spicule is less arched in *D. dipsaci* than in *D. destructor* (Figure 11C).

Remarks. The above characters may vary and it is almost impossible to identify a single specimen to species level. It is recommended that at least one male and one female specimen are examined. Lateral incisures in the male may, for instance, occasionally be reduced to four near the tail, forming a pattern similar to that of *D. dipsaci*.

Table 2. Comparative diagnostic characteristics of *Ditylenchus africanus*, *Ditylenchus destructor*, *Ditylenchus dipsaci*, *Ditylenchus gigas* and *Ditylenchus myceliophagus*

Characters	***D. destructor*** **(after Hooper, 1973)**	***D. africanus*** **(after Wendt *et al.*, 1995)**	***D. myceliophagus*** **(after Hesling, 1974)**	***D. gigas*** **(after Vovlas *et al*., 2011)**	***D. dipsaci*** **(after Hooper, 1972)**
Body length female (mm)	0.8–1.9	0.7–1.1	0.6–1.4	1.6–2.2	1.0–1.7
Number of lateral lines	6	6–15	6	4	4
Form of tail terminus	Rounded	Rounded	Rounded	Pointed to finely rounded	Pointed
c (body length/tail length) of female	14–20	8.8–16.9	8.2–17	15.7–27.6	11–20
Posterior bulb	Short, dorsally overlapping	Short, dorsally overlapping	Short, dorsally overlapping	Slightly overlapping	Not overlapping
Stylet length (µm) of female	10–14	8–10	7–8	10.5–13.0	10–12
PUS/vulva–anus length (%)[1]	53–90	37–85	30–69	About 50[2]	40–70
Spiculum length (µm)	24–27	17–21	15–20	23.5–28	23–28
Bursa length (as % of tail length)	50–70	48–66	20–55	72–76	40–70
Host preference[3]	Higher plants and mycelia of fungi	Groundnuts and fungi	Mycelia of fungi	Higher plants	Higher plants and fungi

[1] PUS, the postvulval part of the uterine sac.

[2] Calculated from species description.

[3] Helpful in case of confusing morphological criteria.

4.2 Molecular identification

When necessary, a molecular identification of the species *D. dipsaci* or *D. destructor* can be conducted, especially when confounding species may occur (e.g. *D. myceliophagus*, *D. africanus* or *D. gigas*) and cannot be distinguished conclusively from the target species morphologically.

In this case, the solution containing the nematode individuals should preferably be stored in cold conditions (i.e. refrigerated) for not more than few days before the DNA is extracted.

In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. 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. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

4.2.1 *Ditylenchus dipsaci*

Various molecular approaches have been developed for *D. dipsaci* identification.

Southern hybridization (Wendt *et al*., 1993) and electrophoresis (Tenente and Evans, 1997; Palazova and Baicheva, 2002) were used to investigate the concept of races within *D. dipsaci* species and the genetic diversity among *Ditylenchus* species.

Molecular approaches have also been thoroughly investigated for specific identification, mostly by PCR or PCR-RFLP, and for population variation detection by sequence analysis (Leal-Bertioli *et al*., 2000; Zouhar *et al*., 2002).

Six molecular tests (PCR, PCR-RFLP) have been published that can be used in the identification of *D. dipsaci*; these are described in sections 4.2.4 to 4.2.9. The specificity of each test is included in the description, as is the nematode genus and species against which each test has been evaluated.

The molecular analysis of ribosomal (r)DNA sequences, including different regions (the internal transcribed spacer (ITS)1-5.8S-ITS2 region, the D2–D3 fragment of the *s8S* gene, the small 18S subunit, the partial mitochondrial gene for *cytochrome c oxidase I* (mitochondrial (mt)DNA) and *hsp90* gene sequences (nuclear (n)DNA)), clearly distinguishes *D. gigas* from *D. dipsaci s.s.* (Vovlas *et al*., 2011).

4.2.2 *Ditylenchus destructor*

Molecular diagnosis of *D. destructor* is based on PCR-RFLP or sequencing of the ITS region of the rRNA gene.

Wendt *et al.* (1993) showed that PCR-RFLP of the ITS region allowed *D. destructor* parasitizing potato to be distinguished from two races of *D. dipsaci* and from *D. myceliophagus.* They published the diagnostic RFLP profiles for these three species. *D. africanus* can be distinguished from *D. destructor* by a combination of the following characters: RFLP generated by seven restriction enzymes on the ITS region of rDNA.

Ji *et al*. (2006) obtained RFLP profiles for several populations of *D. destructor* from sweet potato and revealed some differences in their RFLP profiles.

Powers *et al.* (2001) first sequenced the ITS1 region for *D. dipsaci*, but more than 50 sequence accessions of rRNA fragments obtained from *D. destructor* collected from different localities and host plants are presently available in the GenBank database.

4.2.3 DNA extraction

Several juveniles or adults are transferred to a microtube and DNA is extracted from them. DNA extraction is described by Webster *et al*. (1990).

4.2.4 ITS-rRNA PCR-RFLP test for *D. dipsaci* and *D. destructor*

This test was developed by Wendt *et al.* (1993).

Methodology

The ITS rRNA universal primers (as described in Vrain *et al*. (1992)) used in this test are:

18S: 5′-TTG ATT ACG TCC CTG CCC TTT-3′

26S: 5′-TTT CAC TCG CCG TTA CTA AGG-3′

The amplicons are 900 base pairs (bp) for both *D. dipsaci* and *D. myceliophagus,* and 1 200 bp for *D. destructor*.

Amplification is obtained following the manufacturer's recommendations for PCR kits containing Taq DNA polymerase, nucleotides and reaction buffer.

The PCR cycling parameters[1] consist of a first cycle of 1.5 min at 96 °C, 30 s at 50 °C and 4 min at 72 °C; 40 cycles of 45 s at 96 °C, 30 s at 50 °C and 4 min at 72 °C; and a final cycle of 45 s at 96 °C, 30 s at 50 °C and 10 min at 72 °C. After DNA amplification, 2–5 µl of the product is run on a 1% agarose gel. The remainder is stored at –20 °C and used for RFLP. Several restriction enzymes are useful for distinguishing *D. destructor* and *D. dipsaci* from other *Ditylenchus* species; for example, *Hae*III, *Hpa*II, *Hin*fI and *Rsa*I (Wendt *et al*., 1993). The lengths of the restriction fragments generated by these diagnostic enzymes are given in Table 3.

Table 3. Approximate length (bp) of RFLP fragments of the ITS-rRNA for *Ditylenchus* species generated by four restriction enzymes

Enzyme	*D. destructor*	*D. myceliophagus*	*D. dipsaci*	*D. gigas*[1]	*D. africanus*
Unrestricted PCR product	1 200	900	900	900	1 000
*Hae*III	450, 170	450, 200	900	800, 200	650, 540
*Hpa*II	1 000	900	320, 200, 180	600, 200	950
*Hin*fI	780, 180	630, 310	440, 350, 150	350, 150	450, 340, 150, 130, 100
*Rsa*I	600, 250, 170	900	450, 250, 140	490, 450	690, 450

Source: Wendt *et al.* (1993, 1995).

bp, base pairs; ITS, internal transcribed spacer; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; rRNA, ribosomal RNA.

1 Named in the original paper as *D. dipsaci* giant race.

4.2.5 SCAR PCR test for *D. dipsaci*

This sequence characterized amplified region (SCAR) PCR test developed by Esquibet *et al*. (2003) was designed as a species-specific test for *D. dipsaci* with differentiation between normal and giant

[1] The PCR cycling parameters are those described in the original article (Wendt *et al.*, 1993). Improvement of thermocyclers and reagents for PCR may lead to revision of these cycling parameters.

races. It was evaluated against *D. myceliophagus* (one population), *D. dipsaci* normal race (11 populations from different hosts and locations) and *D. dipsaci* giant race, described as *D. gigas* by Vovlas *et al*. (2011) (11 populations from different locations isolated from *V. faba*).

Methodology

The *D. dipsaci*-specific primers used are:

D. dipsaci (normal race):

H05: 5′-TCA AGG TAA TCT TTT TCC CCA CT-3′

H06: 5′-CAACTG CTA ATG CGT GCT CT-3′

D. dipsaci (giant race, described as *D. gigas* by Vovlas *et al.* (2011)):

D09: 5′-CAA AGT GTT TGA TCG ACT GGA-3′

D10: 5′-CAT CCC AAA ACA AAG AAA GG-3′

The amplicon is approximately 242 bp for *D. dipsaci* (normal race) and 198 bp for *D. dipsaci* (giant race). For both primer sets, no amplification is observed with non-target species and non-target race (Esquibet *et al*., 2003).

The 10 µl PCR mixture is composed of: 1.5 mM $MgCl_2$, 250 µM each dNTP, 690 nM each primer for duplex PCR (H05-H06) or (D09-D10) or 500 nM each primer for multiplex PCR (H05-H06-D09-D10) and 0.5 U Taq DNA polymerase. The cycling parameters are: initial denaturation 3 min at 94 °C; 30 cycles of 1 min at 94 °C, 1 min at 59 °C and 1 min at 72 °C; and final elongation of 10 min at 72 °C. The PCR products are analysed by agarose gel electrophoresis.

4.2.6 18S and ITS1-specific PCR test for *D. dipsaci*

This test developed by Subbotin *et al*. (2005) was designed as a species-specific test for *D. dipsaci s.s.* (normal race only). It was evaluated against *D. destructor* (one population), *D. dipsaci* normal race (18 populations from different hosts and locations) and *Ditylenchus* sp. (12 populations from different hosts and locations).

Methodology

The *D. dipsaci*-specific primers used are:

rDNA2: 5′-TTT CAC TCG CCG TTA CTA AGG-3′ (Vrain *et al*., 1992)

DitNF1: 5′-TTA TGA CAA ATT CAT GGC GG-3′

The amplicon is approximately 263 bp for *D. dipsaci s.s.* (giant race, later called *D. gigas*, not included). No amplification is observed with non-target species.

The 25 µl PCR mixture is composed of: 1× from 10× PCR buffer including 15 mM $MgCl_2$, 0.2 mM each dNTP, 60 nM each primer and 1 U Taq DNA polymerase. The PCR is performed in a 96-well Peltier type thermocycler (PTC100, MJ Research[2]) with the following cycling parameters: initial 4 min at 94 °C; 35 cycles of 15 s at 94 °C, 30 s at 57 °C and 30 s at 72 °C; and final elongation of 10 min at 72 °C. The PCR products are analysed by agarose gel electrophoresis.

[2] In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. 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. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

4.2.7 5.8S rDNA-specific PCR test for *D. dipsaci*

This test developed by Marek *et al*. (2005) was designed as a species-specific test for *D. dipsaci*. It was evaluated against *D. dipsaci* (three European populations from different hosts) and non-target genus populations (*Globodera pallida, Bursaphelenchus xylophilus, Rhabditis* spp.).

Methodology

Two specific primer sets were developed for *D. dipsaci* identification, but the most sensitive (10 pg of target DNA detected) is:

PF1: 5′-AAC GGC TCT GTT GGC TTC TAT-3′

PR1: 5′-ATT TAC GAC CCT GAG CCA GAT-3′

The amplicon with this primer set is approximately 327 bp for *D. dipsaci*.

The 25 µl PCR mixture is composed of: 1× Taq buffer, 1.5 mM $MgCl_2$, 200 µM each dNTP, 10 pmol each primer (PF1-PR1 primer set) and 1.5 U Taq DNA polymerase (Fermentas[2]). The PCR test was developed on a 96-well Peltier type thermocycler (PTC200, MJ Resarch[2]), with the following cycling parameters: 3 min at 94 °C; 30 cycles of 2 min at 94 °C, 30 s at 62 °C and 2 min at 72 °C; and final elongation of 10 min at 72 °C. The PCR products are analysed by agarose gel electrophoresis.

4.2.8 5.8S rDNA and ITS-specific PCR test for *D. dipsaci*

This test developed by Kerkoud *et al*. (2007) was designed as a species-specific test for *D. dipsaci*. It was evaluated against *D. dipsaci* (ten populations from different hosts and locations), *D. africanus, D. destructor, D. myceliophagus, Aphelenchoides ritzemabosi* (one population for each species) and *Ditylenchus* sp. (according to the paper and now described as *D. gigas*) (ten populations from different locations isolated from *V. faba*).

Methodology

Two specific primer sets are used, one for the identification of *D. dipsaci* alone and one for the identification *of D. gigas* and *D. dipsaci*. The use of both primer sets allows separation of *D. gigas* from *D. dipsaci*. The primers are:

First primer set:

DdpS1: 5′-TGG CTG CGT TGA AGA GAA CT-3′

rDNA2: 5′-TTT CAC TCG CCG TTA CTA AGG-3′ (Vrain *et al*., 1992)

The amplicon is approximately 517 bp for *D. dipsaci*. No amplification is observed with non-target species, including *D. gigas*.

Second primer set:

DdpS2: 5′-CGA TCA ACC AAA ACA CTA GGA ATT-3′

rDNA2: 5′-TTT CAC TCG CCG TTA CTA AGG-3′ (Vrain *et al*., 1992)

The amplicon is approximately 707 bp for *D. dipsaci* and *D. gigas.*

The 20 µl PCR mixture is composed of: 1.5 mM amplification buffer with final $MgCl_2$ concentration of 5 mM, 200 µM each dNTP, 0.5 µM each primer (in the simplex PCR with DdpS1-rDNA2 or DdpS2-rDNA2; in the duplex PCR, the final concentration of DdpS1 primer is 0.5 µM whereas it is 1 µM for DdpS2 and rDNA2) and 1 U Taq DNA polymerase (MP Biomedicals[2]). The PCR was developed on a 96-well Peltier type thermocycler (GeneAmp 9600 PCR System, Perkin Elmer[2]), with the following cycling parameters: 1 min at 94 °C; 40 cycles of 30 s at 94 °C, 30 s at 60 °C and 45 s at 72 °C; and final elongation of 10 min at 72 °C. The PCR products are analysed by agarose gel electrophoresis.

4.2.9 SCAR PCR test for *D. dipsaci*

This SCAR PCR developed by Zouhar *et al*. (2007) was designed as a species-specific test for *D. dipsaci*. It was evaluated against only *D. dipsaci* (ten European populations from different hosts).

Methodology

Two specific primer sets were designed for *D. dipsaci* identification:

First primer set:

DIT_2 forward: 5′-GCA ATG CAC AGG TGG ATA AAG-3′

DIT_2 reverse: 5′-CTG TCT GTG ATT TCA CGG TAG AC-3′

The amplicon with this primer set is approximately 325 bp for *D. dipsaci*.

Second primer set:

DIT_5 forward: 5′-GAA AAC CAA AGA GGC CGT AAC-3′

DIT_5 reverse: 5′-ACC TGA TTC TGT ACG GTG CAA-3′

The amplicon with this primer set is approximately 245 bp for *D. dipsaci*.

The 25 µl PCR mixture is composed of: 1× PCR buffer (Fermentas[2]), 1.5 mM $MgCl_2$, 200 µM each dNTP, 10 pmol each primer (either DIT_2 or DIT_5 primer set), 1.5 U Taq DNA polymerase (Fermentas[2]) and 50 ng DNA as template. The PCR is performed in a 96-well Peltier type thermocycler (PTC200, MJ Research[2]), with the following cycling parameters: 3 min at 94 °C; 30 cycles of 1 min at 94 °C, 30 s at 60 °C and 1 min at 72 °C; and final elongation of 10 min at 72 °C. The PCR products are analysed by agarose gel electrophoresis.

4.2.10 Controls for molecular tests

For the test result obtained to be considered reliable, appropriate controls – which will depend on the type of test used and the level of certainty required – should be considered for each series of nucleic acid isolation and amplification of the nucleic acid of the target pest or target nucleic acid. A positive nucleic acid control, a negative amplification control and a negative extraction control are the minimum controls that should be used.

Positive nucleic acid control. This control is used to monitor the efficiency of the amplification (apart from the extraction). Pre-prepared (stored) nucleic acid of the target nematode may be used.

Negative amplification control (no template control).This control is necessary for conventional PCR to rule out false positives due to contamination during preparation of the reaction mixture. PCR-grade water that was used to prepare the reaction mixture is added at the amplification stage.

Negative extraction control. This control is used to monitor contamination during nucleic acid extraction. This control comprises nucleic acid extraction and subsequent amplification of extraction buffer only. Multiple controls are recommended to be included when large numbers of positive samples are expected.

4.2.11 Interpretation of results from 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 target nematode species
- no amplicons of the correct size for the target nematode species are produced in the negative extraction control and the negative amplification control.

5. Records

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

In cases where other contracting parties may be adversely affected by the diagnosis, the records and evidence (in particular preserved or slide-mounted specimens, photographs of distinctive morphological features, DNA extracts and photographs of gels, as appropriate), should be kept for at least one year.

6. Contacts Points for Further Information

Further information on this protocol can be obtained from:

Biosystematics Division, ARC-PPRI, Private Bag X134, Queenswood, 0121 Republic of South Africa (Antoinette Swart; e-mail: SwartA@arc.agric.za).

Plant Pest Diagnostic Center, California Department of Food and Agriculture, 3294 Meadowview Road, Sacramento, CA 95832-1448, United States (Sergei Subbotin; e-mail: subbotin@ucr.edu).

Charlottetown Laboratory – Potato Diseases, Canadian Food Inspection Agency, 93 Mount Edward Rd, Charlottetown PEI, C1A 5T1, Canada (Harvinder Bennypaul; e-mail: bennypaulhs@inspection.gc.ca).

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).

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The description of the molecular techniques was done by Sergei Subbotin (Plant Pest Diagnostic Center, California Department of Food and Agriculture, 3294 Meadowview Road, Sacramento, CA 95832-1448, United States).

The following nematologists improved the protocol by their comments:

- Harvinder Bennypaul (Canadian Food Inspection Agency, Canada)
- Johannes Hallmann (Julius Kühn-Institut, Germany)
- Mikhail Pridannikov (Center of Parasitology, A.N. Severtsov Institute of Ecology and Evolution, Russia)
- P. Castillo (Instituto Agricultura Sostenible, Consejo Superior de Investigaciones Cientificas, Spain).

8. References

The present standard refers to International Standards for Phytosanitary Measures (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. *Vicia faba* seed infected by *Ditylenchus dipsaci* (with nematode wool showing).
Photo courtesy G. Caubel, Nemapix (1999).

Figure 2. *Allium sativum* infected by *Ditylenchus dipsaci*.
Photo courtesy G. Caubel, Nemapix (1999).

Figure 3. Young *Allium cepa* plants infected by *Ditylenchus dipsaci*.
Photo courtesy E. Hennig, State Plant Health and Seed Inspection Service, Torun, Poland.

Figure 4. Garlic bulb infected by *Ditylenchus dipsaci.*
Photo courtesy G. Caubel, Nemapix (2002).

Figure 5. *Narcissus* spp. infected by *Ditylenchus dipsaci.*
Photo courtesy G. Caubel, Nemapix (1999).

Figure 6. Cross-section of *Narcissus* sp. bulb infected by *Ditylenchus dipsaci.*
Photo courtesy C.W. Laughlin, Nemapix (2002).

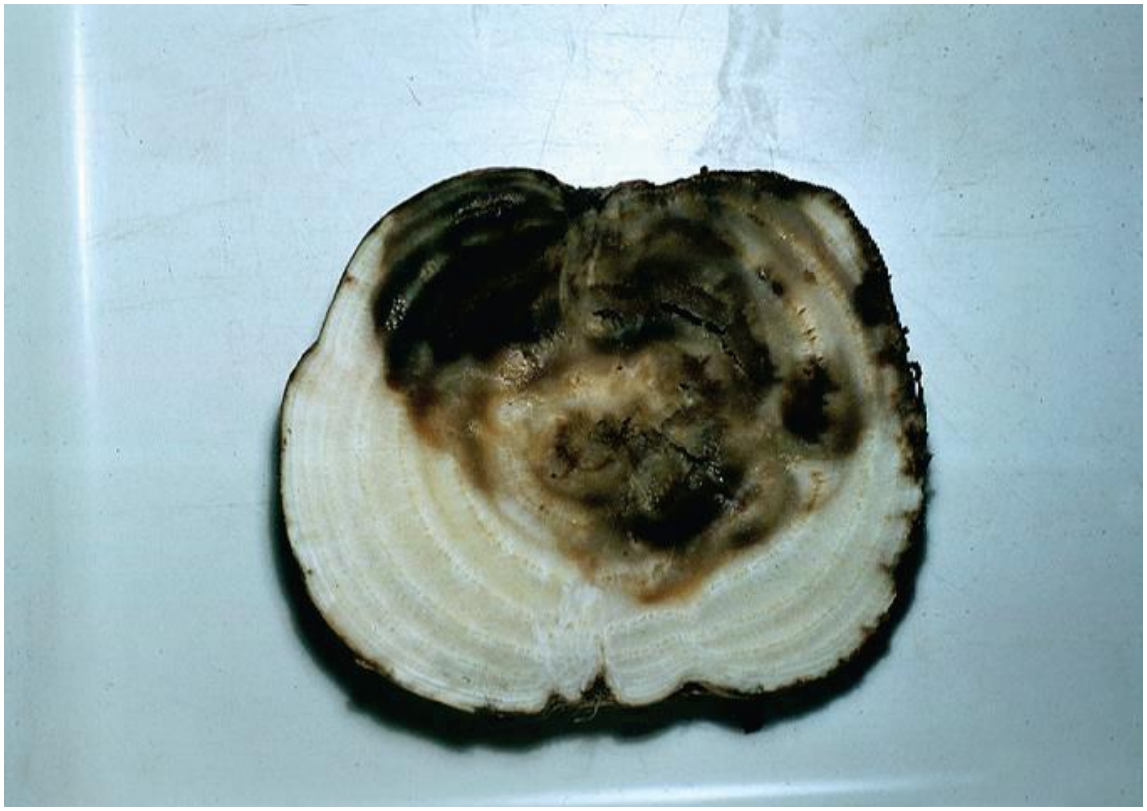

Figure 7. Cross-section of sugar beet infected by *Ditylenchus dipsaci.*
Photo courtesy C. Hogger, Nemapix (1999).

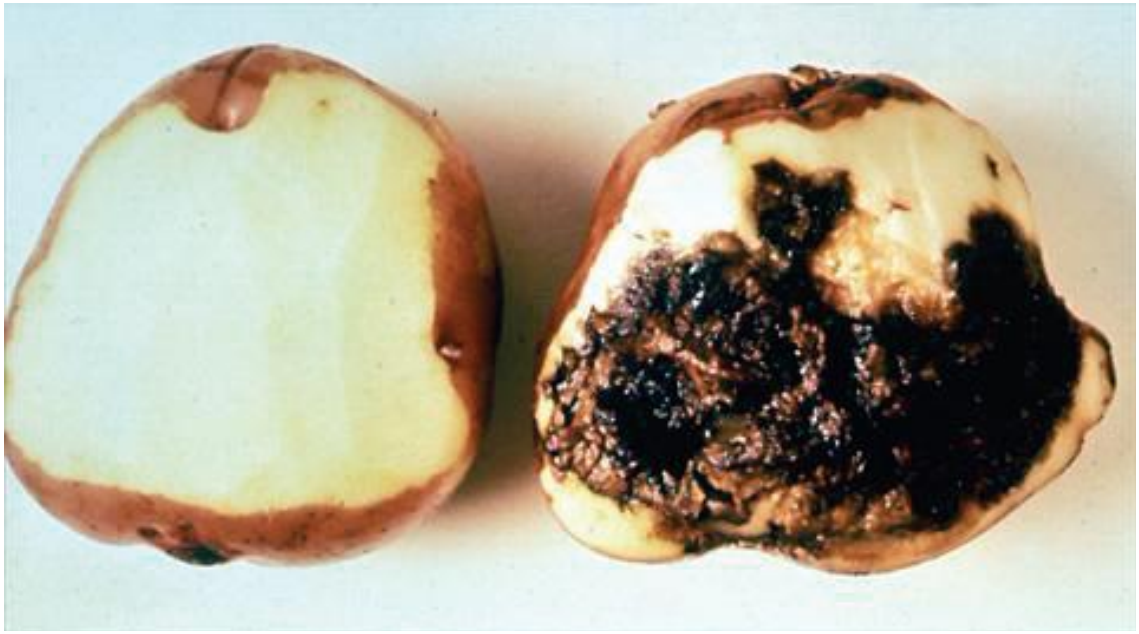

Figure 8. Cross-section of potato infected by *Ditylenchus destructor* compared with non-infected potato. *Photo courtesy S. Ayoub, Nemapix (2000).*

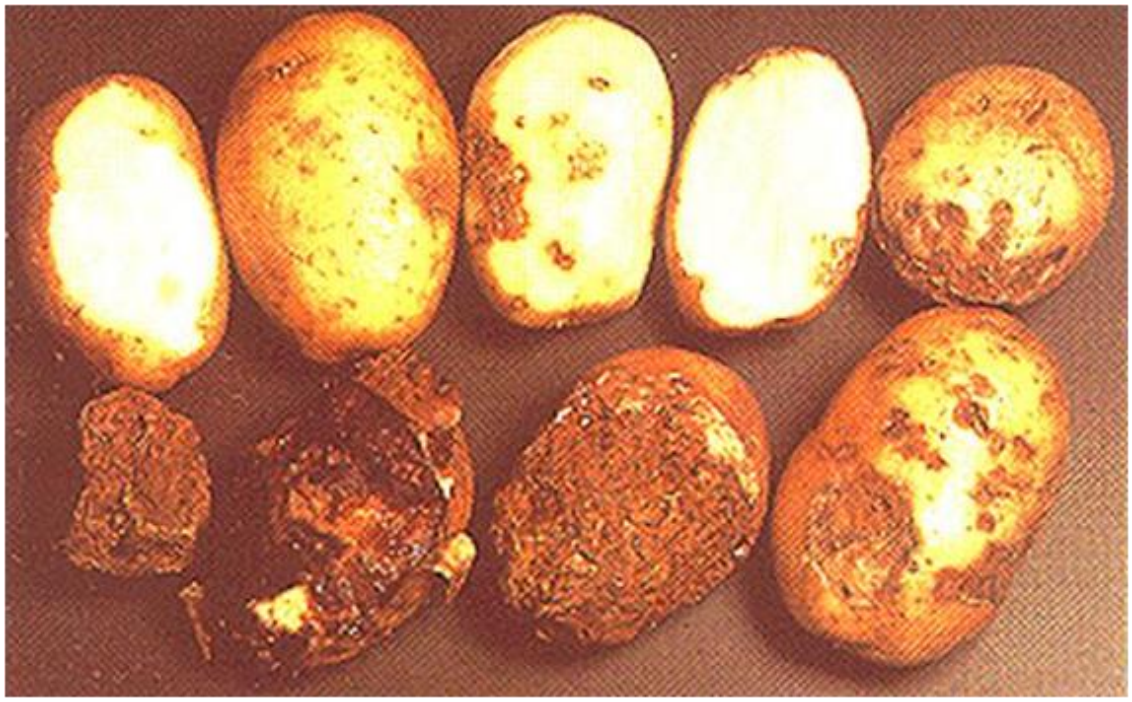

Figure 9. Potatoes of various levels of infestation by *Ditylenchus destructor.* *Photo courtesy H. Andersen.*

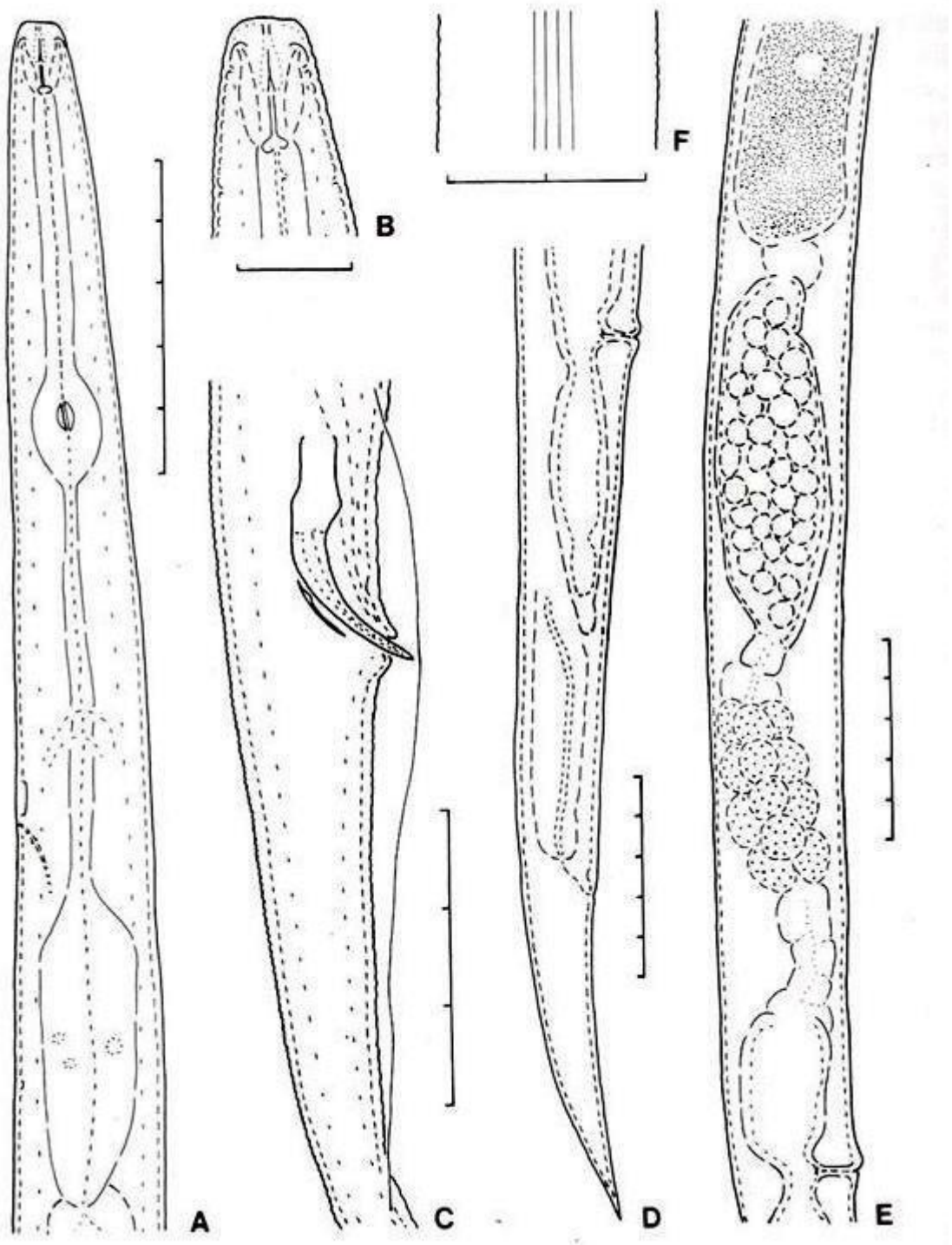

Figure 10 *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 (after Sturhan and Brzeski, 1991). (A) female, oesophageal region; (B) head of female; (C) male, spicule region; (D) female, posterior region; (E) part of female reproductive system; and (F) lateral field at midbody. Each unit marking on scale bars = 10 µm.

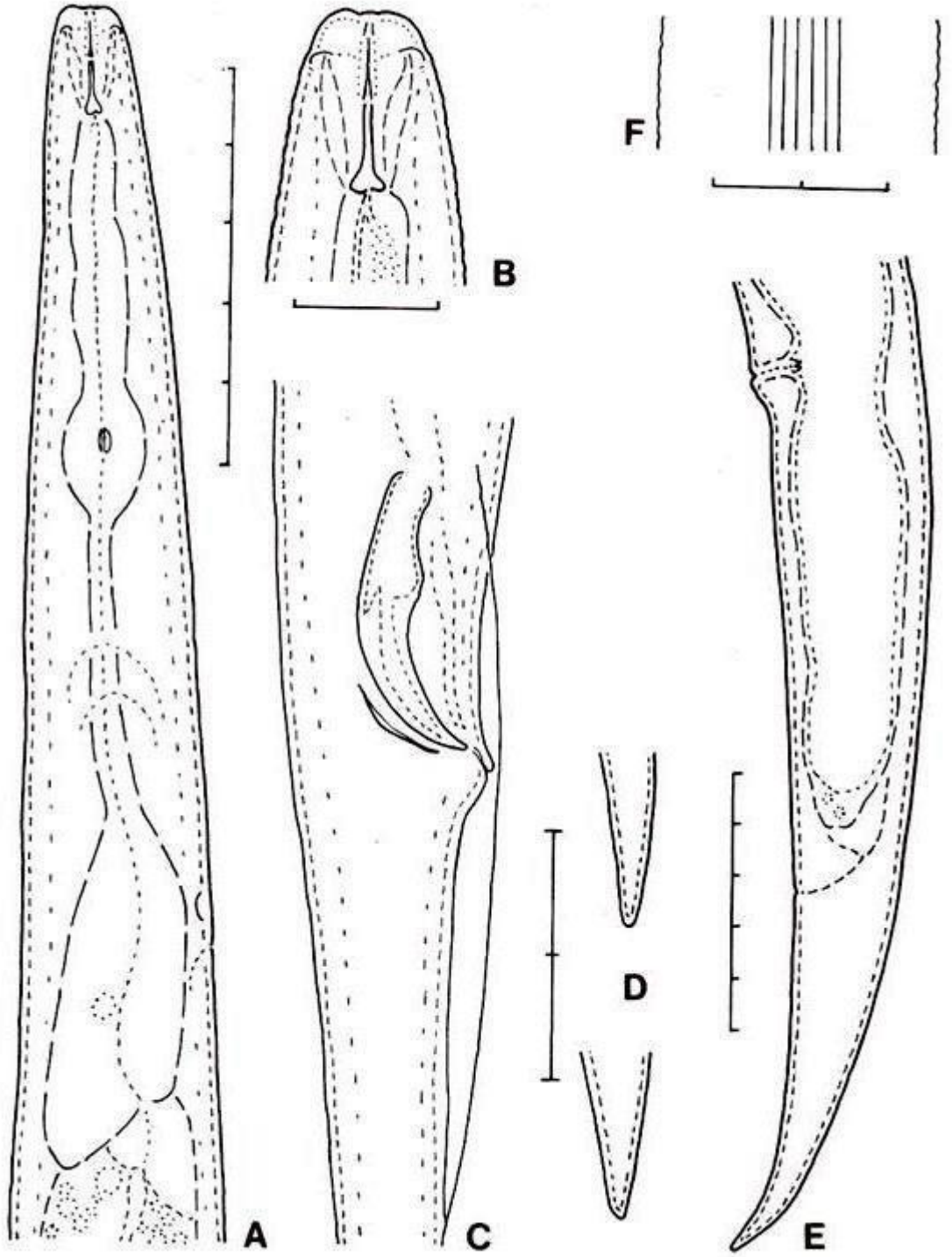

Figure 11. *Ditylenchus destructor* Thorne, 1945 (after Sturhan and Brzeski, 1991). (A) female, oesophageal region; (B) female, head; (C) male, spicule region; (D) tail tips of two females; (E) female, posterior region; and (F) lateral field at midbody. Each unit marking on scale bars = 10 µm.

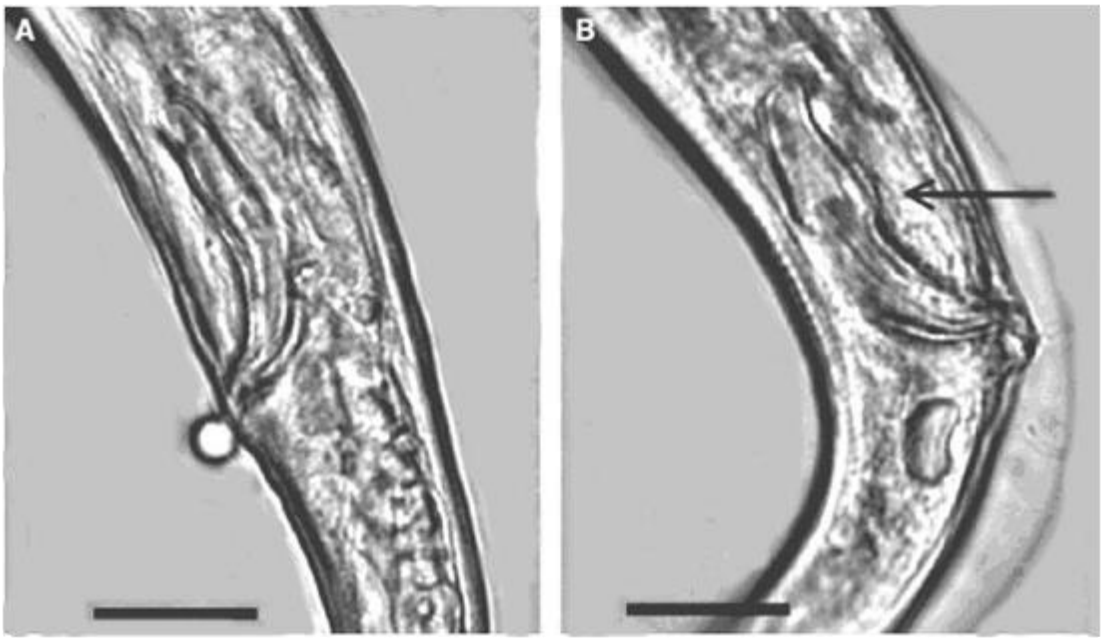

Figure 12. *Ditylenchus* spiculum: (A) *D. dipsaci* and (B) *D. destructor.* Arrow = tumulus. Scale bars = 12 µm. *Photo courtesy Karssen and Willemsen (2010).*

Publication history

This is not an official part of the standard

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2013-06 Draft presented to TPDP meeting.

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ISPM 27. **Annex 8**. *Ditylenchus dipsaci* and *Ditylenchus destructor* (2015). Rome, IPPC, FAO.

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ISPM 27
Diagnostic protocols for regulated pests

DP 9: Genus *Anastrepha* Schiner

Adopted 2015; published 2016

CONTENTS

1. Pest Information

The family Tephritidae comprises about 4 450 species in 500 or so genera (Norrbom *et al.*, 1999a, 1999b; Norrbom, 2004b) (the figure was about 4 700 species in 2014 (A.L. Norrbom, personal communication, 2014)). The Tephritidae are distributed worldwide in temperate, tropical and subtropical regions. *Anastrepha* Schiner (Tephritidae: Toxotrypanini) is the largest genus of Tephritidae in the Americas, and is represented by more than 250 species that occur from the southern United States (Texas and Florida) to northern Argentina (Hernández-Ortiz, 1992; Foote *et al.*, 1993; Hernández-Ortiz and Aluja, 1993; Norrbom, 2004b; Norrbom *et al*., 2012). At least seven species of *Anastrepha* are considered major economic pests because of the great importance of the cultivated fruits they attack (e.g. mango and citrus) and their wide host range. These seven species are *A. fraterculus* (Wiedemann); *A. grandis* (Macquart); *A. ludens* (Loew); *A. obliqua* (Macquart); *A. serpentina* (Wiedemann); *A. striata* Schiner; and *A. suspensa* (Loew). *A. fraterculus* (Wiedemann) has been recognized as a cryptic species complex (Hernández-Ortiz *et al*., 2004, 2012; Selivon *et al.*, 2004, 2005; Vera et al., 2006, Cáceres *et al.*, 2009). This diagnostic protocol for *Anastrepha* covers morphological identification of the genus and the species of major economic importance. For further general information about species of Tephritidae, see Norrbom (2010).

The length of the tephritid life cycle varies according to each species as well as environmental and climatic conditions (Basso, 2003). Female *Anastrepha* deposit their eggs inside fruits. The number of eggs deposited per fruit is variable, and depends mainly on features of the host fruit such as size and ripeness (Malavasi *et al.*, 1983), but each species also seems to have innate limits on the number of eggs laid (Aluja *et al*., 1999). Within several days, deposited eggs hatch and larvae emerge. Larvae usually feed on fruit pulp, but in some cases also or exclusively on seeds. Mature larvae usually leave the fruit to pupate in the ground, but in certain cases pupation can take place within the fruit. Adults usually emerge after a pupal period of 16–25 days, and they require a period of sexual maturation of 5–20 days after emergence. During this process the flies obtain food from homopteran secretions, bird faeces, and juice produced by ripe fruits (Prokopy and Roitberg, 1984).

The relationship between *Anastrepha* species and their host plants is poorly understood. There are more than 330 host species from 48 families, many of them reported for a few generalist *Anastrepha* species (Norrbom and Kim, 1988; Norrbom, 2004a) while food plants for many other *Anastrepha* species remain unknown. Furthermore, current information includes numerous doubtful records, and reports of infestations induced only under laboratory conditions. Restricting the host list to natural infestations, hosts are known for about 39.8% of *Anastrepha* species (Hernández-Ortiz and Aluja, 1993).

The introduction of cultivated exotic species such as *Mangifera indica* and *Citrus* spp. have allowed some pest species of *Anastrepha* to expand their original areas of distribution and enhance their reproductive potential. However, they still have marked preferences for certain native hosts, which is probably indicative of their original host relationships. In this regard, the species *A. suspensa, A. fraterculus* and *A. striata* breed mainly in hosts belonging to the family Myrtaceae, *A. ludens* in the Rutaceae, *A. obliqua* in the Anacardiaceae, *A. serpentina* in the Sapotaceae, and *A. grandis* in the Cucurbitaceae (Norrbom, 2004a).

Among native hosts in the American tropics, there seems to be an ancestral association with plants that produce latex and particularly the family Sapotaceae. Sapotaceous fruits are frequent hosts for the *dentata, leptozona, serpentina, daciformis, robusta* and *cryptostrepha* species groups. Myrtaceous fruits are also very important hosts. Almost 26 *Anastrepha* species, most belonging to the *A. fraterculus* species complex, have been reported feeding on plants of this family (Norrbom and Kim, 1988; Norrbom *et al*., 1999c).

2. Taxonomic Information

Name: *Anastrepha* Schiner, 1868

Synonyms: *Acrotoxa* Loew, 1873; *Pseudodacus* Hendel, 1914; *Phobema* Aldrich, 1925; *Lucumaphila* Stone, 1939

Taxonomic position: Insecta: Diptera: Tephritidae, Trypetinae, Toxotrypanini

Common names: See Table 1.

Table 1. Common names and synonyms of fruit fly species of major economic importance belonging to the genus *Anastrepha*

Common name	*Anastrepha* species	Synonyms
South American fruit fly	*Anastrepha fraterculus* (Wiedemann, 1830) species complex	*Tephritis mellea* Walker, 1837
		Trypeta unicolor Loew, 1862
		Anthomyia frutalis Weyenbergh, 1874
		Anastrepha fraterculus var. *soluta* Bezzi, 1909
		Anastrepha peruviana Townsend, 1913
		Anastrepha braziliensis Greene, 1934
		Anastrepha costarukmanii Capoor, 1954
		Anastrepha scholae Capoor, 1955
		Anastrepha pseudofraterculus Capoor, 1955
		Anastrepha lambayecae Korytkowski and Ojeda, 1968
Melon fruit fly	*Anastrepha grandis* (Macquart, 1846)	*Anastrepha schineri* Hendel, 1914
		Anastrepha latifasciata Hering, 1935
Mexican fruit fly	*Anastrepha ludens* (Loew, 1873)	*Anastrepha lathana* Stone, 1942
West Indian fruit fly	*Anastrepha obliqua* (Macquart, 1835)	*Anastrepha fraterculus* var. *mombinpraeoptans* Sein, 1933
		Anastrepha fraterculus var. *ligata* Lima, 1934
		Anastrepha trinidadensis Greene, 1934
Sapodilla fruit fly	*Anastrepha serpentina* (Wiedemann, 1830)	*Urophora vittithorax* Macquart, 1851
Guava fruit fly	*Anastrepha striata* Schiner, 1868	*Dictya cancellaria* Fabricius, 1805 (see Norrbom *et al.*, 1999b)
Caribbean fruit fly	*Anastrepha suspensa* (Loew, 1862)	*Anastrepha unipuncta* Sein, 1933
		Anastrepha longimacula Greene, 1934

3. Detection

Fruit flies can be detected by inspection as larvae inside fruits and as pupae in the containers in which the fruits are being transported, or they can be captured outdoors as adults by means of trapping systems.

3.1 Inspection of fruits.

Infested fruits can be found in imported or exported shipments, in baggage, and even on aeroplanes or terrestrial transportation vehicles. Fruits with soft areas, dark stains, rot, orifices or injuries that might have originated from female oviposition or larval feeding activities are targeted for inspection. In order to detect punctures made by female flies during oviposition, the visual examination should be done 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 should be cut open to search for eggs or larvae inside. The success of detection depends on careful sampling and examination of fruits.

3.2 Inspection of traps.

Guidance on trapping *Anastrepha* fruit flies is given in Appendix 1 of ISPM 26 *(Establishment of pest free areas for fruit flies (Tephritidae))*. In general, monitoring systems established for the detection of fruit fly adults in trees, either in fruit-growing regions or in border areas between countries, require the utilization of McPhail traps baited with food attractants or synthetic lures. The baits, often with rich sources of ammonium, should be recognized and approved internationally (e.g. ISPM 26). The specific methods of trap deployment and time of service of the traps must be in agreement with the national phytosanitary regulations.

4. Identification

The taxonomy of the genus *Anastrepha* is based on adult external morphology and characters of the female terminalia (Stone, 1942; Hernández-Ortiz, 1992; Zucchi, 2000; Norrbom *et al*., 2012). Because morphological characters of immature stages are not well documented for most *Anastrepha* species, these characters have a more limited utility in species recognition (White and Elson-Harris, 1992) in comparison with adult morphology. However, some information on egg structures and third instar larvae is available in the scientific literature and has diagnostic utility for certain species (Steck and Wharton, 1988; Steck *et al.*, 1990; Frías *et al*., 2006, 2008, 2009; Dutra *et al.*, 2011a, 2011b, 2012, 2013; Figueiredo *et al.*, 2011). Identification keys for the larvae of the seven species of *Anastrepha* known to be of major economic importance (section 1; listed in Table 1) are available (Steck *et al.*, 1990; Carroll *et al.*, 2004) but should be used with consideration of their limits.

Although the third instar larvae of some *Anastrepha* species apparently can be discriminated (Berg, 1979; Steck and Wharton, 1988; Carroll and Wharton, 1989; Steck *et al.*, 1990; White and Elson-Harris, 1992; Carroll *et al*., 2004; Frías *et al*., 2006; Hernández-Ortiz *et al.*, 2010), the available data are based on very limited sampling for most species that have been described. Studies of additional closely related species that have not yet been characterized may also reduce the reliability of the method. For this reason, experts should perform these diagnoses and evaluate all available information. The most reliable method for identification is rearing larvae to the adult stage.

Several pest species of *Anastrepha* are believed to comprise multiple (yet to be described) cryptic species that are morphologically indistinguishable or require morphometric analysis for their recognition (Hernández-Ortiz *et al.*, 2004, 2012).

To study this idea further, the International Atomic Energy Agency (IAEA) has coordinated an international research project to describe the cryptic species in the *A. fraterculus* species complex. As part of this project, molecular methods have been examined for diagnostic utility within the genus. Based on available data, methods such as DNA barcoding using the *cytochrome oxidase I* gene cannot

reliably identify some dipteran species, including several important pest species (Will *et al.*, 2005; Meier *et al.*, 2006; Virgilio *et al.*, 2010; Lopes *et al.*, 2013). Some progress has been made by internal transcribed spacer (ITS)1 analysis (e.g. Sonvico *et al.*, 2004, GenBank accession number AY686689). This information was associated with morphological characterization of specimens and karyotypic analysis, along with cross-mating studies (Basso, 2003).

Consequently, the identification methods included in this diagnostic protocol are based on morphological characters.

4.1 Preparation of adults for identification

4.1.1 Rearing larvae to obtain adults

The fruits are placed in cages covered with cloth or fine mesh and that have a sterile pupation medium (e.g. damp vermiculite, sand or sawdust) at the bottom. Once the larvae emerge from the fruit, they will move to the substratum for pupation. It is recommended to incubate each fruit separately. Each sample must 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 48–72 h to ensure that the tegument and wings acquire the rigidity and characteristic coloration of the species. The adults are then killed and preserved by placing them in 70% ethanol (96% ethanol for molecular studies (DNA) or they are killed with ethyl acetate or another agent and then mounted on pins. For female flies, immediately after killing them (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 (so that it does not need to be dissected later).

4.1.2 Preparation of adults for microscopic examination

For species recognition of adult stages, the entire specimen should be preserved – either dry (pinned) or in 70% ethanol. Examination of the wings and the aculeus is particularly important. Examination of the aculeus must be done at about 400× magnification. The wing and aculeus of each specimen can be mounted under two separate coverslips on the same slide. Dissection and mounting should be done only by someone with experience. Dissecting the female terminalia in *Anastrepha* is difficult and it is easy to damage useful parts.

4.1.2.1 Aculeus

It is preferable to cut off the whole abdomen from a female to dissect the oviscape (syntergosternite 7), the eversible membrane and the aculeus. For preserved dry (pinned) specimens, fine dissection scissors are recommended to remove the abdomen. The abdomen needs to be cleared. This can be accomplished by placing it in a 10% sodium hydroxide (NaOH) or 10% potassium hydroxide (KOH) solution and heating it in a boiling water bath for 10–15 min, washing the structure with distilled water, and then removing internal contents under a stereomicroscope with the help of dissection forceps. The aculeus and the eversible membrane should be exposed. At this step it is possible to examine the aculeus directly in one or two drops of glycerine under a microscope. Afterwards, the structure can be transferred to a microvial with glycerine and pinned under the mounted dry specimen. For permanent slides, proceed as described in section 4.1.2. Mounting the aculeus permanently in the ventral position prevents the observation of some characters better seen in lateral view. For this reason, preservation in glycerine in a microvial is often preferable.

4.1.2.2 Wings

Wing characters can usually be observed without mounting, so mounting is not recommended as a general practice. It may be necessary for morphometric studies, but it is not necessary for observation of the characters used in the key in section 4.3.2. If permanent mounts are made, it is recommended to cut off one of the wings from its base (the right wing is preferred because it facilitates comparison with images reported in the literature and this diagnostic protocol).

4.2 Preparation of larvae for identification

4.2.1 Handling the biological sample

As noted in section 4, observation of adult characters may be necessary to make a definitive identification. If immature stages are found, it is recommended to preserve a few larvae for morphological examination by treating them in hot water (section 4.2.2) and then storing them in 70% ethanol. The remaining larvae and pupae are reared to obtain adult specimens for identification (section 4.1.1).

Morphological examination of larvae (section 4.2.2) can be performed on unmounted larvae using a stereomicroscope, on slide-mounted larvae using a compound microscope, or on critical-point dried larvae using a scanning electron microscope (SEM). Slide mounting larvae can preclude subsequent analysis of morphological characters. On slide-mounted larvae it is possible to examine external morphology (e.g. anterior and posterior spiracles, oral ridges) as well as internal structures such as the cephalopharyngeal skeleton (Figures 21–44), using an optical microscope with objective 20×, 40× or higher. Detailed, high resolution observation of the external morphology of larvae is only possible using an SEM (Figures 45–61). It is therefore not recommended to slide mount all specimens representing a sample or the only larva available for diagnosis; unmounted larvae should be kept for future analysis.

4.2.2 Preparation of larvae for microscopic examination

To prepare specimens for examination the larvae must be treated in hot water, which can be accomplished by placing live larvae in water at approximately 65 °C for 2–4 min. The larvae are cooled to room temperature and then immersed in 50% alcohol for 15–30 min. The specimens are transferred to a hermetic vial (15–25 ml) filled with 70% alcohol. It is advisable to include a label on the vial with all sampling information. These samples are ready for examination under a stereomicroscope or subsequent preparation for slide mounting or examining under an SEM.

To prepare specimens for slide mounting, it is necessary to remove (clean) all the internal contents to allow observation of the cuticle, oral opening, cephalopharyngeal skeleton and anterior spiracles, as well as the posterior spiracular plate and anal lobes. This can be accomplished by making two transverse incisions in the larva, one behind the cephalic region and the anterior spiracles, and one before the caudal segment. The incised larva then needs to be immersed in a test tube containing 10% NaOH or 10% KOH solution and heated in a boiling water bath for 10–15 min. The internal contents can then be carefully removed from the specimen using forceps and distilled water under a stereomicroscope (45× magnification or greater).

Permanent slide mounts can be made using Canada balsam or Euparal. Before doing this, cleaned structures must be dehydrated by placing them for 25 min in each of 50%, 75% and 100% ethanol. For mounting with Canada balsam, the specimens should be transferred to lavender oil for 15 min to clear them and then immediately mounted on a slide with one or two drops of Canada balsam. When Euparal is used as the mounting medium, structures should be transferred from 100% ethanol to clove oil for about 30 min to clear them before mounting. In both cases, slides must be allowed to dry for several days (the time can be reduced by using an oven), but they can be examined under the microscope at low magnification immediately after mounting. Slides should be labelled.

For observation using an SEM, the specimens (stored in alcohol) must first be cleaned in their vials in distilled water with a drop of liquid soap added to serve as a surfactant. Then they should be rinsed thoroughly with distilled water and dehydrated by running through a series of ethanol baths: 70%, 80%, 95%, and three changes of absolute ethanol (15 min each bath). Specimens should then be critical-point dried and coated with gold-palladium (Carroll and Wharton, 1989). Similar techniques can be found elsewhere (e.g. Frías *et al.*, 2006, 2008, 2009).

4.3 Morphological identification of adults

4.3.1 Identification of the genus *Anastrepha* Loew

Adults (Figure 1). Head (Figure 2-A): Usually yellow with two to eight frontal and one or two orbital setae, sometimes posterior orbital seta absent; ocellar seta usually very weak or indistinct; postocellar, medial and lateral vertical setae present. Thorax (Figures 2-B, 3): Macrosetae of thorax usually black, red–brown or orange, rarely golden yellow; scutum usually yellow to orange, occasionally mostly dark brown or sometimes with dark brown or black stripes or spots, always with two to five yellow stripes; mesonotum with the following setae: one postpronotal, two notopleurals, one presutural supra-alar, one postsutural supra-alar, one postalar, one intra-alar, one dorsocentral, one acrostichal (rarely absent) and two scutellars.

Wings (Figure 4): Subcostal break present; crossvein *R-M* placed distal to mid-length of discal cell (*dm*); basal cubital cell (*bcu*) with a well-developed posteroapical extension; vein *M* usually conspicuously curved forwards apically (strongly so in all pest species) and not meeting costa at a 90° angle. Wing pattern with orange to brown coloured bands forming a typical pattern as follows: costal (C)-band on basal costal margin including all of vein R_1, subcostal cell and the pterostigma; S-band extending from apex of cell *bcu* across cell *dm* and crossvein *R-M*, reaching costal margin, and continuing to apex of wing; V-band forming an inverted V shape, comprising the proximal arm (subapical band) along vein *DM-Cu* and the distal arm (posterior apical band) arising from cell *m*, both convergent in cell r_{4+5}; distal arm frequently incomplete or absent. The typical wing pattern is modified in some economically important species (see key to species in Section 4.3.2).

Male terminalia (Figure 5): Epandrium broad in lateral view with lateral surstylus short or elongated; medial surstylus shorter than lateral surstylus with two stout blackish prensisetae apically; proctiger membranous, weakly sclerotized laterally and ventrally; phallus elongated, usually longer than length of oviscape of female; glans weakly sclerotized with an apical T-shaped sclerite, glans sometimes absent in non-pest species.

Female terminalia (Figure 6): Oviscape tube-like, variable in length; eversible membrane (usually inverted inside oviscape) apically with dorsal group of hook-like sclerotized plates (also named raspers); aculeus (usually inverted inside eversible membrane and oviscape) well sclerotized, tip sometimes serrated on lateral margins.

4.3.2 Key to adults of major economically important species of the genus *Anastrepha*

Key adapted from Hernández-Ortiz *et al*. (2010). For additional information on morphological structures and other *Anastrepha* species, see Norrbom *et al.* (2012). See Tables 2 and 4 for diagnostic morphological characters of the genus *Anastrepha.*

1. Wing with C-band interrupted just at end of vein R_1 by a well-delimited hyaline mark in cell r_1; anterior and posterior orbital setae present; distal arm of V-band usually present at least partially, but if absent, wing pattern dark brown to black **2**

– Wing with C-band uninterrupted from wing base to apex, sometimes diffuse in cell r_1; posterior orbital seta often absent; distal arm of V-band absent. All following characters must be present: basal half of S-band continuous from apex of cell *bcu* through crossvein *R-M* and connecting with C-band; cell r_{2+3} completely pigmented in entire length; vein R_{2+3} almost straight in entire length; cell *br* broadly hyaline between veins *BM-Cu* and *R-M* (Figure 7); abdominal tergites yellow; scutum with dark brown dorsocentral stripes; aculeus of female extremely long (5.3–6.2 mm) and usually greater than 0.10 mm wide, aculeus tip with V-shaped ridges, lateral margins non-serrate (Figure 14); glans of male present. (Larvae infest melons.)..... ***Anastrepha grandis* (Macquart)**

2. Scutum predominantly dark brown with brown to black stripes..... **3**

– Scutum yellow or orange, without dark brown markings except sometimes along scuto-scutellar suture …..... **4**

3. Wing pattern mostly dark brown; distal arm of V-band completely absent (Figure 8); abdominal tergites mostly dark brown with T-shaped medial white mark; thoracic pleuron mostly brown, strongly contrasting with yellow markings; female aculeus 2.6–3.8 mm long, aculeus tip 0.37–0.46 mm long, 0.14–0.17 mm wide, lateral margins finely serrated on distal 0.5–0.7 (Figure 15). (Larvae infest sapotaceous fruits.) ..... ***Anastrepha serpentina*** (Wiedemann)

– Wing pattern mostly orange and moderate brown; distal arm of V-band usually present (Figure 9); abdominal tergites and pleuron yellow or orange; scutum with two broad dorsocentral stripes connected on posterior margin to form U-shaped mark, without setulae in a small area along transverse suture, but with dense white microtrichia contrasting with black setulae; female aculeus 1.95–2.30 mm long, tip broad, 0.24–0.31 mm long, 0.17–0.20 mm wide (Figure 16). (Larvae infest guavas.) .... ***Anastrepha striata*** **Schiner**

4. Anterior apical band of wing (=distal section of S-band) narrow to moderately broad, never reaching apex of vein *M*; V-band with arms separated anteriorly or if joined, with large hyaline mark between them and vein *M;* scuto-scutellar suture with or without brown spot medially; aculeus variable..... **5**

– Anterior apical band of wing (=distal section of S-band) extremely wide, reaching apex of vein *M*; V-band broad and complete, with arms widely connected anteriorly, hyaline mark between them and vein *M* small or absent (Figure 10); scuto-scutellar suture usually with large rounded brown spot medially; female aculeus 1.4–1.6 mm long, tip 0.19–0.23 mm long, 0.10–0.13 mm wide, lateral margins serrate on distal 0.50–0.65 (Figure 17)..... ***Anastrepha suspensa*** **(Loew)**

5. Female aculeus length less than 2.0 mm (usually 1.4–1.9 mm), tip short and wide with large teeth on sides; other characters variable..... **6**

– Female aculeus length more than 2.5 mm (usually 3.3–5.8 mm); aculeus tip length 0.28–0.42 mm, with a moderate constriction near mid-length; lateral margins non-serrate or finely serrate on distal 0.55 or less (Figure 18); brown lateral markings of subscutellum always evident and sometimes extended onto mediotergite (Figure 3B); wing pattern as in Figure 11. (Larvae commonly infest citrus and mango.) ..... ***Anastrepha ludens*** **(Loew)**

6. Subscutellum entirely yellow, only mediotergite with brown markings on sides (Figure 3C); brown spot on scuto-scutellar suture absent; aculeus tip 0.16–0.20 mm long, with lateral serrations on distal two-thirds or four-fifths (Figure 19); wing pattern as in Figure 12. (Larvae commonly infest mangos or *Spondias* fruits.) ..... ***Anastrepha obliqua*** **(Macquart)**

– Both mediotergite and subscutellum with broad dark brown to black markings on sides (Figure 3A); brown spot on scuto-scutellar suture usually present; aculeus 1.4–1.9 mm long, aculeus tip 0.20–0.28 mm long, lateral margins with 8 to 14 teeth on distal two-fifths to three-fifths (Figure 20); wing pattern variable (Figure 13)..... ***Anastrepha fraterculus*** **(Wiedemann) species complex**

4.4 Morphological identification of third instar larvae

4.4.1 Key to third instar larvae of major economically important genera of Tephritidae in the Americas

Key adapted from Frías *et al*. (2006). For additional information on larval morphology of this genera and related species, see White and Elson-Harris (1992), Carroll *et al*. (2004) and Frías *et al*. (2006, 2008).

1. Mandible more than 0.3 mm long. Ventral apodeme of mandible broad and rounded apically (Figure 26). Spiracular hairs shorter than width of medial spiracular slit (Figure 49)..... ..... ***Toxotrypana Gerstaecker***

– Mandible less than 0.3 mm long. Ventral apodeme of mandible sharp apically (Figures 22–24). Spiracular hairs longer than width of medial spiracular slit (as in Figure 50) **2**

2. Hypopharyngeal bridge narrow at subapical area of hypopharyngeal sclerite (Figure 21). Preoral and oral teeth present (Figure 47); oral ridges usually non-serrate (Figures 45, 47). Dorsolateral sensilla group equidistant from antenna and maxillary palpus.....***Rhagoletis* Loew**

– Hypopharyngeal bridge narrow at middle of hypopharyngeal sclerite (as in Figures 27–32). Preoral and oral teeth absent; oral ridges usually with serrated margins (Figure 48). Dorsolateral sensilla group closer to maxillary palpus than to antenna. **3**

3. Posterior region of mandible without distinct neck (Figure 23). Caudal ridge lacking (Figure 59)….. .....***Anastrepha* Schiner**

– Posterior region of mandible with distinct neck (Figures 22, 24). Caudal ridge present (Figure 60). **4**

4. Oral ridges with shorter round teeth (Figure 48).....***Ceratitis* McLeay**

– Oral ridges with long, sharply pointed teeth (not as above)..... ***Bactrocera* Macquart**

4.4.2 Key to third instar larvae of major economically important species of the genus *Anastrepha*

Key adapted from Steck *et al*. (1990). See Table 3 for diagnostic morphological characters of third instar larvae of *Anastrepha* species.

Geographic distribution and hosts are quoted only as additional information of the common source of origin for the species.

1. Posterior spiracles prominently raised from the body surface; or most body segments with conspicuous setae or processes; or posterior spiracular openings sinuous..... **Not Tephritidae**

– Posterior spiracles nearly flush with body surface; tubercles, if present, on caudal segment only; posterior spiracular slits elongate or oval (Figures 49–50) (Tephritidae) **2**

2. Prominent chitinized preoral teeth (=stomal guards) adjacent to oral opening, or dental sclerite conspicuous (Figures 45, 47); and/or caudal tubercles strongly developed; or larva taken from papaya with caudal ridges lacking and caudal sensilla strongly reduced. ..... ……..... **Other Tephritidae** (not *Anastrepha*)

– Preoral teeth (=stomal guards) lacking, and dental sclerite lacking or inconspicuous (Figure 48); caudal tubercles at most moderately developed .....**(*Anastrepha*) 3**

3. Dorsal spinules present on at least two or more abdominal segments, separate, conical, in fewer than five to six rows on thoracic segments T2 and T3 (Figure 61); posterior spiracular processes SP-I and SP-IV (Figure 46) with average of six or more trunks with bristle length one-third or more times length of spiracular opening (Figures 40, 44) **4**

– Dorsal spinules absent on all abdominal segments, or if present, only in abdominal segment A1 (some specimens of *A. ludens*)..... **5**

4. Anterior spiracle with 28–37 tubules (Figure 43); cephalopharyngeal skeleton as in Figure 32. (Main hosts: larvae breed in fruits of Cucurbitaceae; distribution: Panama to Argentina.) ..... ……..... ***Anastrepha grandis***

– Anterior spiracle with 12 to 23 tubules (Figure 39); cephalopharyngeal skeleton as in Figure 31. (Main hosts: larvae breed in fruits of Myrtaceae; distribution: tropical Americas.) ..***Anastrepha striata***

5. Dorsal spinules present on thoracic segment T3 (Figure 61) **6**

– Dorsal spinules absent on thoracic segment T3 (not as above).....**7**

6. Oral ridges in 11 to 17 rows, usually with margins entire; anterior spiracles with 12 to 20 tubules (Figures 33, 51); posterior spiracular slits 3.1–4.6 times longer than wide (Figure 34). Cephalopharyngeal skeleton as in Figure 27. (Main hosts: larvae breed in fruits of *Citrus* spp. (Rutaceae) or *Mangifera indica*; distribution: southern Texas in United States to Panama.) ....***Anastrepha ludens***

– Oral ridges in 8 to 11 rows with stout, bluntly rounded, widely spaced teeth; anterior spiracles with 9 to 15 tubules (Figure 41); posterior spiracular slits 2.5–3.5 times longer than wide (Figure 42). Cephalopharyngeal skeleton as in Figure 29. (Main hosts: larvae breed in fruits of Myrtaceae; distribution: Florida in United States and Antilles.) .....***Anastrepha suspensa***

7. Posterior spiracular processes SP-I and SP-IV with 5 to 11 short basal trunks (average, 8) (Figure 36); oral ridges usually in 12 to 14 rows; anterior spiracle with 13 to 19 tubules in a single row (Figure 35); anal lobes usually bilobed (as in Figure 57). Cephalopharyngeal skeleton as in Figure 30. (Main hosts: larvae breed in fruits of Sapotaceae; distribution: tropical Americas.) ....***Anastrepha serpentina***

– Posterior spiracular processes SP-I and SP-IV with 8 to 18 long basal trunks (average, 13); oral ridges in 7 to 10 rows; anterior spiracle with 9 to 18 tubules in a single row (as in Figure 34); anal lobes complete or bilobed (Figures 57, 58).....**8**

8. Posterior spiracular processes SP-II usually with three to six basal trunks; posterior spiracular slits 3.0–4.9 times longer than wide (Figure 38). Cephalopharyngeal skeleton as in Figure 28. (Main hosts: larvae breed in fruits of the Anacardiaceae; distribution: tropical Americas, including Antilles.) ....***Anastrepha obliqua***

– Posterior spiracular processes SP-II usually with four to nine basal trunks; posterior spiracular slits 2.5–4.0 times longer than wide (Figure 46). (Distribution: tropical Americas.)..... .....***Anastrepha fraterculus* (Weidemann) species complex**

Table 2. Diagnostic morphological characters of the genus *Anastrepha* used in the keys of this protocol

Biological stage	Structure	Description
Larva	Mandible	Less than 0.3 mm long; posterior region without distinct neck; preapical tooth lacking
	Posterior spiracles	Spiracular hairs longer than width of medial spiracular slits
	Hypopharyngeal bridge	Narrow, located at middle of hypopharyngeal sclerite
	Preoral and oral teeth	Absent
	Oral ridges	Usually serrated
	Stomal sensory organ	Enlarged
Adult	Head chaetotaxy	Two to eight frontal and one or two orbital setae; ocellar setae very weak or indistinct; postocellars unicolorous
	Mesonotum chaetotaxy	One postpronotal, two notopleurals, one presutural supra-alar, one postsutural supra-alar, one postalar, one intra-alar, one dorsocentral, one acrostichal (rarely absent) and two scutellars
	Wings	Veins: Vein *M* usually conspicuously curved forwards apically (strongly so in all pest species) and meeting costa without 90º angle; crossvein *r-m* placed distal to mid-length of discal cell (*dm*); basal cubital cell (*bcu*) with well-developed posteroapical extension
		Wing pattern: C-band on basal costal margin; S-band (from apex of cell *bcu* across cell *dm* and crossvein *r-m*); V-band forming an inverted V shape comprising the proximal arm (subapical band) on *dm-cu* and distal arm (posterior apical band) arising from cell *m*, both convergent in cell R_{4+5}
	Male genitalia	Lateral surstylus short or elongate; medial surstylus shorter than lateral surstylus with two prensisetae apically; proctiger weakly sclerotized laterally and ventrally; glans weakly sclerotized with an apical T-shaped sclerite, glans sometimes absent in non-pest species
	Female genitalia	Oviscape tube-like, variable in length; eversible membrane apically with dorsal hook-like sclerotized plates (also named raspers); aculeus well sclerotized, length variable, tip sometimes serrated on lateral margins

Table 3. Diagnostic morphological characters of third instar larvae of *Anastrepha* species

Species	Structure	Description
Anastrepha fraterculus species complex	Oral ridges	7 to 10 rows
	Anterior spiracle	9 to 18 tubules in a single row
	Dorsal spinules	Abdominal segments absent
		Thoracic segments absent on T3
	Posterior spiracles	SP-I and SP-IV with 10 to 17 long trunks; SP-II usually with 6 to 9 trunks; slits 2.5–3.5 times longer than wide
	Anal lobes	Entire in some populations, bifid in others
Anastrepha grandis	Oral ridges	8 to 13 rows
	Anterior spiracle	28 to 37 tubules
	Dorsal spinules	Abdominal segments present on two or more segments
		Thoracic segments present on T2 and T3
	Posterior spiracles	SP-I and SP-IV with six or more trunks with bristle length one-third times length of spiracular opening
	Anal lobes	Bilobed
Anastrepha ludens	Oral ridges	11 to 17 rows; margins entire
	Anterior spiracle	12 to 20 tubules
	Dorsal spinules	Abdominal segments present on A1
		Thoracic segments present on T3
	Posterior spiracles	Slits 3.1–4.6 times longer than wide
	Anal lobes	Bilobed
Anastrepha obliqua	Oral ridges	7 to 10 rows
	Anterior spiracle	9 to 18 tubules in a single row
	Dorsal spinules	Abdominal segments absent
		Thoracic segments absent on T3
	Posterior spiracles	SP-I and SP-IV with 10 to 17 long trunks; SP-II usually with 3 to 6 trunks; slits 3–4.5 times longer than wide
	Anal lobes	Entire
Anastrepha serpentina	Oral ridges	12 to 18 rows
	Anterior spiracle	13 to 19 tubules in a single row
	Dorsal spinules	Abdominal segments absent
		Thoracic segments absent on T3
	Posterior spiracles	SP-I and SP-IV with six to nine short trunks

Species	Structure	Description
	Anal lobes	Usually bilobed (occasionally entire)
Anastrepha striata	Oral ridges	5 to 8 rows
	Anterior spiracle	12 to 23 tubules
	Dorsal spinules	Abdominal segments present on two or more segments; thoracic segments present on T2 and T3
	Posterior spiracles	SP-I and SP-IV with six or more trunks, length of hairs one-third or more of the length of the spiracular opening
	Anal lobes	Entire or partially bilobed
Anastrepha suspensa	Oral ridges	8 to 11 rows; margins with stout, bluntly rounded, widely spaced teeth
	Anterior spiracle	9 to 15 tubules
	Dorsal spinules	Abdominal segments absent
		Thoracic segments present on T3
	Posterior spiracles	Slits 2.5–3.5 times longer than wide
	Anal lobes	–

Table 4. Diagnostic morphological characters of adults of *Anastrepha* species

Species	Structure	Description
Anastrepha fraterculus species complex	Head chaetotaxy	Posterior orbital seta present
	Thorax	Both mediotergite and subscutellum with broad brown markings on sides; scuto-scutellar suture usually with medial brown spot
	Wings	Distal arm of S-band normally developed, never reaching apex of vein *M*; V-band connected to or separated from S-band anteriorly
	Female genitalia	Aculeus 1.4–1.9 mm long; aculeus tip 0.20–0.28 mm long; lateral margins with 8 to 14 teeth occupying distal two-fifths to three-fifths
Anastrepha grandis	Head chaetotaxy	Posterior orbital seta usually absent
	Thorax	Scutum with dark brown dorsocentral stripes
	Wings	C-band uninterrupted along costal vein; basal half of S-band (on discal cell) continuous from apex of cell *bcu* through crossvein *R-M* and connecting with C-band above; cell r_{2+3} completely pigmented in entire length; vein R_{2+3} almost straight; cell *br* broadly hyaline between veins *bm-cu* and *r-m*
	Female genitalia	Aculeus extremely long (5.3–6.2 mm), and usually greater than 0.10 mm wide; aculeus tip with V-shaped ridges, lateral margins non-serrate
Anastrepha ludens	Head chaetotaxy	Posterior orbital seta present
	Thorax	Subscutellum always with brown marks laterally, sometimes extending onto mediotergite
	Wings	V-band usually not connected to S-band, and with arms separated anteriorly
	Female genitalia	Aculeus usually 3.3–5.8 mm long; aculeus tip 0.28–0.42 mm long, 0.12–0.14 mm wide, with a moderate constriction near mid-length; lateral margins non-serrate or finely serrate on distal 0.55 or less
Anastrepha obliqua	Head chaetotaxy	Posterior orbital seta present
	Thorax	Subscutellum entirely yellow, only mediotergite with brown markings on sides; scuto-scutellar suture without medial brown spot
	Wings	Distal arm of S-band normally developed, never reaching apex of vein *M*; V-band usually connected anteriorly to S-band
	Female genitalia	Aculeus less than 2.0 mm long; aculeus tip 0.16–0.20 mm long, with lateral serrations on distal two-thirds to four-fifths
Anastrepha serpentina	Head chaetotaxy	Posterior orbital seta present
	Thorax	Thorax mostly brown or red–brown contrasting with yellow markings; scutum

Species	Structure	Description
		mostly brown with three yellow stripes
	Wings	Wing pattern mostly dark brown; distal arm of V-band completely absent
	Female genitalia	Aculeus 2.6–3.8 mm long; aculeus tip 0.37–0.46 mm long, 0.14–0.17 mm wide, lateral margins finely serrated on distal 0.5–0.7
Anastrepha striata	Head chaetotaxy	Posterior orbital seta present
	Thorax	Scutum with two broad dorsocentral stripes connected on posterior margin forming a U-shaped mark, without setulae in a small area along transverse suture
	Wings	Wing pattern mostly orange and brown; distal arm of V-band present or absent
	Female genitalia	Aculeus 1.95–2.30 mm long; aculeus tip broad, 0.24–0.31 mm long, 0.17–0.20 mm wide
Anastrepha suspensa	Head chaetotaxy	Posterior orbital seta present
	Thorax	Scuto-scutellar suture with large rounded brown spot medially; mediotergite entirely yellow or with brown mark on sides
	Wings	Anterior apical band (=distal section of the S-band) extremely wide, reaching the apex of vein *M*; V-band broad and complete, with arms widely connected anteriorly
	Female genitalia	Aculeus 1.4–1.6 mm long; aculeus tip 0.19–0.23 mm long, 0.10–0.13 mm wide, lateral margins serrate on distal 0.50–0.65

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 diagnosis, the records and evidence (in particular, preserved or slide-mounted specimens and photographs of distinctive taxonomic structures, as appropriate) should be deposited in a museum or another permanent collection.

6. Contact Points for Further Information

Further information on this protocol can be obtained from:

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

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8. References

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

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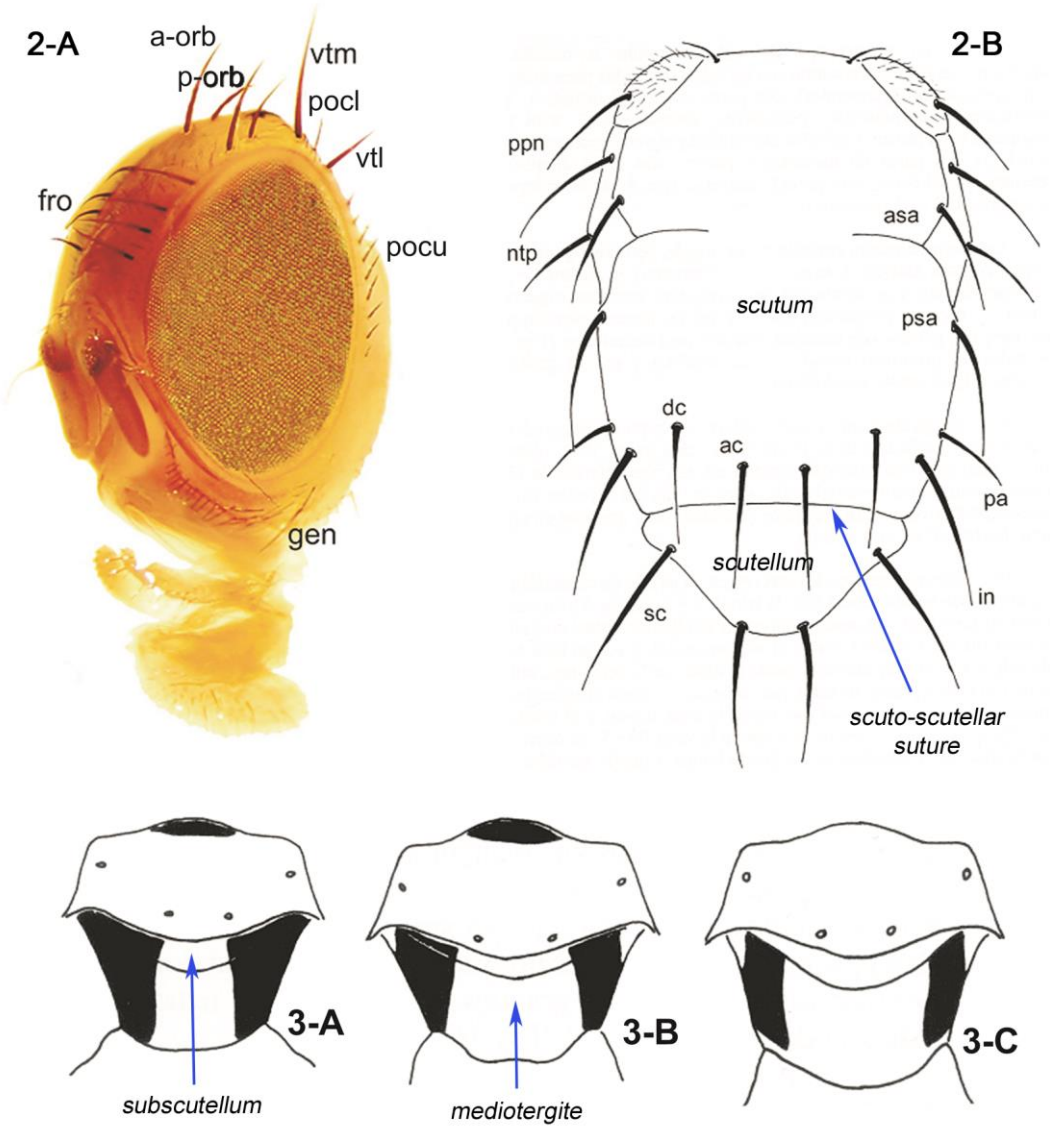
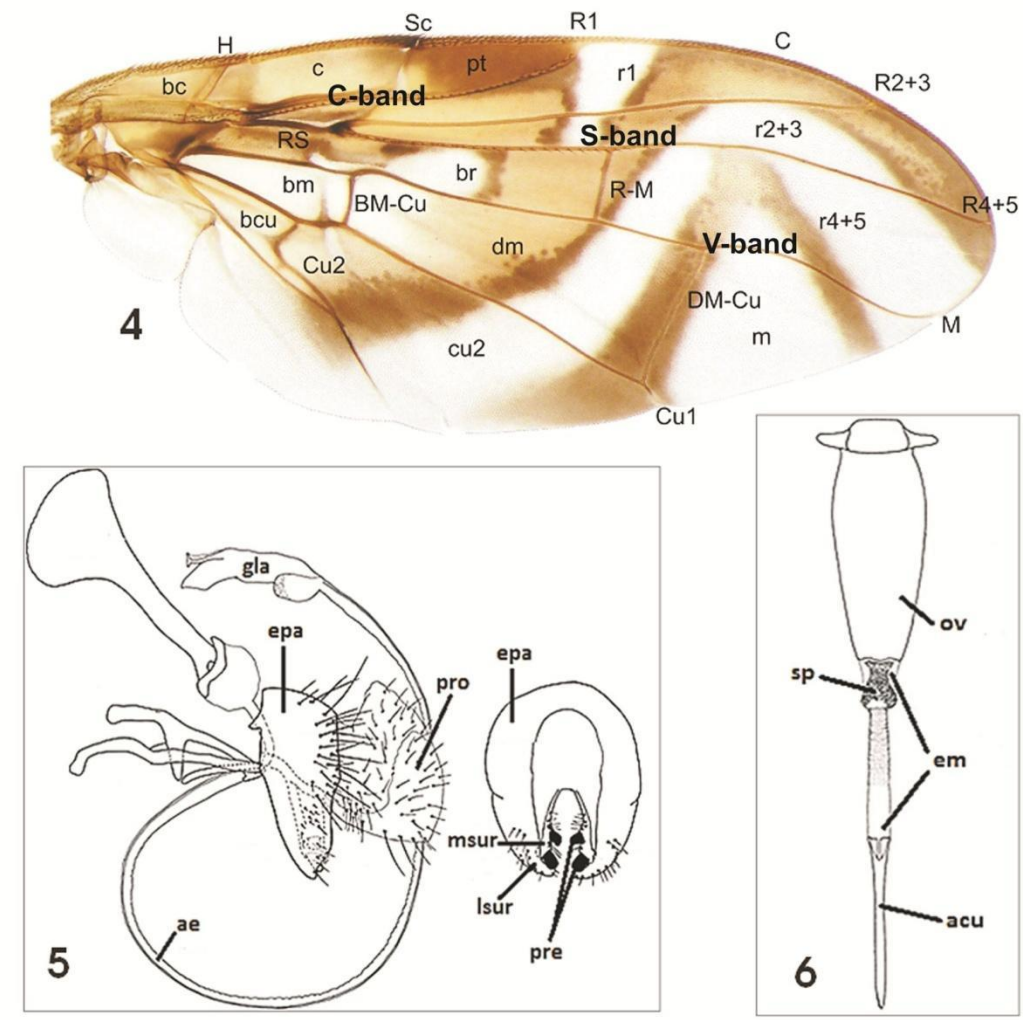
9. Figures

Figure 1. General habitus of the adult female of *Anastrepha ludens* (Mexican fruit fly) in dorsal view. *Micrograph courtesy V. Hernández-Ortiz.*



Figures 2–3. (2-A) Morphology of the head of *Anastrepha* species, fronto-lateral view. *a-orb,* anterior orbital setae; *fro,* frontal setae; *gen,* gena; *pocl,* postocellar setae; *pocu,* postocular setae; *p-orb,* posterior orbital seta; *vtl,* vertical lateral seta; *vtm,* vertical medial seta. **(2-B)** Thorax dorsal view and chaetotaxy. *ac,* acrostichal; *asa,* presutural supra-alar; *dc,* dorsocentral; *in,* intra-alar; *ntp,* notopleurals; *pa,* postalar; *ppn,* postpronotal; *psa,* postsutural supra-alar; *sc,* scutellars. **(3)** Mediotergite and subscutellum, postero-dorsal view: **(3-A)** *A. fraterculus*; **(3-B)** *A. ludens*; and **(3-C)** *A. obliqua.*
Source: Figure 1(A) adapted from Hernández-Ortiz et al. *(2010); Figures 2 and 3 adapted from Hernández-Ortiz (1992).*



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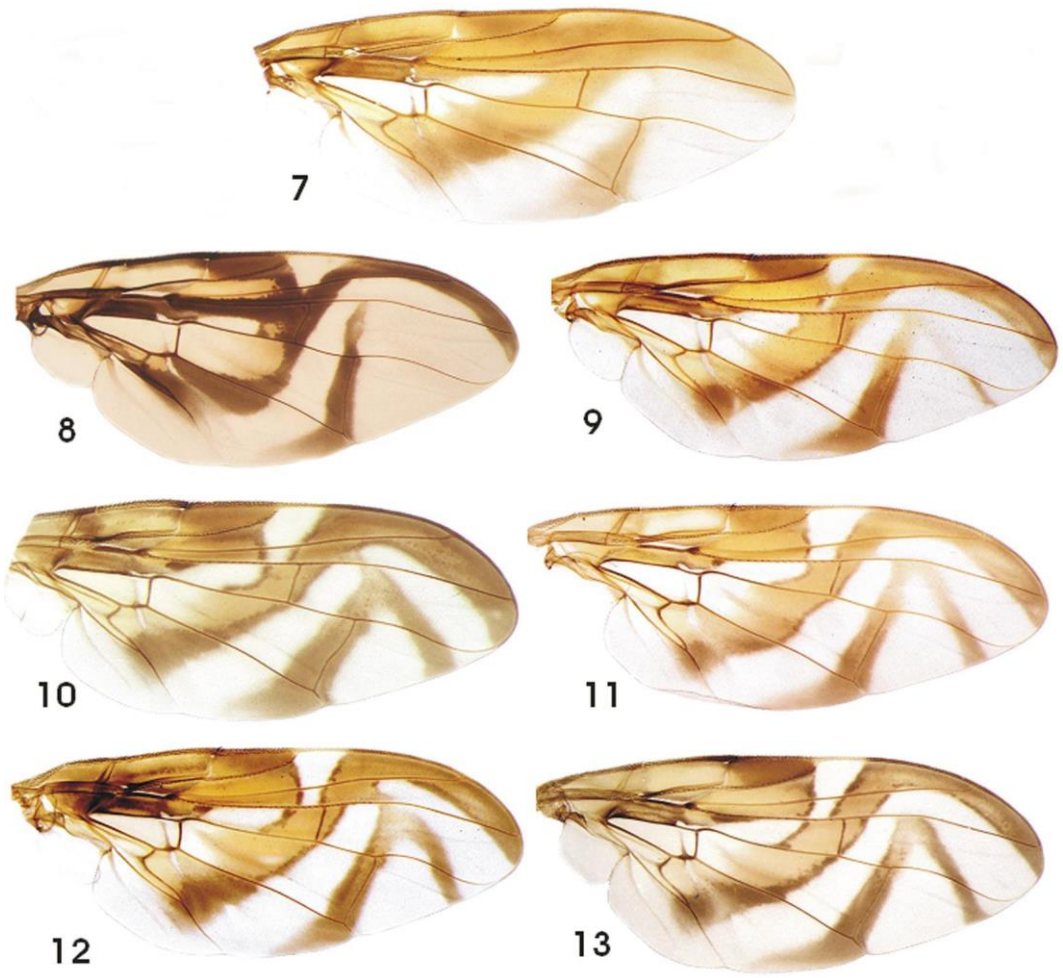
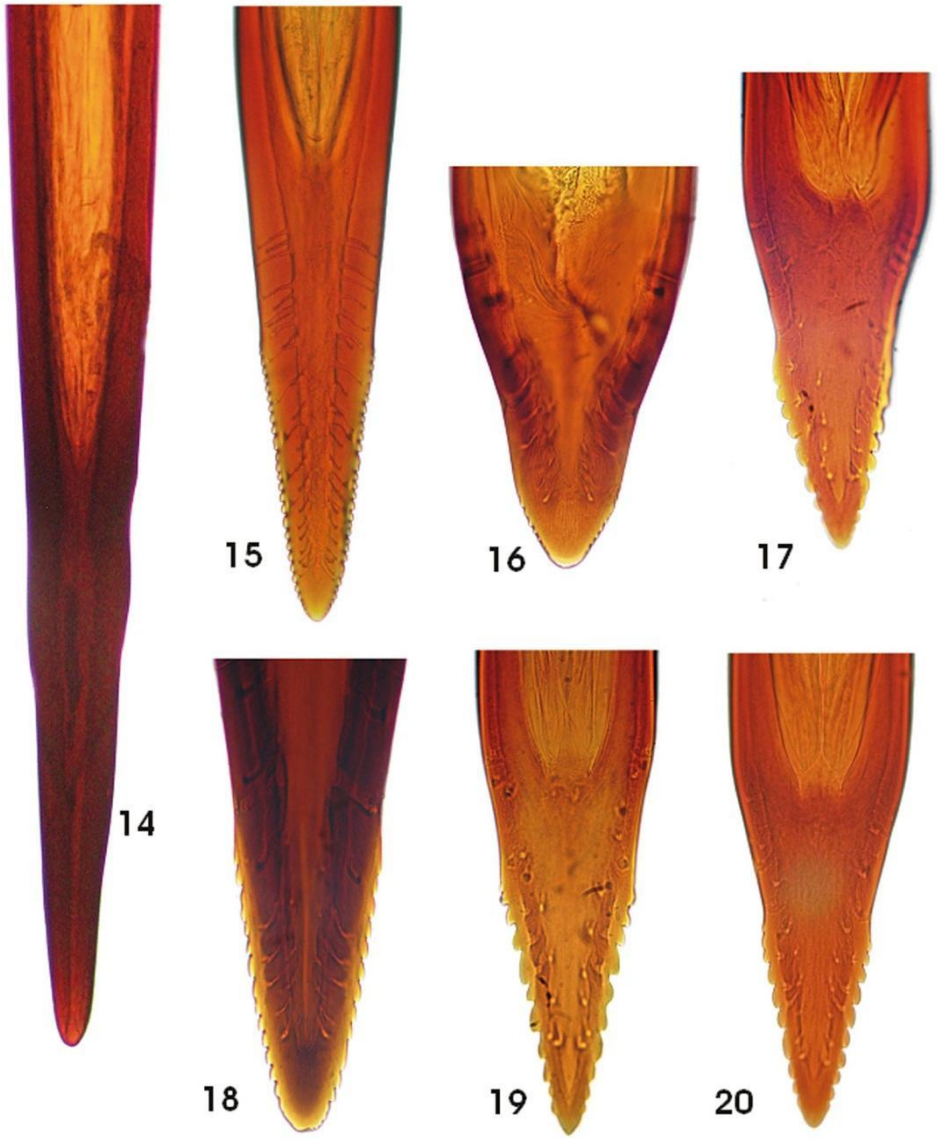
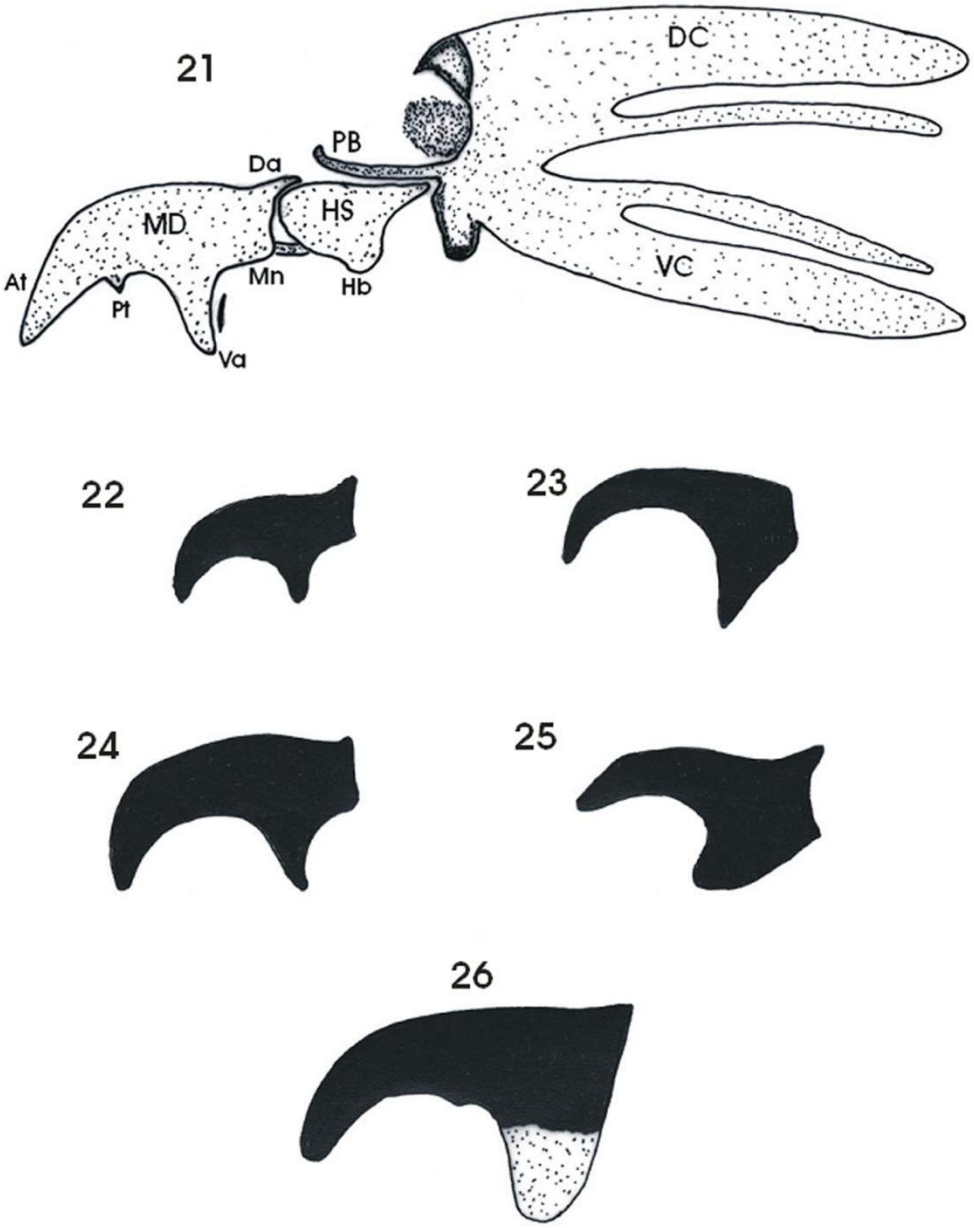
Source: Figure 4 adapted from Hernández-Ortiz et al. *(2010); Figures 5 and 6 adapted from Norrbom* et al*. (2012).*



Figures 7–13. Wing pattern of *Anastrepha* species: **(7)** *A. grandis*; **(8)** *A. serpentina*; **(9)** *A. striata*; **(10)** *A. suspensa*; **(11)** *A. ludens*; **(12)** *A. obliqua*; and **(13)** *A. fraterculus* (Brazil).
Source: All figures adapted from Hernández-Ortiz et al*. (2010).*



Figures 14–20. Morphology of the aculeus tip in *Anastrepha* species of major economic importance: **(14)** *A. grandis*; **(15)** *A. serpentina*; **(16)** *A. striata*; **(17)** *A. suspensa*; **(18)** *A. ludens*; **(19)** *A. obliqua*; and **(20)** *A. fraterculus* (Brazil).
Source: All figures adapted from Hernández-Ortiz et al*. (2010).*



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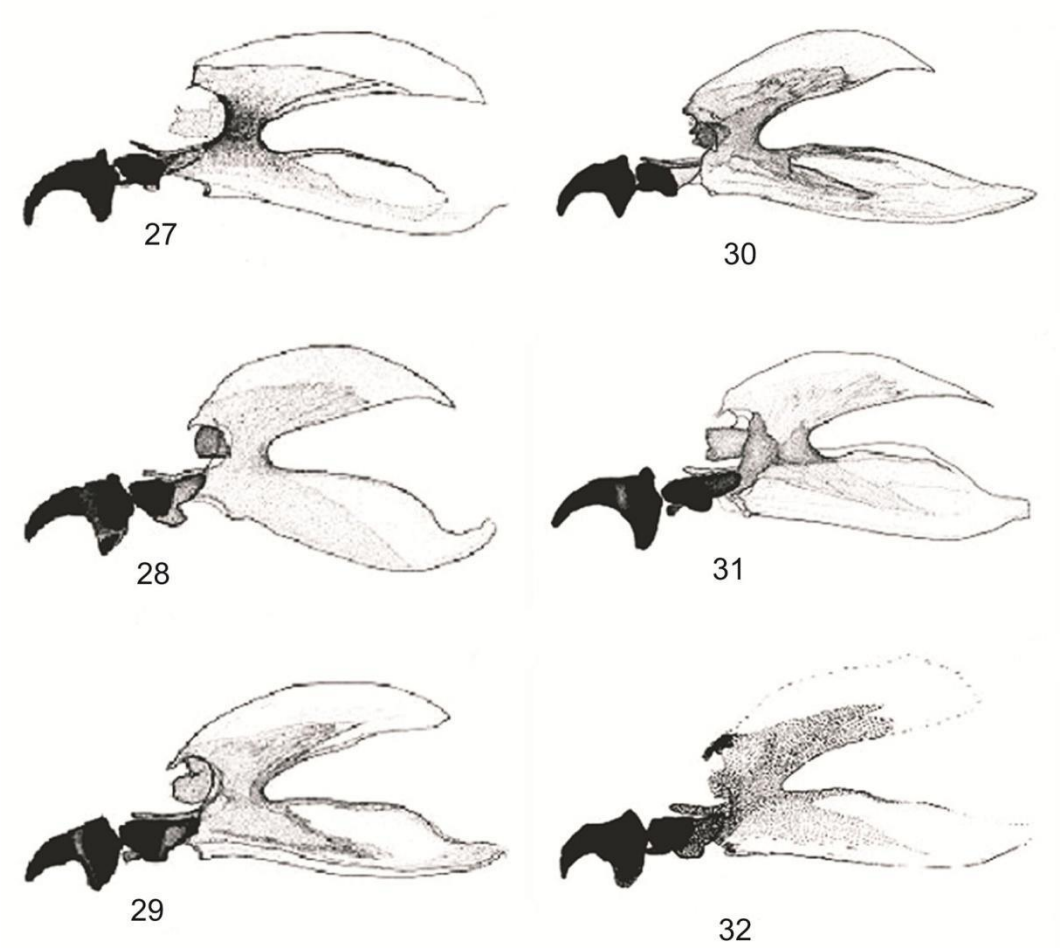
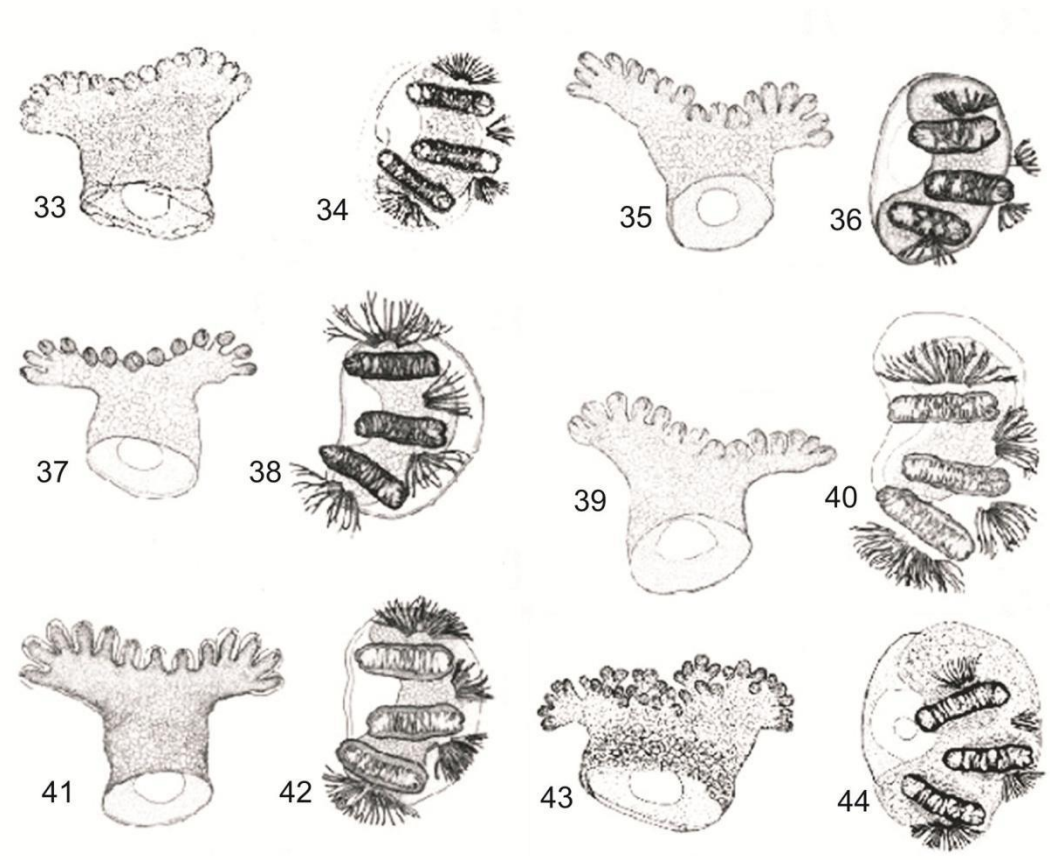
Source: All figures adapted from Frías et al*. (2006).*



Figures 27–32. Cephalopharyngeal skeleton of third instar larvae of *Anastrepha* species: **(27)** *A. ludens*; **(28)** *A. obliqua*; **(29)** *A. suspensa*; **(30)** *A. serpentina*; **(31)** *A. striata*; and **(32)** *A. grandis*.
Source: All figures adapted from Carroll et al*. (2004).*



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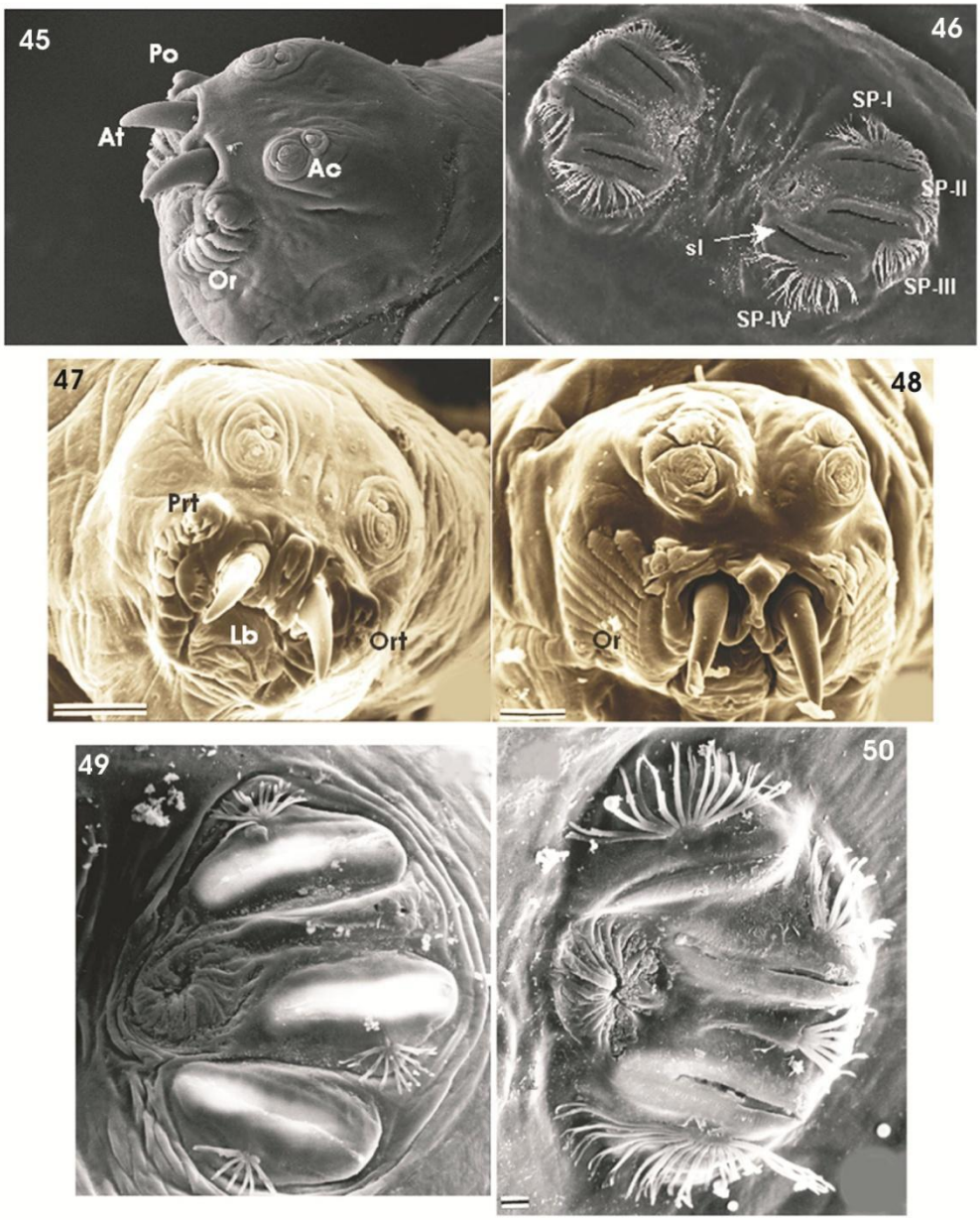
Source: All figures adapted from Carroll et al*. (2004).*



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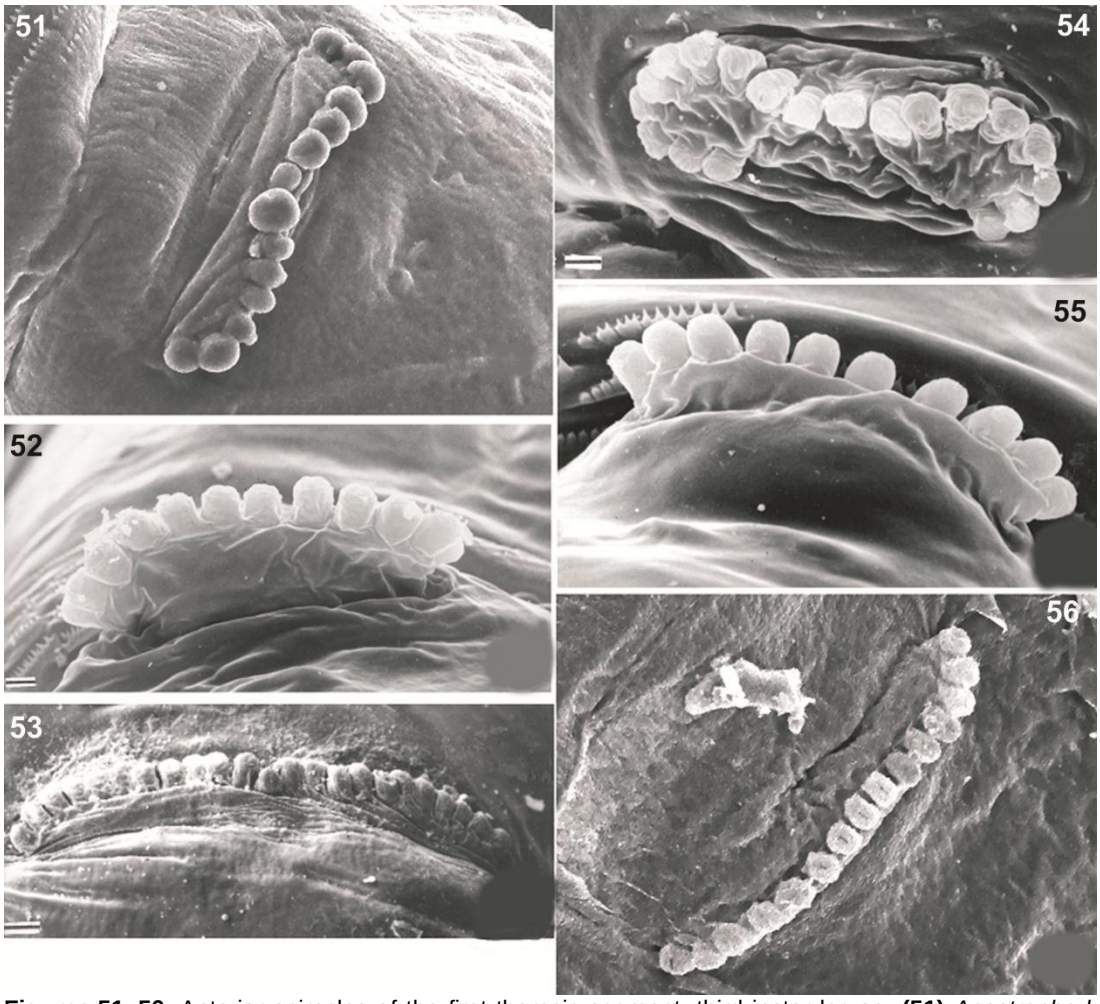
Source: Figures 45 and 47–50 adapted from Frías et al*. (2006); Figure 46 adapted from Hernández-Ortiz* et al*. (2010).*



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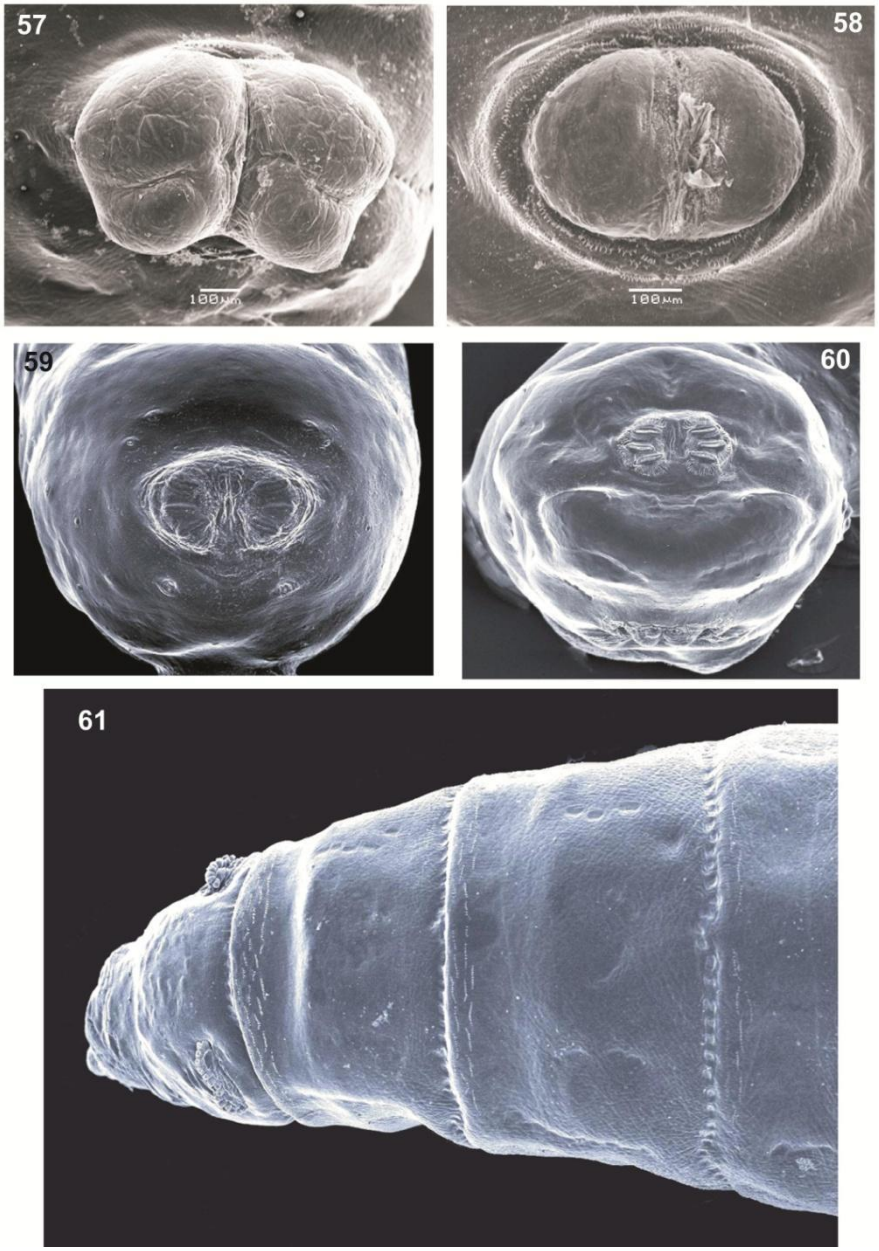
Source: Figures 52–55 adapted from Frías et al. *(2006); Figures 51 and 56 adapted from Hernández-Ortiz* et al. *(2010).*



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Micrographs courtesy G. Steck.

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2013-06 Draft presented to TPDP (meeting).

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ISPM 27. **Annex 9**. Genus *Anastrepha* Schiner (2015). Rome, IPPC, FAO.

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This diagnostic protocol was adopted by the Standards Committee on behalf of the Commission on Phytosanitary Measures in January 2016.
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ISPM 27
Diagnostic protocols for regulated pests

DP 10: *Bursaphelenchus xylophilus*

Adopted 2016; published 2016

CONTENTS

1. Pest Information

The pine wood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer, 1934) Nickle 1970, is the causal agent of pine wilt disease. *B. xylophilus* is believed to be native to North America, where it is widely distributed in Canada and the United States (Ryss *et al.*, 2005) and is apparently of limited distribution in Mexico (Dwinell, 1993). North American pine species are resistant or at least tolerant to *B. xylophilus*, but exotic species planted in North America, especially in the warmer southern areas of the United States, are killed when attacked by the nematode.

B. xylophilus was carried to Japan at the beginning of the twentieth century, presumably on timber exported from North America, and it became one of the most damaging forest pests in the country, where it still causes remarkable losses of pine trees (*Pinus densiflora*, *P. thunbergii* and *P. luchuensis*) today. *B. xylophilus* was also introduced to China (including Taiwan) and Korea; it was found there in the mid to late 1980s. In 1999, *B. xylophilus* was found for the first time in Europe (Portugal) on *P. pinaster*, which is killed by the nematode within a few months after infestation (Mota *et al.*, 1999; Fonseca *et al.*, 2012). *B. xylophilus* has also been detected on *P. nigra* and *P. radiata* in Portugal and Spain, respectively (Inácio *et al.*, 2014; Zamora *et al.*, 2015). In 2008, *B. xylophilus* was found for the first time in Spain (Abelleira *et al*., 2011).

B. xylophilus is transmitted from tree to tree by wood-inhabiting beetles of the genus *Monochamus* (Coleoptera: Cerambycidae) (Linit, 1990; Evans *et al.*, 1996). The nematodes enter the bodies of the insects shortly after the latter emerge from pupation and just before they bore out of the host tree (Wingfield, 1987). The beetles fly to the crown of healthy trees and feed on the young shoots and leaves (maturation feeding). They then mate and the females search for a weakened tree or one that has died recently, or for trunks or bigger branches (including felling debris), depending on the *Monochamus* species, where they lay their eggs through the bark. The beetle larvae that hatch from the eggs feed in the cambial tissues just below the bark for several months. On reaching maturity, they bore deeper into the wood to pupate, and thus their life cycle is completed. *B. xylophilus* takes advantage of this life cycle to obtain transport to new host trees (Wingfield, 1987). Their introduction into the new tree may take place during oviposition by the beetle (this appears to be the only means of transmission for several species of *Bursaphelenchus* that colonize dead trees) (Edwards and Linit, 1992). *B. xylophilus*, however, seems to be unique among these species in that it can also be transmitted to a new tree during maturation feeding by beetles, and the development of pine wilt disease can occur as a consequence of transmission through the young shoots (Wingfield, 1987).

When *B. xylophilus* is transmitted during oviposition, the nematodes remain relatively close to the site of introduction. But when transmission occurs through the young shoots and when the tree succumbs to pine wilt disease, the nematodes distribute throughout the whole tree, destroying wood tissues such as epithelial cells, parenchyma cells of axial and radial resin canals, cambium and phloem. *B. xylophilus* can also be found in roots, even when the above-ground part of the tree is already dead, dried out or felled. Whether the tree develops pine wilt disease depends on the tree species (in general only *Pinus* spp. of non-American origin are affected), its state of health and the climatic conditions (particularly temperature and water supply). These factors also influence the distribution of nematodes throughout the tree: their distribution can be localized or irregular and this needs to be taken into account in sampling strategies (Schröder *et al*., 2009).

B. xylophilus can also be found in dead trees of *Abies*, *Chamaecyparis*, *Cedrus*, *Larix*, *Picea* and *Pseudotsuga* and other conifers (except *Thuja* spp.), but none of these genera is known to be affected by pine wilt disease, although pathogenicity tests on seedlings show remarkable reactions, including death (Evans *et al*., 1996).

B. xylophilus is almost exclusively vectored by *Monochamus* species, with the vector species varying among the geographic regions; for example, *M. alternatus* in China and Japan, *M. saltuarius* in Japan, *M. carolinensis* in North America and *M. galloprovincialis* in Portugal. Occasionally, other beetles of the family Cerambycidae or other Coleoptera have been found to carry "dauer" juveniles of the

nematode on their bodies, but there is no evidence that they play a role as vectors in the dissemination of the nematode (Evans *et al*., 1996).

Human activity is known to be the principal route for dispersal of *B. xylophilus* over greater distances and *B. xylophilus* and its vectors have been intercepted on a number of occasions in the international trade of wood, wood products and, most notably, solid wood packaging made from conifers. Therefore, the risk of further international spread is high.

Though *B. xylophilus* associated with the vector beetles poses the highest risk of spread, movement of *B. xylophilus* from infested wood to non-infested wood or to uninfested trees can occur under specific circumstances: direct contact from donor to receiving wood, high moisture content of receiving wood or wounds on receiving trees (Sousa *et al.*, 2011; Hopf and Schroeder, 2013).

More details about the biology of *B. xylophilus*, its vectors, pine wilt disease, geographical distribution, trade and economic impacts, and management strategies can be found in the following comprehensive books: Kishi (1995); Mota and Vieira (2004); Mota and Vieira (2008); and Zhao *et al.* (2008).

2. Taxonomic Information

Name: *Bursaphelenchus xylophilus* (Steiner and Buhrer, 1934) Nickle, 1970

Synonyms: *Aphelenchoides xylophilus* Steiner and Buhrer, 1934
Paraphelenchoides xylophilus (Steiner and Buhrer, 1934) Haque, 1967
*Bursaphelenchus lignicolu*s Mamiya and Kiyohara, 1972

Taxonomic position: Nematoda, Rhabditida, Tylenchina, Aphelenchoidea, Aphelenchoididae, Parasitaphelenchinae, *Bursaphelenchus*

Common name: Pine wood nematode

3. Detection

B. xylophilus has six life stages: the egg and four juvenile stages preceding the adult. The first juvenile stage (J1) moults to the second juvenile stage (J2) in the egg. J2 hatches from the egg, and there are two more juvenile stages (J3 and J4) preceding the adult. Different types of juvenile stages appear under different conditions. Under favourable conditions at 25 °C *B. xylophilus* develops from the egg through four propagative juvenile stages (J1 to J4) to reach the adult stage within four days (Hasegawa and Miwa, 2008) (Figure 1).

Under unfavourable conditions, the J_{III} dispersal stage develops in place of the J3 stage. J_{III} is probably a non-feeding stage. It has lipids accumulated in the intestinal cells (Kondo and Ishibashi, 1978) and can survive unfavourable conditions such as drought, low temperature or lack of nutrition. Normally this stage moults into the J_{IV} dispersal stage (dauer juveniles), which is transmitted by vector beetles to new trees. Nevertheless, if the conditions become suitable for nematode development, for example by putting the J_{III} stage on fungal culture plates, the nematodes develop to the J4 propagative juvenile stage (Wingfield *et al*., 1982).

Living *B. xylophilus* can be found in various types of wood of host species, including standing or fallen trees, round wood, sawn wood, and wood products such as coniferous wood packaging material, as well as in saw dust, wood chips and particles, wood waste, untreated furniture and handicrafts. The following sections give specific information on detection of *B. xylophilus* in trees, wood and wood products as well as in its vector beetles. Although correct sampling is essential for obtaining material with the highest likelihood of being infested with *B. xylophilus*, guidance on sampling is not part of the current protocol. General guidance on sampling with reference to the European *Monochamus* species as vectors was published by Schröder *et al.* (2009) and EPPO (2012).

3.1 Detection in trees

If it is not known whether *B. xylophilus* occurs in an area, sampling should be focused on trees near high-risk sites; for example, ports handling imports from countries with known *B. xylophilus* infestation, airports, sawmills, wood processing facilities, places where wood is stored, and areas where forest fires have occurred (*Monochamus* is attracted by forest fires).

To have the best chance of detecting *B. xylophilus* in an area, it is advisable to concentrate sampling on pine trees that are dying or have died recently (Figures 2 and 3), both of which may be standing or fallen. Trees and cut waste from a recent felling season (i.e. one to two year old logging sites) that have been colonized after the felling by *Monochamus* beetles may also be used as sampling material. The following symptoms should be searched for: discoloration (e.g. yellowing) of needles, wilting, evidence of insect attack (e.g. wood shavings on the ground or protruding from cracks in the bark, flat-headed larvae of *Monochamus* beneath the bark, surface galleries beneath the bark with oval entrance holes oriented in the longitudinal direction of the stem, the round exit holes of adults), blue stain fungal growth in the wood and lack of oleoresin flow from wounds. The rate of oleoresin flow should be checked while the trees are still green by removing part of the bark from the cambial layer. Healthy trees will cover the wood surface with resin within one hour while no or reduced resin flow will occur in infested trees. However, these symptoms vary between species of pine and are non-specific for *B. xylophilus* as they may be caused by other pathogens or by physical factors. There is currently no method to visually distinguish between trees that are dying from pine wilt disease and those dying for other reasons. Trees to be sampled preferably should be associated with *Monochamus* attack, either maturation feeding or breeding, but at the least, it should be known that *Monochamus* species occur in the area where samples are to be taken.

The distribution of the nematodes can be localized within the trees, especially shortly after they have been introduced by oviposition or by the maturation feeding of the beetle vector. In cases of pine wilt disease, nematodes can spread rapidly to produce large numbers in all parts of the tree except the needles, cones and seeds. *B. xylophilus* also invades the root system and can survive there for a certain period when the tree is already dead and desiccated or has been felled. However, in non-susceptible trees, under unfavourable climatic conditions or in particular physiological states of the tree, *B. xylophilus* attack can remain limited in distribution within the trees; for example, an infestation of *B. xylophilus* may already be established in the crown or parts of the crown without further spread to other tree parts.

3.2 Detection by the use of insect traps, trap logs and in samples from sawmills and timber yards

Insect traps with lures for attracting *Monochamus* species have been developed in recent years and can be used for monitoring as well (Sanchez-Husillos *et al.*, 2015). When using *Monochamus* traps to collect beetles to be investigated for potential *B. xylophilus* infestation, the beetles need to be caught alive and not in a liquid killing agent.

In areas with a known population of *Monochamus* beetles, logs felled during the flight period of the beetles may be used as trap logs. Beetles are attracted to them for oviposition and it has been proven that nematode transmission will take place in such cases (Dwinell, 1997; Luzzi *et al.*, 1984). The wood or the emerged beetles can be sampled to monitor the presence of *B. xylophilus* in a limited area. Beetles can complete their life cycle in such material. It is also possible to accelerate beetle development by taking the trap logs to the laboratory in autumn: beetles will emerge several weeks before they would have emerged under natural conditions.

Collection of wood samples, shavings or wood chips from sawmills and wood yards might be more successful than sampling standing trees. Such samples may have come from a very wide area because large sawmills might obtain their wood from far away and process both domestic and imported wood. But this is also a disadvantage in that a correlation between a positive sample and the area of origin might be difficult to determine.

3.3 Direct detection in wood, wood products and solid wood packaging

All types of coniferous wood, especially solid wood packaging, particularly from countries in which *B. xylophilus* occurs, can be sampled by low-speed drill, borer, saw, axe, hook and so forth. Sampling should be concentrated on pieces with circular grub holes (i.e. the emergence holes of beetles) and oval entrance holes and larval tunnels, which are sometimes blocked with wood particles. Removal of bark when present may help detect galleries. In the case of sawn wood, normally no exit holes will be seen, but larval tunnels may be seen, which are sometimes difficult to detect because they are blocked with shavings. Pieces with fungal growth, especially blue stain fungus, should be sampled. Nevertheless, several interceptions have shown that living *B. xylophilus* can be detected in samples without the above-mentioned indications (EPPO, 2012).

Solid wood packaging (e.g. pallets) can come into contact with soil during service. This may lead to surface contamination with soil and soil-inhabiting nematodes, which can survive desiccation. To avoid a contamination of the extracted wood sample with those nematodes, the sample should be investigated after removal of the outer part of the wood (Schröder *et al*., 2009).

3.4 Extraction of nematodes from wood samples

Living nematodes can be extracted from infested wood using the Baermann funnel technique or the modified Baermann funnel technique (Penas *et al.*, 2002; EPPO, 2013c). In the Baermann funnel technique, a glass or plastic funnel with the narrow tube at the base closed by means of a rubber tube and a clamp is filled with water. The sample consisting of small pieces of wood or wood shavings is supported on a sieve in the funnel. A paper tissue permeable for nematodes is placed on the sieve to avoid contamination of the water with wood debris. The funnel is then filled with water to cover the sample. The sample is left for 24 to 48 h at room temperature or in an incubator (both at approximately 25 °C), during which time nematodes migrate from the wood into the water and fall to the base of the funnel from where they can be collected by releasing a small quantity of the water (approximately 10 ml) into a small dish.

The principle of the Baermann funnel technique is as described above, but several modifications are used in practice (EPPO, 2013c). For instance, wood chips can be directly submerged in water or they can be placed on a cotton wool filter laid in a plastic basket for extraction of nematodes. In addition, each method described in EPPO (2013c) can be combined with a mistifier spray apparatus.

Under a stereoscopic microscope and using a pipette or a needle the nematodes can be transferred from the small Petri dish to a glass slide for examination under a high power microscope.

Nematodes may occur in very low numbers in the sample, so detection might be difficult. It is recommended to allow the nematodes to multiply before extraction. To do this, the moistened wood sample without any bark is sealed in a plastic bag and incubated at approximately 25 °C for two to three weeks. The nematodes are then extracted with the Baermann funnel technique.

The principle of the Baermann funnel technique is based on detecting living nematodes when they exit the wood sample, but within the recommended 24 to 48 h some nematodes die (Baermann, 1917). Nevertheless, one can be sure that those were alive when the extraction was started. This has to be kept in mind when analysing imported wooden material. Some other extraction methods – for example a centrifugation method (not described here; much faster than the Baermann funnel technique) – will also extract nematodes that were already dead in the wood (Moens, 2000). The centrifugation method can be used to monitor an area with *B. xylophilus* infestation but not to prove that wood has undergone a successful phytosanitary treatment (Moens, 2000).

3.5 Extraction of nematodes from vector insects

Beetles of the genus *Monochamus* caught by traps (Pajares *et al*., 2004; Ibeas *et al*., 2007) or trap logs can be assessed for the presence of nematodes (section 3.2). The beetles need to be caught alive and not in a liquid killing liquid agent, unless they are to be used for direct molecular detection.

Nematode juveniles are usually present as J_{IV} dispersal stage (dauer juveniles) in the tracheae and on the body of the beetles. J_{IV} dauer juveniles do not have a stylet. To isolate the nematodes, the beetles are dissected and crushed in an appropriate dish and kept in water for 24 to 48 h at approximately 25 °C (Sousa *et al.*, 2001; EPPO, 2013c). Dauer juveniles will leave the beetles. J_{IV} dauer juveniles need to be transferred to fungal mats of *Botryotinia fuckeliana* (anamorph: *Botrytis cinerea)* grown on malt agar (section 4.1.1) to enter the propagative life cycle because further morphological identification can only be done on adult nematodes. Alternatively they can be used directly for molecular identification. The Baermann funnel technique may also be used to extract the nematodes from the beetles.

Nematodes extracted from wood or insect vectors as described above can be morphologically examined, or molecular testing for *B. xylophilus* can be performed directly on the extracts. EPPO (2013b) reports a screening procedure based on a modified Baermann extraction method followed by a real-time polymerase chain reaction (PCR) test (adapted from François *et al*., 2007).

There are also several reports of molecular detection methods for which DNA from *B. xylophilus* is extracted directly from wood before amplification (Takeuchi *et al*., 2005; François *et al*., 2007; Kikuchi *et al*., 2009; Hu *et al*., 2011; Kanetani *et al*., 2011; Cardoso *et al*., 2012). However, in these reports, the amount of wood used for the DNA extraction ranges from 5 to 120 mg, which is very small compared with the size of the wood samples that are routinely analysed. In addition, this direct detection approach by molecular assay would detect any target nematode, alive or dead. Consequently, users of this approach should have defined procedures in place to confirm the presence of living nematodes in the sample, if appropriate for the aim of the analysis.

4. Identification

To date, about 110 species of the genus *Bursaphelenchus* have been described (Futai, 2013). The latest overviews can be found in Ryss *et al.* (2005), Hunt (2008), Braasch *et al.* (2009) and Futai (2013). *B. xylophilus* can be positively identified by either one of two methods: that based on morphological features and that based on molecular biology techniques. Although the number of *Bursaphelenchus* species described in recent years has increased and some of them have similar morphological characters, a determination based on morphology is possible in most cases. However, identification of the mucronate form of *B. xylophilus* based on morphological characters may be difficult.

Identification based on morphological features requires preparation of good quality microscope slides, access to a high power microscope and considerable experience in nematode taxonomy, especially in the small group of species closely related to *B. xylophilus* (*B. mucronatus mucronatus, B. mucronatus kolymensis, B. fraudulentus* and others). Identification methods based on molecular biology require expensive equipment and reagents, but can be applied with less technical experience (and very little nematological training). Adequate experience is, however, needed to ensure that the limited nematode material is not lost during the procedure. While morphological identification is based on adult specimens, molecular identification can be made even if only juvenile stages or one sex of adults are available, which is an advantage. While DNA-based PCR methods fail to differentiate between dead and living nematodes, new methods based on mRNA can clarify whether the positive detection originates from living nematodes (Leal *et al.*, 2013).

B. xylophilus can be identified by a nematologist or an experienced phytopathologist with a nematological background using morphological features if the specimens are available as male and female adults and in good condition. However, there may be situations where a combination of morphological features and molecular information is recommended to obtain a higher degree of certainty on the identification; for example, when *B. xylophilus* has been detected in a new area, when *B. xylophilus* has been found by a laboratory for the first time, as quality assurance for compliance with certification schemes, and when *B. xylophilus* is found in consignments during import inspection, especially when the exporting country has been declared to be free from *B. xylophilus*. In addition, *B. xylophilus* can show morphological variations that may make the use of molecular biology techniques necessary; for example, round or mucronate tail tip of females (Figure 4) or the position of

the excretory pore. When only a small number of nematodes have been isolated, multiplying them on *B. fuckeliana* before identification is recommended to obtain enough material for a reliable identification (section 4.1.1).

4.1 Morphological identification

Numerous nematode species may be present in an aqueous extract from coniferous wood, especially if decay of the tissues has begun. Some of these will be saprophagous species where adult nematodes lack the stylet that is typical for nematodes of the orders Tylenchida, Aphelenchida and Dorylaimida. *Bursaphelenchus* species belong to the Aphelenchida, which have the dorsal pharyngeal gland opening into the metacorpus, in contrast to the Tylenchida, where the gland opens into the lumen of the pharynx between the bulb and the stylet (Figure 4). If the extract contains only juveniles, morphological identification of *B. xylophilus* will not be possible. In such cases, aphelenchoide species that fall in the range of *B. xylophilus* juvenile size (see, e.g., Penas *et al.*, 2008) should be separated and either multiplied on a culture plate or used directly for molecular identification.

For identification under a light microscope, a magnification of 400× to 1 000× (oil immersion lens) is recommended. Differential interference contrast (DIC) may facilitate observations.

4.1.1 Preparation of specimens

It may be necessary to multiply the extracted nematodes to obtain enough material for identification. Most *Bursaphelenchus* species can be cultured on the sporulating form of the fungus *B. fuckeliana*. Some species, especially those belonging to the *sexdentati* group, require culture on the non-sporulating form. Both fungal forms are cultured on 2% malt extract agar (MEA) medium (15 g agar-agar, 15 g malt extract, 750 ml water; pH 7.0). Petri dishes (90 mm diameter) are filled with 25 ml sterilized MEA. Either fungal spores or pieces of agar with fungal growth are transferred to the Petri dishes in a clean bench unit. Incubation of the fungal plates is recommended at room temperature (approximately 25 °C). Nematodes to be reared are transferred in a small droplet placed on the mycelium using a pipette or other means. Nematode incubation is recommended at approximately 25 °C (based on its biology), which leads to a sufficient reproduction rate to obtain enough adult and juvenile individuals.

4.1.1.1 Temporary preparations

Temporary preparations for quick identification or study of features best seen in unfixed specimens are prepared as follows. Living specimens are transferred to a small drop of water on a glass slide. The slide is briefly heated over a spirit flame, checking frequently for nematode movement. Heating should be stopped as soon as the specimens stop twitching. A coverslip is applied and the slide is ready for study. Fixing the coverslip is not recommended as the body of the male nematodes may have to be moved subsequently into the dorso-ventral position to see the bursa.

4.1.1.2 Permanent preparations

Permanent preparations for identification under light microscopy are prepared as follows. Living nematodes extracted from plant material or nematode rearing are killed by gentle heat, fixed in FAA fixative (35% distilled water, 10% of 40% formalin, 5% glacial acetic acid, 50% of 95% alcohol) (Andrássy, 1984) or triethanolamine and formalin (TAF) fixative (7 ml formalin (40% formaldehyde), 2 ml triethanolamine, 91 ml distilled water), processed to anhydrous glycerine (for long-term storage) and mounted on slides in anhydrous glycerine as described by Seinhorst (1959) and Goodey (1963). A more rapid method (1–1.5 h) to prepare permanent slides was described by Ryss (2003) based on killing the nematodes in hot 4% formaldehyde solution. Fixation then takes place at different temperatures in a programmable thermal controller, followed by processing to glycerine. More details on preparing nematode specimens and permanent slides, including recipes for fixatives, can be found in van Bezooijen (2006), which is freely available on the Internet.

4.1.2 Key to species level

The following key, partly derived from Bongers (1989), is used to determine the subfamily of female specimens. The key within the subfamily Parasitaphelenchinae to determine the genus *Bursaphelenchus* is adapted from Hunt (2008). The key within the genus *Bursaphelenchus* for the *xylophilus* group is cited from Braasch *et al.* (2009). Alternatively, a simple key, which has been established by consensus in the European and Mediterranean Plant Protection Organization (EPPO) region and is widely used, is available in the EPPO diagnostic protocol for *B. xylophilus* (EPPO, 2013b).

Definitions of terminology used in the following sections can be found in EPPO's *Diagnostic protocols for regulated pests: Pictorial glossary of morphological terms in nematology* (EPPO, 2013a).

4.1.2.1 Key to families or subfamilies

1. Nematode with spear or stylet **2**

– Nematode without spear or stylet ..... **NBS**

2. Mouth with tylenchid stylet, pharynx with metacorpus **3**

– Mouth with dorylaimid stylet, pharynx cylindrical or bottle-shaped, without metacorpus ..... **NBS**

3. Metacorpus with metacorpal plates **4**

– Metacorpus without conspicuous metacorpal plates ..... **NBS**

4. Procorpus clearly separated from metacorpus by a constriction **5**

– Procorpus and metacorpus not separated by a constriction, basal bulb strongly reduced, cuticle conspicuously annulated ..... **NBS**

5. One gonad (vulva posterior) **6**

– Two gonads ..... **NBS**

6. Lip region without setae **7**

– Lip region with setae ..... **NBS**

7. Metacorpus strongly muscular and conspicuously well developed, clearly visible at low magnification, ovoid to rounded rectangular, dorsal pharyngeal gland opens into lumen of pharynx within metacorpus **8**

– Metacorpus normal, dorsal pharyngeal gland opens into lumen of pharynx just behind stylet ..... **NBS**

8. Pharyngeal glands overlap intestine dorsally **9**

– Pharyngeal glands within abutting bulb ..... **NBS**

9. Male tail tip enveloped by a small, bursa-like flap of cuticula (seen only when nematode is lying in the dorso-ventral position) **10**

– No bursa-like flap of cuticula ..... **NBS**

10. Stylet knobs usually present, female with anus ..... **Parasitaphelenchinae**

– Stylet knobs usually not present, female without anus ..... **NBS**

4.1.2.2 Key to subfamily Parasitaphelenchinae

11. In most species, J_{III} or J_{IV} dauer juveniles phoretically associated with insects; vulva posterior (usually 60–80% of body length); spicules partially fused or separated; male tail strongly recurved; bursa present in most species ..... ***Bursaphelenchus***

– J_{IV} dauer juveniles; vulva very posterior (80–90% of body length); spicules partially fused; male tail not strongly recurved; bursa present..... **NBS**

4.1.2.3 Key to genus **Bursaphelenchus**

12. Vulva with prominent flap; spicules long, slender and semicircular with angular lamina in posterior third, capitulum flattened with small condylus and distinct rostrum, cucullus usually present; lateral field with four lines ..... ***xylophilus* group**

– Characters different ..... **Not *xylophilus* group**

4.1.2.4 Key to **xylophilus** *group*

Within the *xylophilus* group the following key (amended according to EPPO (2013b, 2014)) can be used to distinguish *B. xylophilus* extracted from wood and bark from other *Bursaphelenchus* species of the same group. More details concerning the other species belonging to the *xylophilus* group can be found in Braasch and Schönfeld (2015). The *xylophilus* group also contains species that do not originate from coniferous wood (e.g. *B. populi*); these can be excluded simply by determining the species of the wood. Rearing nematodes on agar plates with fungi may increase the variability of the female tail.

13. Female tail broadly subcylindrical, with or without mucro (Figures 4 and 5) **14**

– Female tail conical (Figure 6) or strongly tapering, with or without mucro ..... **Not *B. xylophilus***

14. Spicule length <30 µm (measured from condylus to distal end)..... **15**

– Spicule length >30 µm ..... **Not *B. xylophilus***

15. Spicule with long and pointed rostrum, limbs of spicule with an angular curvature (Figures 5(C) and 7)..... **16**

– Spicule with short and pointed rostrum, limbs of spicule with a rounded curvature . **Not *B. xylophilus***

16. Female vulval flap straight, not ending in a deep depression (Figures 5(G) and 8)..... **17**

– Female vulval flap ending in a deep depression (Figure 9(A)) ..... **Not *B. xylophilus***

17. Female tail with mucro >3 µm (Figures 4(c) and 10(d))..... **18**

– Female tail without mucro (Figures 5(H) and 4(a)) and with or without a small projection <2 µm* (Figures 4(b) and 5(I)–(J))..... ***B. xylophilus* (round-tailed form)**

18. Excretory pore at or behind metacorpus..... ..... ***B. mucronatus kolymensis* and *B. xylophilus* (mucronated form**)**

– Excretory pore anterior to metacorpus ..... **Not *B. xylophilus***

NBS, not *Bursaphelenchus* species.

* In some populations of *B. fraudulentus*, females with a small projection or even without mucro may be found (Figure 9(B)). If the wood species where nematodes occur is not certain (*B. fraudulentus* occurs in deciduous wood but has also been found in larch, though not in pine) molecular testing is recommended.

** The mucronated form of *B. xylophilus* is mainly found in North America and molecular tests (Gu *et al.*, 2011) are recommended for a reliable separation of this form from the "'European type"' of *B. mucronatus*; that is, *B. mucronatus kolymensis* (Braasch *et al.*, 2011).

If the position of the excretory pore is not discernible, an identification based on morphological characters may be incorrect. In such cases, molecular tests should be performed.

B. xylophilus has the general characters of the genus *Bursaphelenchus* (Nickle, 1970; Hunt 2008): about 1 mm in length, slender; cephalic region high, offset by a constriction, and with six lips; stylet well developed, usually with small basal thickenings; metacorpus well developed (Figures 11 and 5(F)); male tail terminus strongly curved ventrally, conoid, with a small terminal bursa that can be seen in the dorso-ventral position (Figure 12); spicules robust, rose thorn-shaped, usually with a prominent apex and rostrum; gubernaculum absent (Figures 7 and 10); vulva 70–80% of the body length; post-uterine sac well developed (Figure 5(A)).

Most populations of *B. xylophilus* are round-tailed and can be distinguished from other *Bursaphelenchus* species by the presence of the following three characters (Figure 10). (1) Males of *B. xylophilus* (Figure 7) have relatively large spicules, evenly arcuate, with a sharply pointed prominent rostrum and cucullus (disc-like projection) at the distal ends of the spicules. (2) The tail of the females is subcylindrical with a broadly rounded terminus (Figure 4(a)), normally without a mucro (small projection), but occasionally females of round-tailed populations have a mucro on their tail terminus, which is usually less than 2 μm (Figure 4(b)). (3) The vulva has a long, overlapping anterior lip (Figure 8).

However, females of the mucronate populations generally have a mucro (1.5–4.2 μm) at the tail terminus (Figure 4(c)).

Characters best seen by scanning electron microscopy are four incisures (Figure 13) in the lateral field, and the number and position of caudal papillae in males (Figure 14): an adanal pair just before the anus, two post-anal pairs just before the origin of the bursa, and a single median papilla just preanal. These characters sometimes can barely be seen by light microscopy. Figures 13 and 14 are electron micrographs illustrating these two characters as they are cited in section 4.1.3 for grouping *Bursaphelenchus* species in the *xylophilus* group.

Measurements of morphological characters of *B. xylophilus* are given in Table 1.

Table 1. Measurements (mean, and range in parentheses) of *Bursaphelenchus xylophilus* characters

Males					
Author Character	Nickle *et al.* (1981) (*n* = 5) (United States)†	Mamiya and Kiyohara (1972) (*n* = 40) (Japan)†	Mota *et al.* (1999) (*n* = 12) (Portugal)†	Penas *et al.* (2008) (*n* = 20) (Portugal)†	Penas *et al.* (2008) (*n* = 20) (Portugal)‡
Length (L), mm	0.56 (0.52–0.60)	0.73 (0.59–0.82)	1.03 (0.80–1.30)	0.57 (0.45–0.69)	1.04 (0.87–1.17)
a (body length / greatest body diameter)	40.8 (35–45)	42.3 (36–47)	49.4 (44–56)	46.0 (40.2–58.5)	45.7 (41.3–48.9)
b (body length / distance from anterior to pharyngo-intestinal valve)	9.4 (8.4–10.5)	9.4 (7.6–11.3)	13.3 (11.1–14.9)	9.6 (8.2–10.7)	13.7 (11.6–15.4)
c (body length /	24.4 (21–29)	26.4 (21–31)	28.0 (24–32)	21.6 (19.1–24.6)	26.8 (23.6–31.4)

Males					
Author Character	Nickle *et al.* (1981) (*n* = 5) (United States)†	Mamiya and Kiyohara (1972) (*n* = 40) (Japan)†	Mota *et al.* (1999) (*n* = 12) (Portugal)†	Penas *et al.* (2008) (*n* = 20) (Portugal)†	Penas *et al.* (2008) (*n* = 20) (Portugal)‡
tail length)					
Stylet, µm	13.3 (12.6–13.8)	14.9 (14–17)	12.6 (11–16)	11.0 (10–14)	14.0 (12–15)
Spicules, µm	21.2 (18.8–23.0)	27.0 (25–30)	24 (22–25)	19.3 (16.5–24.0)	30.4 (25.0–33.5)

Females					
Author Character	Nickle *et al.* (1981) (*n* = 5) (United States)†	Mamiya and Kiyohara (1972) (*n* = 30) (Japan)†	Mota *et al.* (1999) (*n* = 12) (Portugal) †	Penas *et al.* (2008) (*n* = 20) (Portugal) †	Penas *et al.* (2008) (*n* = 20) (Portugal)‡
Length (L), mm	0.52 (0.45–0.61)	0.81 (0.71–1.01)	1.05 (0.89–1.29)	0.58 (0.51–0.66)	1.13 (0.91–1.31)
a (body length / greatest body diameter)	42.6 (37–48)	40.0 (33–46)	50.0 (41–58)	41.9 (32.8–50.6)	45.6 (39.4–50.3)
b (body length / distance from anterior to pharyngo-intestinal valve)	9.6 (8.3–10.5)	10.3 (9.4–12.8)	13.8 (12.7–16.4)	10.1 (9.1–11.2)	14.7 (11.6–16.8)
c (body length / tail length)	27.2 (23–31)	26.0 (23–32)	26.6 (22–32)	25.4 (20.2–29.0)	28.1 (21.9–34.4)
Stylet, µm	12.8 (12.6–13.0)	15.9 (14–18)	12.3 (11–15)	11.2 (10.0–12.5)	14.4 (12–16)
Vulva position (V), % of L	74.7 (73–78)	72.7 (67–78)	73.3 (70–76)	71.5 (70.1–72.9)	72.6 (70.4–74.5)

† Nematodes after extraction from natural host substrate.

‡ Nematodes grown on fungal culture for one year.

4.1.3 Comparison of *Bursaphelenchus xylophilus* with similar species

Keys for the determination of *Bursaphelenchus* species are available (e.g. Ryss *et al.*, 2005), but both of those in Ryss et al. (2005) suffer from the disadvantage that early descriptions of *Bursaphelenchus* species are incomplete or based on few specimens. See Vieira *et al.* (2003) for the original descriptions of 74 *Bursaphelenchus* species.

B. xylophilus is one species of the *xylophilus* group *sensu* Braasch (2001). Although there is current debate among taxonomists on the number of species within this group, at least 15 species or subspecies (as at April 2015) belong to the *xylophilus* group based on the number of lateral lines (Figure 9), the number and position of caudal papillae and spicule characteristics, and the large vulval flap (Gu *et al*., 2005; Ryss *et al*., 2005; Braasch *et al*., 2009; Braasch and Schönfeld, 2015). At least two *Bursaphelenchus* species (*B. trypophloei* Tomalak & Filipiak, 2011 and *B. masseyi* Tomalak, Worrall & Filipiak, 2013) were recently proposed to be added to the *xylophilus* group; however, this protocol follows the last grouping of Braasch and Schönfeld (2015), who did not consider these species to be valid members of the group because of their spicule morphology. Therefore, the members of the *xylophilus* group are:

- *B. xylophilus* (Steiner & Buhrer, 1934) Nickle, 1970
- *B. fraudulentus* Rühm, 1956 (Goodey, 1960)
- *B. mucronatus mucronatus* (Mamiya & Enda, 1979) Braasch, Gu & Burgermeister, 2011
- *B. mucronatus kolymensis*, Braasch, Gu & Burgermeister, 2011
- *B. conicaudatus* Kanzaki, Tsuda & Futai, 2000
- *B. baujardi* Walia, Negi, Bajaj & Kalia, 2003
- *B. luxuriosae* Kanzaki & Futai, 2003
- *B. doui* Braasch, Gu, Burgermeister & Zhang, 2004
- *B. singaporensis* Gu, Zhang, Braasch & Burgermeister, 2005
- *B. macromucronatus* Gu, Zheng, Braasch & Burgermeister, 2008
- *B. populi* Tomalak & Filipiak, 2010
- *B. paraluxuriosae* Gu, Wang & Braasch, 2012
- *B. firmae* Kanzaki, Maehara, Aikawa & Matsumato, 2012
- *B. koreanus* Gu, Wang & Chen, 2013
- *B. gillanii* Schönfeld, Braasch, Riedel & Gu, 2013

B. xylophilus can be separated into two forms or populations: round-tailed and mucronated (Gu *et al.*, 2011) (Figure 4). Mucronated populations are mainly found in North America and are very similar to *B. mucronatus kolymensis*.

The 15 species or subspecies of the *xylophilus* group can be distinguished from all other *Bursaphelenchus* species by the shape of the male spicules and by the presence in the female of a vulval flap with a characteristic shape. To separate *B. xylophilus* from the 14 other species in the group, the female tail shape (subcylindrical to cylindrical with a normally round terminus, and absence of a mucro) can be used. A detailed key to all species of the *xylophilus* group, including drawings of the main characters, can be found in Braasch and Schönfeld (2015). All other species of the *xylophilus* group have either a conical or a mucronate female tail. However, a few mucronate populations of *B. xylophilus* exist in North America and are difficult to differentiate morphologically from other mucronate species (Figure 4). In addition, *B. xylophilus* females from laboratory cultures normally show a typical round tail terminus, whereas strains obtained from infested or artificially inoculated trees may contain females with mucros of variable length beside round-tailed females (Figure 4). More details on this subject can be found in Gu *et al.* (2011).

The most widespread species in the *xylophilus* group are *B. mucronatus mucronatus* and *B. mucronatus kolymensis*. They are distributed throughout Europe and Asia and also occur in Canada

(Ryss *et al*., 2005). Therefore, it is probable that the most frequent differentiation will be between *B. xylophilus* and *B. mucronatus mucronatus* or *B. mucronatus kolymensis* (Figures 6 and 10).

Reference cultures of 50 *Bursaphelenchus* species, including 41 *B. xylophilus* strains from different origins across the world, are available in the *Bursaphelenchus* culture collection at the Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for National and International Plant Health, Braunschweig, Germany.

4.2 Molecular identification

This section provides information on molecular tests that allow the identification of *B. xylophilus* from isolated nematodes. The tests are generally performed following a morphological examination in order to confirm the results obtained. In the following subsections different types of tests are presented that address specific issues, as described at the beginning of each section.

Many methods are available for the identification of *B. xylophilus*. The molecular tests described hereafter are those recommended at the time of drafting the protocol. Other tests may be performed. Molecular identification can be performed by conventional PCR (section 4.2.2) or by real-time PCR (section 4.2.3) methods. All these techniques, particularly internal transcribed spacer (ITS)-restriction fragment length polymorphism (RFLP) (section 4.2.1), have been used successfully in laboratories throughout the world, but have not, so far, been evaluated by a ring test. A loop-mediated isothermal amplification (LAMP) test (section 4.2.5) was developed for direct detection and identification of the target nematode from wood.

The most recent approach for molecular identification relies on sequencing and barcoding analysis (section 4.2.8). This approach requires access to sequencing facilities and to reliable sequences (such as those found in Q-bank, (http://www.q-bank.eu/Nematodes/) as well as highly skilled staff to analyse the sequences in such a way as to avoid false results.

When molecular techniques are used to detect *B. xylophilus* in wood products for quarantine purposes, it is critical to distinguish between living and dead nematodes. Several phytosanitary treatments kill *B. xylophilus* in wood, and current DNA-based detection methods are unable to differentiate whether a positive result is due to living nematodes or DNA remnants of dead nematodes. The use of molecular methods based on RNA that can distinguish between living and dead nematodes present in wood is preferable for questions of quarantine regulation (Leal *et al*., 2013) (section 4.2.4). This problem needs to be taken into account when choosing the nematode extraction method (e.g. the Baermann funnel technique relies on living nematodes; see sections 3.4 and 3.5) and the molecular technique for determination. Whenever possible, a positive molecular result should be validated by morphological identification.

In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. 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. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

4.2.1 ITS-PCR RFLP

Burgermeister *et al*. (2005, 2009) used a PCR-based ITS-RFLP technique for differentiating *B. xylophilus* from 43 other *Bursaphelenchus* species. Almost all descriptions of new *Bursaphelenchus* species published after 2009 contain the ITS-PCR RFLP patterns on the basis of the method developed by Burgermeister *et al*. (2009). Of all the molecular techniques in this protocol, this is the one that has been shown to be effective for the widest range of *Bursaphelenchus* species.

DNA is extracted from mixed life stages of nematodes (adult females and males, juveniles) using the QIAamp DNA Micro Kit (Qiagen[1]). Nematode samples (1 to 30 specimens) are placed in 5 µl water in Eppendorf[1] tubes and frozen at –20 °C until extraction. Before extraction, the sample is thawed, mixed with 10 µl ATL buffer (Qiagen[1]) and homogenized in the Eppendorf[1] tube using a micropestle (Eppendorf[1]). Then the DNA extraction process is conducted according to the manufacturer's recommendations (QIAamp DNA Micro Kit Handbook, Qiagen: "Isolation of genomic DNA from tissues"[1]), except for the following steps. For step 4, the incubation lasts 3 h. For step 12 (elution), 20 µl (for single nematode extraction) to 100 µl (for extraction of up to 30 nematodes) of AE buffer (Qiagen[1]) is applied to the membrane. The eluate containing extracted DNA is stored at –20 °C until use.

ITS-PCR RFLP analysis is carried out by performing PCR on the extracted DNA followed by RFLP on the PCR product. A segment of nematode ribosomal (r)DNA containing the ITS regions ITS1 and ITS2 is amplified by PCR using the following primer pair:

ITS1-forward (F): 5′-CGT AAC AAG GTA GCT GTA G-3′ (Ferris *et al*., 1993)

ITS2-reverse (R): 5′-TTT CAC TCG CCG TTA CTA AGG-3′ (Vrain, 1993)

The PCR mixture (50 µl) contains 0.6 µM of each primer, 2 U Taq DNA polymerase (Stratagene[1] or Fermentas[1]), 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM dNTPs and 2 ng DNA template. Amplification is carried out using a thermal cycler, with the following cycling parameters: denaturation at 94 °C for 2.5 min, 40 reaction cycles of (94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min) and a final extension at 72 °C for 5 min. After completion of the PCR, 5 µl aliquots of the PCR product are analysed by gel electrophoresis. Suitable aliquots of the amplified DNA are digested with 3 U restriction endonucleases *Alu*I, *Hae*III, *Hinf*I, *Msp*I and *Rsa*I, following the manufacturer's instructions.

B. xylophilus is identified on the basis of the species-specific DNA restriction fragment patterns (Figure 15). Numbers and sizes of DNA restriction fragments at least for the following *Bursaphelenchus* species have been described (Gu, 2014): *B. abietinus*, *B. abruptus*, *B. africanus*, *B. anamurius*, *B. andrassyi*, *B. antoniae*, *B. arthuri*, *B. arthuroides*, *B. braaschae*, *B. burgermeisteri*, *B. chengi*, *B. conicaudatus*, *B. corneolus*, *B. doui*, *B. eggersi*, *B. eremus*, *B. fraudulentus*, *B. fuchsi*, *B. fungivorus*, *B. gerberae*, *B. gillanii*, *B. hellenicus*, *B. hildegardae*, *B. hofmanni*, *B. hylobianum*, *B. koreanus*, *B. leoni*, *B. luxuriosae*, *B. macromucronatus*, *B. masseyi*, *B. mucronatus mucronatus* (previously *B. mucronatus* East Asian type), *B. mucronatus kolymensis* (previously *B. mucronatus* European type), *B. obeche*, *B. paraburgeri*, *B. paracorneols*, *B. paraluxoriosae*, *B. paraparvispicularis*, *B. parathailandae*, *B. parvispicularis*, *B. pinasteri*, *B. pinophilus*, *B. poligraphi*, *B. populi*, *B. posterovolvus*, *B. rainulfi*, *B. seani*, *B. sexdentati*, *B. silvestris*, *B. sinensis*, *B. singporensis*, *B. thailandae*, *B. tusciae*, *B. vallesianus*, *B. willibaldi*, *B. xylophilus*, *B. yongensis* and *B. yuyaoensis*.

B. hunanensis and *B. lini* are proposed to be regrouped and therefore no longer belong to the genus *Bursaphelenchus*. Burgermeister *et al*. (2009) give a comprehensive summary of the patterns and ITS-RFLP DNA fragment sizes for 44 *Bursaphelenchus* species. An example of species differentiation by ITS-RFLP restriction fragment patterns for *B. xylophilus*, *B. mucronatus mucronatus* and *B. mucronatus kolymensis* isolates is provided in Table 2.

[1] In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and/or reproducibility achieved. 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. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

Table 2. Restriction fragment length polymorphism (RFLP) patterns of *Bursaphelenchus* species

Species	PCR product (base pairs)	Restriction fragments (base pairs) produced by restriction enzyme				
		***Rsa*I**	***Hae*III**	***Msp*I**	***Hinf*I**	***Alu*I**
B. mucronatus East Asian type = *B. mucronatus mucronatus*	920	486 412 12	621 299	355 302 263	408 232 121 86 49 24	674 246
B. mucronatus European type = *B. mucronatus kolymensis*	925	413 263 227 22	625 195 105	356 303 266	412 232 121 87 49 24	678 247
B. xylophilus	925	483 420 22	728 197	562 363	263 232 142 139 125 24	433 256 142 96

Source: Burgermeister *et al.* (2009).

4.2.2 Conventional PCR

The following PCR tests allow the species-specific identification of *B. xylophilus* but will not determine whether any other *Bursaphelenchus* species are present.

4.2.2.1 Conventional PCR targeting ITS rDNA

A species-specific method to identify *B. xylophilus* targeting the ITS1–ITS2 region of rDNA was described by Matsunaga and Togashi (2004). This method was evaluated against five and four Japanese populations of *B. xylophilus* and *B. mucronatus*, respectively. The experimental protocol is as follows.

Nematodes are individually placed in 5 µl lysis buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.2), 2.5 mM $MgCl_2$, 0.45% (w/v) Nonidet P-40, 0.45% (w/v) Tween 20, 0.01% (w/v) gelatin and 0.06 mg/ml proteinase-K) in 0.2 ml MicroAmp reaction tubes (Applied Biosystems[1]) and placed at −70 °C or below for 10 min (DNA extraction adapted from Barstead *et al*., 1991). After thawing at room temperature, the DNA solution is heated at 60 °C for 1 h and then at 95 °C for 15 min. The resulting crude DNA extract is used as a template in a specific PCR. PCR is performed using the following primer pair:

X-F: 5′-ACG ATG ATG CGA TTG GTG AC-3′

X-R: 5′-TAT TGG TCG CGG AAC AAA CC-3′

PCR is carried out in a 10 µl reaction mixture containing the previously prepared template DNA (5 µl crude DNA extract), 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM $MgCl_2$, 0.001% gelatin, 200 µM each dNTP, 5 pmol each primer and 0.25 U Taq DNA polymerase (AmpliTaq Gold, Applied Biosystems[1])

using a Perkin Elmer GeneAmp PCR System 9600 thermal cycler (Applied Biosystems[1]). After denaturation at 94 °C for 5 min, cycling is performed for 35 cycles of (94 °C for 30 s, 55.9 °C for 30 s and 72 °C for 1 min), with a final extension at 72 °C for 6 min.

This reaction produces a DNA amplicon of 557 base pairs (bp) from all *B. xylophilus* isolates tested.

4.2.2.2 Conventional PCR targeting satellite DNA

A species-specific method to identify *B. xylophilus* using a satellite DNA-based PCR technology was described by Castagnone *et al.* (2005). Its specificity was evaluated against non-target *Bursaphelenchus* species (*B. leoni*, *B. mucronatus* and *B. tusciae*) as well as one Japanese and two Canadian populations of *B. xylophilus.*

Amplification is performed on individual nematodes, prepared according to a PCR procedure modified from Williams *et al.* (1992). Briefly, single nematodes are transferred to a PCR tube and covered with 2.5 µl lysis buffer (50 mM KCl, 10 mM Tris (pH 8.2), 2.5 mM $MgCl_2$, 60 mg/ml proteinase-K, 0.45% Nonidet P-40, 0.45% Tween 20 and 0.01% gelatin). Tubes are placed at –80 °C for 45 min, and immediately transferred to 60 °C for 60 min and then 95 °C for 15 min in a thermal cycler. The resulting crude DNA extract is used as a template in a specific PCR.

PCR primers used in the reaction are designed close to both ends of the sequence of the 160 bp monomer of the satellite DNA family previously characterized in *B. xylophilus* (Tarès *et al.*, 1993; GenBank accession number L09652):

J10-1: 5′-GGT GTC TAG TAT AAT ATC AGA G-3′

J10-2Rc: 5′-GTG AAT TAG TGA CGA CGG AGT G-3′

PCR is carried out in a 25 µl reaction mixture containing the previously prepared template DNA (5 µl crude DNA extract), 50 mM KCl, 10 mM Tris (pH 8.2), 2.5 mM $MgCl_2$, 200 µM each dNTP, 250 ng each primer and 1 U Taq DNA polymerase (QBiogene[1]). After denaturation at 94 °C for 5 min, cycling is performed for 25 cycles of (94 °C for 30 s, 64 °C for 1 min and 72 °C for 1 min), with a final extension at 72 °C for 5 min.

Because the satellite DNA family has been shown to be constituted of repeats organized in tandem arrays (Tarès *et al.*, 1993), the amplification of a ladder of multimers of the 160 bp monomer is obtained after a PCR containing *B. xylophilus* DNA as template. Conversely, in the case of other *Bursaphelenchus* species, no amplification is detected, which provides a simple and reliable result of either clearly positive or clearly negative for *B. xylophilus* (Castagnone *et al.*, 2005).

4.2.3 Real-time PCR

Real-time PCR tests can be performed for specific identification of *B. xylophilus*. This type of test is generally more sensitive and less time-consuming than the conventional PCR techniques described in sections 4.2.1 and 4.2.2.

4.2.3.1 Real-time PCR targeting satellite DNA sequences

A species-specific method to identify *B. xylophilus* using satellite DNA sequences was described by François *et al*. (2007). This method is highly sensitive, detecting as little as 1 pg genomic DNA and single nematodes in mixed samples in which *B. xylophilus* was associated with the closely related species *B. mucronatus*, up to the limit of 0.01% and 1% of the mixture, respectively. This method also detected *B. xylophilus* directly from 100 mg wood.

DNA is extracted from isolated nematodes originating from pure cultures using a simplified procedure, as previously described (Castagnone *et al.*, 2005), with a slight modification: the volume of the lysis buffer used is not constant but adapted to the number of nematodes (i.e. 3 µl for one to four nematodes and 20 µl for a larger number of nematodes).

DNA extraction from *B. xylophilus*-infested wood is performed using a ChargeSwitch genomic DNA Plant Kit (Invitrogen[1]). Approximately 0.1 g infested wood is cut into small pieces and placed in a plastic bag with 5 ml CST Lysis Buffer containing 1% polyvinylpyrrolidone and 20 mM calcium chloride. The sample is lightly disrupted using a hammer, then 1 ml lysate is removed and processed according to the manufacturer's instructions. Briefly, 100 µl sodium dodecyl sulphate is added to the lysate after which it is incubated at room temperature for 5 min, then 400 µl precipitation buffer is added and it is centrifuged at maximum speed (approximately 18 000 *g*) for 5 min. Approximately 1 ml supernatant is removed, and 100 µl CST detergent and 40 µl CST beads are added to the supernatant. A PickPen 8-M (Bio-Nobile[1]) is used to transfer the CST beads and bound DNA through two washing steps (each with 1 ml CST Wash Buffer) and into 150 µl CST Elution Buffer in a 2.2 ml deep-well plate. The magnetic particles are then removed. The DNA is either tested immediately or stored at –20 °C for future analysis.

The primers and TaqMan probe used in this method are:

BsatF: 5′-TGA CGG AGT GAA TTG ACA AGA CA-3′

BSatRV: 5′-AAG CTG AAA CTT GCC ATG CTA AA-3′

Fluorogenic TaqMan probe BSatS: 5′-FAM-ACA CCA TTC GAA AGC TAA TCG CCT GAG A-TAMRA-3′

PCR is carried out in a total volume of 25 µl containing 1 µl genomic DNA. Each reaction contains 2.5 µl of 10× reaction buffer (qPCR Core Kit, Eurogentec[1]), 5 mM $MgCl_2$, 200 µM each dNTP, 0.5 U Taq polymerase (qPCR Core Kit[1]) and 200 nM each primer and probe. Real-time PCR tests are performed in a DNA Engine Opticon 2 thermal cycler (MJ Research[1]). Cycling parameters are 95 °C for 10 min, followed by 30 cycles of (95 °C for 15 s and 59 °C for 30 s). Data are analysed using the Opticon 2 Monitor software version 3.1[1] according to the manufacturer's instructions. Extracts are tested undiluted and diluted 1:10 in nuclease-free water.

Real-time PCR testing of wood extracts is performed on a SmartCycler II (Cepheid[1]). Each reaction consists of 0.025 U/µl Hot Taq (Biogene[1]), 1× PCR buffer, 0.2 mM each dNTP, 5.5 mM $MgCl_2$, 5% trehalose (w/v), 300 nM each primer and 100 nM probe. Cycling conditions are 95 °C for 10 min, followed by 40 two-step cycles of (95 °C for 15 s and 60 °C for 1 min). Data are analysed using the default threshold setting of the SmartCycler II software[1] (30 fluorescence units). Extracts are tested undiluted and diluted 1:10 in nuclease-free water.

4.2.3.2 Real-time PCR test targeting a **hsp70** *gene sequence*

A real-time PCR method based on a heat shock protein gene (*hsp70*) was developed by Leal *et al.* (2007). This method was shown to be specific for *B. xylophilus* (it was tested on five isolates of *B. xylophilus*), with no amplification observed for seven non-target *Bursaphelenchus* species. This *hsp70* PCR is sensitive enough to detect at least 0.005 ng *B. xylophilus* genomic DNA, as well as DNA extracted from single nematodes.

For DNA extraction, the method of Burgermeister *et al.* (2005) is used with the following changes: (1) incubation of sample homogenate is at 56 ºC overnight instead of for 3 h; (2) carrier RNA is used only when DNA is extracted from single nematodes; (3) elution buffer (10 mM Tris-HCl, pH 8.0) is applied to the membrane of the mini-column and incubated for 5 min before centrifugation to elute the sample DNA; (4) DNA extracts are heated at 55 ºC for 5 min to remove any residual ethanol that could later affect the measurement of DNA quantity and quality and PCR amplification; and (5) samples are eluted in 30 µl (for single nematodes) and 50 µl (for samples containing more than one nematode).

The primers and TaqMan probe used in this method are (lower case letters indicate the locked nucleic acids):

BxLNAF: 5′-TAA GAT GTc TTT tAc AGA TGc CAA G-3′

BxLNAR: 5′-GCc TGG ACG AcC TTG AAT-3′

Dual-labelled TaqMan probe BxLNAP: 5′-FAM-AtT GgC CGC AAA TtC GaT GAa CC-IAblkFQ-3′

PCR is carried out in a 20 µl reaction volume containing 5 µl template, 50 mM Tris (pH 8.3), 0.25 mg/ml non-acetylated bovine serum albumin (BSA) (Sigma[1]), 0.1 µM probe, 0.7 µM forward primer, 0.5 µM reverse primer, 0.4 mM each dNTP (Roche[1]), 5.0 mM $MgCl_2$ and 1.0 U FastStart Taq DNA Polymerase (Roche[1]). Amplification is performed in the LightCycler 1.5 thermal cycler (Roche Diagnostics[1]), using the following parameters: initial denaturation and activation of the FastStart Taq DNA Polymerase (Roche Diagnostics[1]) at 95 ºC for 10 min, followed by 45 cycles of (denaturation at 94 ºC for 5 s, annealing at 62 ºC for 20 s and extension at 72 ºC for 10 s). Data are analysed using LightCycler version 3.5 software[1].

To confirm the quality of the purified nematode genomic DNA used in this test, amplification with the control primers ITS1-F and ITS2-R (primers described in section 4.2.1) is performed by conventional PCR. The 25 µl PCR reaction mixture consists of 5 µl template, 2.5 µl of 10× reaction buffer (50 mM Tris-HCl, 10 mM KCl, 5 mM $(NH_4)_2SO_4$; pH 8.3), 1.5 mM $MgCl_2$, 1 µM each primer, 1.6 µg BSA, 0.2 mM each dNTP and 1 U FastStart Taq DNA Polymerase (Roche[1]). The cycling parameters include an initial denaturation at 94 ºC for 5 min, followed by 40 cycles of (94 ºC for 1 min, 55 ºC for 1 min and 72 ºC for 2 min), with a final elongation at 72 ºC for 5 min.

4.2.4 RNA-based molecular tests for detection of living *Bursaphelenchus xylophilus*

The following tests detect only living nematodes. Options are given for conventional and real-time reverse transcription (RT)-PCR.

4.2.4.1 Conventional RT-PCR targeting a hsp70 *DNA sequence*

A conventional RT-PCR method for the detection of living *B. xylophilus* based on an *hsp70* gene sequence was described by Leal *et al*. (2013). In this test, the forward and reverse primers are placed on either side of the *hsp70* intron so that genomic DNA can be easily differentiated from cDNA by amplicon size. The specificity of the test was evaluated against six non-target *Bursaphelenchus* species and six isolates of *B. xylophilus*. The limit of detection of this test is 0.4 nematodes per reaction, measured in three of three replicates.

The RNA and genomic DNA are extracted from at least 20 nematodes. The simultaneous extraction of RNA and genomic DNA is performed using the AllPrep DNA/RNA Mini Kit (Qiagen[1]) following the manufacturer's protocol with the following modifications: nematode pellets that had been stored at –80 °C are ground using a Kontes Pellet Pestle (Kimble Chase Life Science and Research Products[1]), and 350 µl lysis buffer RLT (from the Qiagen[1] extraction kit) is added to each pellet containing the nematodes. The homogenization step is completed using QIAshredder Mini Spin Columns (Qiagen[1]). The RNA is eluted from a column using 20 µl RNase-free water and the DNA is eluted using 50 µl pre-warmed EB buffer (from the Qiagen[1] extraction kit). The eluate is allowed to sit on the column membrane for approximately 3 min to facilitate maximum elution with a single centrifugation.

B. xylophilus-specific primers used in this test are as follows, and the amplicon produced from cDNA template is 473 bp:

Hsp23F1: 5′-ACC CAA GTT TGA GTT GTA TTG TTT-3′

Hsp19R2: 5′-ACG GTA ACA ACG GCA TCC T-3′

The following control primers target the actin gene and can be included to ensure the test performs as expected when testing isolated genomic DNA. They produce an amplicon of 228 bp:

BxActF3: 5′-TCG TCA CCA ACT GGG ATG ATA-3′

BxActR3: 5′-CAC CAG TGG TAC GAC CG-3′

A two-step RT-PCR protocol is employed. The RT reaction is completed using the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics[1]) with the anchored-oligo(dT)18 primer protocol. For

cDNA synthesis, 12 µl RNA is used as starting material. The optional step suggested by the manufacturer of the kit to denature the RNA and the primers at 65 °C for 10 min is included, followed by immediate cooling on ice. After cDNA synthesis is complete, samples are stored at –20 °C for later use as template.

The 25 µl PCR reaction mixture contains 2 µl cDNA as template, 19 µl GoTaq Flexi PCR buffer (Promega[1]), 1.5 mM $MgCl_2$, 0.20 mM each dNTP (Roche Diagnostics[1]), 1.25 U GoTaq Flexi DNA Polymerase (Promega[1]) and 0.4 µM each primer (Hsp23F1 and Hsp19R2). Amplification is performed according to the following cycling parameters: initial denaturation at 95 °C for 5 min, followed by 35 cycles of amplification (denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 1 min) and a final elongation at 72 °C for 5 min. For the amplification with control primers, the 25 µl PCR reaction mixture is the same as above, except that 1 µl genomic DNA (40 ng/µl) and 1 µM each primer (BxActF3 and BxActR3) are used. Amplification is performed with the following cycling parameters: initial denaturation at 95 °C for 5 min, followed by 35 cycles of (95 °C for 30 s, 52 °C for 30 s and 72 °C for 1 min), with a final elongation at 72 °C for 5 min.

4.2.4.2 Real-time RT-PCR targeting a **hsp70** *cDNA sequence*

A SYBR Green real-time RT-PCR test to identify living *B. xylophilus* exclusively by detecting the presence of *hsp70* mRNA as a viability marker was described by Leal *et al.* (2013). This test detects the specific amplification of reverse transcribed *B. xylophilus hsp70* cDNA as the reverse primer binds across an exon–intron junction, thereby eliminating the amplification of genomic DNA. Its specificity was evaluated against six non-target *Bursaphelenchus* species and six isolates of *B. xylophilus.* The limit of detection of this test is 0.25 nematodes per reaction, measured in three of three replicates.

The protocol for the simultaneous extraction of RNA and genomic DNA is carried out as in the conventional PCR method (section 4.2.4.1).

Primers used in this test are:

HspexF3: 5′-AGA ACC ACT CCC TCG TAT GTC-3′

HspexR3: 5′-TCA AAC GCT TGG CAT CAA-3′

The following internal control primers may be included to ensure the test performs as expected:

BxActF3: 5′-TCG TCA CCA ACT GGG ATG ATA-3′

BxActR3: 5′-CAC CAG TGG TAC GAC CG-3′

A two-step RT-PCR protocol is used, and the cDNA synthesis is performed as for the conventional PCR method (section 4.2.4.1), with the exception that either the anchored-oligo(dT)18 primer or the sequence-specific primer (HspexR3) is used. After cDNA synthesis is complete, samples are stored at –20 °C for later use as template.

The 20 µl PCR reaction mixture is composed of 5 µl cDNA template (diluted 1:10 in 10 mM Tris, pH 8.0), 0.6 µM forward primer (HspexF3) and 0.4 µM reverse primer (HspexR3), and 4 µl of 5× LightCycler FastStart DNA MasterPLUS SYBR Green 1 Mix (Roche Diagnostics[1]). Real-time amplification is carried out in a LightCycler 2.0 (Roche Diagnostics[1]) using LightCycler version 4.1 software[1] with the following parameters: initial denaturation and activation at 95 °C for 10 min followed by 40 cycles of (95 °C for 15 s, 66 °C for 10 s and 72 °C for 15 s). For the amplification with control primers, the 20 µl PCR reaction mixture is the same as above, except that 0.5 µM each primer (BxActF3 and BxActR3) is used. Amplification is performed with the following cycling parameters: initial denaturation and activation at 95 °C for 10 min followed by 45 cycles of (95 °C for 15 s, 52 °C for 10 s and 72 °C for 15 s).

4.2.5 LAMP

A method for detecting *B. xylophilus* from wood samples by LAMP was described by Kikuchi *et al.* (2009). These authors developed the method to detect *B. xylophilus* faster and with higher sensitivity

than a TaqMan probe real-time PCR test also developed by their group. Specificity of the primers and the LAMP test was confirmed using DNA from non-target material: ten nematode species related to *B. xylophilus*, six non-target nematode genera, *P. thunbergii*, *P. densiflora* and *B. fuckeliana*. The sensitivity of the LAMP test was defined as ten copies of target gene (ITS) and as 2.5×10^{-5} of a nematode isolated from pure culture.

Wood samples (approximately 0.12 g wood in the experimental procedure) are incubated at 55 °C for 20 min in 800 µl extraction buffer, which contains proteinase-K and dithiothreitol supplied with the *B. xylophilus* detection kit (Nippon Gene[1]), followed by incubation at 95 °C for 10 min.

This method uses the following LAMP primers:

ITS(ID19) F3: 5′-GCA GAA ACG CCG ACT TGT-3′

ITS(ID19) B3: 5′-TCA TCC GAA CGT CCC TGA C-3′

ITS(ID19) FIP: 5′-CGC GGA ACA AAC CGC GTA AAA C-CG TTG TGA CAG TCG TCT C G-3′

ITS(ID19) BIP: 5′-AGA GGG CTT CGT GCT CGA TTGGCC GTT GAA ACA ACA TCA CC-3′

ITS(ID19) LF: 5′-AGA TGG TGC CTA ACA TTG CG-3′

The LAMP reaction is performed as described by Notomi *et al*. (2000) with the Loopamp DNA Amplification Kit (Eiken Chemical[1]). The 25 µl reaction mixture contains 2 µl extracted DNA, 5 pmol each F3 and B3 primers, 40 pmol each FIP and BIP primers, 20 pmol LF primer, 12.5 µl of 2× reaction mix, 1 µl Bst DNA polymerase and 1 µl fluorescent detection reagent (Eiken Chemical[1]). The reaction mixture is incubated at 63 °C for 60 to 120 min and terminated by incubation at 80 °C for 2 min. LAMP amplicons are detected by colour changes of the reaction solution under ultraviolet light.

Amplified products can be evaluated optionally with a probe-based detection system. The 5′-biotinylated form of the FIP primer is used for the LAMP reaction. After the LAMP reaction, 10 µl fluorescein isothiocyanate (FITC)-labelled probe (10 pmol/µl; 5′-GGC GAG AGG GCT TCG TGC TCG ATT GTC GTG C-3′) designed to hybridize to an internal region of the target sequence is added to the reaction mixture and incubated at 95 °C for 5 min, then slowly cooled to 25 °C. The reaction mixture is diluted with 100 µl running buffer (phosphate-buffered saline with 3% Tween) and applied directly to HybriDetect strips (Milenia Biotec[1]) according to the manufacturer's instructions. HybriDetect strips detect fragments containing both biotin and FITC resulting from specific amplification. In contrast, when non-specific amplification has occurred, no signal is observed at the test band line.

4.2.6 Controls for molecular tests

For the test result obtained to be considered reliable, appropriate controls – which will depend on the type of test used and the level of certainty required – should be considered for each series of nucleic acid isolation and amplification of the target pest nucleic acid. For molecular tests, a positive nucleic acid control, a negative amplification control (no template control) and, when relevant (e.g. direct detection of the nematode), an internal control are the minimum controls that should be used. For RT-PCR (conventional or real-time), a positive RT control should be included.

Positive nucleic acid control. This control is used to monitor whether or not the test performed as expected under the experimental conditions and parameters. A positive control can be any nucleic acid that contains the target sequence of the test; that is, *B. xylophilus* nucleic acid that has previously tested positive; a plasmid containing the cloned target sequence; *in vitro* transcribed RNA; a product from a previous amplification reaction; or synthetic double stranded (ds)DNA or a long oligonucleotide.

Negative amplification control (no template control). This control is necessary for PCR to rule out false positives due to contamination during preparation of the reaction mixture or non-specific amplification. PCR-grade water that was used to prepare the reaction mixture is added at the amplification stage.

Internal control. For conventional PCR, real-time PCR and LAMP, endogenous controls such as the ITS region, 18S rRNA, or β-actin or COX genes can be used to eliminate the possibility of PCR false negatives due to nucleic acid extraction failure or degradation or the presence of PCR inhibitors.

For RT-PCR, a no reverse transcriptase control should be included to verify that RNA samples are not contaminated with genomic DNA. This control contains all the RT-PCR reagents except the reverse transcriptase enzyme. In the absence of genomic DNA contamination, this control should generate no signal after amplification.

For RT-PCR, a positive reverse transcriptase control should be included to verify that the reverse transcriptase enzyme operates correctly. This control contains all the RT-PCR reagents and a RNA extract that includes the target sequence of the test (e.g. an RNA extract prepared by the laboratory and confirmed previously as positive). This control should generate a signal after amplification.

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

4.2.7 Interpretation of results from PCR

4.2.7.1 Conventional PCR

The pathogen-specific PCR test is considered valid only if:

- the positive control produces an amplification product of the expected size for the target nematode
- the negative extraction control and the negative amplification control do not produce an amplification product of the expected size for the target nematode.

If internal control primers are used, for simplex reactions, positive controls, as well as each of the test samples, should produce an amplification product of the expected size. For multiplex reactions, all negative samples should produce an amplification product of the expected size. In some cases positive samples for the nematode can also produce an amplification product of the expected size with the internal control primers.

The test on a sample will be considered positive if it produces an amplification product of the correct size.

4.2.7.2 Real-time PCR

The real-time-PCR is considered valid only if:

- the positive control produces an amplification curve with the target nematode-specific primers
- the negative controls do not produce an amplification curve.

If internal control primers are used, the positive control and each of the test samples should produce an amplification curve.

4.2.8 Sequencing

Several genomic regions have been directly sequenced from isolated nematodes (single for Wu *et al*. (2013) or bulk from cultures on fungus for Ye *et al.* (2007)) for the purpose of species identification of *B. xylophilus* and differentiation of different *Bursaphelenchus* species. These regions include internal transcribed spacers (ITS-1, ITS-2, 5.8S) of rDNA (Abelleira *et al*., 2011; Wu *et al*., 2013) or the D2–D3 region of the 28S rRNA gene (Ye *et al.*, 2007). The targeted region is amplified by PCR, and the amplicons are sequenced either directly or after they are cloned. Sequence data can then be analysed

using the Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) and compared with *Bursaphelenchus* sequences available in the NCBI database (e.g. accession numbers HQ646254 and KC460340 for the above-mentioned ITS region and AY508105 to AY508109 for the 28S rRNA region).

For the ITS gene, if the sample's pairwise sequence divergence compared with known *B. xylophilus* sequences is less than 2% but more than 2% with all other species, it is identified as *B. xylophilus*. For the 28S gene, if the sample's pairwise sequence divergence compared with known *B. xylophilus* sequences is less than 0.5% but more than 0.5% with all other species, it is identified as *B. xylophilus*. Any other results should be further investigated.

The Cytochrome Oxidase Subunit I COI region can also be used for species identification. Guidance on methodology and a reference sequence obtained from reference material (sequence Q38) is available at Q-bank (http://www.q-bank.eu/Nematodes/), including BLAST.

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 *B. xylophilus* 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:

- A sample of nematodes either mounted as a permanent slide, or fixed in TAF fixative or in a glycerine solution. For cases where *B. xylophilus* is found in an area for the first time, it would be helpful for further investigations of the pathway to establish a culture of living *B. xylophilus* multiplied on *B. cinerea.* Keeping specimens or DNA for molecular testing at a later stage may also be useful, even in the case of morphological identification.
- If the identification was based on molecular techniques, extracted DNA may be kept at –20 °C and extracted RNA at –80 °C.
- For cases of occurrence of *B. xylophilus* in wood or wood products, including wood packaging, instead of the geographical information on sampling, data concerning the origin, material (e.g. round wood, wood packaging) and import conditions (e.g. simultaneous occurrence of vector beetles) should be kept. Note that wood packaging is not necessarily of the same origin as the consignment. According to ISPM 15 (*Regulation of wood packaging material in international trade*), wood packaging in international trade should bear a mark in which the two first letters represent the ISO code of the country where the wood packaging was produced.

6. Contact Points for Further Information

Further information on this organism or this protocol can be obtained from:

Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Institute for National and International Plant Health, Messeweg 11-12, D-38104 Braunschweig, Germany (Thomas Schröder; e-mail: thomas.schroeder@jki.bund.de).

Technical Center, Ningbo Entry-Exit Inspection and Quarantine Bureau, No. 9 Mayuan Road, Ningbo, 315012 China (Jianfeng Gu; e-mail: jeffgu00@qq.com).

ANSES Plant Health Laboratory, 7 rue Jean Dixméras, 49044 Angers Cedex 01, France (Geraldine Anthoine; e-mail: geraldine.anthoine@anses.fr).

Canadian Forest Service, 506 West Burnside Road, Victoria, BC V8Z 1M5, Canada (Isabel Leal; e-mail: ileal@nrcan.gc.ca).

Canadian Food Inspection Agency, 3851 Fallowfield Road, Ottawa, ON K2H 8P9, Canada (Fencheng Sun; e-mail: sunfc@inspection.gc.ca).

In addition to the experts mentioned above, regional experts on this nematode are listed in Table 3.

Table 3. List of regional and national experts on *Bursaphelenchus xylophilus* (not exhaustive)

Region or country	Contact details of expert
Africa	Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa (Michael J. Wingfield; e-mail: mike.wingfield@fabi.up.ac.za)
Australia	CSIRO Ecosystem Sciences-Black Mountain Laboratories, Clunies Ross Street, Black Mountain, ACT 2601, Australia (Mike Hodda; e-mail: Mike.Hodda@csiro.au)
China	Department of Forest Protection, Nanjing Forestry University, No. 159 Longpan Road, Nanjing, 210037 China (Boguang Zhao; e-mail: 13505186675@126.com)
European Union	NemaLab-ICAM, Departamento Biologia, Universidade de Évora, 7002-554 Évora, Portugal (Manuel Mota; e-mail: mmota@uevora.pt)
Japan	Forest Pathology Laboratory, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan (Mitsuteru Akiba; e-mail: akiban@ffpri.affrc.go.jp)
Republic of Korea (South Korea)	Division of Forest Insect Pests and Disease, Korea Forest Research Institute, 207 Cheongnyangni 2-dong, Dongdaemun-gu, Seoul 130-712, Korea (ROK) (Hyerim Han; e-mail: hrhan@forest.go.kr)

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 diagnostic protocol was written by Thomas Schröder (JKI, Federal Research Centre for Cultivated Plants, Institute for National and International Plant Health, Germany (see preceding section)), Geraldine Anthoine (ANSES Plant Health Laboratory, France (see preceding section)), Isabel Leal (Canadian Forest Service, Canada (see preceding section)), Jianfeng Gu (Technical Center, Ningbo Entry-Exit Inspection and Quarantine Bureau, China (see preceding section)) and Fengcheng Sun (Canadian Food Inspection Agency, Canada (see preceding section)).

Vladimir Gaar (Diagnostic Laboratory, State Phytosanitary Administration, Czech Republic) and David McNamara (formerly EPPO) contributed to the protocol at an early stage.

The description of the ITS-RFLP technique was initially prepared by Wolfgang Burgermeister (Institut für Pflanzenvirologie, Mikrobiologie und Biologische Sicherheit, JKI, Germany). The initial description of the conventional PCR targeting satellite DNA method to identify *B. xylophilus* was provided by Philippe Castagnone-Sereno (UMR1064 INRA/UNSA/CNRS, Interactions Plantes-Microorganismes et Sante Vegetale, France).

The text of this diagnostic protocol is based partly on the EPPO diagnostic protocol for *B. xylophilus* (EPPO, 2001, 2013b).

8. References

The present annex may refer to international standards for phytosanitary measures (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

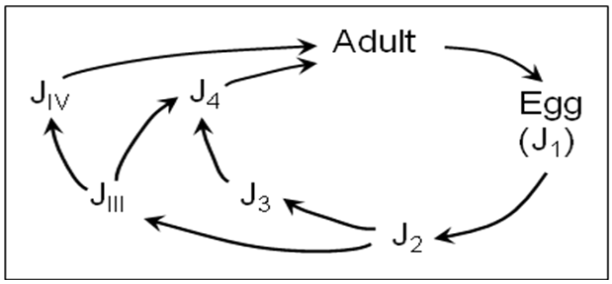

Figure 1. Life cycle of *Bursaphelenchus xylophilus* from egg to adult nematodes.
JX, juveniles of X-stage.
Source: Modified from Wingfield et al*. (1982).*

Figure 2. Evolution of symptoms of pine (*Pinus pinaster*) infested by *Bursaphelenchus xylophilus*, from healthy tree to dead.
Photos courtesy T. Schröder, Julius Kühn-Institut, Germany.

Figure 3. Symptoms of pine wilt disease on *Pinus pinaster* caused by *Bursaphelenchus xylophilus*.
Photo courtesy T. Schröder, Julius Kühn-Institut, Germany.

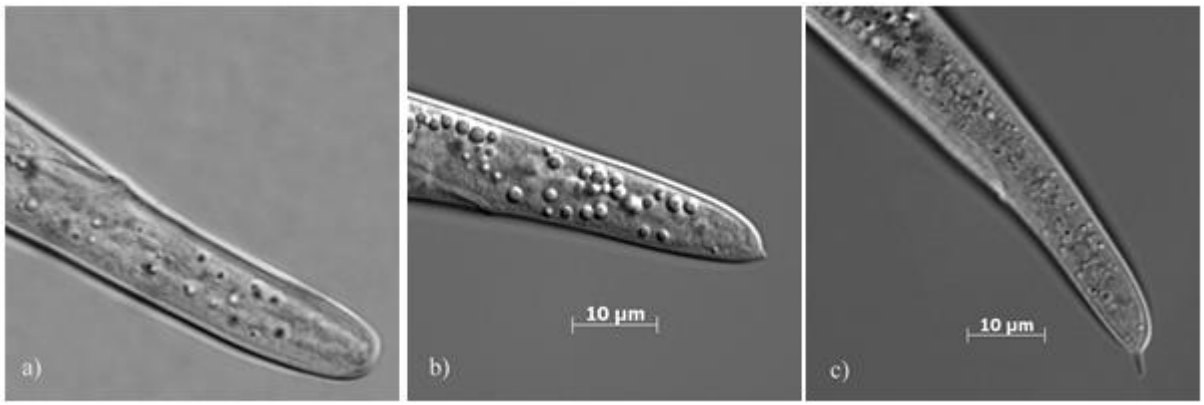

Figure 4. *Bursaphelenchus xylophilus* female tails: (a) round (×1 000 magnification); (b) with small projection; and (c) mucronate form.
Photos courtesy (a) T. Schröder, Julius Kühn-Institut, Germany and (b, c) J. Gu, Ningbo Entry-Exit Inspection and Quarantine Bureau, China.

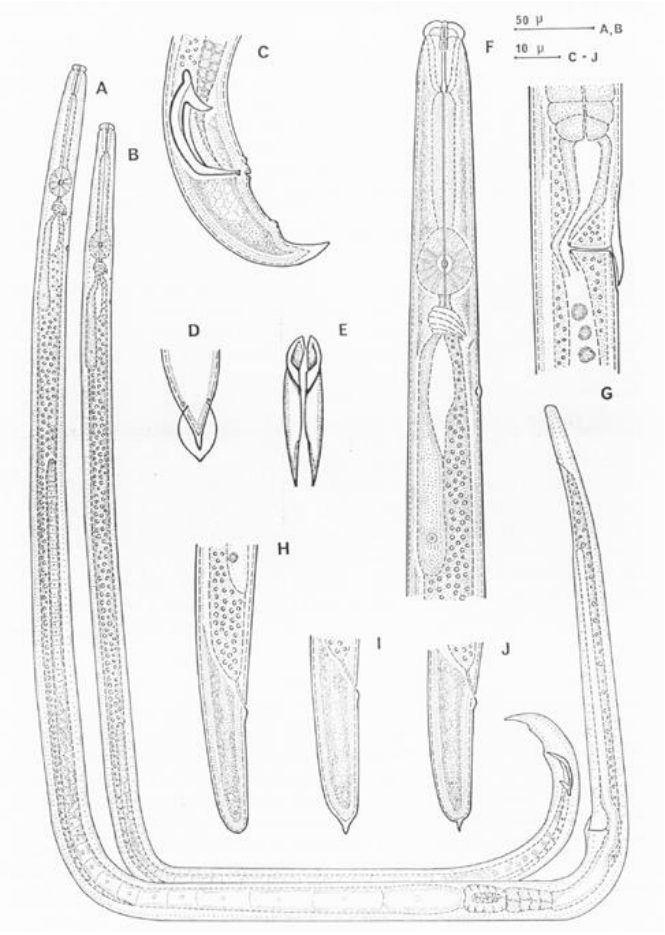

Figure 5. *Bursaphelenchus xylophilus*: (A) female; (B) male; (C) male tail; (D) ventral view of male tail, tip with bursa; (E) ventral view of spicules; (F) female, anterior portion; (G) female vulva; and (H), (I) and (J) female tail. *Source: Mamiya and Kiyohara (1972).*

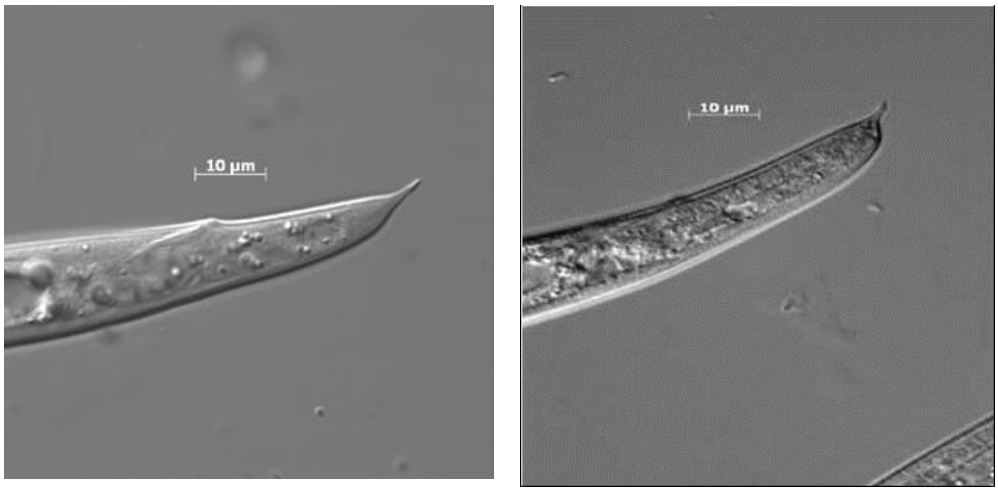

Figure 6. Female tail of *Bursaphelenchus mucronatus mucronatus* (left) and *B. mucronatus kolymensis* (right). *Photos courtesy J. Gu, Ningbo Entry-Exit Inspection and Quarantine Bureau, China.*

Figure 7. *Bursaphelenchus xylophilus* male tail with spicules (×1 000 magnification).
Photo courtesy T. Schröder, Julius Kühn-Institut, Germany.

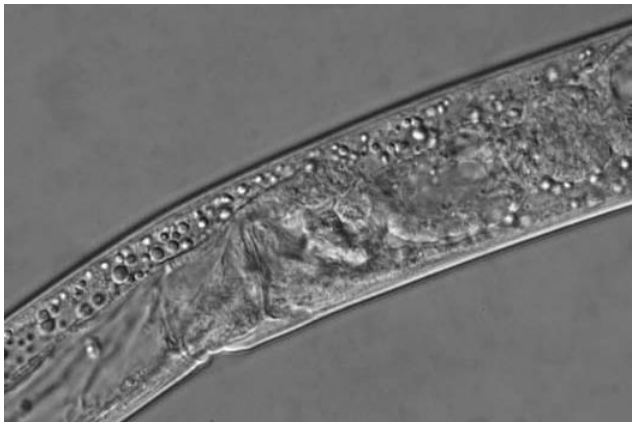

Figure 8. *Bursaphelenchus xylophilus* female with vulval flap (×640 magnification).
Photo courtesy T. Schröder, Julius Kühn-Institut, Germany.

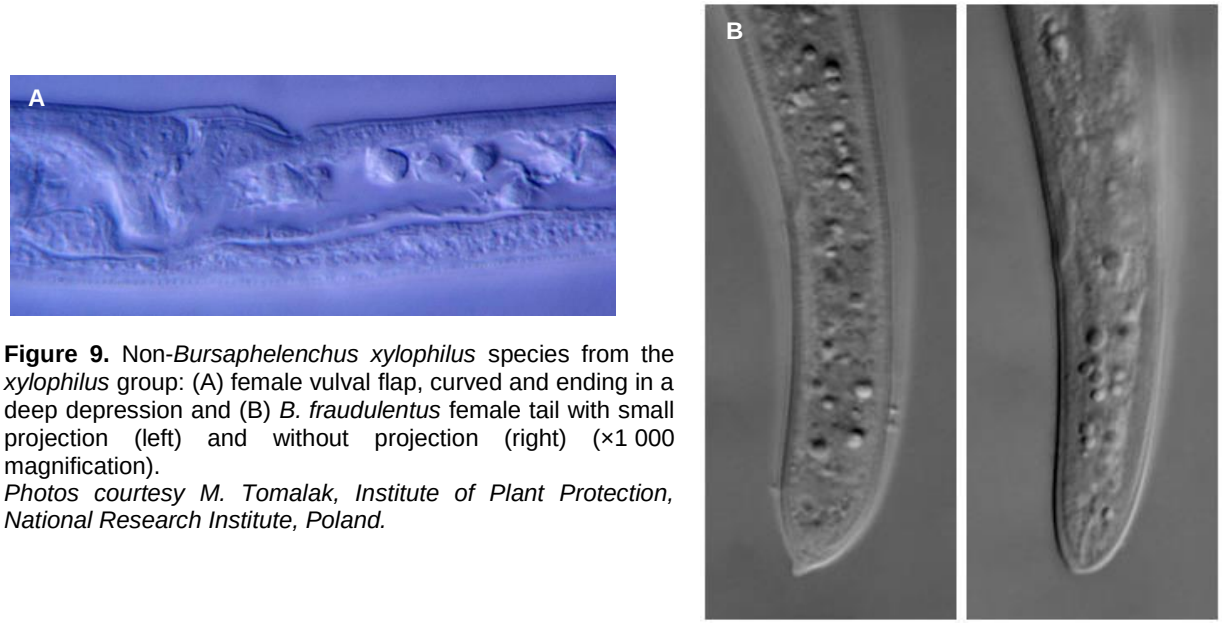

Figure 9. Non-*Bursaphelenchus xylophilus* species from the *xylophilus* group: (A) female vulval flap, curved and ending in a deep depression and (B) *B. fraudulentus* female tail with small projection (left) and without projection (right) (×1 000 magnification).
Photos courtesy M. Tomalak, Institute of Plant Protection, National Research Institute, Poland.

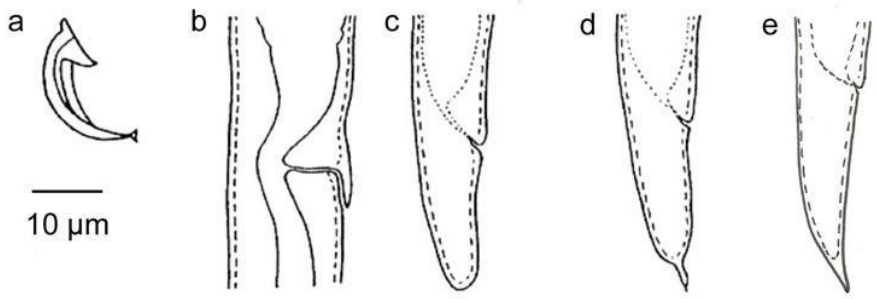

Figure 10. Diagnostic characters of *Bursaphelenchus xylophilus*, *B. mucronatus mucronatus* and *B. mucronatus kolymensis*: (a) spicules of all three species; (b) vulval flap of all three species; (c) female tail terminus of *B. xylophilus*, round form; (d) female tail terminus of *B. mucronatus kolymensis*; and (e) female tail terminus of *B. mucronatus mucronatus.*
Source: Modified from EPPO/CABI (1996).

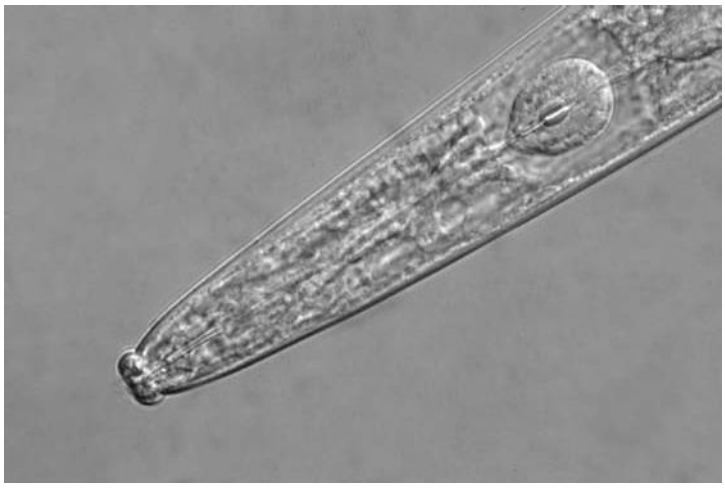

Figure 11. *Bursaphelenchus xylophilus* anterior region with stylet and metacorpus (×640 magnification).
Photo courtesy T. Schröder, Julius Kühn-Institut, Germany.

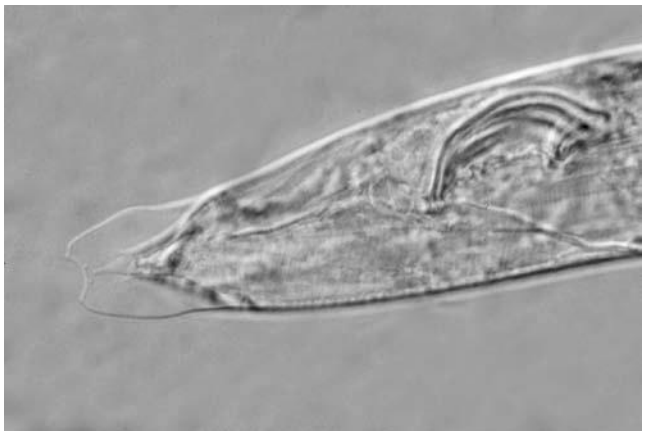

Figure 12. *Bursaphelenchus xylophilus* view of male tail in dorso-ventral position with bursa (×1 000 magnification).
Photo courtesy T. Schröder, Julius Kühn-Institut, Germany.

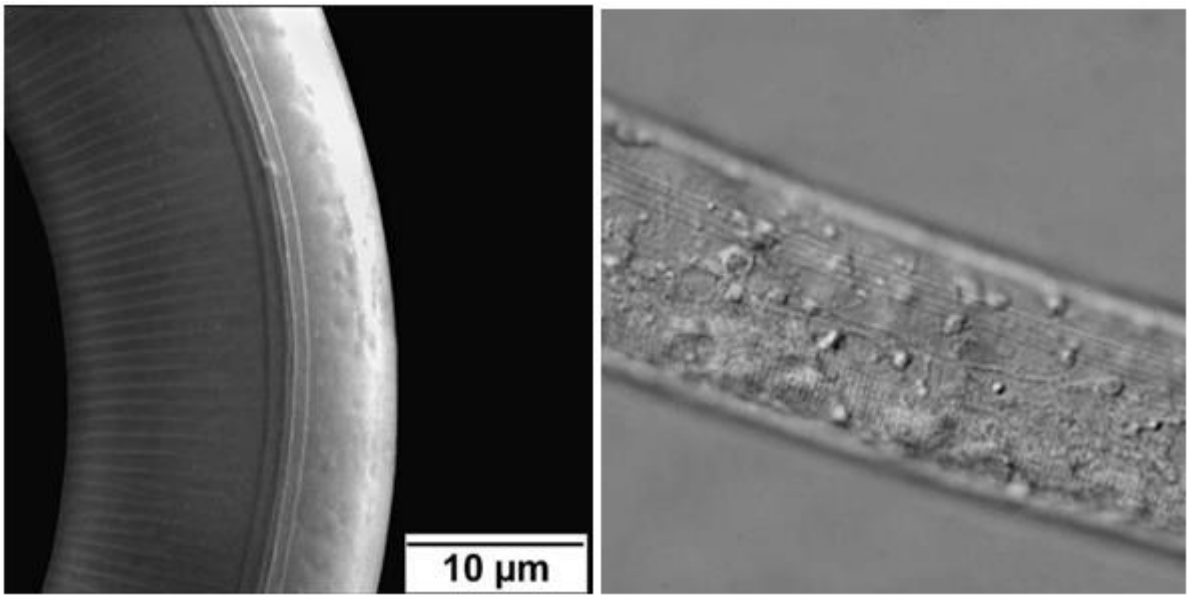

Figure 13. *Bursaphelenchus xylophilus* under lateral field scanning electron microscope (left) and light microscope (right (×1 600 magnification)).
Photos courtesy (left) M. Brandstetter, Austrian Research Centre for Forests, Austria and (right) T. Schröder, Julius Kühn-Institut, Germany.

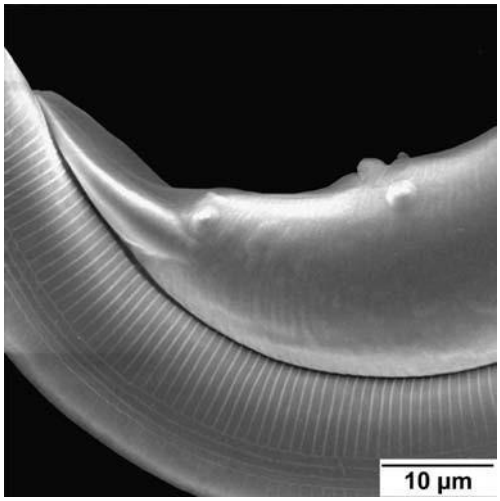

Figure 14. *Bursaphelenchus xylophilus* caudal papillae, scanning electron micrograph.
Photo courtesy M. Brandstetter, Austrian Research Centre for Forests, Austria.

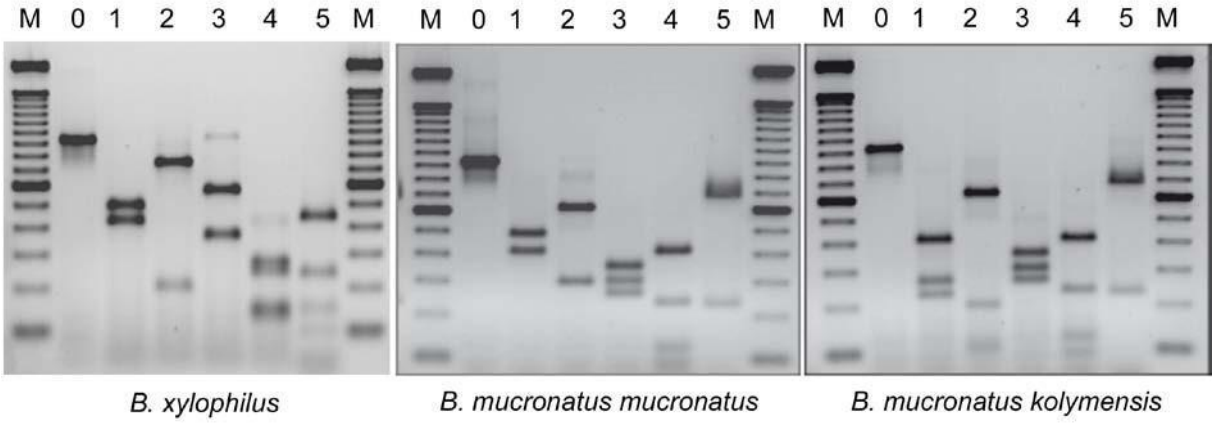

Figure 15. Internal transcribed spacer (ITS)-restriction fragment length polymorphism (RFLP) patterns of *Bursaphelenchus xylophilus* (left), *B. mucronatus mucronatus* (middle) and *B. mucronatus kolymensis* (right). Restriction fragments were obtained by digestion of the amplified ribosomal (r)DNA fragment (0) with *Rsa*I (1), *Hae*III (2), *Msp*I (3), *Hinf*I (4) and *Alu*I (5).
M, DNA marker (100 base pair ladder).
Photos courtesy W. Burgermeister, Julius Kühn-Institut, Germany.

Publication history

This is not an official part of the standard

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2006-04 CPM-1 (2006) added work programme topic: Nematodes (2006-008).

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2008-06 TPDP discussed draft with lead author.

2013-09 New DP drafting group formed.

2014-03 Expert consultation.

2014-10 SC approved for member consultation (2014_eSC_Nov_11).

2015-02 Member consultation.

2015-10 TPDP approved to submit to SC for adoption e-decision (eTPDP_Oct_02).

2015-11 SC approved for DP notification period (2015_eSC_Nov_08).

2016-01 SC adopted DP on behalf of CPM (with no formal objections received).

ISPM 27. Annex 10. *Bursaphelenchus xylophilus* (2016). Rome, IPPC, FAO.

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The annex is a prescriptive part of ISPM 27.

ISPM 27
Diagnostic protocols for regulated pests

DP 11: *Xiphinema americanum sensu lato*

Adopted 2016; published 2016

CONTENTS

1. Pest Information

The group known as *Xiphinema americanum sensu lato (s.l.)* is considered to comprise 56 nominal species (T. Prior, personal communication, 2014). Both morphologically and biochemically, most members of the group are difficult to distinguish. As certain putative species have been shown to transmit a range of economically important viruses, countries that have not recorded the presence of species in this group have included them all on their quarantine lists. However, there has been pressure among trading partners for more clarity on identification to be provided by researchers in an attempt to ease restrictions on trade.

Investigations into the identity of *X. americanum* started in 1979 when Lamberti and Bleve-Zacheo studied populations from disparate geographical areas and concluded that there were in fact 25 different species, 15 regarded as new. Subsequently, new studies and standard virus transmission tests were required to confirm the identity of those species that transmitted viruses (Trudgill *et al*., 1983). Despite several morphological and molecular studies on *X. americanum s.l.*, taxonomic debate about the number of species in the group continues (Coomans *et al*., 2001). This diagnostic protocol presents a considered approach to the identification of, and pest information for, *X. americanum s.l.*

Nematodes belonging to *X. americanum s.l.* occur in Africa and widely in Asia, Central and South America, Europe and North America, but have been found infrequently in Australasia and Oceania (Hockland and Prior, 2009; CABI, 2013). These species have a very wide host range of both herbaceous and woody plants in agriculture, horticulture and forestry. As free-living ectoparasites they are found in soil or growing media, and some species can overcome dry periods and survive for years in soil even in the absence of host plants. These species can therefore be moved in trade with soil associated with plants for planting, plant products (such as potato tubers contaminated with soil), bulk soil and any other goods contaminated with soil. Bare rooted plants free from soil are unlikely to present a pathway for entry of these species. When consignments of ornamental plants are sampled for plant-parasitic nematodes, the growing media from the rhizosphere of the plant should be analysed and evidence of possible re-potting before export should be looked for.

In the absence of virus infection, the aerial parts of plants grown in soil infested with *X. americanum s.l.* show no symptoms unless population levels are high, when roots exhibit swellings close to the root tips, and typical symptoms of root damage (such as reduction in vigour or signs similar to those that occur when a plant is under limited water conditions) may be observed. In the United States, direct damage by *X. americanum sensu stricto (s.s.)* appears to be economically important in several states (CABI, 2013). However, the importance of the group overall is due to the ability of some species to transmit economically important nepoviruses.

Brown et al. (1994) reported that *X. americanum s.s.*, *X. californicum* and *X. rivesi* transmit *Cherry rasp leaf virus* (CRLV) (*Cheravirus*), *Tobacco ringspot virus* (TRSV) (*Nepovirus*) and *Tomato ringspot virus* (ToRSV) (*Nepovirus*) and noted the broad spectrum virus transmission capabilities of these North American populations compared with the relatively narrow specificity of transmission that exists between indigenous European nepoviruses and their vector species. *X. bricolense* was shown to transmit only the two serologically distinguishable strains of ToRSV but was a more efficient vector of the peach stem pitting (PSP) strain than the prune line (PBL) strain of the virus. *X. tarjanense* and *X. intermedium* are both reported to vector TRSV and ToRSV, and *X. inaequale* has been shown to vector ToRSV (Verma et al., 2003).

CRLV, *Peach rosette mosaic virus* (PRMV) (*Nepovirus*), TRSV and ToRSV are listed as recommended for regulation by the European and Mediterranean Plant Protection Organization (EPPO). Until recently, no European populations of *X. americanum s.l.* had been shown to transmit these European quarantine viruses, but in 2007 Širca and colleagues reported transmission of TRSV and ToRSV to bait plants by a Slovenian population of *X. rivesi* with no known links to imported consignments. Auger *et al.* (2009) have also recorded Chilean populations of *X. rivesi* as a vector of ToRSV to cucumber. Although none of the South African *X. americanum s.l.* has been shown to

transmit these viruses, CRLV, *Arabis mosaic virus* (ArMV) and *Grapevine fanleaf virus* (GFLV) have all been reported from South Africa (A. Swart, personal communication, 2014).

2. Taxonomic Information

Name: *Xiphinema americanum (sensu lato)*

Type species: *Xiphinema americanum (sensu stricto)* Cobb, 1913

Synonyms: *Tylencholaimus americanus* (Cobb, 1913) Micoletzky, 1922 (of *X. americanum sensu stricto*)

Taxonomic position: Nematoda, Adenophorea, Dorylaimida, Longidoridae, Xiphinematinae (after Coomans *et al*., 2001)

Common names: American dagger nematode, tobacco ring spot nematode. Other common names in various languages are listed in the CABI Crop Protection Compendium (CABI, 2013).

3. Detection

Xiphinema spp., as with most ectoparasitic plant-parasitic nematodes, can be detected by extraction from soil or growing media. Nematode extraction techniques, such as the Flegg-modified Cobb technique (Flegg, 1967) or Oostenbrink (Oostenbrink, 1960) or other suitable elutriation methods can be used for extraction of longidorid nematodes. Migratory endoparasites may also be present in soil residues adhered to plant roots, bulbs and tubers. Consequently *Xiphinema* spp. may be found following processing of plant material using methods such as modified Baermann processes (EPPO, 2013a).

To extract longidorid nematodes from soil using the Flegg-modified Cobb technique, the following methodology can be followed. A 1 litre beaker is filled with 250 ml water and a soil sample (approximately 200 ml) is added to the water and soaked for approximately 30 min (loamy soil) to 60 min (clay soil); the suspension is stirred two or three times during the soaking period. A 2 mm aperture sieve is placed on a 5 litre plastic bucket and the soil suspension is washed through the sieve into the bucket. The sieve is removed and the bucket is topped up with water, then the solution is agitated by stirring. After 25 s sedimentation time, the supernatant suspension is decanted through a bank of three 150 µm aperture sieves, ensuring that the sediment remains in the bucket. The residue on the sieves is gently washed with a delicate stream of water (such as from a wash bottle) to a clean 1 litre beaker. The bucket containing the soil residue is topped up again with water and swirled thoroughly. After 15 s sedimentation, the supernatant is decanted through the same bank of three 150 µm aperture sieves (again ensuring the sediment remains in the bucket) and the residue is added to that collected previously. The contents of the 1 litre beaker are poured in entirety onto a 90 µm aperture sieve (with a maximum thickness of soil layer about 2–3 mm), and the sieve is placed onto an appropriately sized, supported glass funnel. Water is added from the side until the bottom of the sieve just touches the water. Nematodes are collected after 24–72 h in a glass beaker by opening the spring or screw clip on the funnel stem. The nematodes are examined under a dissecting microscope.

Detailed descriptions of extraction equipment and procedures can be found in the EPPO standard on nematode extraction (EPPO, 2013a).

4. Identification

There are, at present, no appropriate polymerase chain reaction (PCR) protocols for the identification of *X. americanum s.l.* or for the identification of those species that have been acknowledged as virus vectors. Hence there remains the need to rely on morphological identification. Reference material for many of the species of *X. americanum s.l.* is in very short supply, and the contact points listed in section 6 should be consulted for assistance with identification.

4.1 Preparation of material

As with other species of plant-parasitic nematodes, morphological observation should be carried out on as many adult specimens as possible. There are numerous published methods for fixing and processing nematode specimens for study, most recently summarized in Manzanilla-López and Marbán-Mendoza (2012). Nematodes processed to anhydrous glycerol are recommended for examination as important taxonomic features can be obscured if specimens are not cleared sufficiently.

Temporary microscope slide preparations can be made quickly for instant examination but such slides may remain usable for only several weeks.

If possible, permanent slides should be prepared for future reference and deposited in nematode reference collections. Methods of preparing permanent slide mounts of nematodes have been described in detail elsewhere (Seinhorst, 1962; Hooper, 1986). The slow evaporation method as described by Hooper (1986) is outlined in section 4.1.2.

In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. 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. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

4.1.1 Temporary preparations

Place a small drop of water on a glass cavity slide, enough to fill the well. Transfer the nematode specimens to the water and place the slide on a hotplate set at 65 °C. It is vital that the heating should be just sufficient to kill the nematodes, as prolonged heating will result in distortion and deterioration of the specimens. In practice, 10–15 s on a hotplate will be sufficient time for most species, but check the slide at intervals to monitor progress and remove it from the heat only when movement of all the nematodes has ceased.

Select a glass slide, ensure that it is dust free and put it on the side of the microscope stage. Place a small drop of single strength triethanolamine and formalin (TAF) fixative (7 ml formalin (40% formaldehyde), 2 ml triethanolamine, 91 ml distilled water) or another appropriate fixative in the centre of the slide and position an appropriate amount of paraffin wax shavings around the drop (the wax will help support the coverslip and seal it to the slide).

Transfer the nematodes from the cavity slide to the TAF fixative and ensure they are positioned beneath the meniscus in the centre of the drop and not overlapping one another. The number of specimens able to fit on a slide will vary according to the size of the nematodes.

Carefully clean an appropriately sized coverslip with lens tissue. Lower it gently onto the wax shavings so that contact is made with the drop of TAF fixative. Place the slide on a hotplate and leave it there until the wax has just melted, gently tapping the slide to remove air that may be lodged under the coverslip. Remove from the heat and examine.

There should be a clear area of TAF fixative containing the nematodes in the centre and a complete ring of wax to seal the slide.

Should the seal be broken or the nematodes become embedded in the wax, heat the slide again, carefully remove the coverslip, recover the nematodes and remount them on a new slide. If the wax has spread beyond the coverslip, clear this away with a fine blade.

Seal the coverslip with a ring of clear nail varnish. When the varnish has dried, the specimens are ready for study.

4.1.2 Permanent preparations

Place a small drop of water on a glass cavity slide, enough to fill the well. Transfer the nematode specimens to the water and place the slide on a hotplate set at 65 °C. It is vital that the heating should be just sufficient to kill the nematodes, as prolonged heating will result in distortion and deterioration of the specimens. In practice, 10–15 s on a hotplate will be sufficient time for most species, but check the slide at intervals to monitor progress and remove it from the heat only when movement of all the nematodes has ceased.

Transfer the nematodes to an embryo dish or suitable watchglass half full of single strength TAF fixative (see section 4.1.1 for composition). Cover and leave to fix for a minimum of one week.

Transfer the specimens to a watchglass containing a 3% glycerol solution with a trace amount of TAF fixative. Ensure the nematodes are submerged. Place a coverslip over the watchglass and leave overnight.

Move the coverslip slightly so that a small gap is produced to allow evaporation, and leave the watchglass in an incubator (approximately 40 °C) until all the water has evaporated (this will take at least one week). At the same time, leave a small beaker of glycerol in the incubator to ensure it becomes anhydrous.

Using a syringe or dropper, dispense a small drop of the anhydrous glycerol onto the centre of a glass slide and transfer the nematodes to this, arranging them centrally.

Carefully select three coverslip supports, such as glass beads, of similar diameter to that of the nematodes, and place them at intervals in the margin of the glycerol drop, so that they form an even support.

Place small amounts of paraffin wax shavings at regular intervals around the circumference of the glycerol drop.

Heat a coverslip on a hotplate for a few seconds. Clean the coverslip with lens tissue and gently lower it onto the wax, so that contact is just made between coverslip and glycerol.

Place the slide on the hotplate and as soon as the wax has melted and any air bubbles have been expelled by the settling coverslip, remove the slide from the heat and allow the wax to reset.

When the wax is completely hard, remove any excess wax from around the coverslip with a scalpel.

Seal the coverslip with a ring of sealant such as Glyceel or clear nail varnish. Label the slide with an indelible marker, or affix a slide label to it. Include classification, date of slide preparation, host, locality, sample number (if applicable) and method of preservation used.

4.2 Identification of the genus *Xiphinema*

Definitions of terminology used in the following sections can be found in EPPO's *Diagnostic protocols for regulated pests: Pictorial glossary of morphological terms in nematology* (EPPO, 2013b).

Diagnosis of the genus *Xiphinema* has been described by Coomans *et al*. (2001). *Xiphinema* (Cobb, 1913) is among the largest genera in the family Longidoridae, which are migratory, polyphagous root ectoparasites. In summary, members of *Xiphinema* have: a body length of 1.2–7.3 mm; habitus straight to spiral; lip region varying from well offset and knob-like to continuous with body contour, and from low to high; amphidial aperture slit-like; stylet composed of needle-like, heavily sclerotized odontostyle with forked base and odontophore with sclerotized basal flanges; guiding apparatus consisting of folded tube between guide ring and odontophore; dorsal pharyngeal gland nucleus round, larger than those of the ventrosublateral glands and located adjacent to orifice; variable female reproductive system but typically amphidelphic-didelphic; tail shape varying from elongate filiform to short and bluntly rounded; and tails usually similar in shape in both sexes.

4.3 Identification of *Xiphinema americanum sensu lato*

Loof and Luc (1990) defined the particular features of *X. americanum s.l.*, but the characters were slightly amended by Lamberti *et al*. (2000) and Coomans *et al.* (2001). The following combination of characters distinguishes members of *X. americanum s.l.* from other *Xiphinema* species; however, characters marked with an asterisk (*) are seldom observed in those species considered to be part of a *X. pachydermum* group based on morphology (this group is described in more detail following the list of characters):

- body length small to medium (L varies from 1.2 to 3.0 mm)
- body shape assumes a more or less open C to spiral shape when heat-relaxed (Figure 1(a))
- lip region rarely continuous, usually demarcated by a shallow depression or deep constriction (Figure 1(b))
- guide ring more anterior and the folded part of the guiding sheath shorter than in other *Xiphinema* species (Figure 1(b))
- odontostyle robust, length rarely exceeds 150 μm
- pharyngeal bulb usually with thick platelet reinforcements of the lumen wall (Figure 1(c)); bulb not offset from the rather wide slender part
- nuclei in the pharyngeal bulb: dorsal nucleus is often recorded as further from the dorsal orifice and the subventral nucleus is placed more posteriorly than in other *Xiphinema* species
- V% around or behind the middle of the body (V% = 42–65)
- female genital branches equally developed but generally short (Figure 1(d)); short or very short uteri without Z-differentiation or spines and usually with weakly developed sphincter muscles*
- compact ovaries, comprising rather few and narrow germ cells and typically associated with verrucomicrobial endosymbionts (Figures 1(e) and 2(d), (e))*
- tail short, conoid, rounded to slightly digitate, rarely broadly rounded; tail terminus generally pointed or rounded
- males rare, females devoid of sperm*
- male usually with 5–11 ventromedian supplements, with the most posterior lying closer to the paired precloacal papillae (adanal papillae) than in other *Xiphinema* species (i.e. within spicula range) (Figure 1(f))
- three or four juvenile stages.

Detailed descriptions and observations on verrucomicrobial bacteria present in the ovaries of *Xiphinema* can be found in Coomans *et al*. (2000) and Vandekerckhove *et al*. (2000).

Lamberti and Ciancio (1993) distinguished five species subgroups based on hierarchical cluster analysis of morphometrics, among them a *X. pachtaicum* group, which included *X. pachydermum*. *X. pachydermum* and related (mostly Portuguese) species differ from typical *X. americanum s.l.* in females possessing ovaries without associated symbiotic bacteria (except in *X. mesostilum*, where the bacteria are arranged in parallel strands in the wall of the ovaries), a well-developed sphincter muscle and longer uteri, as well as in males being common in most species (Luc *et al*., 1998; Coomans *et al*., 2001; Decraemer and Geraert, 2013). Based solely on morphological characters, the *X. pachydermum* group comprises the following species: *X. brevisicum*, *X. duriense*, *X. exile*, *X. lafoense*, *X. longistilum*, *X. mesostilum*, *X. microstilum*, *X. opisthohysterum*, *X. pachydermum*, *X. parapachydermum* and *X. paratenuicutis*. Following recent molecular work (He *et al*., 2005; Gutiérrez-Gutiérrez *et al.*, 2012), phylogenetic relationships based on sequence comparison of the D2–D3 and internal transcribed spacer (ITS)1 regions partially support the hypothesis that the *X. pachydermum* subgroup is a subgroup outside *X. americanum s.l.*; however, the group does not cluster separately and includes other species such as *X. pachtaicum*. Consequently, the relationships within this subgroup and with other species of *X. americanum s.l.* remain unclear and additional

sequences are required for a larger analysis, which may allow the construction of a more complete and precise phylogeny in this group.

4.4 Identification of species within *Xiphinema americanum sensu lato*

Identification to species level within *X. americanum s.l.* is of particular importance for phytosanitary regulation because of the risk these nematodes pose as virus vectors, but it is problematic as a result of the general similarity of the morphology of the putative species, the high number of putative species (56 at present), weak differences reported between many species, lack of data on intraspecific morphological and morphometric variability, and insufficient illustrations for many populations.

The number of putative species included in this group is constantly under review. The existence of 56 species is considered here. Some authorities regard several species (*X. diffusum*, *X. incognitum*, *X. parvum*, *X. pseudoguirani*, *X. sheri* and *X. taylori*) to be synonymous with *X. brevicolle* (Coomans *et al.*, 2001). As yet, no reliable molecular tests to distinguish between members of *X. americanum s.l.* can be recommended.

Lamberti and Carone produced the first dichotomous key for the identification of species within *X. americanum s.l.* in 1991. Lamberti *et al.* (2000) presented a series of regional polytomous identification keys together with a combined polytomous key to the species occurring worldwide. These keys provided the first comprehensive attempt to resolve the problems with the identification of the *X. americanum s.l.* species. The polytomous key is most useful when some characters are difficult to observe or measure. Luc and Baujard (2001) stated that dichotomous keys can be used to complement a polytomous key in which several species share the same code for one or more characters. In both the dichotomous and polytomous keys, priority was given to quantitative morphological characters to minimize subjective evaluation of qualitative characters. Lamberti *et al.* (2000) listed species authorities and stated that odontostyle length, ratio *c* and V% appeared more reliable for examining intra- and inter-population relationships. When ratio *c* and V% were used as principal discriminants, relatively small groups of species were formed, within which demarcation of the individual species could be made using less robust characters such as body length, ratio *a* and tail length and also using subjective characters such as lip region and tail shape. Although ratio *c′* was considered reliable for identification by Lamberti, other authors (e.g. Griesbach and Maggenti, 1990) have found it to be of little significance. The polytomous key (Tables 1 to 4) was revised by Lamberti *et al*. (2004), with the characters as defined by the author, but unfortunately with few definitions or drawings. There has been confusion regarding the definition of the lip region and tail shape as well as the arbitrary division of morphometric data, thus the current morphological characters used to describe species are under review (T. Prior and S. Hockland, personal communication, 2014).

The amended key included in this diagnostic protocol incorporates all putative species described to date, with updated morphometric data and redefinition of the lip region and of tail shape. The key is useful in assigning a provisional identification to species that can then be checked with reference to the original description and finally by an expert.

The two *species inquirendae*, *X. neoamericanum* and *X. sharmai*, have been omitted from the key. This is because of the poor quality of their original descriptions and the fact that neither species has been unequivocally identified after the publication of their original description. They are considered to have little relevance for phytosanitary regulation.

4.4.1 Polytomous key identification codes

(After Yeates *et al.*, 1997; Coomans *et al*., 2001; Lamberti *et al*., 2004; Gozel *et al.*, 2006; Barsi and De Luca, 2008; Gutiérrez-Gutiérrez *et al.*, 2012.)

The polytomous key described in section 4.4.2 uses the following characters with different possible values (coded as 1 to 6) to describe the nematode observed.

Characters used in the polytomous key and their codes

A	**1**	Females without verrucomicrobial bacteria present in the ovaries, or if present, arranged in parallel strands in the wall of the ovaries (Figure 2(a), (b)) (Table 1 and dichotomous key (section 4.4.3))
	2	Females with verrucomicrobial bacteria present in the ovaries, embedded in the epithelial wall cells of the ovaries at the apex, in the multiplication zone and in the distal part of the growing zone, often compressing the developing oocytes (Figure 2(c)–(e)) (Tables 2 to 4)
B	**1**	Lip region greatly expanded or separated by a deep constriction (Figure 2(l)–(n))
	2	Lip region demarcated by a weak depression or shallow constriction, to almost continuous with the rest of the body (Figure 2(o)–(q))
C	**1**	Tail dorsally convex-conoid (conoid in two species), terminus acute to slightly sub-digitate (Figure 2(r)–(t))
	2	Tail dorsally convex-conoid, ventrally straight; terminus rounded (Figure 2(u)–(v))
	3	Tail broadly convex-conoid, tapering to a broadly rounded terminus with main curvature on dorsal contour (Figure 2(w))
D	**1**	Odontostyle length ≤70 µm
	2	Odontostyle length 71–80 µm
	3	Odontostyle length 81–90 µm
	4	Odontostyle length 91–100 µm
	5	Odontostyle length 101–120 µm
	6	Odontostyle length >120 µm
E	**1**	Vulva (V%) ≤50%
	2	Vulva 51–54%
	3	Vulva 55–58%
	4	Vulva >58%
F	**1**	Value of *c′* ratio (defined as tail length / body width at anus) ≤1.0
	2	Value of *c′* ratio 1.1–1.4
	3	Value of *c′* ratio 1.5–1.8
	4	Value of *c′* ratio >1.8
G	**1**	Value of *c* ratio (defined as body length / tail length) <60
	2	Value of *c* ratio 60–80
	3	Value of *c* ratio >80
H	**1**	Body length <1.5 mm
	2	Body length 1.5–2.0 mm
	3	Body length >2.0 mm
I	**1**	Value of *a* ratio (defined as body length / greatest body diameter) <60
	2	Value of *a* ratio 61–80

3 Value of *a* ratio >80

J **1** Tail length <27 µm

2 Tail length 27–32 µm

3 Tail length >32 µm

4.4.2 Polytomous key code to valid species

Table 1. Species of *Xiphinema americanum sensu lato* without verrucomicrobial bacteria embedded in the epithelial wall cells of the ovaries

Species	Identification code									
	A	B	C	D	E	F	G	H	I	J
exile	1	1	1	1	23	4	12	3	23	2
brevisicum	1	1	1	1	234	4	12	23	23	2
duriense	1	1	1	12	34	34	12	123	23	12
microstilum	1	1	1	12	34	34	23	3	23	2
opisthohysterum	1	1	1	12	4	234	12	12	12	12
parapachydermum	1	1	1	123	34	34	12	123	12	123
pachydermum	1	1	1	23	234	23	23	23	123	12
paratenuicutis	1	1	1	23	34	123	12	23	12	123
mesostilum	1	1	1	34	234	23	23	3	3	12
longistilum	1	1	1	5	23	23	23	3	23	2
lafoense	1	1	2	23	12	2	3	3	3	12

Species included in this list possess relatively long uteri, clearly differentiated oviducts with well-developed sphincters not embedded in surrounding cell bodies, and compact ovaries without the presence of symbiotic bacteria (refer to Jairajpuri and Ahmad (1992) and Coomans *et al*. (2001) for descriptions of the female reproductive system). Males are common within the population for the majority of species included here.

An additional dichotomous key for these 11 species is provided after Table 4.

Table 2. Species of *Xiphinema americanum sensu lato* with verrucomicrobial bacteria embedded in the epithelial wall cells of the ovaries; lip region greatly expanded or separated by a deep constriction; and tail dorsally convex-conoid with terminus acute to slightly sub-digitate

Species	Identification code									
	A	B	C	D	E	F	G	H	I	J
lambertii	2	1	1	1	12	34	1	1	1	MD
simile†	2	1	1	12	1234	1234	123	23	123	123
parasimile†	2	1	1	12	1234	34	12	23	12	123
pachtaicum‡	2	1	1	12345	234	1234	123	123	123	123
kosaigudense	2	1	1	2	1	MD	1	1	1	MD
citricolum	2	1	1	23	123	34	12	12	1	23
pacificum	2	1	1	23	23	34	12	23	12	3

Species	Identification code									
	A	B	C	D	E	F	G	H	I	J
tarjanense	2	1	1	234	123	23	12	12	1	123
floridae¶	2	1	1	2345	12	12	12	123	1	123
californicum	2	1	1	2345	123	234	123	23	12	123
neoelongatum§	2	1	1	4	23	23	1	12	1	MD
fortuitum	2	1	1	45	123	34	23	3	23	23
madeirense	2	1	1	45	234	34	12	23	123	23
georgianum¶	2	1	1	456	123	234	12	23	12	123
*incertum**	2	12	2	34	23	23	23	23	12	123

MD, missing data.

† For detailed comparison of these species, refer to Barsi and Lamberti (2004), Barsi and De Luca (2008), and Lazarova *et al.* (2008).

‡ *X. pachtaicum* has relatively long uteri compared with those of the other species listed in this table.

¶ The tail shape of these two species is regularly conoid rather than dorsally convex-conoid (Figure 2(t)).

§ Considered to be a junior synonym of *X. pachtaicum* by Luc *et al.* (1984).

* Expanded lip region less pronounced in some specimens (Gutiérrez-Gutiérrez *et al.*, 2012). The validity of *X. incertum* was questioned by Barsi and Lamberti (2002).

Table 3. Species of *Xiphinema americanum sensu lato* with verrucomicrobial bacteria embedded in the epithelial wall cells of the ovaries; lip region demarcated by a weak depression or shallow constriction, to continuous with the rest of the body; and tail dorsally convex-conoid with terminus acute to slightly sub-digitate

Species	Identification code									
	A	B	C	D	E	F	G	H	I	J
pakistanense	2	2	1	1	12	2	1	12	1	123
minor	2	2	1	12	12	3	1	12	1	123
intermedium	2	2	1	12	123	23	1	12	1	32
americanum	2	2	1	123	123	234	1	123	12	123
tenuicutis	2	2	1	2	12	23	12	2	1	123
santos	2	2	1	23	123	1234	12	123	1	123
bricolense	2	2	1	234	12	234	12	23	123	23
peruvianum	2	2	1	234	123	23	12	123	1	123
laevistriatum	2	2	1	234	123	234	12	12	1	123
oxycaudatum	2	2	1	234	123	234	12	123	12	123
franci	2	2	1	34	23	23	1	12	1	123
inaequale	2	2	1	345	12	23	12	23	1	23
rivesi	2	2	12	2345	123	1234	12	123	1	123

Table 4. Species of *Xiphinema americanum sensu lato* with verrucomicrobial bacteria embedded in the epithelial wall cells of the ovaries; lip region demarcated by a weak depression or shallow constriction, to continuous with the rest of the body; and tail dorsally convex-conoid, ventrally straight, with terminus rounded or broadly convex-conoid, tapering to a broadly rounded terminus with main curvature on dorsal contour

Species	Identification code									
	A	B	C	D	E	F	G	H	I	J
rivesi	2	2	12	2345	123	1234	12	123	1	123
occiduum	2	2	2	1234	123	23	12	23	12	23
thornei	2	2	2	23	12	23	123	23	1	213
diffusum	2	2	2	234	123	12	123	123	1	123
taylori	2	2	2	234	123	12	23	23	1	123
incognitum	2	2	2	34	123	12	123	123	1	123
utahense	2	2	2	34	123	12	12	23	12	123
parvum	2	2	2	34	23	12	12	12	1	12
brevicolle	2	2	2	345	123	12	123	123	1	123
paramanovi	2	2	2	3456	123	2	12	23	1	3
luci	2	2	2	4	12	12	123	2	1	12
sheri	2	2	2	45	23	1	12	2	1	1
parabrevicolle	2	2	2	45	23	1	23	23	1	12
pseudoguirani	2	2	2	45	234	1	3	23	1	12
himalayense	2	2	2	5	2	12	3	3	1	2
waimungui	2	2	2	56	23	12	123	3	12	23
silvaticum	2	2	23	56	23	1	23	23	1	12
bacaniboia	2	2	3	6	23	1	3	3	1	12

A morphological and molecular review of *X. diffusum* and related species is currently in preparation (S.S. Lazarova, personal communication, 2014).

4.4.3 Dichotomous key to species of *Xiphinema americanum sensu lato* without verrucomicrobial bacteria embedded in the epithelial wall cells of the ovaries (polytomous key code A1)

Because of the almost continuous overlap in morphometric characters between species, morphological features have been used as far as is possible. However, the use of male characters could not be avoided.

1. Mature females with sperm present in uteri or oviduct, body length 1.4–4.4 mm, males common in population.....**3**

– Mature females without sperm present in uteri or oviduct, body length 1.3–2.1 mm, males absent or rare in population **2**

2. Female odontostyle 54–72 µm, guide ring 49–51 µm from oral aperture.....***X. opisthohysterum***

– Female odontostyle 68–74 µm, guide ring 53–60 µm from oral aperture.....***X. duriense***

3. Posteriormost ventromedian supplement in the male distinctly anterior to the level of the spicule head (>25 µm) (Figure 2(f), (g)) **4**

– Posteriormost ventromedian supplement in the male at level of or just anterior to level of the spicule head (<20 µm) (Figures 1(f) and 2(h)).....**6**

4. Female tail dorsally convex-conoid, with a rounded terminus (Figure 2(i)).....***X. lafoense***

– Female tail dorsally convex-conoid, with terminus acute to sub-digitate (Figure 2(j)) **5**

5. Male with three ventromedian supplements preceding the cloacal pair.....***X. exile***

– Male with four to five ventromedian supplements preceding the cloacal pair.....***X. brevisicum***

6. Verrucomicrobial bacteria present and arranged in parallel strands in the wall of the ovaries .....***X. mesostilum***

– No verrucomicrobial bacteria present in the wall of the ovaries.....**7**

7. Female odontostyle >100 µm.....***X. longistilum***

– Female odontostyle <100 µm.....**8**

8. Uteri relatively short (45–56 µm).....***X. parapachydermum***

– Uteri longer (≥75 µm) **9**

9. Spicule with capitulum simple, not differentiated from lamina, lamina with short ventral expansion (Figure 2(k-a) .....***X. pachydermum***

– Spicule with capitulum almost cephalated, demarcation on the dorsal limb, lamina with gradual ventral expansion (Figure 2(k-b).....***X. microstilum***

– Spicule with capitulum elongated, slight demarcation on the dorsal limb, lamina with prominent ventral expansion (Figure 2(k-c) .....***X. paratenuicutis***

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, the following records and evidence and additional material should be kept for at least one year in a manner that ensures traceability: preserved or slide-mounted specimens, photographs of distinctive taxonomic structure.

For morphological evidence, critical features as outlined in the diagnostic keys should be drawn or photographed while fresh material is available, and relevant measurements should be included.

Good photomicrographs (or scanning videos) of key morphological features are likely to be important for record keeping.

6. Contact Points for Further Information

Further information on this protocol can be obtained from:

Nematology Unit, The Food and Environment Research Agency Science (Fera), Sand Hutton, York YO1 1LZ, United Kingdom (Thomas Prior; e-mail: thomas.prior@fera.co.uk; tel.: +44 1904 462206).

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Nematology Unit, Biosystematics Division, Agricultural Research Council – Plant Protection Research Institute (ARC-PPRI), Private Bag X134, Queenswood, 0121 South Africa (Antoinette Swart; e-mail: SwartA@arc.agric.za).

Agricultural Institute of Slovenia, Hacquetova ulica 17, 1000 Ljubljana, Slovenia (Sasa Širca; e-mail: sasa.sirca@kis.si).

Laboratorio de Nematología, INTA-Estación Experimental de Balcarce, Casilla de Correo 276, 7620 Balcarce, Argentina (Eliseo Jorge Chaves; e-mail: eliseo_chaves@yahoo.com.ar).

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

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8. References

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

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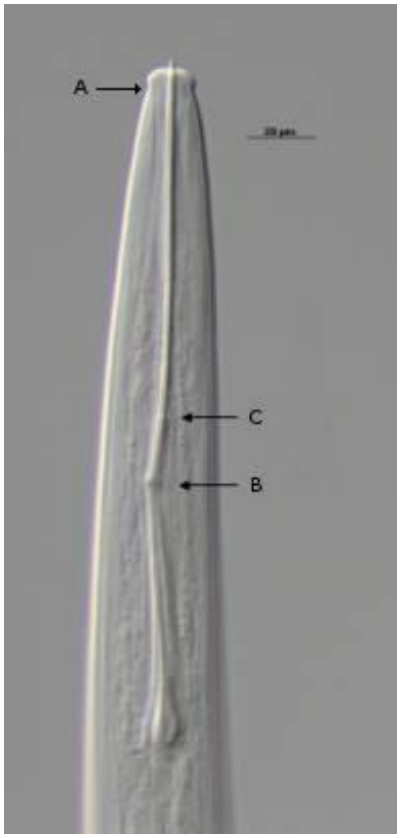
9. Figures

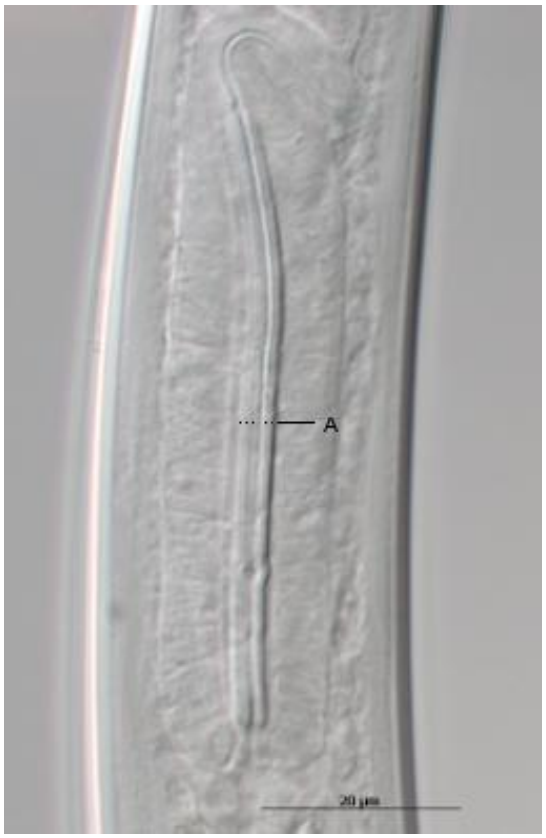
Figure 1. Diagnostic morphological characters of *Xiphinema americanum sensu lato (s.l.).*
Images courtesy The Food and Environment Research Agency, Crown Copyright, except drawing 1(a), reproduced from Lamberti et al. *(1991), courtesy* Nematologia Mediterranea*.*

1

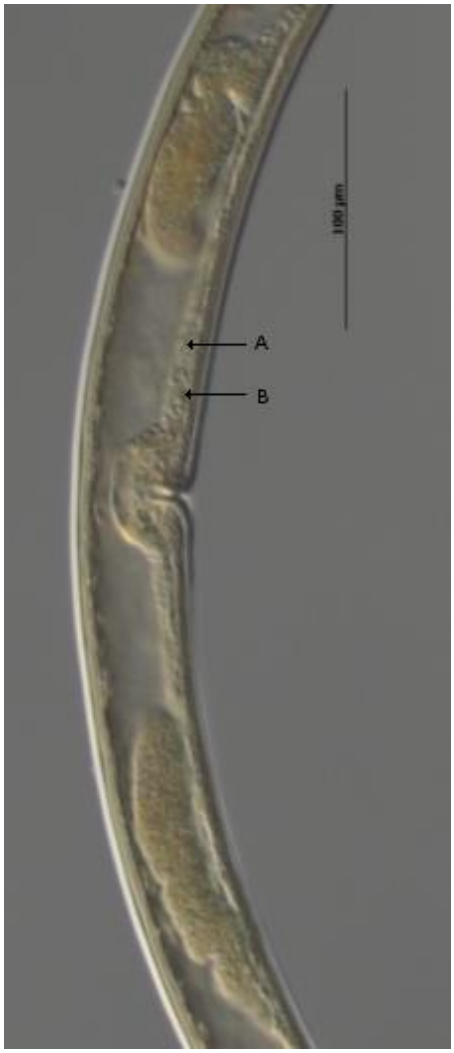
1a. Habitus of *X. americanum s.l.*: (left to right) *X. pachtaicum*, *X. parvum*, *X. pseudoguirani* and *X. taylori*.



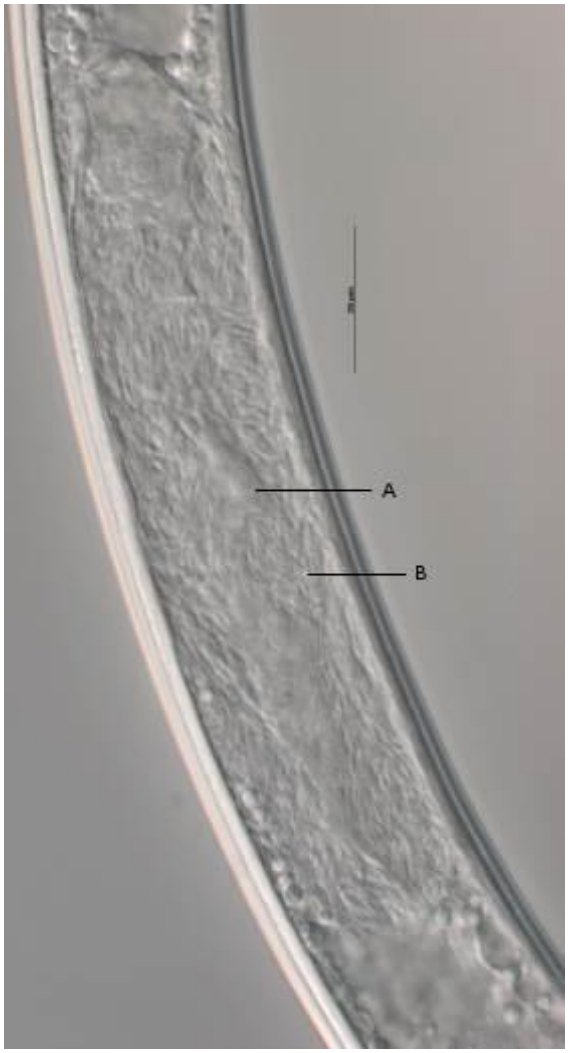
1b. *X. pachtaicum*, anterior. Lip region demarcated by a constriction (A) and relative position of guide ring (B) and anterior part of guiding sheath (C).



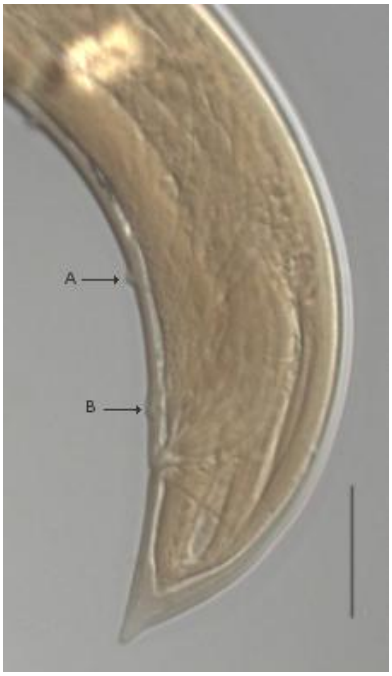
1c. *X. peruvianum*, pharyngeal region. Pharyngeal bulb showing platelet reinforcements of the lumen wall (A).



1d. *X. citricolum*, vulval region. Female genital branches equally developed but relatively short. Uteri without Z-differentiation or spines (A) and usually with weakly developed sphincter muscles (B).

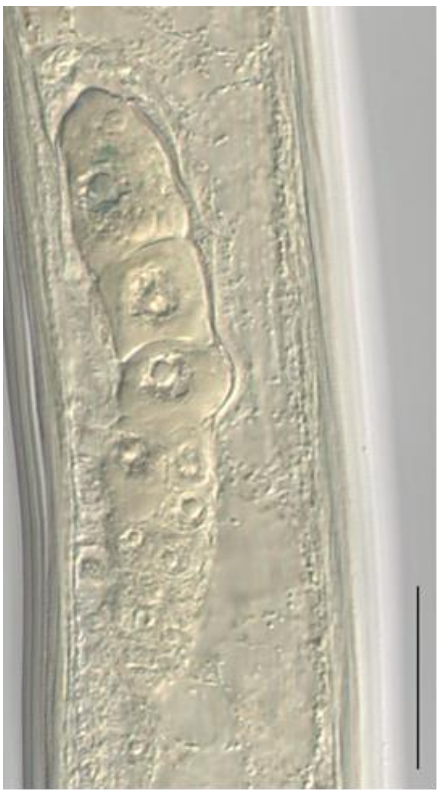


1e. *X. incognitum*. Compact ovaries, comprising rather few and narrow germ cells (A), and typically associated with verrucomicrobial endosymbionts (B).

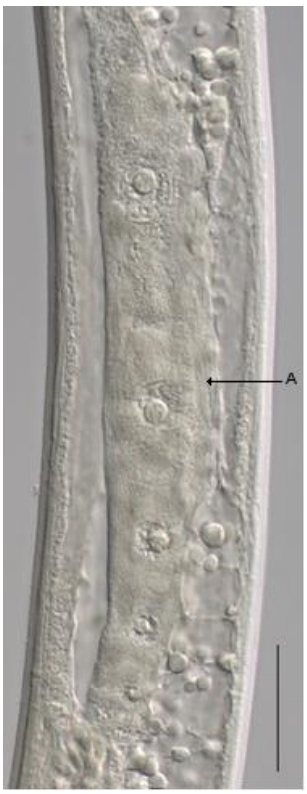


1f. *X. pachtaicum* male (*X. mediterraneum* allotype). Spicular region and posterior ventromedian supplements, with posteriormost (A) lying closer to the precloacal papillae (adanal papillae (B)) (within spicula range) (scale bar: 20 µm).

Figure 2. Diagnostic morphological characters of *Xiphinema americanum sensu lato (s.l.)* for use with identification keys.
Images courtesy The Food and Environment Research Agency, Crown Copyright, except drawings 2(e), adapted from Vandekerckhove et al. *(2002), courtesy* Applied and Environmental Microbiology, *and 2(k), adapted from Gutiérrez-Gutiérrez* et al. *(2012), courtesy* European Journal of Plant Pathology**.**

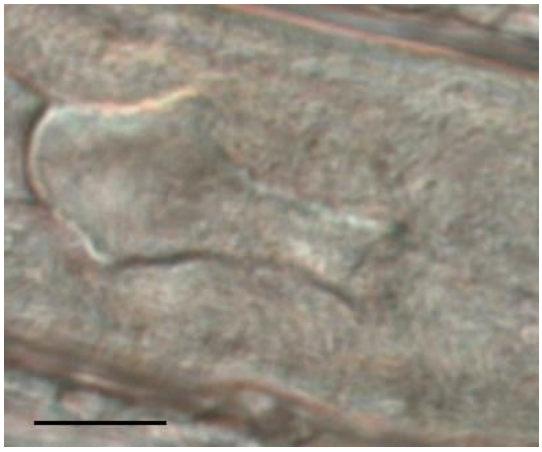


2a. Anterior ovary of *X. longistilum* with no verrucomicrobial bacteria present (scale bar: 20 µm).

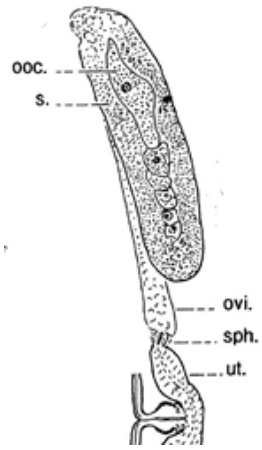


2b. Anterior ovary of *X. mesostilum* with verrucomicrobial bacteria arranged in parallel strands (A) (scale bar: 20 µm).

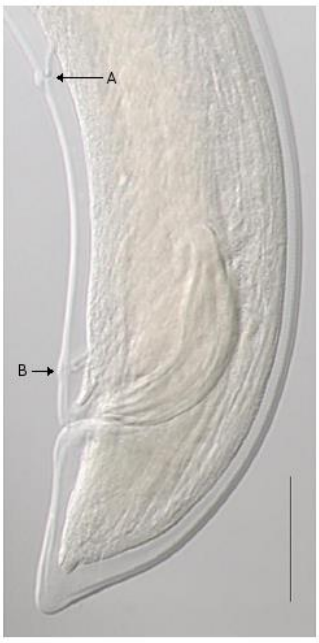
2c. Anterior ovary of *X. incognitum* with verrucomicrobial bacteria present (B) compressing the developing oocytes (C) (scale bar: 20 µm).



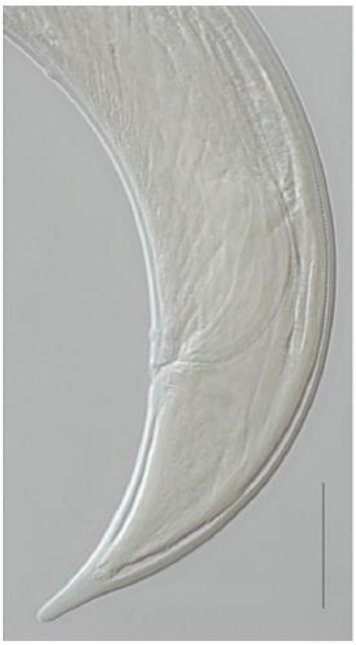
2d. Section of the posterior ovary of *X. incognitum*, with verrucomicrobial bacteria present compressing the developing oocyte (scale bar: 10 µm).



2e. Anterior branch of the reproductive system of an *X. americanum s.l.* female.
ooc., oocyte; ovi., oviduct; s., symbiotic bacteria; sph., sphincter; ut., uterus.

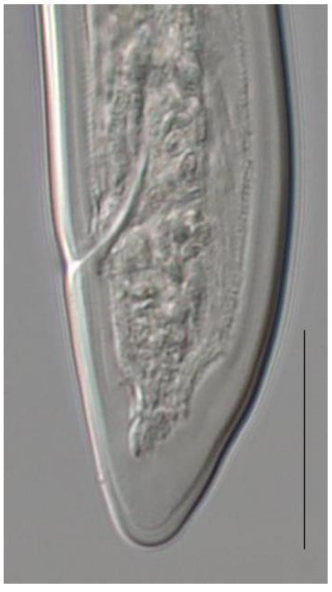


2f. *X. lafoense*, male, posterior. Spicular region and posterior ventromedian supplements, with posteriormost (A) lying further from the precloacal papillae (adanal papillae (B)) (not within spicular range) (scale bar: 20 µm).

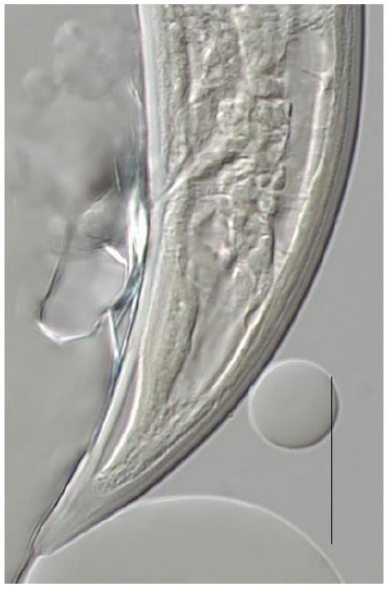


2g. *X. exile*, male, posterior (scale bar: 20 µm).

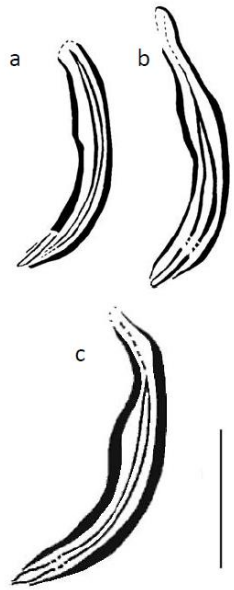
2h. *X. longistilum*, male, posterior (scale bar: 20 µm).



2i. *X. lafoense*, female, tail (scale bar: 20 µm).

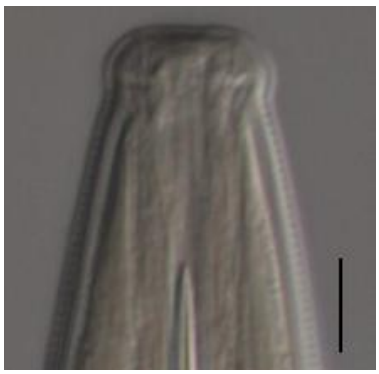


2j. *X. exile*, female, tail (scale bar: 20 µm).

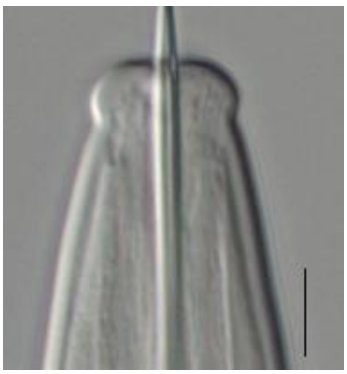


2k. (*a*) *X. pachydermum*, spicule; (*b*) *X. microstilum*, spicule; and (*c*) *X. paratenuicutis*, spicule (scale bar: 15 µm).

2l. *X. californicum*, lip region (paratype) (scale bar: 5 µm).



2m. *X. citricolum*, lip region (paratype) (scale bar: 5 µm).



2n. *X. pachtaicum*, lip region (scale bar: 5 µm).

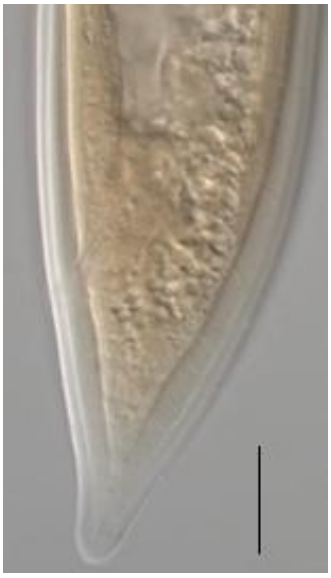
2o. *X. santos*, lip region (paratype) (scale bar: 5 µm).

2p. *X. bricolense*, lip region (paratype) (scale bar: 5 µm).

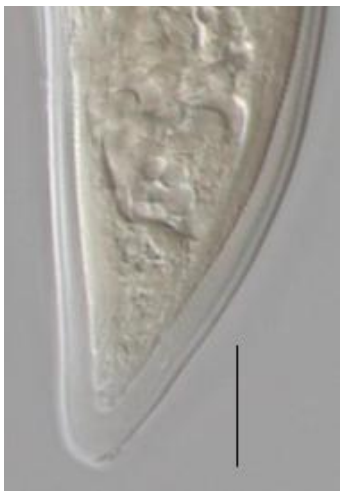
2q. *X. diffusum*, lip region (paratype) (scale bar: 5 µm).

2r. *X. citricolum*, posterior (scale bar: 10 µm).

2s. *X. santos*, posterior (paratype) (scale bar: 10 µm).



2t. *X. floridae*, posterior (paratype) (scale bar: 10 µm).



2u. *X. utahense*, posterior (paratype) (scale bar: 10 µm).

2v. *X. silvaticum*, posterior (topotype) (scale bar: 10 µm).

2w. *X. bacaniboia*, posterior (paratype) (scale bar: 10 µm).

Publication history

This is not an official part of the standard

2004-11 SC introduced original subject: *Xiphinema americanum* (2004-025).

2005-12 First draft presented to TPDP.

2006-04 CPM-1 (2006) added work programme topic: Nematodes (2006-008).

2014-02 Expert consultation.

2014-10 SC approved for member consultation (2014_eSC_Nov_14).

2015-02 Member consultation .

2015-10 TPDP approved to submit to SC for adoption (eTPDP_Oct_01).

2015-11 SC approved for DP notification period (2015_eSC_Nov_11).

2016-01 SC adopted DP on behalf of CPM (with no formal objections received).

ISPM 27. Annex 11. *Xiphinema americanum* (2016). Rome, IPPC, FAO.

Publication history last updated: 2016-04

This diagnostic protocol was adopted by the Standards Committee on behalf of the Commission on Phytosanitary Measures in January 2016.
The annex is a prescriptive part of ISPM 27.

ISPM 27
Diagnostic protocols for regulated pests

DP 12: Phytoplasmas

Adopted 2016; published 2016

CONTENTS

1. Pest Information

Phytoplasmas were first discovered by Doi *et al*. (1967) during their search for the agent of aster yellows. The unicellular organisms were called mycoplasma-like organisms because of their morphological similarity to animal mycoplasmas and their sensitivity to tetracycline antibiotics (Ishiie *et al*., 1967). Phytoplasmas are obligate prokaryotic plant pathogens that do not possess cell walls, and they are pleiomorphic in profile, with a mean diameter of 200–800 nm. They inhabit the phloem sieve cells of their plant hosts. Phytoplasmas have genomes ranging in size from around 550 to 1 500 kb – a relatively small genome compared with other prokaryotes – and they lack several biosynthetic functions (Marcone *et al*., 1999; Davis *et al*., 2005; Bai *et al*., 2006; Oshima *et al*., 2013).

Phytoplasmas are associated with a wide variety of symptoms in a diverse range of plant hosts (Lee *et al*., 2000). Characteristic symptoms associated with phytoplasma infection include virescence (the development of green flowers and the loss of normal flower pigments); phyllody (the development of floral parts into leafy structures); witches' broom (proliferation of auxiliary or axillary shoots) and other abnormal proliferation of shoots and roots; foliar yellowing, reddening and other discoloration; reduced leaf and fruit size; phloem necrosis; and overall decline and stunting (Davis and Sinclair, 1998). Some plant species are tolerant or resistant to phytoplasma infections; when infected, these plants may be asymptomatic or exhibit mild symptoms (Lee *et al*., 2000).

Seemüller *et al*. (2002) estimated that about 1 000 plant species are affected by phytoplasmas. Most of the phytoplasma host plants are dicotyledons. Fewer phytoplasmas have been detected in monocotyledons; such hosts are mainly from the Palmae and Poaceae families (Seemüller *et al*., 2002).

Phytoplasmas occur worldwide. The geographical distribution and impact of phytoplasma diseases depends on the host range of the phytoplasma as well as on the presence and the feeding behaviour of the insect vector. Some phytoplasmas have a broad range of plant hosts and polyphagous vectors and therefore have a wide distribution. Other phytoplasmas have restricted host ranges and oligophagous or monophagous insect vectors, which restrict their geographical distribution. For a review of the geographic distribution of the main phytoplasma taxonomic groups, see Foissac and Wilson (2010).

Phytoplasmas can be transmitted by insect vectors, dodders and grafting and can be spread by vegetative propagation of infected plant parts. Insect vectors of phytoplasmas, responsible for much of their natural spread, are restricted to phloem-feeding leafhoppers, plant hoppers and psyllids (Hemiptera, Auchenorrhyncha). They transmit the pathogen in a persistent manner. Weintraub and Beanland (2006) list more than 90 species that are known to be vectors, some of which are capable of vectoring more than one phytoplasma. Other methods of transmission of phytoplasmas include dodder and graft transmission. Dodders (*Cuscuta* and *Cassytha* spp.) are parasitic vines that develop vascular connections with their hosts through haustoria. When a bridge is established between a healthy plant and a phytoplasma-infected plant, the phytoplasma will transfer to the healthy plant via the connecting phloem elements. Graft transmission and micropropagation of plants in tissue culture can be used to maintain phytoplasmas for reference purposes (IPWG, n.d.).

Further information on phytoplasmas, including photos showing disease symptoms, a list of insect vectors and a phytoplasma classification database, can be found at the following websites: COST Action FA0807 Integrated Management of Phytoplasma Epidemics in Different Crop Systems (http://www.costphytoplasma.ipwgnet.org/) and Phytoplasma Resource Center (http://plantpathology.ba.ars.usda.gov/phytoplasma.html).

2. Taxonomic Information

Name: Phytoplasma

Synonyms: Mycoplasma-like organism (MLO), mycoplasma

Taxonomic position: Bacteria, Firmicutes, Mollicutes, Acholeplasmatales, Acholeplasmataceae, '*Candidatus* Phytoplasma'

The International Research Programme on Comparative Mycoplasmology (IRPCM) Phytoplasma/Spiroplasma Working Team – Phytoplasma Taxonomy Group has published guidelines for the description of '*Candidatus* (*Ca.*) Phytoplasma' species (IRPCM, 2004). Delineation of '*Ca.* Phytoplasma' species is based on 16S ribosomal (r)RNA gene sequences as well as on biological characteristics. In general, phytoplasmas within a species are ≥97.5% identical over ≥1 200 nucleotides of their 16S rRNA gene. When a '*Ca.*' species includes phytoplasmas with different biological characteristics (vectors and host plants) they can be taxonomically distinguished following specific rules reported in IRPCM (2004). Descriptions of '*Ca.* Phytoplasma' species are published in the *International Journal of Systematic and Evolutionary Microbiology* and as of March 2015, 37 '*Ca.* Phytoplasma' species have been described.

3. Detection and Identification

Polymerase chain reaction (PCR) techniques are the method of choice for phytoplasma detection. Successful molecular detection of phytoplasmas is dependent on appropriate sampling of plant tissue and reliable nucleic acid extraction methods (Palmano, 2001; Firrao *et al.*, 2007). Phytoplasmas can be unevenly distributed and in an uneven titre throughout a plant, particularly in woody hosts, and symptomatic tissue is optimal for phytoplasma detection (Constable *et al*., 2003; Garcia-Chapa *et al*., 2003; Christensen *et al.*, 2004; Necas and Krska, 2006). Symptomless infection can occur in some plant hosts and if this is suspected it is important to thoroughly sample different tissues of the plant.

Phytoplasma titre in the plant host affects the reliability of the PCR test (Marzachì, 2004). Phytoplasma titre can be affected by phytoplasma strain or species, host plant species, timing of infection and climatic conditions. The timing for sampling plant tissues is important as location in the plant and titre of phytoplasmas may be affected by seasonal changes (Seemüller *et al*., 1984; Jarausch *et al*., 1999; Berges *et al*., 2000; Constable *et al*., 2003; Garcia-Chapa *et al*., 2003; Prezelj *et al*., 2012).

For most phytoplasma diseases, leaves with symptoms are the best sources of samples for diagnosis. Phytoplasmas reside in the phloem sieve elements of infected plants and therefore the leaf petioles and midveins, stems or inner bark are often used for DNA extraction. In some cases (e.g. X-disease phytoplasma), fruit peduncles contain the highest phytoplasma titre (Kirkpatrick, 1991). Although phytoplasmas can be detected in roots and bark scrapings of dormant trees, generally it is best to test for phytoplasmas at the end of summer. Collected plant samples can be stored at −20 °C for up to six months before testing. Longer term storage is at −80 °C, or the plant material can be freeze-dried or dried over calcium chloride and stored at 4 °C.

Various nucleic acid extraction methods have been reported for phytoplasma detection by PCR. A number of methods use an enrichment step to concentrate the phytoplasmas before nucleic acid extraction (Kirkpatrick *et al*., 1987; Ahrens and Seemüller, 1992; Prince *et al*., 1993). These techniques can be useful for hosts in which phytoplasmas are found in low titre, such as woody perennial plants, or for "difficult" hosts from which high levels of compounds such as polysaccharides and polyphenols that can inhibit PCR are often co-extracted with the nucleic acid. In some simplified methods, plant tissue is ground directly in a commercially available lysis buffer or in cetyl trimethylammonium bromide (CTAB)-based buffer. Typically, a 2% CTAB buffer is used (it has been shown that a 3% solution is more reliable for grapevines) (Daire *et al*., 1997; Angelini *et al*., 2001). The DNA is then extracted directly from the lysate using commercially available silica spin columns (Green *et al*., 1999; Palmano, 2001) or magnetic beads (Mehle *et al*., 2013), or with organic solvents

(Daire *et al*., 1997; Zhang *et al*., 1998). The method of using magnetic beads is generally performed on an automated nucleic acid extraction instrument (e.g. KingFisher from Thermo Scientific[1]). Most extraction methods are well validated for a variety of plant host species. The choice of method is dependent on the host being tested and the availability of facilities and equipment. It may be practical to use a method incorporating a phytoplasma enrichment step for woody perennial hosts and a simplified method for herbaceous hosts. For routine diagnostics it is important to validate an extraction method for a particular host to ensure reliability.

A number of universal PCR primers have been designed that allow amplification of the 16S rRNA gene of any known phytoplasma. The most commonly used primers are the P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995) and R16F2n/R16R2 (Lee *et al*., 1993; Gundersen and Lee, 1996) primer pairs, which can be used in a nested PCR protocol. The P1/P7 primer pair amplifies a PCR product that contains the entire 16S rRNA gene as well as the 16S/23S rRNA spacer region. Real-time PCR has been reported to be more than or as sensitive as nested PCR, depending on the host–phytoplasma combination (Christensen *et al.*, 2004), and is more amenable to high throughput analysis because post-amplification processing is not required. Real-time PCR using TaqMan probes is also more specific and there is less chance of cross-contamination than with conventional PCR, especially nested PCR. False positives with closely related bacteria can occur with the PCR assays recommended in this protocol – a necessary compromise for a universal assay (Fránová, 2011; Pilotti *et al*., 2014). It is possible to run more specific PCR assays or if the outcome is critical (e.g. post-entry quarantine samples, new host record, new distribution), the conventional PCR product should be sequenced.

As well as amplification of the 16S rRNA gene, PCR methods have also been used to amplify other genome regions for phytoplasma detection and classification, including ribosomal protein genes (Lim and Sears, 1992; Jomantiene *et al*., 1998; Lee *et al*., 1998; Martini *et al*., 2007), the *tuf* gene (Schneider *et al*., 1997; Makarova *et al*., 2012), the 23S rRNA gene (Guo *et al*., 2003) and the *secY* gene (Lee *et al*., 2010; Davis *et al*., 2013; Quaglino *et al*., 2013). These primers may be useful when a second independent region of the phytoplasma genome is required.

Samples may contain compounds that are inhibitory to PCR depending on the host species and type and age of the tissue. Therefore it is important to check the PCR competency of the DNA extractions using internal control primers that amplify a gene from the plant host. Inhibitory effects of the host can be overcome by further purifying the DNA through a sephacryl spin column or by adding bovine serum albumin (BSA) to the PCR mixture to a final concentration of 0.5 mg/ml (Kreader, 1996).

In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. 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. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

3.1 Conventional nested PCR

The PCR primers used in this assay are P1 (Deng and Hiruki, 1991) and P7 (Schneider *et al*., 1995) for the first-stage PCR:

P1 (forward): 5′-AAG AGT TTG ATC CTG GCT CAG GAT T-3′

P7 (reverse): 5′-CGT CCT TCA TCG GCT CTT-3′

[1] In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. 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. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

The second-stage PCR primers are R16F2n (Gundersen and Lee, 1996) and R16R2 (Lee *et al*., 1993):

R16F2n (forward): 5′-GAA ACG ACT GCT AAG ACT GG-3′

R16R2 (reverse): 5′-TGA CGG GCG GTG TGT ACA AAC CCC G-3′

The 20 µl reaction mixture consists of 1× Taq DNA polymerase buffer containing 1.5 mM $MgCl_2$, 0.5 µM of each primer, 200 µM dNTPs, 1 U Taq DNA polymerase and 2 µl DNA template. The amplification conditions are an initial denaturation step of 94 °C for 2 min followed by 40 cycles of 94 °C for 30 s, 53 °C (P1/P7 primers) or 50 °C (R16F2n/R16R2 primers) for 30 s and 72 °C for 1 min, and a final extension step of 72 °C for 10 min. For nested PCR, 1 µl of the first-stage PCR products is used either undiluted or at up to a 1:30 dilution as the template for the second-stage PCR. The PCR products are analysed by gel electrophoresis. The P1/P7 and R16F2n/R16R2 primers produce a 1 800 base pair (bp) and 1 250 bp amplicon, respectively.

The presence of PCR-competent DNA in the extracts is confirmed using the universal eukaryotic 28S rRNA gene primers of Werren *et al*. (1995):

28Sf (forward): 5′-CCC TGT TGA GCT TGA CTC TAG TCT GGC-3′

28Sr (reverse): 5′-AAG AGC CGA CAT CGA AGG ATC-3′

The reaction mixture for the 28S rRNA assay has the same components and is cycled under the same conditions as the phytoplasma assay, so that the two assays can be run simultaneously in separate tubes. The 28Sf/28Sr primer pair produces a 500–600 bp amplicon.

Other primer pairs can also be used to check that the DNA is PCR-competent.

3.2 Real-time PCR

Real-time PCR is performed using the TaqMan assay designed for the 16S rRNA gene by Christensen *et al.* (2004):

Forward primer: 5′-CGT ACG CAA GTA TGA AAC TTA AAG GA-3′

Reverse primer: 5′-TCT TCG AAT TAA ACA ACA TGA TCC A-3′

TaqMan probe: 5′-FAM-TGA CGG GAC TCC GCA CAA GCG-BHQ-3′

Alternatively, the real-time PCR of Hodgetts *et al*. (2009) designed for the 23S rRNA gene can be used:

JH-F 1 (forward primer): 5′-GGT CTC CGA ATG GGA AAA CC-3′

JH-F all (forward primer): 5′-ATT TCC GAA TGG GGC AAC C-3′

JH-R (reverse primer): 5′-CTC GTC ACT ACT ACC RGA ATC GTT ATT AC-3′

JH-P uni (TaqMan probe): 5′-FAM-MGB-AAC TGA AAT ATC TAA GTA AC-BHQ-3′

The 25 µl reaction mixture consists of 1× TaqMan real-rime PCR master mix, 300 nM forward primer, 300 nM reverse primer, 100 nM FAM probe and 2 µl DNA template. All samples are tested in duplicate. The amplification conditions are an initial denaturation step of 95 °C for 3 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. These cycling conditions may vary depending on the type of master mix used (e.g. some mixes require a polymerase activation step at 95 °C for 10 min and mixes that contain uracil-DNA glycosylase (UDG) require an initial hold at 50 °C for 2 min). Real-time PCR results are analysed with the manufacturer's software provided with the instrument.

The real-time PCR assay of Christensen *et al*. (2004) uses 900 nM of the reverse primer, and this was updated to 300 nM in a later report (Christensen *et al*., 2013). This assay will work equally well with either concentration of reverse primer.

The 16S rRNA real-time PCR method was evaluated by testing phytoplasmas from 18 subgroups and was found to be as sensitive as or up to ten times more sensitive than conventional nested PCR, depending on the host–phytoplasma combination (Christensen *et al.*, 2004). A ring test for the detection of fruit tree phytoplasmas involving 22 laboratories suggested that the Christensen *et al*. (2004) and Hodgetts *et al*. (2009) assays are similar in terms of sensitivity and specificity (EUPHRESCO FruitPhytoInterlab Group, 2011).

The presence of PCR-competent DNA in the extracts is confirmed using the COX assay of Weller *et al*. (2000), which amplifies the cytochrome oxidase gene:

COX-F (forward primer): 5′-CGT CGC ATT CCA GAT TAT CCA-3′

COX-R (reverse primer): 5′-CAA CTA CGG ATA TAT AAG AGC CAA AAC TG-3′

COX-P (TaqMan probe): 5′-FAM-TGC TTA CGC TGG ATG GAA TGC CCT-BHQ-3′

Alternatively, the 18S rRNA gene assay of Christensen *et al*. (2004) can be used to confirm that the DNA is PCR-competent and is recommended for monocotyledons, for which the COX assay is less efficient:

Forward primer: 5′-GAC TAC GTC CCT GCC CTT TG-3′

Reverse primer: 5′-AAC ACT TCA CCG GAC CAT TCA-3′

TaqMan probe: 5′-FAM-ACA CAC CGC CCG TCG CTC C-BHQ-3′

The reaction mixtures for the COX and the 18S rRNA gene assays have the same components and are cycled under the same conditions as the phytoplasma real-time assay, so that the two assays can be run simultaneously in separate tubes. Alternatively, the internal control assay can be multiplexed in the same tube as the phytoplasma assay if the probe is labelled with a different reporter dye and the primer and probe concentrations have been optimized to prevent low phytoplasma levels being outcompeted by high levels of plant DNA used as the internal control.

3.3 Controls for molecular tests

For the test result obtained to be considered reliable, appropriate controls – which will depend on the type of test used and the level of certainty required – should be considered for each series of nucleic acid isolation and amplification of the target pest nucleic acid. For PCR a positive nucleic acid control, an internal control and a negative amplification control (no template control) are the minimum controls that should be used.

Positive nucleic acid control. This control is used to monitor the efficiency of the test method (apart from the extraction), and specifically the amplification. Phytoplasma DNA extracted from an infected plant, whole genome amplified DNA or a synthetic control (e.g. a cloned PCR product) may be used.

Internal control. For conventional and real-time PCR, a plant housekeeping gene such as the universal eukaryotic 28S rRNA gene (see section 3.1 for its use in the conventional nested PCR) or the COX gene (see section 3.2 for its use in the real-time PCR) should be incorporated into the protocol to eliminate the possibility of PCR false negatives due to nucleic acid extraction failure or degradation or 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 due to contamination during preparation of the reaction mixture. PCR-grade water that was used to prepare the reaction mixture is added at the amplification stage.

Positive extraction control. This control is used to ensure that phytoplasma nucleic acid is of sufficient quantity and quality for PCR and that the pathogen is detected. Phytoplasma DNA is extracted from infected host tissue or healthy plant tissue that has been spiked with the phytoplasma.

The positive control should be approximately one-tenth of the amount of leaf tissue used per plant for the DNA extraction. If bulking of samples is done, then the quantity of positive control should be adjusted accordingly (e.g. ten lots of 20 mg sample bulked for DNA extraction, 2 mg infected leaf + 198 mg healthy plant tissue). If the positive control is not detected then the test should be repeated or the bulking rate reduced until reliable detection is achieved.

For PCR, care needs to be taken to avoid cross-contamination due to aerosols from the positive control or from positive samples. The positive control used in the laboratory should be sequenced so that this sequence can be readily compared with 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 contamination during nucleic acid extraction and/or cross-reaction with the host tissue. The control may be the extraction buffer or may comprise a nucleic acid that is extracted from uninfected host tissue and subsequently amplified. In cases where large numbers of positive samples are expected, it is recommended that negative extraction controls are included between the samples for testing.

3.4 Interpretation of results from PCR

3.4.1 Conventional nested PCR

The pathogen-specific PCR will be considered valid only if:

- the positive control produces the correct size amplicon for the target pathogen
- the negative extraction control and the negative amplification control produce no amplicons of the correct size for the target pathogen.

For internal controls targeting plant DNA, the healthy control (if used), positive control and each of the test samples must produce the amplicon of the expected size. 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.

The test on a sample will be considered positive if it produces an amplicon of the correct size. To identify the phytoplasma present in positive samples, the amplicon will need to be sequenced (see section 3.5). In some cases, more specific PCR assays are available.

3.4.2 Real-time PCR

Real-time PCR will determine if a sample is positive or negative for phytoplasma. To identify the phytoplasma present in positive samples, a conventional PCR will need to be performed to obtain at least the 1 250 bp length of the 16S rRNA gene generated from the R16F2n/R16R2 primer pair for sequence analysis (see section 3.5). Alternatively, for some phytoplasmas it may be possible to use specific real-time PCR assays; for example, 16SrX (apple proliferation) group (Torres *et al*., 2005) and flavescence dorée (Pelletier *et al*., 2009).

3.5 Sequence analysis

PCR products should be sequenced either directly or by first cloning them into a PCR cloning vector. Sequence data can be analysed using the Basic Local Alignment Search Tool, BLASTN, available at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). If the sequence shares less than 97.5% identity with its closest relative, the phytoplasma is considered to be a new '*Ca.* Phytoplasma' species. In this case, the entire 16S rRNA gene should be sequenced and phylogenetic analysis performed. Sequencing a separate region of the genome such as the 16S/23S rRNA spacer region, *secY* gene, ribosomal protein genes or the *tuf* gene is also desirable.

4. Records

Records and evidence should be retained as described in 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 and where the phytoplasma 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:

- The original sample, kept frozen at −80 °C, or freeze-dried or dried over calcium chloride and kept at 4 °C.
- If relevant, DNA extractions should be kept at −20 °C or at −80 °C. Plant extracts spotted on membranes should be kept at room temperature.
- If relevant, PCR amplification products should be kept at −20 °C or at −80 °C.

5. Contact Points for Further Information

Further information on this protocol can be obtained from:

Plant Health and Environment Laboratory, Ministry for Primary Industries, PO Box 2095, Auckland 1140, New Zealand (Lia W. Liefting; e-mail: lia.liefting@mpi.govt.nz; tel.: +64 9 9095726; fax: +64 9 9095739).

Department of Economic Development, Jobs, Transport and Resources, Victoria, AgriBio, 5 Ring Road, Bundoora, VIC 3083, Australia (Fiona Constable; e-mail: fiona.constable@ecodev.vic.gov.au; tel.: +61 3 9032 7326; fax: + 61 3 9032 7604).

Department of Territory and Sustainability, Av. Diagonal 525, 08029 Barcelona, Spain (Ester Torres; e-mail: ester.torres@gencat.net).

Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Protection and Fruit Crops, Schwabenheimer Str. 101, D-69221 Dossenheim, Germany (Wilhelm Jelkmann; e-mail: wilhelm.jelkmann@jki.bund.de).

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).

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7. References

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

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2013-04 Expert consultation.

2013-06 Draft presented to TPDP meeting.

2014-05 SC approved for member consultation (2014_eSC_May_07).

2014-07 Member consultation.

2015-03 TPDP approved to submit to SC for approval for adoption (2015_eTPDP_May_01).

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2015-08 Formal objection received.

2015-09 TPDP virtual meeting.

2015-10 TPDP analysis and revision of the formal objection (2015_eTPDP_Oct_03).

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