



*REPORT*

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# Technical Panel on Diagnostic Protocols June, 2013



Food and Agriculture Organization of the United Nations

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## CONTENTS

1. Opening of the meeting .....	4
2. Administrative Matters .....	4
3. Reports.....	4
4. Update on the Development of Diagnostic Protocols.....	6
5. Scrutiny of Draft Diagnostic Protocols.....	9
6. Procedures and Guidance Related to TPDP .....	15
7. Priorities for Additional Diagnostic Protocols .....	19
8. Update on the Work of Other Organisations, International Organization for Standardization (ISO).....	20
9. TPDP Work Plans.....	20
10. Date and Location of Next Meeting .....	20
11. Other Business.....	21
12. Close of the Meeting.....	21
APPENDIX 1 - Agenda .....	22
APPENDIX 2: Documents list.....	25
APPENDIX 3: Participants list .....	27
APPENDIX 4: Instructions to Authors: Standardized template for diagnostic protocols.....	29
APPENDIX 5: Study on the utility of IPPC diagnostic protocols .....	38
APPENDIX 6: TPDP Medium Term Plan .....	41

## **1. Opening of the meeting**

### **1.1 Welcome**

- [1] The International Plant Protection Convention (IPPC) Secretariat opened the meeting, welcomed the participants and presented apologies from the members who were not able to attend.
- [2] The European and Mediterranean Plant Protection Organization (EPPO) Secretariat also welcomed all the participants. Participants introduced themselves briefly.
- [3] During the week, the Technical Panel on Diagnostic Protocols (TPDP) members had the opportunity to visit the Botanical Garden Department of the National History Museum.

### **1.2 Selection of the Chair and Rapporteur**

- [4] Mr Delano JAMES (Canada) was selected as chairperson and Mr Brendan RODONI (Australia) as rapporteur.

### **1.3 Review and adoption of agenda**

- [5] The TPDP adopted the agenda presented as Appendix 1 to this report.

## **2. Administrative Matters**

### *Local information*

- [6] The organiser provided local information, meeting logistics and arrangements.

### *Documents list*

- [7] The list of documents is attached as Appendix 2 to this report. The diagnostic protocols (DPs) on *Sorghum halepense* (2006-027) and *Erwinia amylovora* (2004-009) had been made available at the meeting. Due the proximity of the expert consultation on draft diagnostic protocols (ECDP) on draft DPs to the 2013 TPDP meeting, some documents were made available only a few days prior to the meeting. The Secretariat noted this can be an issue and the TPDP committed to submitting working papers in advance for future meetings.

### *Participants list*

- [8] The list of participants and their contact information is presented as Appendix 3 of this report.

## **3. Reports**

### *TPDP November 2012 meeting report*

- [9] There was no comment on the report<sup>1</sup> and it was adopted.

### *TPDP February 2013 virtual meeting report*

- [10] There was no comment on the report<sup>2</sup> and it was adopted.
- [11] The panel agreed that the virtual meetings were useful and allowed members to frequently update each other.

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<sup>1,2</sup> 2012 TPDP November meeting report and 2013 TPDP February virtual meeting report: <https://www.ippc.int/core-activities/standards-setting/expert-drafting-groups/technical-panels/technical-panel-diagnostic-protocols>

### *Updates from other relevant IPPC meetings*

- [12] The TPDP Steward presented a summary of the Standards Committee (SC) discussion points of relevance for the TPDP arising from the 2013 May SC meeting<sup>2</sup>. The main points were:
- The DP adoption process (DPs are now adopted by the SC) and the 45-day notification period, (where countries can present formal objections on DPs). Dates for the notification period were set for 1 July to 15 August and 15 December to 30 January.
  - The criteria, and accompanying flow charts, on how to determine if a formal objection is technically justified had been adopted at the Eight Session of the Commission on Phytosanitary Measures (CPM-8 (2013)).
  - Engaging experts in the standards setting process. A questionnaire aimed at National Plant Protections (NPPOs) and Regional Plant Protections (RPPOs) was being developed.
  - A possible study on the utility of DPs was reviewed by the SC (this will be discussed under agenda item 6.5).
  - Two draft DPs were approved by the SC for the 2013 member consultation (MC):
    - Draft Annex to ISPM 27:2006. Diagnostic protocol for *Potato spindle tuber viroid* (2006-022)
    - Draft Annex to ISPM 27:2006. Diagnostic protocol for *Xanthomonas citri* subsp. *citri* (2004-011).
  - The SC reviewed the TPDP recommendations to the SC and agreed to add the following subjects to the TPDP work programme:
    - *Anguina* spp. (nematode) with priority 3 (2013-003)
    - *Conotrachelus nenuphar* (insect) with priority 2 (2013-002)
    - *Liberibacter solanacearum* (bacteria) with priority 1 (2013-001).
  - A second term of TPDP membership was proposed to Ms Géraldine ANTHOINE (France), whose first term would expire in April 2014, which she accepted via email.
  - SC e-decisions for the approval for adoption of two DPs are scheduled to be submitted to the SC before the its next meeting:
    - Draft Annex to ISPM 27:2006. Diagnostic protocol for *Tilletia indica* Mitra (2004-014)
    - Draft Annex to ISPM 27:2006. Diagnostic protocol for *Phyllostica citricarpa* (McAlpine) Aa on fruit (2004-023)
- [13] The TPDP Steward also made an update on the status of the draft DP for *Tilletia indica* (2004-014). Many member comments had been received from 2012 MC period, and the discipline lead and author responded to the comments, which were then approved by the TPDP electronically. The draft DP and responses to comments were sent to the SC with the TPDP recommendation for the adoption of the draft protocol (2013\_eSC\_May\_06), but the SC did not reach a consensus and the decision was to send the DP back to the TPDP for technical revision.
- [14] Two major comments had been made by SC members regarding the detection of *Tilletia indica*: United States of America (USA) did not agree that a wash test was necessary if no bunted kernels are detected in the sample, and Canada did not agree that actions required a threshold of 10 teliospores. The draft was adjusted by the discipline lead and author, and sent to USA and Canada Contact Points. Canada agreed with the revised version of the draft but the USA still had concerns.
- [15] The TPDP noted that DPs provide the minimum requirements for a reliable diagnosis and are based on scientific evidence. A minimum number of 10 teliospores are needed to be sure of the diagnosis, but countries may take a precautionary position as they wish, if justified. A wash test is recommended for detection, and this was covered in the text. However, some cases countries may choose to not carry out a wash test.

<sup>2</sup> 2013 May SC meeting report: <https://www.ippc.int/core-activities/standards-setting/standards-committee>

- [16] A statement had been added that in the absence of bunted kernels, *T. indica* may be considered not to be present. However, the only thing which can be said is that it is not detected based on absence of kernels. The level of confidence of the non-detection is not the same as a result based on the absence of teliospores in a wash test.
- [17] The TPDP thought that it would be difficult to leave the possibility that the non-detection be made on the absence of bunted kernels. The draft DP specifies that direct visual examination is determined to not be reliable for phytosanitary purposes.
- [18] The TPDP proposals were sent to the author and discipline lead and they both agreed with the revised text. Thus, the TPDP agreed that the revised draft DP on *T. indica* will be submitted to the SC with the recommendation for approval for adoption.
- [19] One member wondered whether responses to member comments were made available. The steward noted that the compiled IPPC members comments are made publically available on the International Phytosanitary Portal (IPP)<sup>3</sup> but the responses to the comments were available only to the SC. The normal procedure would be for IPPC members to contact an SC member to obtain detailed responses to comments.
- [20] The TPDP:
- [21] agreed to recommend the revised draft DP on *Tilletia indica* (2004-014) to the SC for adoption.

## 4. Update on the Development of Diagnostic Protocols

### 4.1 General overview of status of protocols

- [22] The Secretariat made a presentation on the current status of the 32 DPs under the TPDP work programme. In the presentation, the Secretariat highlighted the dates where it is expected that the DPs will reach the main steps in the Standard Setting process (i.e. expert consultation, MC, submission to the SC for approval for adoption). The Secretariat also reinforced that engaging experts in the DP drafting groups is crucial to reach the established deadlines and thus facilitate the adoption process.

### 4.2 General overview and reports on status of individual DPs and review of experts associated with the work programme

- [23] The TPDP reviewed the status of protocols<sup>4</sup> and the expertise of the authors in the DP drafting groups. Two draft DPs under the topic Fungi and fungus-like organisms (2006-006) will be recommended to the SC for adoption: (*Phyllostica citricarpa* (2004-023) and *Tilletia indica* (2004-014)).
- [24] The Secretariat mentioned that the two DPs for MC in 2013 (*Xanthomonas citri* subsp. *citri* (2004-011) and *Potato spindle tuber viroid* (2006-022)), will need further work from the DP drafting group once the member consultation period closes (31 November 2013). The intention is to recommend the revised drafts to the SC for approval for adoption in the first quarter of 2014.
- [25] It was proposed to change the title of the draft DP on Tosspoviruses (TSWV, INSV, WSMV) (2004-019) to be in accordance with the scientific convention naming of virus species<sup>5</sup> to *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV). The TPDP agreed with this change.
- [26] The draft DPs that had been subject to the ECDP and revised during this meeting, are expected to be recommended to the SC for approval for MC in the first quarter of 2014. These DPs are:

<sup>3</sup> Webpage on the IPP for the compiled comments on draft standards from member consultation period: <https://www.ippc.int/core-activities/standards-setting/compiled-member-comments-draft-standards>

<sup>4</sup> List of topics for IPPC standards: <https://www.ippc.int/core-activities/standards-setting/list-topics-ippc-standards>

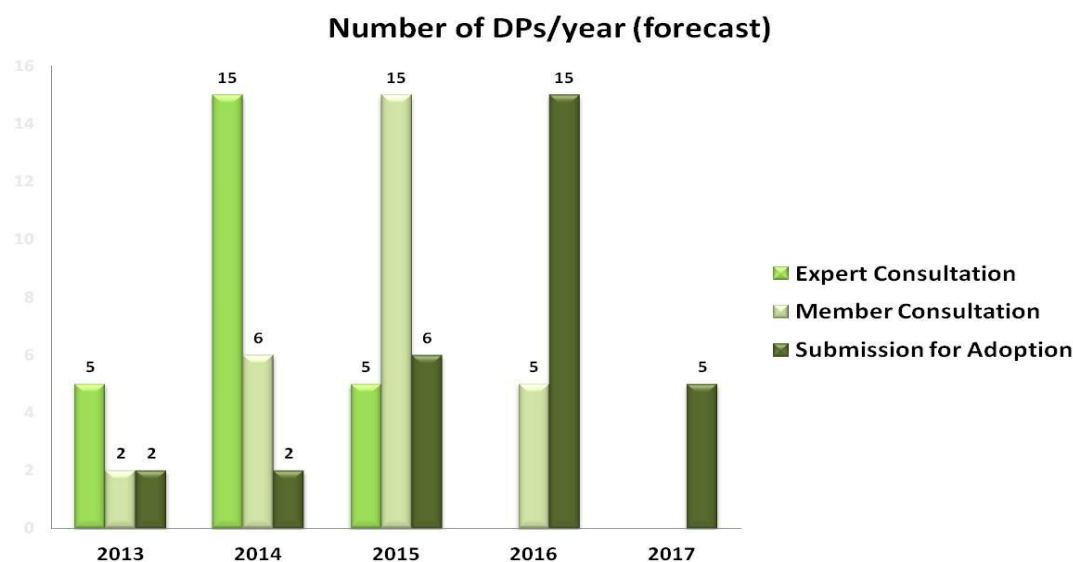
<sup>5</sup> International Committee on Taxonomy of Viruses (ICTV): <http://www.ictvonline.org/>

- *Anastrepha* spp. (2004-015)
- *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV) (2004-019)
- Phytoplasmas (*general*) (2004-018)
- *Ditylenchus destructor* / *D. dipsaci* (2004-017)

[27] Also, the draft DP on *Erwinia amylovora* (2004-009) is expected to be recommended to the SC for approval for MC in the first quarter of 2014.

[28] The general status of the protocols under the TPDP work programme, except for two draft DPs with “pending status”, is represented in Figure 1. The number of DPs per discipline is indicated in Table 1. The goal is to have all 30 DPs, currently active under the TPDP work programme, submitted for adoption by 2017.

[29] **Figure 1.** Number of diagnostic protocols under the TPDP work programme per year (forecast). Thirty DPs in total, excluding the DPs with “pending status” in the *List of topics for IPPC standards*.



[30]

[31] **Table 1.** Number of DPs per discipline under the TPDP work programme forecast by year and step in the Standard Setting process (excluding the DPs with “pending status” in the *List of topics for IPPC standards*)

	2013			2014			2015			2016			2017		
	EC	MC	SA	EC	MC	SA	EC	MC	SA	EC	MC	SA	EC	MC	SA
<b>Bacteriology</b>	-	1	-	3	1	1	1	3	1	-	1	3	-	-	1
<b>Mycology</b>	-	-	2	3	-	-	-	3	-	-	-	3	-	-	-
<b>Entomology</b>	1	-	-	3	1	-	3	3	1	-	3	3	-	-	3
<b>Nematology</b>	1	-	-	3	1	-	1	3	1	-	1	3	-	-	1
<b>Botany</b>	1	-	-	1	1	-	-	1	1	-	-	1	-	-	-
<b>Virology &amp; Phytoplasmas</b>	2	1	-	2	2	1	-	2	2	-	-	2	-	-	-
<b>TOTAL</b>	5	2	2	15	6	2	5	15	6	-	5	15	-	-	5

Legend: **EC** = Expert Consultation; **MC** = Member Consultation; **SA** = Submission for Adoption

- [32] **Review of experts associated with the work programme.** The TPDP members provided updates on their DP drafting groups and it was noted that some authors have not been in contact with the discipline leads. It was agreed that the panel members will try to establish contact with these authors by the beginning of August and follow up on this with the Secretariat if they had difficulties establishing contact.
- [33] **Call for authors.** There had been a call for authors for *Bursaphelenchus xylophilus* (2004-016) under the Nematodes topic (2006-008). The Secretariat received a total of seven nominations: 2 nominations from Canada, 1 from USA, 1 from Poland, 1 from Italy and 2 from China. The TPDP noted that all but one of the candidates were very well qualified with experience with the pest.
- [34] The selection criteria mentioned in the call letter as well as the following items were used by the TPDP to select authors:
- The TPDP prioritized experts from regions other than Europe since the lead author is from this region, and whenever possible regions where the pine wood nematode (*B. xylophilus*) is present.
  - As two co-authors had resigned, two new co-authors could be selected. However, the TPDP selected three new co-authors based on their expertise. It was not desirable to have too many co-authors, as the draft is very advanced and its final version is expected by the end of 2013. Non selected experts will be contacted to take part in the ECDP and their contribution will be recognized as appropriate.
  - The main need for co-authors concerned molecular detection and selection of adequate tests, so co-authors with the most relevant and current experience in this field and in relation with the pinewood nematode were selected.
- [35] The TPDP selected 2 authors from North America region, as representing a “contaminated region” and because they had different profiles: Ms Isabel LEAL (CA) with very active CV in *Bursaphelenchus* molecular identification and Mr Fengcheng SUN (CA) involved in routine analysis and in quarantine pests management. The third author selected was from China, Mr Jianfeng GU (CN), as he also had considerable expertise with the pest.
- [36] Regarding the need for other calls for authors, the TPDP agreed on the need to open a call for authors to the draft DP on *Liberibacter* spp. / *Liberobacter* spp. on *Citrus* spp. (2004-010) under the topic Bacteria (2006-005).

### 4.3 Subjects added by the SC: further steps

- [37] The Secretariat mentioned that for the three subjects, added by the SC to the TPDP work programme, the first draft of the DP, according to the TPDP working procedure, after the DP drafting group is established it is expected to be presented in 12 months (). The TPDP approved the new DP leads and referees assigned to these new DPs as outlined in Table 2. Also, the TPDP agreed on the need to open a call for authors for these DPs.
- [38] **Table 2.** Assigned discipline leads and referees to the subjects added to the TPDP work programme by the SC May 2013 meeting

Draft DP (topic number)	Discipline lead	Referee
<i>Liberibacter solanacearum</i> (2013-001)	Taylor, Robert (New Zealand)	Rodoni, Brendan (Australia)
<i>Conotrachelus nenuphar</i> (2013-002)	Barr, Norman (USA)	Terra, Ana Lía (Uruguay)
<i>Anguina</i> spp. (2013-003)	Anthoine, Geraldine (France)	Taylor, Robert (New Zealand)

- [39] The TPDP:
- [40] *agreed* to change the title of the draft DP for Tospoviruses (TSWV, INSV, WSMV) (2004-019) to *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV).



[41] *agreed* on adding the following experts to the *Bursaphelenchus xylophilus* (2004-016) DP drafting group:

- Ms Isabel LEAL (Canada)
- Mr Fengcheng SUN (Canada)
- Mr Jianfeng GU (China)

[42] *requested* the Secretariat to open a call for authors for the following draft DPs:

- *Liberibacter* spp. / *Liberobacter* spp. on *Citrus* spp. (2004-010)
- *Liberibacter solanacearum* (2013-001)
- *Conotrachelus nenuphar* (2013-002)
- *Anguina* spp. (2013-003)

## 5. Scrutiny of Draft Diagnostic Protocols

[43] The TPDP revised the draft DPs that had been submitted for the meeting (reported in the individual sections below). The draft DPs on *S. halepense* (2006-027) and *Erwinia amylovora* (2004-009) were discussed by the TPDP as the discipline lead requested further guidance. Discipline leads will work with the respective DP drafting groups to further develop the draft DPs.

[44] The panel considered that the ECDP was a very good step for the technical improvement of the DPs and the TPDP will therefore aim to continue using this step during the drafting stage.

[45] For all the protocols revised, the following general comments were made and the *Instructions for authors changed* accordingly (see section 6.1):

- (1) DPs should not be drafted following a Standard Operational Procedure (SOP) format.
- (2) A section on controls for molecular methods, minimum requirements for controls and interpretation of results should be added.
- (3) Information on symptoms should be included only if important for the diagnosis.
- (4) The use of disclaimers and brand names should be avoided, unless extremely necessary for the test performance. When possible, to avoid disclaimers and brand names, terminology should be changed to generic wording as per *Instructions for authors*:

In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. Use of names of reagents chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.

- (5) Experts that contributed to the draft should be acknowledged by adding their names to the MC cover page and those experts, who made major contributions, as determined by the panel, should be added in the *Acknowledgements* section.

### 5.1 *Anastrepha* spp. (2004-015)

[46] The discipline lead for Insects and mites (2006-007), Ms Ana Lía TERRA, was unable to attend this meeting. Thus, the other discipline lead for insects and mites, Mr Norman B. BARR, participated virtually and introduced the draft DP for *Anastrepha* spp and reviewed the compiled comments from three experts received during the ECDP and the checklist for discipline leads and referees. He mentioned that most of the comments had been incorporated into the draft.

[47] He also mentioned that in the DNA section of this draft DP, the explanation was too long and that the discipline leads will redraft it in order to reduce the wording. He noted that the protocol uses not just morphological information, but also host and geographical information and that they will also redraft these sections to reflect the importance of such information.

- [48] The discipline lead explained that, at this time, it is not possible to identify species reliably with DNA barcoding for taxonomic purposes. Some countries are adding barcoding data which has not been very well proved and the lead reinforced that the DP should provide minimum information that is reliable. Also, no molecular tool currently available can differentiate *Anastrepha* genus from other Tephritidae species and this will be added to the *Introduction* section of the DP to save member comments on this. Consequently, the identification methods included in this first version of the DP are based on morphological identification. The DP covers the determination of the genus and some species of economic concern belonging to genus *Anastrepha*.
- [49] Some comments from the TPDP were made on the need to gather more inputs from experts, and the TPDP agreed that the discipline leads should consult experts with regard to taxonomic information and molecular data. It was noted by the Steward that there is no need for another formal ECDP since there were no major comments.
- [50] It was recalled that, despite the high number of species, only a few are considered important economic pests, because of either the cultivated fruits they can attack in certain conditions or their wide host range. Therefore, the discipline lead proposed a new title to this draft DP to “Identification of genus *Anastrepha* with an emphasis on seven species of economic importance”.
- [51] The TPDP *agreed* to change the title of this draft DP to “Identification of genus *Anastrepha* with an emphasis on seven species of economic importance (2004-015)” and to send this draft to the DP drafting group for further adjustments. Then the draft DP will be revised via TPDP e-decision and recommended to the SC for approval for member consultation (MC).

## 5.2 Tospoviruses (TSWV, INSV, WSMV) (2004-019)

- [52] One of the discipline lead for Viruses and phytoplasmas (2006-009) and lead for this draft DP, Mr Delano JAMES, introduced the draft DP and reviewed the compiled experts’ comments received during the ECDP from eight experts and the checklist for discipline leads and referees.
- [53] The discipline lead mentioned that some comments questioned the scope of this draft DP and its title. In order to be clear about the scope the title was changed to include the names of the three viruses: *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV) (2004-019).
- [54] The referee of this draft DP raised the concern that the biological indexing was given too much weight as tospoviruses are very labile and a negative result in a bioassay could be misleading. Also, he noted that the distribution of the virus needs a better explanation.
- [55] The discipline lead mentioned that alternative symptoms are not addressed and that this information should to be included.
- [56] The main discussion points made by the TPDP were as follows:
- The information on the virus genome is to be kept because it is important to the virus taxonomy.
  - It was mentioned that there is a study indicating that some tospoviruses are pollen transmitted, thus seed transmitted. A TPDP member will follow up on this issue and report back to the panel on the scientific findings.
  - It was noted that negative biological indexing concluded that the virus is not present. Concerns were expressed that this is not always the case, especially in case of irregular distribution of the virus in a plant, and that a negative result should be confirmed by Polymerase Chain Reaction (PCR) or other appropriate method.
  - The criteria for bioassays were discussed, such as photoperiod and the stage of the development of a plant to perform these tests. It was stressed that photoperiod is essential for symptoms expression by tospoviruses and the draft should clearly state this. The DP drafting should address the more appropriate photoperiod for the 3 tospoviruses. Also, the stage of development of the plants, which is a 3 true leaves stage. It was also noted that symptom development may

occur after seven days, but usually the symptoms observation requires a period of four weeks and it is preferable to perform this bioassay in a constant photoperiod with 12 hours light and 12 hours dark.

- The draft should include a flow diagram for detection of the tospovirus in symptomatic material to address the minimum requirements for the diagnosis. The minimum requirements for diagnosis from asymptomatic material could be described in the text.

[57] Comments were made on the incomplete information on the descriptions of methods, as for example for biological assays regarding the symptomatology. It was noted that symptoms on pepper plants will depend on the variety and may not reflect those that are described. However, it was pointed out that not all contracting parties will have much experience with the pest and descriptions of symptoms might be helpful. It was pointed out that it would be useful to cross reference some web sites with pictures and also publications with these descriptions.

[58] In discussions on whether the lateral flow test (LFT) should be included in the draft as a preliminary screening test, it was noted that the LFT may give false positives and negatives. Some members commented that LFT might be useful for inspectors to be able to take action in the field on the basis of a positive reaction. The TPDP decided that the LFT should be fully described in the draft but not included in the flow diagram.

[59] Also, in the flow diagram, comments highlighted the need for a better use of negative indicator plants that should be used in all serological (Enzyme-Linked ImmunoSorbent Assay - ELISA) or molecular tests.

[60] The TPDP discussed whether the one-step Reverse Transcriptase (RT)-PCR is publicly available. It was agreed that the discipline lead will follow-up on this with the lead author and if so it should be included in the draft DP.

[61] Discussions were made on Real-time PCR assays and whether they should be used for detection. It was pointed out that the author only mentioned this method and that there might not be a need for a section on it. It was noted that in general it is not a good detection method since it can have cross-reactions and also, when the sample has a low virus titre, the result cannot be confirmed by a conventional PCR. It was also noted that some hosts may not be expected to have the cross-reacting viruses (e.g. groundnut virus might not be present in another host). It was agreed by the TPDP that the *Identification* section in the draft DP will be reinstated and cross referenced to Real-time PCR, having this method as a tool for identification. Also, the interpretation of results for Real-time PCR should be completed by the DP drafting group.

[62] Discussion on the record keeping for the samples storage was raised since *Tospovirus* lose infectivity if they are freeze dried and kept at  $-20^{\circ}\text{C}$  or warmer. Based on scientific evidences from literature, the TPDP agreed that for *Topovirus* samples should be kept at  $-80^{\circ}\text{C}$  to retain infectivity, as this temperature is the next temperature (after  $-20^{\circ}\text{C}$ ) that laboratories are used to work with.

[63] The TPDP agreed to return the draft DP on *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV) (2004-019) to the DP drafting group for further adjustments. Then the draft DP will be revised via TPDP e-decision for the recommendation to the SC for approval for MC.

### 5.3 Phytoplasmas (general) (2004-018)

[64] One of the discipline lead for Viruses and phytoplasmas (2006-009) and lead for this draft DP, Mr Brendan RODONI, introduced the draft DP and reviewed the compiled experts' comments received during the ECDP from six experts and the checklist for discipline leads and referees. Most of the comments provided during the ECDP were incorporated into the draft.

- [65] The discipline lead pointed out concerns on the current taxonomic situation with *Candidatus Phytoplasma* ('*Ca. Phytoplasma*') as described in some recent scientific papers. He will check the correct taxonomy of phytoplasmas and reflect it in the draft DP.
- [66] The main discussion points made by the TPDP were:
- The first section of the draft DP should be *Detection* and then the *Identification* section should follow.
  - The DP needs a standard section for all the controls used in the tests.
  - A general disclaimer for brand names as per the *Instructions for authors* should be included.
  - It was noted that there was a comment from the ECDP on the dilution proportion of a PCR product to perform the nested-PCR on 1:10 to 1:25 that could be ambiguous. The discipline lead will address this with the lead author.
  - Biological detection was not included in the draft as it is not considered applicable for detection.
  - Comments were raised regarding the validity using the primer pairs P1/P7 and R16F2n/R16R2 for detection of new phytoplasma groups. References will be added to improve this statement and to clearly demonstrate that these primers are still used and valid for new described phytoplasma groups.
  - The use of Real-time PCR and sequencing was discussed. It was noted that for phytoplasmas, the risk to obtain false-positive results are high due to the taxonomic proximity with Bacteria, and in those cases sequencing would be needed. It was also noted that for nested-PCR, if the appropriate controls are not used, the risk to obtain false-positives may be high.
  - For the *Identification* section, it was noted that a general statement on sequencing is needed. On this, it was explained that in cases where there is a possibility of contamination or cross reaction with Bacteria, there would be a need to do sequencing. However, for identification of which phytoplasma is in the sample, there are some specific Real-time PCRs that have been tested in a EUPHRESKO project which did not cross react with some Bacteria. EUPHRESKO is a European Research Area Network (ERA-NET) project for research policy development and implementation in the field of statutory and emerging plant pests, diseases and invasive species. So, the TPDP agreed that it would be worth mentioning that these exist. It was also noted that identification can be done using barcoding, but the results are not published yet so should not be included in the draft DP.
  - Regarding the record keeping, there was a need for better clarification on spotted plant extracts. Also, it was highlighted that keeping the samples at -20 °C is preferable if using automatic DNA extraction methods but not for cetyltrimethyl ammonium bromide extraction. It was agreed to have the wording "usually kept at -80 °C" so laboratories that do not have sophisticated facilities can still store at -20 °C.
- [67] The TPDP *agreed* to send the draft DP on Phytoplasmas (*general*) (2004-018) back to the DP drafting group for further adjustments. Then the draft DP will be revised via TPDP e-decision for recommendation to the SC for approval for MC.

#### **5.4 *Ditylenchus destructor* / *D. dipsaci* (2004-017)**

- [68] The Chairperson welcomed the lead author of the draft DP on *Ditylenchus destructor* / *D. dipsaci* (2004-017), Ms Antoinette SWART.
- [69] The nematology discipline lead for this draft DP explained briefly the development history of this draft DP and mentioned that in 2010 two draft protocols had been presented to the TPDP, and one TPDP remark had been that they should be combined. A new species was described, *D. gigas*, which also had to be included in the protocol. Molecular methods were also included, although they are not widely used in practice, thus general information is provided in the draft.
- [70] The Secretariat noted that there were no comments received during the ECDP before the start of this meeting, but as the deadline for commenting was 27 June 2013, comments could still come in.

- [71] The *Checklist for discipline lead* on this draft DP was presented and the main points addressed were:
- There was a need to review the molecular information and place it in the appropriate location.
  - The *Identification* section was improved to highlight that identification can be done by several techniques, but that morphological identification is the minimum requirement.
  - Accuracy in the identification tests was already addressed, by checking the tests, since there are a lot of synonymies for *D. destructor* and *D. dipsaci*.
  - Revision on the disease symptomatology still needs to ensure that those described are enough for detection.
  - The inclusion of some figures showing typical symptoms is needed to reduce the text description of them.
  - The number of references still needs to be reduced.
- [72] The lead author introduced the draft DP and it was noted that *D. dipsaci* infects predominantly cultivated plants. She mentioned that the information available on taxonomy is not fully complete, since *D. dipsaci* and *D. gigas* were originally considered the same species. In the current taxonomy, there is no *D. dipsaci* in *strictus sensus*. She mentioned that she will reword the *Scope* section to reflect this taxonomy information.
- [73] The lead author also noted that this draft DP describes the detection of the *Ditylenchus* complex by molecular and morphological tests. Thus, the information on the phylogenetic analysis can become confusing because the information on phylogeny is still not confirmed. However, this information does not really affect the diagnosis directly but it is important for defining the scope.
- [74] A member noted that similar issues occurred regarding the taxonomy of *Xanthomonas citri* subsp. *citri* and this was included in the taxonomic information of the draft DP for this referred pest.
- [75] It was discussed whether the taxonomic authorship should be included in the species name. It was agreed that all authors should be mentioned because this is scientifically correct. The Secretariat noted that the IPPC scientific editor would be informed about this and guidance provided in the IPPC Style Guide.
- [76] In the revision of the draft, the main points of discussion were as follows:
- The section on bionomics was deleted because it related to biology rather than diagnostics. Readers can refer to pest data sheets if required.
  - A new publication on extraction methods for nematodes is expected in December 2013 and should be included in this draft DP, if appropriate.
  - In the *Preservation of specimens* section, there is a need to reduce the text; however, some information is still needed because this section is especially important for quarantine labs.
  - The inclusion of sensitivity information of the tests is needed, especially on the possibility to have cross reactions with *D. gigas*.
  - The inclusion of commercial kits and brand names should be included only if extremely necessary to the tests performance and should have general disclaimers.
  - The need to include a flow diagram with the decision schemes should be considered.
  - The inclusion of scientific names for the common names of the diseases should be consistent with the *Instructions for authors*.
  - There is a need to include an identification key for *Ditylenchus* genus level.
  - There is a need to include a table with the features to identify at species level.
- [77] The TPDP agreed to send the draft DP on *Ditylenchus destructor* / *D. dipsaci* (2004-017) back to the lead author and DP drafting group for further adjustments. Then, it will be revised and submitted for TPDP approval, via TPDP e-decision, for recommendation to the SC for approval for MC.

## 5.5 *Erwinia amylovora* (2004-009)

- [78] One of the discipline lead for Bacteria (2006-005) and lead for this diagnostic protocol, Mr Brendan RODONI, introduced the draft DP. He acknowledged that this draft was thoroughly discussed during the 2012 TPDP June meeting and it was intended to go to the 2013 member consultation. He added that the draft was not ready by the deadline, thus he was presenting it again to the TPDP for a final check.
- [79] The discipline lead was reported that the DP drafting group found that the adoption process for DPs is too long and this caused lack in motivation in drafting. However, it was highlighted by the discipline lead that the IPPC is trying to streamline the process.
- [80] The main points made by the discipline lead were:
- The first draft was presented in 2005. The lead author pursued international ring tests and modifications resulting from them had been incorporated to the draft.
  - A flowchart of the process is going to be developed to simplify the information in the draft.
  - The discipline lead will work with the lead author to finalize the draft by the December 2013.
- [81] The TPDP *agreed* to send this draft DP on *Erwinia amylovora* (2004-009) back to the lead author and to the DP drafting group for further adjustments. Then, it will be revised and submitted for TPDP approval, via TPDP e-decision, and then recommended to the SC for approval for MC.

## 5.6 *Sorghum halepense* (2006-027)

- [82] The discipline lead for Plants (2007-001), introduced the draft DP. She acknowledged that this draft was discussed during the 2012 TPDP June meeting and it was intended to go to the ECDP during 2013. She thanked panel members who had helped improve the protocol after the last meeting.
- [1] The discipline lead informed that a ring test had been carried out in China by six laboratories using 12 samples and three different methods for identification of *S. halepense*. The findings of this ring test demonstrated that the internal transcribed spacer method was the least good and so this was not included in the draft protocol. She also reported that the scope was limited to identification of *S. halepense* in seed and grain consignments.
- [83] Some members inquired if the results of this ring test were published. The discipline lead mentioned that they will be published soon, and the references will be included in the draft protocol.
- [84] One member noted that this draft includes information on identification of plants and that in the last TPDP meeting it was suggested that the scope should be limited to seed. However, it was explained that some countries might want to be able to identify grown plants of *S. halepense*, which could be a target for inspections, and this was the reason that it was included in this draft. In this regard, the TPDP advised that this draft protocol should include information on where and how to perform the sampling in a consignment and also should indicate the type of commodities that the pest can be associated with, e.g. seed and grain, which are the main pathways for *S. halepense*. This information would be included in the *Pest information* section.
- [85] Other points of discussion were:
- The synonymies for *S. halepense* still need to be verified.
  - For sampling, the draft needs to include information about the amount collected, taking into account that this is an international standard and it might have implication for the inspection of a consignment.
  - The term “inspection” should be avoided; “examination” should be used.
  - Citing “personnel communications” should be avoided and only published references added.
  - For the *Detection* and *Identification* sections, there is a need for a link since these are two separate steps but both sections are necessary for the proper diagnosis. Information on the type of sample, the minimum amount of seeds needed for a reliable diagnosis.

- For the morphological identification, information is needed for both seeds and plants.
- References for the source of information must be included in the text for every table and keys if presented in the draft DP. Also, a reference to a glossary for plants terms should be included.
- For the identification of seeds, this draft protocol still needs to address the information on the length of time needed to grow the plant. Also, it should state if it is an annual or a perennial and, if it normally or occasionally cultivated. This should be scientifically based and take into consideration the minimum time to perform the identification and its impact on trade.
- For molecular assays, it is needed to improve the information on the necessity to perform molecular tests for each individual plant / sample. Also, there is a need to add information on the DNA extraction method.
- For the *Records* section, more information on how to store the samples and what to store (if seeds or plants or both) should be added.

[86] The TPDP *agreed* to send the draft DP on *S. halepense* (2006-027) back to the lead author and to the DP drafting group for further adjustments. Then, it will be revised and submitted for TPDP approval, via TPDP e-decision, to be subject to the expert consultation on draft DPs in September 2013. This DP will be discussed again during the 2014 TPDP face-to-face meeting.

## 6. Procedures and Guidance Related to TPDP

### 6.1 TPDP procedures:

[87] The following procedures were discussed and revised. These procedures can be found in the Standard Setting Procedure Manual<sup>6</sup> as per noted by SC May 2013.

#### TPDP Working procedure

[88] Some members suggested including flow diagrams showing the movement of the DPs through the 19 stages status according to the *List of topics for IPPC standards* and presented in the paper presented at this meeting.

#### TPDP Instructions for authors

[89] The panel reviewed the Instructions for authors and minor adjustments were made, mainly for better clarification of the text. Some points added were as follows:

- DPs are reviewed on a regular basis (every 5 years unless a specific issue was raised). Authors should be aware that this will be done;
- DPs should not instruct NPPOs on the methods to use;
- Interpretation of methods results may be made within the section for each method. In some cases, a specific section may be needed (for example, for molecular methods);
- Line drawings, if included, should be sufficient for diagnosis. If original illustrations are included, the author should be named;
- Pest information content should follow examples of adopted DPs;
- For taxonomic information, the date of authority should be included, but for viruses and viroids no authority/date is required;
- For taxonomy information of fungi, macro- or micro-conidial states should also be presented under synonyms. Also, for fungi, a reference to Mycobank may be included under Reference;
- For detection section, the example of “minimum sample size” was added to the additional information. Also, in the detection section, in some cases where methods can be used for both detection and identification (e.g. virology) the methods should be described in the Detection section and cross-referenced in the Identification section;

<sup>6</sup> Standard Setting Procedure Manual IPP link: <https://www.ippc.int/core-activities/ippc-standard-setting-procedure-manual>

- Contact points for further information, wording from ISPM 27 on requests for revision to the DP should also be added;
- For the acknowledgements section, if drawings or illustrations were produced especially for the protocol, they can be acknowledged here. In addition, special contributions may be mentioned here, for example those experts that made extensive comments on the draft or when the draft protocol made extensive use of work done by others (e.g. ring-testing).

[90] It was noted that some changes would also be done in response to the discussion on the draft standardized template for draft diagnostic protocols (see agenda point 6.2).

#### **Checklist for discipline leads and referees**

[91] No major comments were made.

#### **Checklist for authors**

[92] No major comments were made and no further changes were proposed to the 2012 TPDP November meeting version. It will be added to the *Instructions to authors*.

#### **Criteria for prioritization of protocols**

[93] There were no comments.

[94] The TPDP:

[95] *invited* the SC to note the revised TPDP *Instructions for authors* that will be presented to the SC in its next May meeting.

### **6.2 Draft standardized template for draft diagnostic protocols**

[96] The Secretariat presented the template and the main points of discussion were:

- The standardized template for draft DPs should be an appendix of the *Instruction to authors*.
- It was acknowledged that DPs are subject to review every five years and it was agreed there would be a sentence in the *Instructions to authors* on this and it was agreed that TPDP members should review the diagnostic protocols in their discipline on an annual basis or as determined by the TPDP.
- Reference should be made to adopted DPs (Annexes to ISPM 27:2006) as models for upcoming protocols.

[97] Cases where methods can be used for both detection and identification (e.g. virology) the methods should be described in the *Detection* section and cross-referenced in the *Identification* section.

[98] In the *Sampling* and sample preparation section, add a sentence that there is no need to include instructions on sampling for inspectors; for seeds and grain the information of the sample size is only needed if it is related to the sensitivity of the testing.

[99] The following wording was added: “A request for a revision to a diagnostic protocol may be submitted by NPPOs, RPPOs or CPM subsidiary bodies through the IPPC Secretariat ([ippc@fao.org](mailto:ippc@fao.org)), which will be forwarded it to the TPDP”.

[100] Proper wording on molecular methods and molecular controls should be included as standardized text and how molecular controls fit with quality assurance terms. These wordings were incorporated to the draft standardized template for DPs.

[101] The TPDP:

[102] *agreed* to append the revised draft standardized template for DPs to the Instructions to authors as presented in Appendix 4 of this report.



### 6.3 Draft table template format for PCR reaction conditions

- [103] The nematology discipline lead introduced a paper outlining issues related to PCR reactions. It was mentioned that a proposed template for PCR reactions is intended to be added to the draft standardized template for DPs.
- [104] The TPDP discussed whether to include PCR cycling parameters (denaturation, annealing and extension) as the TPDP wants to avoid standard operating process format in the protocols. The TPDP agreed that this table template is a suggestion for authors to use and not mandatory. It was also agreed that it was premature to add a table format for PCR reaction conditions to the *Instructions to authors*. The nematology discipline lead will redraft this document and present it at the next TPDP face-to-face meeting.

### 6.4 Quality assurance issues

- [105] The nematology discipline lead introduced the paper related to quality assurance and mentioned that a small group composed of Mr Mallik MALIPATIL, Ms Ana Lía TERRA, Mr Norman BARR and Ms Geraldine ANTHOINE had been formed some years ago because the TPDP had identified a need to address some quality assurance terms.
- [106] It was noted that some terms defined in the quality assurance document presented did not reflect the same definition as used in the DPs. The small group also checked relevant terms in the Glossary of Phytosanitary Terms (ISPM 5).
- [107] Some members found that part of the wording in the quality assurance document, for example on controls, could be made more generic or give examples (e.g. for bioassays or ELISA rather than for PCR only). Also, for water control and negative control, no reaction and no cross reaction is expected, and this should be stated.
- [108] It was noted that, the implementation of IPPC DPs and other international standards does not require accreditation from ISO.
- [109] The TPDP:
- [110] *agreed* that the TPDP quality assurance document with the main definitions related to diagnostics be made available to TPDP members on IPP in the restricted work area.
- [111] *asked* Mr Norman BARR to redraft the document and circulate it by email to the TPDP members before the next TPDP face-to-face meeting.

### 6.5 Study on the utility of IPPC diagnostic protocols

- [112] The Secretariat introduced the paper noting that this subject was discussed by the Strategic Planning Group (SPG) and at the IPPC regional workshops, and SC had considered it further in its 2012 November meeting. During the 2013 SC May meeting, the SC requested the TPDP review the final format of the questionnaire for the study on the utility of DPs that the Secretariat presented, and adjust it if necessary. Also, the SC requested the Secretariat to present the study to the TPDP at its next meeting for further elaboration. However, the SC also agreed that such a study may be premature because there were currently only three adopted DPs. The study should be finalized and used again when more protocols are adopted.
- [113] It was reported that two SC members had provided comments to the Secretariat to be considered by the TPDP. The comments provided were considered and the TPDP discussions were as follows:
- One SC member pointed out that the survey should only be for NPPOs and not also for RPPOs, because some countries will receive it more than once as being part of more than one RPPO. The TPDP did not think it was a problem to receive it more than once because it was up to each country to decide whether to answer the questionnaire just once or several times.

- The TPDP agreed with the SC member comment that the survey text should be more driven to gather NPPOs information on the reasons if a DP was changed.

[114] To SC member suggestion to include an additional question to the NPPOs asking to list a maximum of three things that the NPPO would like to see improved in ISPM 27:2006, the TPDP found that this was not for the TPDP to discuss.

[115] Regarding the SC member suggestion to delete all the listed possible users of DPs in some questions, the TPDP agreed to delete the mention of “quarantine officers”, “PRA teams” etc. and simply say “others” allowing NPPOs to state who uses the protocols.

[116] The TPDP agreed to add an extra box at the end of the survey to allow NPPOs or RPPOs state general comments.

[117] The TPDP:

[118] *agreed* to present to the SC and invite the SC to *note* the modified version of *Study on the utility of IPPC diagnostic protocols* as presented in Appendix 05.

## **6.6 New tools: Virtual meeting tool, IPP/TPDP forum page, Expert Consultation System, Possible improvements to the development of diagnostic protocols**

[119] The Secretariat introduced the paper mentioning that the IPPC Secretariat has been using some new virtual tools for the TPDP and DP drafting groups in order to help improve the development of diagnostic protocols and to ensure transparency in the process. The new tools were: virtual meeting tools, TPDP forum webpage and the ECDP on draft diagnostic protocols.

[120] For the virtual meetings tool, the Secretariat had changed to Adobe Connect® and this will be used for the next TPDP meeting because it is less expensive and technical support is more widely available in FAO. It was explained that this tool is useful for meetings with a small agenda and for information on administrative issues, updates and progress reports of the DP drafting groups that require TPDP fast decisions.

[121] For the TPDP forum page, the Secretariat explained that this proposal is based on the model used by the SC for e-decisions. It was also mentioned that the restricted area for TPDP e-decisions<sup>7</sup> is intended to facilitate the TPDP decision process and that TPDP members can access and contribute to forum discussions and express their opinions through polls at any time, from all over the world, while the e-decision is open.

[122] The ECDP on draft DPs<sup>8</sup> has the objective of ensuring quality improvement for the DP development as discussed in other agenda items. The panel agreed that this system would be for the DPs to be discussed in the next TPDP face-to-face meetings.

[123] On possible improvements to the development of DPs, the TPDP discussed the possibility of having two member consultation periods of draft DPs starting in 2015, because in 2014 a high number of drafts DPs will be discussed during the TPDP face-to-face meeting and will therefore become ready for MC.

[124] The TPDP:

[125] *noted* the new web-based tools implemented by the Secretariat.

[126] *invited* the SC to consider having two member consultation periods on draft DPs starting in 2015.

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<sup>7</sup> TPDP Forum page: <https://www.ippc.int/work-area-pages/tpdp-forum>

<sup>8</sup> Expert consultation system on draft DPs on the IPP: <https://www.ippc.int/core-activities/expert-consultation-draft-diagnostic-protocols>

## 7. Priorities for Additional Diagnostic Protocols

### 7.1 Consideration of proposals in 2007 call for topics, as requested by the SC

[127] The Secretariat introduced the paper and explained the main background points justifying a DP for *Boeremia foveata*. Because the TPDP, in its 2012 November meeting, did not reach consensus in adding the *B. foveata* to the work programme, the evaluation against the criteria was being presented again. It was also noted that the biannual IPPC call for topics was open from 20 May to 31 August 2013<sup>9</sup> and that IPPC members and Technical Panels could submit detailed proposals for new topics or for the revision of existing ISPMs to the IPPC Secretariat.

[128] The discipline lead for fungus did not have any further update since the last TPDP meeting because no information on this fungus had been published since then. Some TPDP members believe that this pest was important for some regions, such as South America and Asia. However, no available data was presented.

[129] The TPDP:

[130] asked Ms Ana Lía TERRA and Ms Liping YIN to gather available information on the importance of *B. foveata* and submit the information to the discipline lead on fungus and to the Secretariat in order to be discussed in a virtual meeting.

### 7.2 Specific discussions on the scope and status of protocols: *Bactericera cockerelli* (vector of *Liberibacter solanacearum*)

[131] One of the discipline lead for Insects and mites (2006-007), Mr Norman B. BARR, introduced the document and reported on his findings on the criteria for the inclusion of a pest to the TPDP work programme. It was mentioned that during the 2012 November meeting<sup>10</sup> the TPDP agreed to recommend the subject *Liberibacter solanacearum* be added to the work programme. Thus, some members also proposed the inclusion for the potato psyllid *B. cockerelli*, which is the vector for *L. solanacearum*, under the topic Insects and mites.

[132] The discipline lead for entomology explained that there is not much active research on diagnostic techniques for *B. cockerelli* and that diagnostic features are documented to distinguish the species from other psyllids based on morphology, geography and hosts. Identification can be difficult where other species of *Trioza* and *Bactericera* are common, such as in areas of the United States.

[133] The discipline lead did not recommend the inclusion of *B. cockerelli* to the TPDP work programme because options for regulating commodities and the ability to confirm morphological identifications by experts already exist.

[134] Some members mentioned that this pest is present in some Pacific regions (e.g. New Zealand), but the presence of it does not represent a problem in identification.

[135] It was also pointed out that preventing the spread of the psyllid should help slow the spread of *Candidatus Liberibacter solanacearum* and because of the importance of the disease, countries may want regulation and therefore need a good DP. The Secretariat remarked that the IPPC makes a biannual call for topics and that IPPC members can submit proposals for new topics.

[136] It was also noted that in there is a reference to an Australian protocol posted on the IPPC under the Phytosanitary resources page<sup>11</sup>.

[137] The TPDP:

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<sup>9</sup> IPPC 2013 Call for topics: <https://www.ippc.int/core-activities/calls-topics>

<sup>10</sup> TPDP 2012 November meeting report: <https://www.ippc.int/core-activities/standards-setting/expert-drafting-groups/technical-panels/technical-panel-diagnostic-protocols>

<sup>11</sup> <http://www.phytosanitary.info/>

[138] agreed not to add *Bactericera cockerelli* to the TPDP work programme.

### **7.3 Discussion of proposals for 2013 call for topics**

[139] The TPDP steward stressed that the IPPC biannual call for topics is open from 20 May to 31 August 2013 and that the TPDP will need to review proposed subjects after the 2013 SC November meeting, if appropriate.

[140] It was noted that as three new DPs had been added to the TPDP work programme by the SC at its 2013 May meeting.

## **8. Update on the Work of Other Organisations, International Organization for Standardization (ISO)**

[141] One of the discipline lead for Viruses and phytoplasmas (2006-009), Mr Delano JAMES, mentioned that there had been no major activities since the last update provided during the 2012 TPDP meeting. He mentioned that the ISO president resigned last year and a call for experts had been made. It was reported that a meeting in April 2013 was held to deal with the call for experts and the next call for experts was extended to EPPO and IPPC.

[142] Mr Delano JAMES will continue the liaison with ISO and will inform the TPDP if a call for experts will be take in place.

### **Global Taxonomy Initiative (GTI)**

[143] The Secretariat introduced the papers noting that this subject on the GTI had been deferred from the 2012 TPDP face-to-face meeting. The panel was informed of the Convention of Biological Diversity (CBD) and wondered how to get involved with it.

[144] One member informed that EPPO receives updates on the GTI projects and activities, especially on molecular tools by GTI notifications. Some members suggested that this could be a good resource and that the TPDP would benefit to be kept informed on the GTI activities.

[145] Some members alluded that the outputs of the GTI projects would be a standardize terminology and this may be useful for the TPDP. It was mentioned that, for this initiative, the most contributive players would be the taxonomists not really the TPDP.

[146] The TPDP acknowledged there was interest in knowing the results of the GTI, but no need to take part in their taxonomy work programme.

## **9. TPDP Work Plans**

### **9.1 TPDP 2013-2014-2015 work plan and TPDP medium term plan**

[147] IPPC Secretariat introduced the TPDP 2013-2014-2015 work plan, which had been updated based on decisions taken during the meeting. The TPDP agreed to the work plan for 2013-2014-2015, which will be made available at the TPDP restricted work area on the IPP and with the TPDP Medium Term Plan, which is presented as Appendix 06 of this report.

## **10. Date and Location of Next Meeting**

[148] The next TPDP meeting is scheduled for 7-12 July 2014 and will again be hosted at EPPO headquarters. It will be as a six days meeting due the high number of draft DPs expected to be discussed.

## 11. Other Business

### The use of digital keys in DPs

- [149] One of the discipline lead for Insects and mites (2006-007), Mr Norman B. BARR, introduced the document on the use of digital keys and explained that this topic was identified by Mr Hume DOUGLAS, the lead author for the draft DP on *Ips* spp. (2006-020). He explained that this tool is like a searchable database and that interactive digital keys/tools for species diagnosis are common for many large taxonomic groups of insects. It was also described that rather than using a dichotomous key that follows a pre-planned order, an interactive key allows the user to select the order of the matrix character entry (i.e. taxonomic structure).
- [150] In spite of the disadvantages in using digital keys, a printed version of the DP would produce a big document (100 pages length). Some members queried the possibility that DPs could refer to these keys, but not specifically give instructions as how to use them. Also, there is still a need to clarify the approximate accessibility of the keys, how information is updated and the ownership of the data.
- [151] Some members did not find that it would be appropriate to have digital keys as formal parts of the DPs, but that they could refer to them. It should be clear, if referred to in a DP, that the use of digital keys is not a requirement, but can be an additional resource for consultation.

## 12. Close of the Meeting

- [152] On behalf of the TPDP, the Chairperson thanked EPPO for hosting the meeting and for the hospitality provided.
- [153] The IPPC Secretariat thanked the whole panel and the Chairperson for their work. The Secretariat also thanked the EPPO Secretariat for hosting the TPDP meeting. The TPDP steward thanked EPPO and all the panel members for the work, and, in particular Ms Fabienne GROUSSET (IPPC Secretariat), because she would be no longer the IPPC Secretariat support for the TPDP. The panel thanked her for all the assistance she had provided to the TPDP.

**APPENDIX 1 - Agenda****Technical Panel on Diagnostic Protocols****EPPO Headquarters, Paris, France****24-28 June 2013***Opening: Monday 24 June 2013 at 10:00**Daily Schedule: 08:30-12:30 and 13:30-17:30***AGENDA**

<b>AGENDA ITEM</b>	<b>DOCUMENT NO.</b>	<b>PRESENTER</b>
<b>1. Opening of the meeting</b>		IPPC Secretariat
1.1 Welcome	-	EPPO Secretariat
1.2 Selection of the Chairperson and Rapporteur	-	IPPC Secretariat/Chairperson
1.3 Review and adoption of the agenda	TPDP_2013_Jun_01	Chairperson
<b>2. Administrative Matters</b>		
- Local information	TPDP_2013_Jun_02	EPPO Secretariat / IPPC Secretariat
- Documents list	TPDP_2013_Jun_03	IPPC Secretariat
- Participants list (and membership)	TPDP_2013_Jun_04	IPPC Secretariat
<b>3. Reports</b>		
- TPDP November 2012 meeting report - TPDP February 2013 virtual meeting report	<a href="https://www.ippc.int/core-activities/standards-setting/expert-drafting-groups/technical-panels/technical-panel-diagnostic-protocols">https://www.ippc.int/core-activities/standards-setting/expert-drafting-groups/technical-panels/technical-panel-diagnostic-protocols</a>	IPPC Secretariat
- Updates from other relevant IPPC meetings	TPDP_2013_Jun_12	Steward
<b>4. Update on the development of diagnostic protocols</b>		
4.1 General overview of status of protocols (Including update on DPs subject to SC for adoption (on behalf of CPM) and proposed time scale for adoption)	TPDP_2013_Jun_11	IPPC Secretariat
4.2 General overview and reports on individual DPs status by discipline leads (scope and status of protocols) and review of experts associated with the work programme	TPDP_2013_Jun_18 TPDP_2013_Jun_28 2004-009	Discipline leads, IPPC Secretariat
4.3 Subjects added by the SC: further steps	TPDP_2013_Jun_19	IPPC Secretariat
<b>5. Scrutiny of draft diagnostic protocols</b>		
5.1 <i>Anastrepha</i> spp. (2004-015) - Comments from expert consultation system - Checklist for discipline leads and referees	2004-015 TPDP_2013_Jun_26 TPDP_2013_Jun_25	Entomology discipline lead (Mr Barr)

AGENDA ITEM	DOCUMENT NO.	PRESENTER
5.2 Tospoviruses (TSWV, INSV, WSMV) (2004-019) - Comments from expert consultation system - Possible checklists by discipline lead and referee	2004-019 - TPDP_2013_Jun_23 and TPDP_2013_Jun_29	Virology discipline lead (Mr James)
5.3 Phytoplasmas (general) (2004-018) - Comments from expert consultation system - Possible checklists by discipline lead and referee	2004-018 TPDP_2013_Jun_24 TPDP_2013_Jun_30	Virology discipline lead (Mr Rodoni)
5.4 <i>Ditylenchus destructor</i> / <i>D. dipsaci</i> (2004-017) - Possible comments from expert consultation system - Possible checklists by discipline lead and referee	2004-017	Nematology discipline lead (Ms Anthoine)
5.5 <i>Erwinia amylovora</i> (2004-009)	2004-009	Virology and bacteriology back-up discipline lead (Mr Rodoni)
5.6 <i>Sorghum halepense</i> (2006-027)	2006-027	Botany discipline lead (Ms Yin)
<b>6. Procedures and guidance related to TPDP</b>		
6.1 TPDP procedures: - TPDP Working procedure - TPDP Instructions for authors - Checklist for discipline leads and referees - Checklist for authors - Criteria for prioritization of protocols	TPDP_2013_Jun_10 TPDP_2013_Jun_09 TPDP_2013_Jun_06 TPDP_2013_Jun_07 TPDP_2013_Jun_08	IPPC Secretariat, Steward
6.2 Draft standardized template for draft diagnostic protocols	TPDP_2013_Jun_15	Steward, IPPC Secretariat
6.3 Draft table template format for PCR reaction conditions	TPDP_2013_Jun_16	Nematology discipline lead (Ms Anthoine)
6.4 Quality Assurance issues	TPDP_2013_Jun_17	Entomology discipline lead (Ms Anthoine)
6.5 Study on the utility of IPPC diagnostic protocols	TPDP_2013_Jun_05	Steward, IPPC Secretariat
6.6 New tools: - Virtual meeting tool - IPP/TPDP forum page - Expert consultation system - Possible improvements to the development of diagnostic protocols	TPDP_2013_Jun_20	IPPC Secretariat
<b>7. Priorities for additional diagnostic protocols</b>		
7.1 Consideration of proposals in 2007 call for topics, as requested by the SC (Remaining: <i>Boeremia foveata</i> (syn. <i>Phoma foveata</i> , <i>Phoma exigua</i> var. <i>foveata</i> ))	TPDP_2013_Jun_21	IPPC Secretariat (document prepared by discipline leads on Mycology and Entomology)

AGENDA ITEM	DOCUMENT NO.	PRESENTER
7.2 Specific discussions on the scope and status of protocols - <i>Bactericera cockerelli</i> (vector of <i>Liberibacter solanacearum</i> )	TPDP_2013_Jun_22	Entomology discipline lead (Mr Barr)
7.3 Discussion of proposals for 2013 call for topics	-	Steward, IPPC Secretariat
<b>8. Update on the work of other organisations</b>		
- ISO (especially regarding draft ISO standard 13484) - Global Taxonomy Initiative (GTI)	- TPDP_2013_Jun_13 TPDP_2013_Jun_14	Virology discipline lead (Mr James) (IPPC Secretariat)
<b>9. TPDP work plans</b>		
- TPDP 2013-2014 work plan - TPDP Medium Term Plan	(To be prepared during the meeting)	IPPC Secretariat
<b>10. Date and location of next meeting</b>		IPPC Secretariat
<b>11. Other business</b>		IPPC Secretariat Chairperson
- The use of Digital Keys in Diagnostic Protocols	TPDP_2013_Jun_27	Entomology discipline lead (Mr Barr)
<b>12. Close of the meeting</b>	-	EPPO Secretariat IPPC Secretariat Chairperson



**APPENDIX 2: Documents list****Technical Panel on Diagnostic Protocols****EPPO Headquarters, Paris, France***24-28 June 2013**Opening: Monday 24 June 2013 at 10:00***Documents list***(By agenda number)*

DOCUMENT NO.	AGENDA ITEM	DOCUMENT TITLE
<b>Draft Diagnostic Protocols</b>		
2004-015	05.1	<i>Anastrepha</i> spp. (2004-015)
2004-019	05.2	Tospoviruses (TSWV, INSV, WSMV) (2004-019)
2004-018	05.3	Phytoplasmas (general) (2004-018)
2004-017	05.4	<i>Ditylenchus destructor</i> / <i>D. dipsaci</i> (2004-017)
2004-009	05.5	<i>Erwinia amylovora</i> (2004-009)
2006-027	05.6	<i>Sorghum halepense</i> (2006-027)
<b>Other documents (By agenda number)</b>		
TPDP_2013_Jun_01	01.3	Agenda
TPDP_2013_Jun_03	02	Documents list
TPDP_2013_Jun_04	03	Participants list
TPDP_2013_Jun_02	02	Local information
TPDP_2013_Jun_12	03	Updates from other relevant IPPC meetings
TPDP_2013_Jun_11	04.1	General overview of status of protocols
TPDP_2013_Jun_18	04.2	General overview and reports on individual DPs status and review of experts associated with the work programme
TPDP_2013_Jun_19	04.3	Subjects added by the SC: further steps
TPDP_2013_Jun_25	05.1	TPDP procedures: Checklist for discipline leads and referees - <i>Anastrepha</i> spp. (2004-015)
TPDP_2013_Jun_26	05.1	Comments from expert consultation system – <i>Anastrepha</i> spp. (2004-015)
TPDP_2013_Jun_23	05.2	TPDP procedures : Checklist for discipline leads and referees

DOCUMENT NO.	AGENDA ITEM	DOCUMENT TITLE
TPDP_2013_Jun_24	05.3	Comments from expert consultation system - Phytoplasmas (general) (2004-018)
TPDP_2013_Jun_10	06.1	TPDP Procedure: Working procedures
TPDP_2013_Jun_09	06.1	TPDP Procedure: Instructions to authors
TPDP_2013_Jun_06	06.1	TPDP Procedures: checklist for discipline leads and referees
TPDP_2013_Jun_07	06.1	TPDP Procedures: checklist for authors
TPDP_2013_Jun_08	06.1	TPDP Procedures: criteria for the prioritisation of DPs
TPDP_2013_Jun_15	06.2	Draft standardized template for draft diagnostic protocols
TPDP_2013_Jun_16	06.3	Draft table template format for PCR reaction conditions
TPDP_2013_Jun_17	06.4	Quality assurance issues associated with DPs for regulated pests
TPDP_2013_Jun_05	06.5	Study on the utility of IPPC diagnostic protocols
TPDP_2013_Jun_20	06.6	New tools for the TPDP and DP drafting groups
TPDP_2013_Jun_21	07.1	Consideration of proposals in 2007 call for topics, as requested by the SC (Remaining: <i>Boeremia foveata</i> (syn. <i>Phoma foveata</i> , <i>Phoma exigua</i> var. <i>foveata</i> ))
TPDP_2013_Jun_22	07.2	<i>Bactericera cockerelli</i> (vector of <i>Liberibacter solanacearum</i> ): study against the criteria
TPDP_2013_Jun_13	08	Global Taxonomy Initiative (GTI)
TPDP_2013_Jun_14	08	Global Taxonomy Initiative (GTI) – Attachment 1
TPDP_2013_Jun_27	11	The use of Digital Keys in Diagnostic Protocols

**APPENDIX 3: Participants list****2013 MEETING TECHNICAL PANEL ON DIAGNOSTIC PROTOCOLS****24-28 June 2013****EPPO Headquarters, Paris, France****PARTICIPANTS LIST***A check (✓) in column 1 indicates confirmed attendance at the meeting.*

	Participant role	Name (Country)	Email address	Term begins	Term ends
<b>TPDP members<sup>12</sup></b>					
✓	Steward	Ms Jane CHARD (United Kingdom)	<a href="mailto:jane.chard@sasa.gsi.gov.uk">jane.chard@sasa.gsi.gov.uk</a> ;		
✓	Bacteriology	Mr Robert TAYLOR (New Zealand)	<a href="mailto:Robert.Taylor@maf.govt.nz">Robert.Taylor@maf.govt.nz</a>	May 2011	May 2016
✓	Nematology	Ms Géraldine ANTHOINE (France)	<a href="mailto:geraldine.anthoine@anses.fr">geraldine.anthoine@anses.fr</a> ;	April 2009	April 2019 (2 <sup>nd</sup> term)
✓	Virology	Mr Delano JAMES (Canada)	<a href="mailto:Delano.James@inspection.gc.ca">Delano.James@inspection.gc.ca</a>	Nov. 2010	Nov. 2015
✓	Virology and backup bacteriology	Mr Brendan RODONI (Australia)	<a href="mailto:Brendan.Rodoni@dpi.vic.gov.au">Brendan.Rodoni@dpi.vic.gov.au</a>	July 2012	July 2017
✓	Botany	Ms Liping YIN (China)	<a href="mailto:yinlp@shciq.gov.cn">yinlp@shciq.gov.cn</a> ; <a href="mailto:yinliping@yahoo.com">yinliping@yahoo.com</a> ;	April 2008	April 2018 (2 <sup>nd</sup> term)
	Entomology	Ms Ana Lía TERRA (Uruguay)	<a href="mailto:aterra@mgap.gub.uy">aterra@mgap.gub.uy</a> <a href="mailto:alt2912@live.com">alt2912@live.com</a>	April 2008	April 2018 (2 <sup>nd</sup> term)
	Entomology	Mr Norman B. BARR (USA) <sup>13</sup>	<a href="mailto:Norman.B.Barr@aphis.usda.gov">Norman.B.Barr@aphis.usda.gov</a>	July 2012	July 2017
	Mycology	Mr Johannes DE GRUYTER (The Netherlands)	<a href="mailto:j.de.gruyter@minlnv.nl">j.de.gruyter@minlnv.nl</a> ;	April 2008	April 2018 (2 <sup>nd</sup> term)

<b>Other participants</b>					
✓	Host	Ms Françoise PETTER European and Mediterranean Plant Protection Organization (EPPO) 21 boulevard Richard Lenoir 75011 Paris <b>France</b> Tel: +33 1 45 20 77 94 / Fax: +33 1 70 76 65 47	<a href="mailto:petter@epo.int">petter@epo.int</a>		
✓	Host - Invited expert	Ms Natasa MEHLE National Institute of Biology, Vecna pot 111, SI-1000 Ljubljana, <b>Slovenia</b> Tel: +386-59232808	<a href="mailto:natasa.Mehle@nib.si">natasa.Mehle@nib.si</a>		

<sup>12</sup> For contact details please access the TPDP membership list on <https://www.ippc.int/core-activities/standards-setting/expert-drafting-groups/technical-panels/technical-panel-diagnostic-protocols>

<sup>13</sup> Attendance via virtual tool (Skype).

Other participants			
✓	Invited expert: Lead author ( <i>Ditylenchus destructor</i> / <i>D. dipsaci</i> )	Ms Antoinette SWART National Collection of Nematodes, Biosystematics Division, ARC - Plant Protection Research Institute, Private Bag X134, Queenswood 0121 <b>South Africa</b>	<a href="mailto:SwartA@arc.agric.za">SwartA@arc.agric.za</a>
✓	IPPC Secretariat	Ms Adriana MOREIRA Standard Setting - IPPC Secretariat <b>FAO</b> , Viale delle Terme di Caracalla 00153 Rome, Italy Tel: +39 06 570 55809	<a href="mailto:Adriana.Moreira@fao.org">Adriana.Moreira@fao.org</a>
✓	IPPC Secretariat	Ms Fabienne GROUSSET Standard Setting - IPPC Secretariat <b>FAO</b> , Viale delle Terme di Caracalla 00153 Rome, Italy	<a href="mailto:Fabienne.Grousset@fao.org">Fabienne.Grousset@fao.org</a>

## APPENDIX 4: Instructions to Authors: Standardized template for diagnostic protocols [as revised in meeting – 26 June 2013]

(TPDP, June 2013)

This standardized template is intended to help authors of diagnostic protocols (DPs) when drafting an IPPC diagnostic protocol. The *Instructions to authors* contain information and guidance on the content and formatting of protocols, as well as combination of methods in DPs. Required text is provided in black. Text to be completed by the author and guidance on how to complete it is between square brackets with guidance in italics. Text for completion by the Secretariat or TPDP lead is in square brackets and highlighted in grey. Examples are in boxes in pale green. Authors may use this file to write their draft protocol and can then remove all italics, boxed and highlighted text. A checklist for authors is included as Appendix 3 of the *Instructions to authors*, to cross-check the content of the draft once written.

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**DRAFT ANNEX to ISPM 27:2006 – [Pest name] [(Topic number)]** (*Add the scientific name of the pest and authority where required (no authority should be listed for viruses and viroids). Note: the year of naming is not relevant in the title. Secretariat will add the topic number of the subject from the List of topics for IPPC standards*)

### Examples

**DRAFT ANNEX to ISPM 27:2006 - *Tilletia indica* Mitra (2004-014)**

**DRAFT ANNEX to ISPM 27:2006 – *Potato spindle tuber viroid* (2006-022)**

**DRAFT ANNEX to ISPM 27:2006 – *Erwinia amylovora* (Burrill) Winslow *et al.* (2004-009)**

### Publication information

(Include the table below and complete relevant parts. Secretariat and TPDP lead to complete additional parts as appropriate.)

<b>Date of this document</b>	[to be completed by the Secretariat]
<b>Document category</b>	Draft new annex to ISPM 27:2006 ( <i>Diagnostic protocols for regulated pests</i> )
<b>Current document stage</b>	[to be completed by the Secretariat]
<b>Origin</b>	Work programme topic: [Topic (date of addition by CPM)] Original subject: [Name (number)]
	<b>Example</b> Work programme topic: Fungi and fungus-like organisms, CPM-1 (2006) Original subject: <i>Tilletia indica</i> / <i>T. controversa</i> (2004-014)
<b>Major stages</b>	[to be completed by the Secretariat]
<b>Consultation on technical level</b>	The first draft of this diagnostic protocol was prepared by: [first name, family name of lead author (unit, institute, city, country) and co-authors] ( <i>List the lead author and co-authors – complete addresses are not needed, but the unit, institute, city, country should be mentioned</i> )
	<b>Example</b> Dominie Wright (Department of Agriculture and Food of Western Australia, Perth, Australia); Guiming Zhang (Laboratory of Plant Inspection and Quarantine, Shenzhen Entry-Exit Inspection and Quarantine Bureau, Shenzhen City, China).
	( <i>Add, as appropriate, name of all experts who, although not part of the initial DP drafting group, contributed to the drafting or commented on the draft, as follows:</i> )  - In addition, [names of experts (first name, family name (unit, institute, city, country))] [was/were] significantly involved in the development of this protocol.

	<p>- This protocol has been commented upon by: [names of experts (first name, family name (unit, institute, city, country))]</p> <p>(Also, other relevant information can be mentioned here, for example:)</p> <p>- This draft protocol was adapted from a protocol originally drafted by: [names of experts (first name, family name (unit, institute, city, country))]</p> <p>- It was presented at the [e.g. conference/symposium on (name, place), date], and further comments were provided by: [names of experts (first name, family name (unit, institute, city, country))]</p>
<p><b>Main discussion points during development of the diagnostic protocol</b> [to be updated throughout DP development]</p>	<p>[to be completed by the TPDP lead]</p> <ul style="list-style-type: none"> <li>•</li> <li>•</li> </ul> <p>(Note: Especially after experts have been consulted at early stages of development, the cover note should indicate substantial comments that were not incorporated in the draft. Include as bullet points)</p>
<b>Notes</b>	[to be completed by the Secretariat]

## 1. Pest Information

[Insert pest information text] (See section 4.1 of the Instructions to authors)

### Example. *Thrips palmi*

*Thrips palmi* Karny (Thysanoptera: Thripidae) is a polyphagous plant pest, especially of species in the Cucurbitaceae and Solanaceae. It appears to have originated in Southern Asia and to have spread from there during the latter part of the twentieth century. It has been recorded throughout Asia and is widespread throughout the Pacific and the Caribbean. It has been recorded locally in North, Central and South America and Africa. For more general information about *T. palmi*, see EPPO/CABI (1997) or Murai (2002); online pest data sheets are also available from the Pests and Diseases Image Library (PaDIL, 2007) and EPPO (EPPO, 2008).

The species causes economic damage to plant crops both as a direct result of its feeding activity and from its ability to vector tospoviruses such as *Groundnut bud necrosis virus*, *Melon yellow spot virus* and *Watermelon silver mottle virus*. It is extremely polyphagous, and has been recorded from more than 36 plant families. It is an outdoor pest of, amongst others, *Benincasa hispida*, *Capsicum annum*, *Citrullus lanatus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita* spp., *Glycine max*, *Gossypium* spp., *Helianthus annuus*, *Nicotiana tabacum*, *Phaseolus vulgaris*, *Pisum sativum*, *Sesamum indicum*, *Solanum melongena*, *Solanum tuberosum* and *Vigna unguiculata*. In glasshouses, economically important hosts are *Capsicum annum*, *Chrysanthemum* spp., *Cucumis sativus*, *Cyclamen* spp., *Ficus* spp., Orchidaceae and *Solanum melongena*. The thrips may be carried on plants for planting, cut flowers and fruits of host species, as well as on or associated with packing material, and in soil.

*Thrips palmi* is almost entirely yellow in coloration (Figures), and its identification is hampered by both its small size (1.0–1.3 mm) and its great similarity to certain other yellow or predominantly yellow species of *Thrips*.

## 2. Taxonomic Information

(Use the standardised text below and see section 4.2 of the Instructions to authors). Note: Species names are always italicised, family and other names are not (apart from family names for viruses and viroids, which are italicised).

**Name:** [Scientific name, authority and date]  
**Synonym (or) Synonyms:** [Scientific name, authority and date.]  
(delete as appropriate)  
**Taxonomic position:** [insert taxonomic information]  
**Common name** [English common name(s), and reference, where available, to  
(or)Common names: names in other languages]  
(delete as appropriate)  
**Reference:** [for fungi a reference to Mycobank may be included]

**Examples - Insects**

**Name:** *Thrips palmi* Karny, 1925  
**Synonyms:** *Thrips gossypicola* Ramakrishna & Margabandhu, 1939  
**Taxonomic position:** Insecta, Thysanoptera, Terebrantia, Thripidae  
**Common name:** melon thrips

**Name:** *Trogoderma granarium* Everts, 1898  
**Synonyms:** *Trogoderma khapra* Arrow, 1917  
*Trogoderma koningsbergeri* Pic, 1933  
*Trogoderma afrum* Priesner, 1951  
*Trogoderma granarium* ssp. *afrum* Attia and Kamel, 1965  
**Taxonomic position:** Insecta: Coleoptera: Dermestidae.  
**Common names:** khapra beetle (English)

**Examples – Virus and viroids**

**Name:** *Plum pox virus* (acronym PPV)  
**Synonym:** Sharka virus  
**Taxonomic position:** *Potyviridae*, *Potyvirus*  
**Common names:** Sharka, plum pox.

**3. Detection**

[Insert text on detection of the pest] (*See sections 3 and 4.3 of the Instructions to authors*)

After the main heading, **3. Detection**, insert introductory paragraphs, and organise the methods using the structure below. The headings should be used as required (numbering for illustrative purposes only). It is not possible to provide standardized text in this section, but examples can be found in adopted protocols.

Where detection and/or identification methods are different for plants with symptoms and plants without symptoms, consider separating 3. into “3.1 Detection in symptomatic plants” and “3.2 Detection in asymptomatic plants”, and use the structure below for each of them.

**3.1 Symptoms****3.2 Sampling and sample preparation [symptomatic and asymptomatic material]**

[Insert text on sampling and sample preparation]

(If methods for preparation of material are generic for all methods, it may be appropriate to include text on preparation of material in a general section at the beginning. Alternatively, if preparation of material relates to a group of methods it may be appropriate to include text associated with each type of methodology. Otherwise, where preparation of material is specific to a method, it should be included with the method description. See also section 4.3 of the Instructions to authors.)

**3.3 Isolation [and culturing/growing] [from symptomatic material /from asymptomatic material]**

3.3.1 [Name of method] **e.g. Enrichment isolation**

3.3.2 [Name of method] etc.

**3.4 Biological detection****3.5 Serological detection**

3.5.1 *Preparation of material*

(If relevant, see note at 3.2)

3.5.2 [Name of method] *e.g. Double antibody sandwich indirect enzyme linked immunosorbent assay (ELISA)*

3.5.3 [Name of method] *e.g. Immunofluorescence (IF)*

### 3.6 Molecular detection

#### 3.6.1 Preparation of material

(If relevant, see note at 3.2)

#### 3.6.2 Nucleic acid extraction

3.6.3 [Name of method] *e.g. Conventional reverse transcription-polymerase chain reaction using the primers of Verhoeven et al. (2004)*

3.6.4 [Name of method] *e.g. Immunocapture reverse transcription-polymerase chain reaction*

#### 3.6.5 Controls for molecular tests

[Insert the following standardized text]

For a reliable test result to be obtained the following controls should be considered for each series of nucleic acid isolations, amplification of the target pest or target nucleic acid depending on the test used and the level of certainty required. As a minimum, for [method name] the [name minimum controls, e.g. positive nucleic acid control, internal control and negative amplification control (no template control)] should be used.

*(The rest of this section should provide a brief description of the controls. The minimum controls should be listed first, in the same order as they are named previously. Additional controls, if any, should be at the end. For each control, give additional details as necessary, e.g. specific controls named in individual methods in the protocol, etc.)*

#### **Positive nucleic acid control**

This is used to monitor the efficiency of the test method (apart from the extraction) [and with RT-PCR, the amplification]. [Description of the controls, e.g. Pre-prepared (stored) viroid nucleic acid, whole genome amplified DNA or a synthetic control (e.g. cloned PCR product)] may be used.

#### **Internal control**

For [method name(s)], plant internal controls [name(s) of gene(s) e.g. House Keeper Gene (HKG) such as COX or NAD] should be incorporated into the protocols to eliminate the possibility of PCR false negatives due to extraction failure, nucleic acid degradation or the presence of PCR inhibitors. Preferably the internal control primers should be used [add details, e.g. in a duplex reaction with the pospiviroid/PSTVd primers].

*(Add any qualifying information e.g. difficulties that may be encountered, effects on sensitivity, notes on the part of the assay that the gene acts as a control for e.g. with RT-PCR assays. Also examples of successful use of internal controls if known or relevant and not already referred to in the method descriptions in other sections.)*

When the internal control [name of gene] is not mentioned in the description of a PCR method, the laboratory should choose an internal control and validate it.

#### **Negative amplification control (no template control)**

This is necessary with conventional and real-time RT-PCR to rule out false positives due to contamination during the preparation of the reaction mix. PCR grade water that was used to prepare the reaction mix is added at the amplification stage.



### Positive extraction control

This is used to ensure that nucleic acid from the target is of sufficient quantity and quality and that the target is detected. Nucleic acid is extracted from infected host tissue or healthy plant tissue that has been spiked with the target.

The positive control should be approximately 1/10 of the amount of [type of material e.g. leaf tissue] used per plant for the [RNA/DNA] extraction. (*Add any other relevant elements, on e.g. adjustments to quantity, amounts of control material to use for different bulking rates etc. and if this control is not detected, provide guidance on repeating tests or adjusting the bulking rate until reliable detection is achieved.*)

For [PCR/RT-PCR], care needs to be taken to avoid cross contamination due to aerosols from the positive control or from positive samples. The positive control used in the lab should be sequenced so that this sequence can be readily compared to sequence obtained from PCR amplicons of the correct size. Alternatively, synthetic positive controls can be made with a known sequence which again can be compared to PCR amplicons of the correct size.

### Negative extraction control

This is used to monitor contamination during nucleic acid extraction and/or cross-reactions with the host tissue. This requires nucleic acid extraction and subsequent amplification of uninfected host tissue. It is recommended to include multiple controls when large numbers of positives are expected.

#### 3.6.6 Interpretation of results from [Name of methods]

*(Insert as a separate section only if necessary)*

## 4. Identification

[Insert text on identification methods] (*See Section 3 and 4.4 of the Instructions to authors*)

*It is not possible to provide standardized text in this section, but examples can be found in adopted protocols.*

*After the main heading, 4. Identification, insert introductory paragraphs, and use the structure below. Use the following headings as required (numbering for illustrative purposes only).*

### 4.1 Morphological identification (Note: for insects, fungi, nematodes, plants)

4.1.1 *Preparation of [developmental stage e.g. larvae, adults, seeds, plant material, teliospores] for examination (If necessary, normally for insects.)*

4.1.2 *Isolation [and culturing/growing] of [name of pest]*

4.1.2.1 *[Name of method] e.g. Germination of teliospores, Germination of similar Tilletia species*

4.1.3 *Identification of [developmental stage e.g. larvae, adults of] [family, genus, name of pest]*

4.1.4 *[Differentiation of / morphological comparison with] [developmental stage e.g. larvae, adults of][family, genus, name of pest] from similar species*

[Insert simple key, table or text with relevant details]

4.1.5 *Discriminating features of [developmental stage e.g. larvae, adults, name of pest] [of family, genus, name of pest]*

[Insert checklist of key diagnostic features]

(Add additional sections (and renumber) depending on the level of discrimination e.g. family, genus, species.)

**e.g. Table 1: Family Thripidae – shared characteristics**

Body part	Characteristic
Antennae	seven or eight segments (occasionally six or nine)
	segments III–IV have emergent sense cones (sensoria)
Forewings (if fully developed)	usually slender, with two longitudinal veins each bearing a series of setae
Abdomen – female	with a serrated ovipositor, which is turned downwards at the apex
Median sternites – male	with or without glandular areas

**Examples of structure of 4. Identification - *Thrips palmi* (morphological section only)**

General introductory paragraphs

- 4.1 Morphological identification of the adult thrips
  - 4.1.1 Preparation of thrips for microscopic examination
  - 4.1.2 Identification of the family Thripidae
    - Table 1: Family Thripidae – shared characteristics
    - Table 2: Genus *Thrips* – shared characteristics, adult specimens
  - 4.1.4 Identification of *Thrips palmi*
    - 4.1.4.1 Morphological characteristics of *Thrips palmi*
      - Table 3: A list of morphological characteristics that collectively distinguish *Thrips palmi* from other species in the genus *Thrips*
    - 4.1.4.2 Comparison with similar species (species that are yellow without darker body markings, or predominantly yellow, or sometimes yellow)
      - Table 4: Simplified checklists of the diagnostic features for quick recognition: (a) the genus *Thrips*; (b) *Thrips palmi* (See Figure 4 for the location of the various features.)

**4.2 Biological identification of [name of pest, strains, pathotypes]**

(For subsequent sections (Biological identification, Serological identification and Molecular identification) follow the same structure as in given in section 3. In addition sections on Identification using Nutritional and enzymatic tests or Biochemical identification methods may be required. If some elements are already described adequately in 3 (e.g. preparation of material, nucleic acid extraction, specific methods), do not repeat but cross-refer to the relevant subsection number.)

**4.2.1 Pathogenicity tests**

**4.3 Serological identification**

**4.3.1 Preparation of material**

(If relevant, see note at 3.2)

**4.3.2 [Name of method] (insert new section for each method)**

**4.4 Molecular identification**

**4.4.1 Preparation of material**

(If relevant, see note at 3.2)

#### 4.4.2 Nucleic acid extraction

#### 4.4.3 [Name of method] (insert new section for each method)

#### 4.4.4 Controls for molecular tests

*[Insert standardized text from 3.6.5 with appropriate modification] Insert this section only if necessary i.e. if controls used for detection tests are different to those for identification.)*

#### 4.4.5 Interpretation of results from [Name of methods]

*(Insert text only if necessary and if interpretation of results is different when methods are used for identification rather than detection.)*

### 5. Records

*(Include the following standardized text:)*

Records and evidence should be retained as described in section 2.5 of ISPM 27:2006.

*(Add additional paragraph(s) as required in individual DPs. For example:)*

In cases where other contracting parties may be affected by the results of the diagnosis[, in particular in cases of non-compliance (ISPM 13:2001, *Guidelines for the notification of non-compliance and emergency action*) and where [the pest, name of pest] 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, larvae and adults, preserved or slide-mounted specimens, culture(s) of the pest, [RNA, DNA] extracts, printed tissue sections and/or spotted plant extracts on paper or nylon membranes, PCR amplicons or test materials (e.g. photographs [of distinctive taxonomic structures, fungal structures, symptoms and signs], ELISA plate results printouts and photographs of gels].

*(Additional specific text may be added. For example details on of sample and records may be required e.g. storage temperature (at  $-80^{\circ}\text{C}$  or freeze-dried and stored at room temperature) or culture conditions (e.g. mycelium from broths or mycelial plugs from agar plates can be stored frozen at  $-80^{\circ}\text{C}$ ). Guidance may be included on handling isolates shown to have different molecular or biological characteristics compared to previously recorded isolates (e.g. offered to a national pest herbarium). Also, if there is evidence of any of the tests described failing to detect an isolate, authors may propose that details should be sent to the IPPC Secretariat.*

*In some cases, records of the number of positive subsamples and the estimated number of [telio]spores detected in each positive subsample may need to be kept and, for fungi, records of colony morphology, especially any pigmentation and growth rate under defined conditions, may need to be kept.)*

### 6. Contact Points for Further Information

*(Add the following standardized text. See section 4.6 of the Instructions to Authors.)*

Further information on this protocol can be obtained from [name of institutes and contacts in the format: Unit, institute, complete mailing address, country (full name of expert; e-mail; Tel +XX etc.; Fax: +XX etc.)].

A request for a revision to a diagnostic protocol may be submitted by NPPOs, RPPOs or CPM subsidiary bodies through the IPPC Secretariat (ippc@fao.org), which will in turn forward it to the TPDP.

#### Examples

Faculty of Horticultural Science, Department of Plant Pathology, Corvinus University, Villányi út 29-43, H-1118 Budapest, Hungary (Laszlo Palkovics, e-mail: laszlo.palkovics@uni-corvinus.hu; tel.: +36 14825438; fax: +36 14825023).

Department of Agriculture and Food Western Australia, Biosecurity & Research Division, Plant Biosecurity Branch, Entomology Unit, 3 Baron-Hay Court, South Perth, WA 6151, Australia (Andreas Szito, -e-mail: [aszito@agric.wa.gov.au](mailto:aszito@agric.wa.gov.au); tel: +61 8 9368 3248, +61 8 9368 3965; fax: +61 8 9368 3223, +61 8 9474 2840).

Pest and Disease Identification Team, The Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, United Kingdom. (Dom Collins; e-mail: [dom.collins@fera.gsi.gov.uk](mailto:dom.collins@fera.gsi.gov.uk); tel: +44 1904 462215; fax: +44 1904 462111).

## 7. Acknowledgements

*(Add the following standardized text indicating the experts that first drafted the text and those that made significant contributions. If the address was already mentioned in section 6, add “(see preceding section)”*)

The first draft of this protocol was written by [initials, family name (unit, institution, country, (see preceding section))]. In addition, the following experts were significantly involved in the development of this protocol [initials, family name (unit, institution, country, (see preceding section))].

(as relevant, use standardized text below – See section 4.7 of the Instructions to authors)

[Line drawings, Illustrations] for Figure [number] were produced by [name and address of expert]. The methods included in the protocol were ring tested by [names of experts or project and date] financed by [name of country organization and date].

*(if relevant add other acknowledgements as necessary – see examples below)*

### **Example**

The first draft of this protocol was written by M. Cambra, IVIA, Spain (see preceding section); N.L. Africander, Department of Agriculture, Forestry and Fisheries, Private Bag X 5015, Stellenbosch, 75999, South Africa; L. Levy, USDA, USA (see preceding section); S.L. Lenardon, IFFIVE-INTA, Cno. 60 Cuadras Km 51/2, Córdoba X5020ICA, Argentina. In addition, the following experts were significantly involved in the development of this protocol: A. Olmos and N. Capote, IVIA, Spain (see preceding section); G. Clover, Plant Health & Environment Laboratory, Ministry of Agriculture and Forestry, PO Box 2095, Auckland 1140, New Zealand; and D. Wright, Plant Health Group, Central Science Laboratory, Sand Hutton, York YO41 1LZ, United Kingdom. Line drawings for Figure 5 were produced by S. Kobro, Norwegian Crop Protection Institute, Norway.

### **Additional acknowledgements:**

#### ***Tilletia indica*** [from draft DP]

The basis of this protocol was originally drafted by A.J. Inman, K.J.D. Hughes and R.J. Bowyer (2003), Food and Environment Agency, York, UK. That protocol was ring-tested in different European laboratories (Riccioni *et al.*, 2002), and has formed the basis of the EPPO protocol PM 7/29(1) (EPPO, 2004).

The protocol has been enhanced by D.G. Wright, Department of Agriculture and Food, Western Australia, Australia; K.J.D Hughes, Food and Environment Agency, Sand Hutton, York, United Kingdom; and Guiming Zhang, Laboratory of Plant Inspection and Quarantine, Shenzhen City, China. V. Cockerell, Science and Advice for Scottish Agriculture, Edinburgh (United Kingdom) reviewed the protocol.

#### ***Erwinia amylovora*** [from draft DP]

Most techniques described were ring tested in a DIAGPRO project financed by the EU, in an EUPHRESKO project in 2009, and in a Spanish project in 2010.

#### **PSTVd** [from draft DP]

Thanks are due to S.L. Nielsen (Denmark), L. Seigner, S. Winter, M. Wassenegger (Germany), H. Koenraadt (The Netherlands), A. Fox, T. James, W. Monger, V. Mulholland (UK) for helpful comments during development of this protocol.

## 8. References

[Insert references]

(Provide a list of scientific references and other publications referred to in the protocol (see 4.8 in the Instructions to Authors).)

## 9. Figures

[Insert figures if necessary]

(See section 3 in the Instructions to Authors, as well as Appendix 3.)

### Examples of figure legends

**Figure 1:** *Thrips palmi*, female (left) and male (photo: A. J. M. Loomans, PPS, Wageningen, the Netherlands; scale bar = 500  $\mu\text{m}$  = 0.5 mm)

**Fig. 5.11(a), (b):** Abdominal tergite IX (dorsal), two pairs of campaniform sensilla (scale bar: 30  $\mu\text{m}$ )

**Figure 2: *Trogoderma granarium*:** (A) adult, female; (B) comparison of shape of female (left) and male (right); (C) young larva; (D) mature larva. Scale bar: (A), (B), (D) = 2 mm; (C) = 1 mm. ((A), Tomasz Klejdysz, Instytut Ochrony Roślin - Państwowy Instytut Badawczy, Poznań, Poland; (B), (D), Ya.B. Mordkovich and E.A. Sokolov, All-Russian Plant Quarantine Centre, Bykovo Russia); (C), Cornel Adler, Julius Kühn-Institut; (JKI) Germany))

**Figure 1.** Flow diagram showing the process to be used for the detection and identification of *Tilletia indica* in seed and grain samples

**APPENDIX 5: Study on the utility of IPPC diagnostic protocols****ANNEX 1. DRAFT SURVEY: IPPC DIAGNOSTIC PROTOCOLS**

<b>1.</b> Is your NPPO/RPPO aware of the adopted IPPC diagnostic protocols?	<input type="checkbox"/> YES <input type="checkbox"/> NO
<b>2.</b> Who uses or who would use adopted diagnostic protocols in your NPPO/RPPO?	(Please select one or more, as applicable) <input type="checkbox"/> Lab technicians <input type="checkbox"/> Diagnosticians <input type="checkbox"/> Researchers <input type="checkbox"/> Other (please list them) _____ _____
<b>3.</b> Does your NPPO/RPPO use any adopted diagnostic protocol?	<input type="checkbox"/> YES <input type="checkbox"/> NO
<b>3. a)</b> If so, then in which context?	(Please select as many as apply) <input type="checkbox"/> Official analysis <input type="checkbox"/> Surveillance <input type="checkbox"/> Monitoring <input type="checkbox"/> Post-entry quarantine <input type="checkbox"/> Training <input type="checkbox"/> Research <input type="checkbox"/> Other (please list them) _____ _____
<b>3. b)</b> If not, why are diagnostic protocols not used?	(Please list at least three main reasons) _____ _____ _____ _____
<b>4.</b> Do the protocols used in your NPPO/RPPO have any modification?	<input type="checkbox"/> YES* <input type="checkbox"/> NO *If YES, please list the modifications and the reasons for them: _____ _____
<b>5.</b> Which language version does your NPPO/RPPO use the protocol (or will probably be used when a protocol is implemented)?	<input type="checkbox"/> English <input type="checkbox"/> Spanish <input type="checkbox"/> French <input type="checkbox"/> Chinese <input type="checkbox"/> Arabic <input type="checkbox"/> Russian

<p><b>6.</b> If the protocol is available in English only, would it be useful or would it limit the usefulness of the protocol?</p>	<p><input type="checkbox"/> YES, the English version would be useful and would not limit the usefulness of the protocol*.</p> <p><input type="checkbox"/> NO, the English version would not be useful and would limit the usefulness of the protocol*.</p> <p>*Please, list the reasons of your answer:</p> <p>_____</p> <p>_____</p>
<p><b>7.</b> Do you think there is a need for the development of other DPs or is information on management of the pest more relevant?</p>	<p><input type="checkbox"/> YES, there is a need for the development of other diagnostic protocols.</p> <p><input type="checkbox"/> NO, there is no need for the development of other diagnostic protocols but there is a need of information on management of the pest.</p> <p><input type="checkbox"/> NO, there is no need for the development of other diagnostic protocols and information on management of the pest.</p>
<p><b>8.</b> Do you have any suggestions for improvement of the protocols? Please, list them.</p>	<p>(Please list three main suggestions)</p> <p>_____</p> <p>_____</p> <p>_____</p>
<p><b>9.</b> Which other DPs, on the work programme, should be developed as a priority?</p>	<p>Please list a maximum of five and indicate the reasons (the currently <i>criteria</i> used to prioritize proposals is annexed to this survey):</p> <p>_____</p> <p>_____</p> <p>_____</p> <p>_____</p> <p>_____</p>
<p><b>10.</b> Are there any other criteria for prioritisation of DPs development that you want to suggest?</p>	<p><input type="checkbox"/> YES*</p> <p><input type="checkbox"/> NO</p> <p>*If YES, please list your suggestions to the criteria for prioritisation of DPs development:</p> <p>_____</p> <p>_____</p>
<p><b>11. Any other comment</b></p>	<p>_____</p> <p>_____</p> <p>_____</p> <p>_____</p> <p>_____</p>

## ANNEX 2 TO THE SURVEY - STUDY ON THE UTILITY OF IPPC DIAGNOSTIC PROTOCOLS

### Criteria for the prioritisation of diagnostic protocols

(From IPPC Procedure Manual for Standard Setting (2012): <https://www.ippc.int/core-activities/ippc-standard-setting-procedure-manual>).

*The criteria are not in order of priority.*

Need for international harmonization of the diagnostic techniques for the pest (e.g. due to difficulties in diagnosis or disputes on methodology).
Relevance of the diagnosis to the protection of plants including measures to limit the impact of the pest.
Importance of the plants protected on the global level (e.g. relevant to many countries or of major importance to a few countries).
Volume/importance of trade of the commodity that is subjected to the diagnostic procedures (e.g. relevant to many countries or of major importance to a few countries).
Other criteria for topics as determined by CPM that are relevant to determining priorities.
Balance between pests of importance in different climatic zones (temperate, tropics etc) and commodity classes.
Number of labs undertaking the diagnosis.
Feasibility of production of a protocol, including availability of knowledge and expertise.



**APPENDIX 6: TPDP Medium Term Plan**

<b>TPDP Medium Term Plan</b>	
<b>Year</b>	<b>Activities</b>
<b>2013 (after June)</b>	<ul style="list-style-type: none"> <li>• Recommend 2 DPs for SC approval (e-decision) for adoption (contracting parties notification period)</li> <li>• Expert consultation period on draft DPs: 1 draft DPs</li> <li>• Call for authors</li> <li>• Call for topics</li> </ul>
<b>2014</b>	<ul style="list-style-type: none"> <li>• Recommend 2 DPs for SC approval (e-decision) for adoption (contracting parties notification period)</li> <li>• Recommend 5 DPs for SC approval (e-decision) for member consultation</li> <li>• Expert consultation period: 14 draft DPs</li> <li>• Call for authors</li> <li>• Meeting preparation: Forecast of 15 draft DPs discussion</li> <li>• Meeting (07-12 July, Paris, France)</li> </ul>
<b>2015</b>	<ul style="list-style-type: none"> <li>• Recommend 6 DPs for SC approval (e-decision) for adoption (contracting parties notification period)</li> <li>• Recommend 15 DPs for SC approval (e-decision) for member consultation</li> <li>• Expert consultation period: 5 draft DPs</li> <li>• Call for authors: Possible</li> <li>• Call for experts – Viruses and phytoplasmas: Possible</li> <li>• Meeting preparation: Forecast of 5 draft DPs discussion</li> <li>• Meeting (Tentative: August 2015, China)</li> </ul>
<b>2016</b>	<ul style="list-style-type: none"> <li>• Recommend 15 DPs for SC approval (e-decision) for adoption (contracting parties notification period)</li> <li>• Recommend 5 DPs for SC approval (e-decision) for member consultation</li> <li>• Meeting</li> </ul>
<b>2017</b>	<ul style="list-style-type: none"> <li>• Recommend 5 draft DPs for SC approval (e-decision) for adoption (contracting parties notification period )</li> </ul>