



*REPORT*

Rome, Italy  
13-17 February 2017

# **Technical Panel on Diagnostic Protocols (TPDP) February, 2017**



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

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**Appendices**

## OPENING OF THE MEETING

### 1.1 Welcome

- [1] The International Plant Protection Convention (IPPC) Secretariat (hereafter “Secretariat”), welcomed the participants of the twelfth meeting of the Technical Panel on Diagnostic Protocols (TPDP) to Rome and to the Food and Agriculture Organization of the United Nations (FAO) headquarters. The Standards Officer thanked the TPDP members for the work done over the years on the development of diagnostic protocols (DPs) and congratulated them on the recent adoption of five DPs<sup>1</sup>, some of which were only added to the *List of topics for IPPC standards* in 2013. He emphasized that the panel should strive to have all draft DPs currently on the TPDP work programme submitted for adoption in 2018, as work of the Secretariat will be refocused on other priority areas. Over the next few years, the IPPC community will be able to assess the use of the suite of adopted DPs.
- [2] In relation to the second objection received to the adoption of the draft DP on *Tomato spotted wilt virus*, *Impatiens necrotic spot virus* and *Watermelon silver mottle virus* (2004-019) the Standards Officer encouraged improved communication between the TPDP members and known experts to help prevent repeated objections in the future.
- [3] The Standards Officer informed the TPDP that, according to the decisions of the Commission on Phytosanitary Measures (CPM) Bureau, the limited resources of the IPPC Secretariat should primarily be dedicated to the development of phytosanitary treatments (PTs) and commodity standards. In this context, the work of the TDPD should slow down (see section 4.1 of this report). Development of new DPs may become possible again when the Secretariat’s standard setting unit is allocated additional resources (including staff), coming either from the FAO regular budget or from external donors (such as contracting parties). For these reasons, the 2018 TPDP face-to-face meeting may be the last one for some time. Nevertheless, the work on the DPs that are on the TPDP work programme should be finalized. He did not see the need to disband the TPDP, although he felt that new TPDP members would not be needed at this stage. Instead, he suggested the SC may be requested to extend the memberships of the current members and in this way they could work virtually to help respond to comments.
- [4] TPDP members stressed the importance of correct diagnostics to underpin decisions taken by national plant protection organizations (NPPOs). They also mentioned that DPs get outdated quickly and need revisions, especially as new technologies are coming in (e.g. new generation sequencing (NGS)). The TPDP discussed options for its work on the revisions of adopted DPs, including drafting and discussing them at virtual meetings. They also considered what types of manuals and other guidance material may be needed to help implement DPs.

### 1.2 Election of the Chairperson

- [5] Ms Juliet GOLDSMITH (Jamaica) was elected Chairperson.

### 1.3 Election of the Rapporteur

- [6] Ms Géraldine ANTHOINE (France) was elected Rapporteur.

### 1.4 Review and adoption of the agenda

- [7] The TPDP adopted the Agenda (Appendix 1).

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<sup>1</sup> <https://www.ippc.int/en/news/global-diagnostics-for-plant-pests-five-new-ippc-diagnostic-protocols-adopted/>

## 2. Administrative Matters

- [8] The Secretariat introduced the Documents list (Appendix 2) and the Participants list (Appendix 3). The participants were reminded to update their contact information as it will be reflected in the TPDP membership list<sup>2</sup> on the International Phytosanitary Portal (IPP – [www.ippc.int](http://www.ippc.int)).
- [9] The Secretariat presented the local information document<sup>3</sup>.

## 3. Scrutiny of draft diagnostic protocols

### 3.1 Revision of DP 2: *Plum pox virus* (2016-007) (Priority 1)

- [10] The discipline lead introduced the draft DP<sup>4</sup> and the checklist for discipline leads and referees<sup>5</sup>. He recalled that the revision of this DP was approved by the SC in November 2016<sup>6</sup> to update it indicating the new strains of *Plum pox virus* (PPV) described recently; CR (Cherry Russian) and An (Ancestor Marcus). The TPDP discussed the following main issues.
- [11] A TPDP member raised the point whether and to what extent the specificity of the molecular tests proposed for the identification of the new PPV strains had been tested. It was pointed out that this was clear for the Double-antibody sandwich indirect enzyme-linked immunosorbent assay (DASI-ELISA), but for other tests, such as real-time reverse transcription-polymerase chain reaction (real time RT-PCR) the specific data was incomplete. It was noted that the publication by Glasa *et al.* (2013)<sup>7</sup> mentions that there was no amplification for other strains except for CR strains. A TPDP member explained that it is normal practice and necessary, when developing a testing method for a particular strain, to test it against other strains to check for any possible cross-reaction. Another TPDP member suggested that the details could be requested from the authors of the scientific paper. The TPDP decided that the specificity was sufficiently reflected in the description of the real-time RT-PCR. The TPDP also noted that, for some strains, validation data may not be available, but that this does not invalidate the DP.
- [12] The TPDP agreed that the revision of the entire DP was not planned at this time, and modifications should be restricted only to the detection and identification of new strains of PPV.
- [13] A TPDP member requested clarification on the use of the terms “isolate”, “strain” and “type”. After further discussion, the TPDP decided to use “strain” instead of “type” as it was more appropriate and made this change throughout the draft DP for consistency.
- [14] One TPDP member asked clarification of the name of the monoclonal antibody used in the DASI-ELISA tests (5B-IVIA). It was explained that the antibody is provided by a private company (IVIA), and would be appropriately footnoted regarding the use of brand names. It was also noted that in the “contact points” section, interested parties may request information on how to obtain the antibody.
- [15] It was noted that primers described for the reverse transcriptase-polymerase chain reaction (RT-PCR) allowed for successful detection of all strains of PPV, so this test (RT-PCR) should be considered a PPV-specific detection tool.
- [16] One member suggested that the description of the SYBR Green I method for the simultaneous detection of PPV and identification of D and M strains should be placed in the section on identification of strains.

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<sup>2</sup> TPDP membership list: <https://www.ippc.int/en/publications/81560/>

<sup>3</sup> Local information: 03\_TPDP\_2017\_Feb

<sup>4</sup> 2016-007

<sup>5</sup> 25\_TPDP\_2017\_Feb

<sup>6</sup> 06\_SC\_2016\_Nov and SC November 2016 meeting report: <https://www.ippc.int/en/publications/83881/>

<sup>7</sup> Glasa, M., Prikhodko, Y., Predajna, L., Nagyová, A., Shneyder, Y., Zhivaeva, T., Šubr, Z., Cambra, M. & Candresse, T. 2013. Characterization of sour cherry isolates of *Plum pox virus* from the Volga basin in Russia reveals a new cherry strain of the virus. *Phytopathology*, 103: 972-979.

The TPDP agreed but added a note to clarify that this method cannot be used for identification of all strains, but only the two specific strains.

- [17] A TPDP member proposed that the names of primers in the flowchart on identification of strains be replaced with references to the relevant sections of the draft DP. Considering the full DP was not open for revision, the TPDP did not agree to this, as it would require too many text changes in the draft.
- [18] The TPDP noted that information on controls was missing in the draft DP and discussed whether a reference to another DP (e.g. DP 15: *Citrus tristeza virus*) or a new section should be added. As adding the reference would mean that the two DPs would have to be used at the same time, the TPDP decided to add a new section on controls.
- [19] In this regard, the TPDP agreed that guidance on the controls for the immunocapture RT-PCR should be drafted by the discipline lead and the DP drafting group, with the purpose of adding it to the Instructions to Authors<sup>8</sup>.
- [20] The TPDP reviewed and adjusted the contact points for the draft DP.
- [21] The TPDP:
- (1) *requested* the discipline lead and the DP drafting group to revise the draft DP and send it to the Secretariat by 17 March 2017.
  - (2) *agreed* to submit the revised draft Revision of the DP2: *Plum pox virus* (2016-007) to the SC with the recommendation that it be submitted to the 2017 consultation.

### 3.2 *Bactrocera dorsalis* complex (2006-026) (Priority 2)

- [22] The discipline lead introduced the draft DP<sup>9</sup>, summary of comments from the Expert Consultation<sup>10</sup> and the checklist for discipline lead and referees<sup>11</sup>. He explained that the development of this draft DP had been delayed because of the need to clarify the taxonomy of *B. dorsalis*. He mentioned that recently a paper by Schutze *et al.* (2015)<sup>12</sup> had been published on the synonymization of the species within the genus *Bactrocera*, which helped to move along the work on this draft DP. The discipline lead noted that the paper was supported by many fruit fly experts but that the International Entomological Society was still discussing the issue. The *B. dorsalis* complex currently includes a large number of species and no known diagnostic method allows for their precise identification at the species level. The TPDP discussed the following main issues.
- [23] Pest information: Some members suggested clarifying the scope of the DP because *B. dorsalis* may still be difficult to identify due to other “confusing” species. The TPDP agreed that the scope of the draft DP should be more precise, especially in terms of reasons for the selection of the six *Bactrocera* species described in detail in the draft DP. The TPDP agreed that the wording from adopted protocols should be used, as the six *Bactrocera* species were considered of economic importance as agricultural and quarantine pests in various countries. The discipline lead noted that *B. invadens* is not part of the *B. dorsalis* complex, according to Schutze *et al.* (2015), but it was included in the draft DP to differentiate this species with other in the *B. dorsalis* complex. The TPDP noted that diverse opinions on the taxonomy of *B. dorsalis* complex may reappear when the draft is submitted to the consultation period.

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<sup>8</sup> The TPDP Instructions to Authors: <https://www.ippc.int/en/publications/83612/>

<sup>9</sup> 2006-026 and 2006-026\_Figures

<sup>10</sup> 05\_TPDP\_2017\_Feb

<sup>11</sup> 06\_TPDP\_2017\_Feb

<sup>12</sup> Schutze *et al.* (2015). Synonymization of key pest species within the *Bactrocera dorsalis* species complex (Diptera: Tephritidae): taxonomic changes based on a review of 20 years of integrative morphological, molecular, cytogenetic, behavioural and chemoecological data. *Systematic Entomology*, Volume 40, April 2015, Pages 456–471.

- [24] Some experts, during the Expert Consultation suggested using the lengths of the genitalia as a diagnostic character. It was explained that genitalia identification is not useful for international identification, because the ranges overlap between the species, so it can only be useful for the species identification when found to be at the extreme ends of the ranges. Thus, it was clarified that the recommendation in this draft DP is that genitalia morphometrics are reliable diagnostic characters under very specific circumstances.
- [25] A TPDP member queried the meaning of *sibling*. The lead explained that a species may be referred to as a “sibling species” when it belongs to a group of species that are very closely related e.g. *B. dorsalis*, *B. invadens* and *B. papayae*. He explained that these species can cross breed with each other (at least under laboratory conditions). The lead added that some species may also be cryptic, which means that genetically and morphologically they may be the same, but because of their behaviors in the field they should not be classified as one species. He noted that siblings are not cryptic species. Regarding “hybrids” of *Bactrocera*, the discipline lead noted that in some cases when species are able to cross in the wild, hybrids may be found. However, under laboratory conditions, using Internal transcribed spacer (ITS), it has not been possible to find gene introgressions and the frequency of hybrids between *Bactrocera* species in nature has therefore not been estimated. As “hybrids” may occur under natural conditions, due to various factors, but this cannot be scientifically proved under laboratory conditions, text was provided in the draft DP to clarify the possibility of detection of “hybrids”.
- [26] In reply to one comment during the Expert Consultation, the discipline lead mentioned that it is not possible to identify the subgenera of *Bactrocera* based on the morphology of the larvae, so no method for larval identification was included in the draft DP.
- [27] One TPDP member suggested adding a table with information on the hosts for the *Bactrocera* species as there are many host possibilities and to improve the flow of the text. One TPDP queried the selection of hosts, as the common name given in the draft DP was for one species within a genus. It was clarified that those selected species may actually be the only host species in the genus. Additionally, some *Bactrocera* species in some geographical regions may attack only a specific host within the plant genus. The TPDP agreed to clarify the wording.
- [28] Taxonomic information: The TPDP agreed to have the taxonomic information in a table for easier reference.
- [29] The discipline lead explained that *complex* is not a taxonomic unit. The TPDP agreed that this explanation should be included as additional taxonomic information, as well as the information that the draft DP considers *B. invadens*, *B. papayae*, and *B. philippinensis* as part of *Bactrocera dorsalis sensu lato*.
- [30] Detection: It was agreed to simplify the text on detection to reflect that *Bactrocera* fruit flies are detected by trapping or by inspection of fruits. The TPDP recommended to the DP drafting group adjusting the figure captions to better explain the characters.
- [31] Inspection of fruits: The TPDP discussed whether the information in this section should follow other adopted DPs or if a reference to an adopted DP (e.g. DP 9: Genus *Anastrepha*) would suffice. The TPDP agreed that references to other DPs should not be used because the DPs could be revised in the future making the reference incorrect. Instead, the TPDP agreed that repeating some text from other DPs, if needed, was a better way forward. The TPDP removed text related to where infested fruits could be found (e.g. shipments, baggage, airplanes, terrestrial vehicles, etc.) to avoid direct instructions to NPPOs.
- [32] Identification: The TPDP agreed to use the word “to identify (a pest)” instead of “to diagnose (a pest)” throughout the text. It was highlighted that once detected, immature larvae could be reared to adults for identification. The TPDP noted that the minimum requirement for proper identification in this draft DP was adult identification.

- [33] The TPDP recommended that the references to the use of molecular methods should be clarified to reflect that they are supplementary to the morphological identification.
- [34] One TPDP member queried the difference between the two tables containing information on the adults' diagnostic characters. The discipline lead explained that one table was intended to contain information for the six economically important *Bactrocera* species, while the other table was for the entire *B. dorsalis* complex. He agreed that the tables could be merged and the TPDP asked the DP drafting group to redraft this information.
- [35] Preparation of adults for identification: The TPDP agreed that the appropriate stereoscope magnification level should be specified for the figures. The text on limited usefulness of the lengths of the aedeagus and aculeus for identification should be modified to better reflect that these are helpful only if their lengths are outside the overlapping area. Guidance on measuring the width of lateral vitae should be verified.
- [36] Rearing larvae to obtain adults: The TPDP agreed that the text on the pupation media should be modified to make it clear that the pupation happens in soil; text was adjusted on the use of killing agents to make it broader as there are several killing agents for adult insects that can be used.
- [37] Character to identify the genus *Bactrocera* and subgenus *Bactrocera* (*Bactrocera*): One member queried whether both terms - *setae* and *bristles* - were correct and suggested that only one be used to avoid confusion. The discipline lead explained that either term could be used, but agreed to check which one to use in the draft with the DP drafting group.
- [38] Morphological identification of six economically important species of *B. dorsalis* complex: One member queried the use of the term "character state" in the text. The lead explained that it was used to express the levels of variation of a character. The TPDP felt that it was confusing and agreed to delete the word "state" throughout the text.
- [39] The TPDP recommended that the guidance on the use of morphometric examination of genitalia to distinguish between *B. dorsalis* and *B. carambolae* should be checked for consistency with the section on preparation of adults for identification.
- [40] Molecular identification of six economically important species of *Bactrocera dorsalis* complex: The TPDP agreed to modify the title of this section as it relates to the molecular identification of *B. carambolae* only, and how to differentiate it from *B. dorsalis sensu lato* (*s.l.*). It was stressed that the draft DP did not recommend molecular methods for the identification of all six species of the *B. dorsalis* complex, but it does describe the molecular method to distinguish *B. dorsalis* from *B. carambolae*.
- [41] The TPDP noted that the information in this section could lead to some confusion on the taxonomy and should be rewritten and retaining the most useful information. Paragraphs were reorganized to first mention the useful methods and later explain why some methods cannot be used. The TPDP agreed to create a new section (on "Other methods for identification") containing information on molecular methods for the identification of the *Bactrocera* species and its limitations.
- [42] DNA extraction for molecular tests: The TPDP agreed on adjustments to the text to clarify that the identification of species using morphological methods was fundamental, i.e. the minimum requirement, while molecular methods were supplementary.
- [43] Interpretation of molecular test results: One member queried whether the 99% level of similarity of the tested sequence to the reference sequence was satisfactory. The discipline lead explained that this requirement refers to the whole genome, while actual identification of *B. carambolae* is based on the presence of a 44-bp insertion, thus differentiating from *B. dorsalis s.l.*
- [44] The TPDP:
- (3) *requested* the discipline lead and the DP drafting group to revise the draft DP and send it to the Secretariat by 17 March 2017.

- (4) *agreed* to submit the revised draft DP for *Bactrocera dorsalis* complex (2006-026) to the SC with the recommendation to be submitted to the 2017 consultation, by prior approval via a TPDP e-decision.

### 3.3 *Conotrachelus nenuphar* (2013-002) (Priority 2)

- [45] The discipline lead introduced the draft DP<sup>13</sup>, the summary of comments from the Expert Consultation<sup>14</sup> and the checklist for discipline lead and referees<sup>15</sup>. He mentioned that some experts during the Expert Consultation suggested adding identification keys, but the DP drafting group did not agree as they believed listing the characters was more useful. Some experts suggested including identification by genitalia. The discipline lead explained that the relevance of genitalia as a character depended on the range of species concerned. The DP drafting group had gathered some figures and images but genitalia identification had not been included in the text. The TPDP recommended that the DP drafting group should carefully reconsider this issue. The TPDP discussed the following main points.
- [46] Pest information: Mentions of specific countries where the pest is present were removed, as per recommendation in the Instruction to Authors. The TPDP found it was unclear whether the host species names mentioned in the draft DP were examples or if they formed exhaustive lists of hosts. The TPDP asked that this issue be clarified by the discipline lead and DP drafting group. The information on hosts was moved to this section from the section on detection.
- [47] One member noted that the references seemed outdated. The discipline lead explained that all relevant references on diagnostics were included and that the DP drafting group were not aware of any new references.
- [48] The TPDP agreed that the pest information should be supplemented by information on pupation in soil. The description of stages of the life cycle should be put in their natural sequence. The TPDP agreed to use the word “generation” instead of “brood” for clarity.
- [49] Symptoms of eggs and larvae: This section was merged with the section on symptoms of adults to form one section on symptoms. The title was modified to reflect this.
- [50] Methods of insect recovery from plants and plant products: The TPDP discussed whether the title was clear and if it fully reflected the intent of the section. The title was modified to enhance clarity on the purpose of the section (i.e. collection of insects from plants and plant products). The TPDP noted that some figures could be deleted because they did not relate to the diagnostics of the pest, and asked the DP drafting group to reconsider the figures.
- [51] Preparation of adult beetles for microscopic examination: The TPDP discussed again whether more information on the genitalia as a diagnostic character should be included, as there was no description on how to perform identification based on genitalia. The TPDP asked the discipline lead and the DP drafting group to re-consider this carefully.
- [52] Morphological identification of adult *Conotrachelus nenuphar*: The TPDP discussed the possible confusion of the pest with other pests of fruit. They noted that the reference to “other fruit pests” had probably been added due to possible confusion with other species, but agreed it should be removed since the section dealt with the identification of *C. nenuphar* species after the identification at the genus level.
- [53] Some TPDP members queried if only the fruits can be infested, or if the tree or parts of the tree such as branches can also be infested. It was recalled that the pupae can be found in soil, so the TPDP agreed that further clarification was needed from the DP drafting group.

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<sup>13</sup> 2013-002 and 2013-002\_Figures

<sup>14</sup> 07\_TPDP\_2017\_Feb

<sup>15</sup> 08\_TPDP\_2017\_Feb

- [54] The TPDP noted that there were two tables with diagnostic characters for *Conotrachelus* identification. It was explained that one table contained the minimum requirements to a reliable diagnose of *C. nenuphar* and to differentiate it from three other *Conotrachelus* species. The other table contained information on four species related to *C. nenuphar* discussed and illustrated in the draft DP. The TPDP asked that all common names from the tables be removed.
- [55] The discipline lead stressed that for the identification of *C. nenuphar* all characters included in the tables should be present.
- [56] Morphological identification of voltinic strains of adult *Conotrachelus nenuphar*: The TPDP agreed that characters described in this section were not relevant for plant health and phytosanitary diagnostics, and thus decided to delete the section. The TPDP instead agreed that a short mention in the section on pest information could be considered.
- [57] Contact points for further information: The discipline lead noted that there were two calls for authors for this DP and that the current authors had changed positions so experts on the identification of *C. nenuphar* were hard to find. The discipline lead would try to contact some possible experts before consultation and if unsuccessful, it was hoped that experts would be identified during the consultation stage.
- [58] Acknowledgements: The TPDP agreed that Ms Juliet GOLDSMITH should be included as one of the authors of the DP drafting group. Names of organizations should be mentioned for all experts who provided comments to the draft during the Expert Consultation.
- [59] References: The TPDP noted that some of the references (Fabricius, Herbst, LeConte, Peck and Say) should not be listed as references as they are the authorities of the pest name.
- [60] Figures: The TPDP asked that the figures be revised to make sure that arrows point at the characters described in the text. The source of the figures should also be checked. The TPDP asked the DP drafting group to review the figures and to include just the ones relevant to diagnostics.
- [61] The TPDP:
- (5) *requested* the discipline lead and the DP drafting group to revise the draft DP and send it to the Secretariat by 27 March 2017.
  - (6) *agreed* that a TPDP e-decision for final approval of the revised draft DP to the SC should be made only if information on the use genitalia for the pest identification was included in the draft DP.
  - (7) *agreed* to submit the revised draft DP for *Conotrachelus nenuphar* (2013-002) to the SC with the recommendation to be submitted to the 2017 consultation.
  - (8) *agreed* that Ms Juliet GOLDSMITH should be acknowledged as a co-author in the DP drafting group for *Conotrachelus nenuphar* (2013-002).

### 3.4 *Ips* spp. (2006-020) (Priority 4)

- [62] The lead introduced the draft DP<sup>16</sup>, the summary of comments from the Expert Consultation<sup>17</sup> and the checklist for discipline lead and referees<sup>18</sup>. He noted that almost all comments from the Expert Consultation were incorporated.
- [63] The referee of the draft DP mentioned that the taxonomic classification was changed for some species, which were now part of another genus: Pseudo-Ips. She noted, however, that there were still some similarities between the genera and the TPDP agreed that this should be clarified in the draft DP. The TPDP discussed the following main issues.

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<sup>16</sup> 2006-020 and 2006-020\_Figures

<sup>17</sup> 09\_TPDP\_2017\_Feb

<sup>18</sup> 10\_TPDP\_2017\_Feb

- [64] Pest information: The TPDP agreed not to include authorities after the scientific names of the genera, as it was deemed unnecessary. Regarding hosts list, reference to the Abietoideae subfamily was removed as not all mentioned species belonged to this subfamily.
- [65] Information on the new taxonomy changes, moving some previous *Ips* species to other genera was moved into this section from the section on taxonomy information.
- [66] The TPDP requested that the taxonomy of the *Ceratocystis* fungi be checked, because some *Ceratocystis* species have recently been reclassified to the genus *Ophiostoma*. As the draft DP contains information that *Ips* bark beetles can transmit pathogenic fungi belonging to the genus *Ceratocystis*, there may be a need to also mention the genus *Ophiostoma* in this context.
- [67] The TPDP agreed to remove the information on the pest distribution and instead replace this with a reference to an international database; because this section might otherwise need frequent updating.
- [68] The TPDP noted that the draft DP described methods to diagnose the genus *Ips* and 14 *Ips* species. Mention of “species pairs”, which cannot be distinguished from each other using morphological evidence, was moved to the section “identification”.
- [69] Taxonomic information: The TPDP agreed to put the taxonomic information in table format, add information on the subgenera next to each of the species and arrange the species according to the subgenera. The TPDP noted that the validity of common names of *Ips* species should be checked in the database of the Entomological Society of America.
- [70] Collecting specimens from plants and wood products: It was stressed that it is not possible to identify juveniles at species level or genus level. Text was adjusted to better reflect this.
- [71] Identification: It was stressed that the minimum requirement for the identification of Genus *Ips* is examination of morphology. The discipline lead explained that even though DNA sequencing has been used to identify the genus *Ips*, currently there is not enough information available for this method to be recommended for the identification of all species. Also, all the existing information may not have been validated. Nevertheless, the TPDP agreed that some explanatory text would be useful because DNA sequencing may become a good supplementary method of species identification in the future.
- [72] The TPDP noted that *Ips* belongs to the Ipini tribe and the draft included a mention outlining that *Ips* and the most similar genera in this tribe have specific features. The TPDP queried what was meant by “similar” (if morphological or phylogenetic similar), as this could lead to possible confusion. The TPDP proposed some adjusted text, but also asked the DP drafting group to clarify this and adjust the text further, if needed.
- [73] Key to distinguish *Ips* adults from other *Scolytinae*: One TPDP member queried if the key was published and whether a reference should be included. It was explained that in some cases the authors develop identification keys themselves for the purpose of the DP and these are not published. The TPDP agreed to include a note in the Instructions to Authors stating that whenever a key would be described in a DP, a reference to the paper where the key was published or information on its development by authors should be included.
- [74] Identification of other Ipini: The text in this section was moved to the section on identification of adult insects to the genus level. The TPDP agreed that references to “diagnostic features” should be replaced with “identification”, where appropriate for consistency.
- [75] Diagnostic notes on focal species (modified from Cognato, 2015) and description of sub-genera: The TPDP discussed the necessity of having the descriptions of the subgenera, because the identification of subgenera is not a minimum requirement for this DP. The lead explained that these “diagnostic notes” are not necessary to identify the pest, but provide additional important notes for the identification. The TPDP decided to retain the section as it provided additional information that may be helpful for the

identification of the *Ips* species, and text was added to clarify that it is not necessary to identify subgenera to identify *Ips* species.

- [76] Morphological identification of larvae: It was highlighted that larvae identification is possible for genus level, but not for species level. It was pointed out that the key provided may help determine that some larvae are not *Ips*, but it should not be used for positive identification of *Ips*. The TPDP agreed to merge this section with the key to distinguish final instar *Ips* larvae from other Scolytinae. The TPDP deleted the text regarding species level identification of *Ips* larvae because the TPDP had previously agreed that reliable species identification of *Ips* larvae was not possible.
- [77] Contact points for further information: The discipline lead would try to contact some possible experts before first consultation and if unsuccessful, it was hoped that experts be identified during the consultation stage.
- [78] The TPDP:
- (9) *requested* the discipline lead and the DP drafting group to revise the draft DP and send it to the Secretariat by 23 March 2017.
  - (10) *agreed* to submit the revised draft DP for *Ips* spp. (2006-020) complex to the SC with the recommendation to be submitted to the 2017 consultation.

## 4. Updates from relevant IPPC bodies

### 4.1 Relevant updates from other IPPC meetings

- [79] CPM Bureau: The Secretariat and the TPDP Steward updated the TPDP on the CPM Bureau October 2016 meeting<sup>19</sup> where it was decided to slow down the progressing of DPs by spreading the development over a four-year period, to reduce the impact on the Secretariat resources. The CPM Bureau suggested that the next global IPPC survey in the third Implementation Review and Support System (IRSS) cycle should try to gather information on the use of diagnostic protocols (as previously requested).
- [80] SC November 2016: The Secretariat presented an update on the issues relevant for the TPDP discussed by the SC at the November 2016 meeting<sup>20</sup>. During the SC's discussion on the proposed revision of DP 2: *Plum pox virus* (2016-007) the SC queried the consequences of revising vs. not revising the DP. The SC requested that such analysis be presented whenever a revision of a DP is proposed. The Secretariat noted that a detailed report on the activities of the TPDP would be presented to the SC at their May 2017 meeting, which would include the outcome of the current meeting.
- [81] The Secretariat noted that the work on draft DPs that are currently on the TPDP work programme need to be finalized. The Secretariat informed that the next call for topics, a joint one with a call for phytosanitary resources, may be opened in 2018. Currently there are no obstacles to contracting parties proposing new topics, including topics for DPs, however it should be done via the standard setting procedure. The Secretariat explained that every year the SC reviews and adjusts priorities of all topics for ISPMs.
- [82] The TPDP members expressed concerns that halting the development of DPs could lead to losing the team of authors and that, in the least, their motivation, built over many years, would be difficult to maintain in such a situation. The TPDP noted that the panel's future work would depend on the availability of financial and human resources in the Secretariat and priorities set. One member stressed that the TPDP's work would not stop completely because there would other issues to address (e.g. revision of adopted DPs or the advice to the SC or the IPPC community on DP relevant issues such as NGS or taxonomy).
- [83] The TPDP members reiterated that IPPC DPs are broadly used in their countries and regions, where they are relied on to secure coordinated and effective action to prevent or control the introduction and spread

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<sup>19</sup> The CPM Bureau October 2016 report is available at: <https://www.ippc.int/en/publications/83586/>

<sup>20</sup> The SC November 2016 meeting report is available at <https://www.ippc.int/en/publications/83881/>

of pests. The TPDP members also stressed that IPPC DPs are particularly important for those regions that do not have the capacity or resources to develop regional DPs.

[84] The TPDP:

- (11) *agreed* to have a strategic discussion in their next face-to-face meeting on how the TPDP can make a proper transition to a new way of working.

## 5. Overview of the TPDP work programme

### 5.1 General overview of DPs and next steps

[85] The Secretariat presented the 2017-2018 standard setting calendar related to DPs, the timeline of adopted DPs and the current status of the TPDP work programme, including the dates when the DPs would tentatively reach the different steps of the standard setting process. The Secretariat also mentioned that five out of six draft DPs submitted for the DP Notification Period in December 2016 were adopted by the SC on behalf of the CPM<sup>21</sup>. The draft DP for *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV) (2004-019) received an objection<sup>22</sup>. The Secretariat noted that leads for the adopted DPs may be contacted in case of any translation difficulties encountered.

[86] The following draft DPs should, tentatively, be submitted to the DP notification period in July 2017 (1 July – 15 August 2017):

- *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV) and *Watermelon silver mottle virus* (WSMoV) (2004-019)
- *Phytophthora ramorum* (2004-013)

[87] The following draft DPs should, tentatively, be submitted to the consultation in 2017 (1 July – 30 September 2017):

- *Candidatus Liberibacter* spp. on *Citrus* spp. (2004-010)
- *Puccinia psidii* (2006-018)
- *Xylella fastidiosa* (2004-024)
- *Bactrocera dorsalis* complex (2006-026)
- *Conotrachelus nenuphar* (2013-002)
- *Ips* spp. (2006-020)
- Revision of DP 2: *Plum pox virus* (2016-007)

[88] The Expert Consultations on draft DPs have been planned to be opened on 10 August or on 10 September 2017 for the following draft DPs:

- Genus *Ceratitis* (2016-001)
- *Striga* spp. (2008-009)
- Tephritidae: Identification of immature stages of fruit flies of economic importance by molecular techniques (2006-028)
- Begomoviruses transmitted by *Bemisia tabaci* (2006-023).

[89] The Secretariat gave a brief overview on how to respond to consultation comments and noted that the timing of the next DP notification period may change slightly, pending SC approval. The deadline for submitting responses to consultation comments was tentatively set for 26 October 2017. The TPDP noted that they would try to meet this deadline, although it would depend on the number of comments received and on the availability of authors during that period.

[90] The Secretariat highlighted that a face-to-face meeting would be organized in February 2018 only if there were enough draft DPs to be reviewed. It would be optimal to have the draft DP for Tephritidae:

<sup>21</sup> Adopted ISPMs: <https://www.ippc.int/en/core-activities/standards-setting/ispms/>

<sup>22</sup> 2017-01 objection received: <https://www.ippc.int/en/publications/83990/>

Identification of immature stages of fruit flies of economic importance by molecular techniques (2006-028), but that would require that it would be submitted to the Expert Consultation in 2017. The Secretariat recalled that, currently this draft DP is on pending status. The discipline lead for the draft DP noted that this draft DP has become a huge document and he suggested revising the specific fruit fly DPs (e.g. for *Anastrepha* or *Ceratitis*) as an alternative. He noted that another reason to revise the DP for *Anastrepha* in near future was that recently this genus was merged with *Toxotrypana*. As the submission of the draft DP for Tephritidae to the Expert Consultation in 2017 was not realistic, the alternatives for its development may need to be considered at the next TPDP meeting.

- [91] The Secretariat summarized that the plan for the 2018 face-to-face meeting should include at least two draft DPs: Genus *Ceratitis* (2016-001) and *Striga* spp. (2008-009), and a strategic discussion on the next TPDP work cycle. Most likely the meeting would last only four days. One TPDP member suggested having a discussion on the needs for revisions of the adopted DPs. He noted that a previous discussion on this topic took place in July 2016 and that there would be a need to discuss it every year to make sure that all needs for revisions are timely identified. Another TPDP member noted that the Loop-mediated Isothermal Amplification (LAMP) method for *Thrips palmi* had been developed since DP 1 was adopted. The TPDP members agreed that at least one virtual meeting would be needed to prepare for the 2018 face-to-face meeting.

## 5.2 General overview of status of protocols

### Reports on individual DPs status by discipline leads

- [92] The Secretariat presented the document on the status of DPs on the TPDP work programme<sup>23</sup> and invited the discipline leads to present their views on the development of particular drafts.
- [93] One TPDP member suggested that the finalized steps of the standard setting procedure foreseen for the DP, and the lengths of each step (e.g. present the process as a workflow) not be deleted from the DP status document, so the information would be captured in one place and thus provide easier reference for the TPDP members and DP drafting groups to better understand the process. The Secretariat will consider this.
- [94] Genus *Ceratitis* (2016-001): The discipline lead and the DP drafting group should draft the DP and the discipline lead should send it to the Secretariat by 1 September 2017. The discipline lead queried about the possibility of opening a call for authors. The Secretariat suggested that the DP drafting group be the same as for the draft DP for Tephritidae, as there would not be sufficient time to call for authors, if the aim was to discuss this draft DP in the 2018 TPDP face-to-face meeting. The discipline lead would try to contact the Tephritidae authors to ask of their availability.
- [95] *Striga* spp. (2008-009): The discipline lead and the DP drafting group should draft the DP and the discipline lead should send it to the Secretariat by 1 September 2017. The discipline lead noted that development of this draft DP was delayed as the lead author was not responsive to emails and due to the lead author's other assignments. It was suggested that the discipline lead could try to involve other members of the drafting group, however, as a first new approach, the Secretariat would try contacting the lead author and the entire DP drafting group.
- [96] *Phytophthora ramorum* (2004-013): A TPDP e-decision would be opened on 20 February on the recommendation of the draft DP to the SC for its submission to the July 2017 DP notification period.
- [97] *Xylella fastidiosa* (2004-024): The draft DP should return from the editor by the end of February. The editor's comments should be reviewed by the discipline lead and the draft returned to the Secretariat by 15 March 2017 and a TPDP e-decision should be opened on 3 April 2017 on the recommendation of the draft DP to the SC for its submission to the 2017 consultation.

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<sup>23</sup> 26\_TPDP\_2017\_Feb

- [98] *Candidatus Liberibacter spp. on Citrus spp. (2004-010)*: The referee for this draft DP would try to contact the lead author. The draft should be submitted to the Secretariat by 16 March 2017 with the aim to recommend it to the SC for its submission to the 2017 consultation.
- [99] *Puccinia psidii (2006-018)*: A TPDP e-decision would be opened on 20 February 2017 on the recommendation of the draft DP to the SC for its submission to the 2017 consultation.
- [100] *Begomoviruses transmitted by Bemisia tabaci (2006-023)*: The Secretariat would try to contact the DP drafting group to see whether it was possible to receive the finalized draft by 1 August 2017. It would then be submitted to the Expert Consultation on 10 August 2017.

### **Review of DP drafting groups associated with the work programme**

- [101] The Secretariat reminded the TPDP that a document containing the contact details of the DP drafting groups members is publicly available on the IPP<sup>24</sup> and requested the TPDP members to notify the Secretariat about any changes to the experts information so this document can be updated.
- [102] It was noted that the contact information in adopted DPs would change over the years and it would be difficult to keep up to date. The TPDP agreed that a note should be added to the DPs stating that the contact information may have changed since the DP was adopted. As now there are 22 adopted DPs, it was suggested to remove the information on the DP drafting groups for DPs that were adopted as this information would be captured in the adopted DP. This document would only present the information on drafting groups that were still active. The Secretariat would update the document accordingly.

### **Survey results: An introduction for authors of IPPC DPs**

- [103] The Secretariat presented the paper<sup>25</sup> on the results from a survey on the utility of the brochure for DP authors<sup>26</sup>. It was noted that at the moment there are no plans to revise the brochure. The Secretariat requested of the TPDP members to take some time to fully inform their authors about the standard setting procedure, and send them the brochure for authors, as their input is needed at many stages during the development of a draft DP.
- [104] Some members queried the procedure where the authors would not agree between them on a technical issue. The Secretariat explained that the discipline lead would decide when for instance, the authors could not agree on the inclusion of a particular test, but that the TPDP should be informed and the TPDP would make the final decision. It was stressed that an IPPC DP is not a scientific paper, but an international standard based on science.

## **6. Considerations for updating TPDP procedures**

### **TPDP Working procedures**

- [105] The TPDP members had no comments to the TPDP Working Procedures<sup>27</sup>.

### **TPDP Instructions to authors**

- [106] The Secretariat introduced the TPDP Instructions to authors<sup>28</sup> and noted that it would be updated with the decisions taken by the TPDP during this meeting (see sections 3 and 7 of this report).

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<sup>24</sup> IPPC Diagnostic Protocols (DPs) drafting groups: <https://www.ippc.int/en/publications/2582/>

<sup>25</sup> 11\_TPDP\_2017\_Feb

<sup>26</sup> Brochure: An introduction for authors of IPPC DPs:  
[https://www.ippc.int/largefiles/IPPC\\_IntroToAuthors\\_e\\_W.pdf](https://www.ippc.int/largefiles/IPPC_IntroToAuthors_e_W.pdf)

<sup>27</sup> TPDP Working Procedures: <https://www.ippc.int/en/publications/1187/>

<sup>28</sup> TPDP Instructions to Authors (including Checklist for authors, Criteria for prioritization of protocols and draft standardized template for draft diagnostic protocols): <https://www.ippc.int/en/publications/83612/>

## Checklist for discipline leads and referees

[107] The Secretariat introduced the document<sup>29</sup>. A TPDP member queried the role of the referee in the work of a DP. The Secretariat explained that a referee is expected to review the draft after the Expert Consultation and fill in the checklist. Also, referees may be involved at any stage during the development of a DP, if requested by the discipline lead. No changes were made to the document.

## 7. Follow up on actions from the TPDP previous meetings

### 7.1 ELISA controls and interpretation of results

[108] Ms Géraldine ANTHOINE introduced the document<sup>30</sup> mentioning that the initial objective of this document was to explain Enzyme-Linked Immunosorbent Assay (ELISA) controls for virus and bacteria detection and identification and their use in interpreting results. She noted that there are different options for interpreting ELISA test results and in particular to establish positive and negative thresholds, and that this was reflected in the paper. It was recalled that this information was intended to be included in the Instructions to authors. The TPDP reviewed the paper and proposed modifications.

[109] One member thought that the requirement for positive controls on the same matrix should not be needed for all viruses. It was explained that “matrix” was used to indicate the combination of a particular plant material (species, cultivar, part of a plant or tissue) and the virus, which ideally should be the same as the tested sample. Text was adjusted to include examples to enhance clarity on what was meant by “matrix”.

[110] The requirement to have only two wells or two prints for positive controls was maintained to minimize the risk of cross-contaminations.

[111] Regarding “in-house controls”, the text was adjusted to cover more collections of cultures of microorganisms, as it was noted that there are no internationally recognized collections of viruses.

[112] For commercial kits, one TPDP member noted that, in some cases, the formulas for calculation of thresholds or fixed values provided by the suppliers of the kits may differ from those given in the paper. The TPDP agreed that for commercial kits, it would be recommended to follow the instructions of the supplier and appropriate wording was added to the text. The TPDP also agreed to include guidance on this in the Instructions to authors.

[113] The lead explained that guidance on the interpretation of results for bacteria was separate from that for viruses as it is possible to have pure bacteria cultures, which is not possible for viruses.

[114] Some TPDP members felt that this document might be too detailed for the DP authors as it included guidance for laboratories. One TPDP member suggested that the document might be more useful for the TPDP members and help the discipline lead to ensure that the DPs outline the minimum requirements for controls and interpretation results. The TPDP agreed that the paper as modified in this meeting should be appended to the meeting report but that a more standardized text for inclusion in the Instructions to authors should be prepared for discussing in the next TPDP meeting.

[115] The TPDP:

- (12) *agreed* to append the paper ELISA controls and interpretation of results, as modified in this meeting, to this report (Appendix 04).
- (13) *requested* the lead, Ms Géraldine ANTHOINE, to prepare a paper for the next TPDP face-to-face meeting with a standardized text proposition on “ELISA controls and interpretation of results” for inclusion in the TPDP Instruction to authors.

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<sup>29</sup> TPDP Checklist for discipline leads and referees: <https://www.ippc.int/en/publications/81302/>

<sup>30</sup> 12\_TPDP\_2017\_Feb

## 7.2 Control options for molecular tests for pest group categories

- [116] Ms Géraldine ANTHOINE introduced the documents<sup>31</sup>. She mentioned that the discipline for “botany”, plants as pests, was not covered.
- [117] The TPDP reviewed the paper and proposed modifications.
- [118] The TPDP agreed that examples of universal primers should be given throughout the document. One TPDP member mentioned that internal controls may be needed even in cases where universal primers sets are used. This would depend on species-specific PCR failure, for example, and may be applicable for all disciplines.
- [119] The TPDP agreed that the requirements for the negative extraction controls for bacteria should be revised to clarify whether samples previously tested and detected as negative could serve as negative extraction control for further tests, or samples of unknown status could act as negative controls. The TPDP asked the leads of this paper to clarify this issue.
- [120] For entomology, the TPDP noted that there were not many molecular tests for detection and for that reason detection of insects had not been included in this document, although it could be in the future. For the negative extraction controls, the TPDP agreed that they are obligatory to detect cross-contamination during the extraction process.
- [121] With regards to some comments made on the controls for the DP 21 (*Candidatus Liberibacter solanacearum*) (2013-001) during the DP notification period, the TPDP noted the comments and highlighted that the TPDP is discussing control options for molecular tests to ensure that the minimum requirements on controls are clearly outlined in each DP for future development or revision.
- [122] The TPDP agreed that the leads (Ms Geraldine ANTHOINE and Mr Norman BARR) would revise the paper and present it to the TPDP at the next face-to-face meeting. In the meantime, the TPDP members were invited to send any comments to the leads.
- [123] The TPDP:
- (14) *requested* Ms Géraldine ANTHOINE and Mr Norman BARR to revise the document “Control options for molecular tests for pest group categories” for the next TPDP face-to-face meeting.
  - (15) *invited* TPDP members to submit additional comments to the document to the leads by 30 August 2017.

## 7.3 Best practices for sequencing

- [124] Mr Norman BARR introduced the document<sup>32</sup>. The TPDP revised the document. One member queried the change from species to populations in the context of sampling. It was explained that this was to address the fact that if a species has variable populations, taking samples at the species level, disregarding those populations may result in not being able to detect the existing variability within the species but instead may lead to a false conclusion that the tested samples belong to different species. The lead would check if the term “population”, which is most commonly used for insects, could be replaced with a more appropriate term.
- [125] One TPDP member queried whether it was possible to specify the required minimum length of a sequence. The lead explained that it would depend on the situation (i.e. it may vary from test to test and organism to organism) and would be very complicated to describe.

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<sup>31</sup> 13\_TPDP\_2017\_Feb and 14\_TPDP\_2017\_Feb

<sup>32</sup> 15\_TPDP\_2017\_Feb

[126] The TPDP agreed that the paper as modified in this meeting should be appended to the meeting report but that a more standardized text for inclusion in the Instructions to authors should be prepared for discussing in the next TPDP meeting.

[127] The TPDP:

- (16) *agreed* to include the document on “Best practices for sequencing” as appendix to the report (Appendix 05).
- (17) *requested* Mr Norman BARR to prepare a paper for the next TPDP face-to-face meeting with a standardized text proposition on “Best practices for sequencing” for inclusion in the TPDP Instruction to authors.
- (18) *invited* the TPDP members to submit additional comments to the document to the lead by 30 August 2017.

#### **7.4 Quality assurance for diagnostic protocols**

[128] Mr Norman BARR introduced the document<sup>33</sup>. He noted that the document was to be used by the discipline leads. He noted that since the last meeting no changes had been made.

[129] The TPDP agreed that the paper should be discussed again in the next meeting and invited the TPDP to provide comments on the paper.

[130] The TPDP:

- (19) *requested* Mr Norman BARR to revise and update, if needed, the document “Quality Assurance for diagnostic protocols” for the next TPDP face-to-face meeting.
- (20) *invited* TPDP members to submit comments to the document to the lead (Mr Norman BARR) by 30 August 2017.

#### **7.5 Next generation sequencing (NGS) as a diagnostic tool**

[131] Following discussion on this issue at the July 2016 TPDP meeting, the TPDP recognized the potential benefits and challenges associated with the use of NGS technologies in a phytosanitary context. The TPDP Steward suggested that the TPDP could use the document from the previous meeting to draft some recommendations on the use of NGS as a diagnostic tool and present them to the SC. The TPDP agreed that a small group (Robert TAYLOR (lead), Françoise PETTER, Norman BARR, Jane CHARD and Brendan RODONI (by e-mail) would draft a paper in the margins of the TPDP meeting for consideration by the TPDP.

[132] Mr Robert TAYLOR introduced the paper<sup>34</sup> on the use of NGS for the identification of pests prepared by the small group during this meeting. He noted that NGS technologies allow for the sequencing of whole genomes and offer many advantages, but carry the risk of false positives because artefacts of no relevance may be detected. The risk of false positives may lead to assumptions on the pathogenicity (ability to infect).

[133] The NGS technologies are applicable to all organisms (known or unknown), however, they are currently more developed for viruses, due to their relatively short genomes. The proper interpretation of results is the biggest challenge, as it requires very large databases of known pests as the reference for comparisons. Additionally, the databases generated using earlier methods may not be appropriate for NGS. Guidance on the interpretation of the NGS results has not been developed yet. For such reasons, these technologies may currently be used for screening consignments, but not to form the basis for final decisions (e.g. destruction or rejection of consignments).

[134] The differences between NGS and conventional sequencing was emphasized. It was explained that conventional sequencing has a specific guidance based on search for a pest-specific part of DNA,

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<sup>33</sup> 16\_TPDP\_2017\_Feb

<sup>34</sup> CRP\_01\_TPDP\_2017\_Feb

whereas NGS is non-targeted (multiplex) and can detect “everything” (i.e. NGS allows to see the whole genome and compare it with other genomes). Thus, NGS may lead to misinterpretation, depending on the reliability of the interpretation of results. As an advantage, NGS also allows for testing and detecting of presence of foreign DNA in an asymptomatic plant.

- [135] If NGS was to be used for phytosanitary purposes, significant validation data would have to be available and criteria for its use and for the interpretation of the results would need to be developed. The TPDP noted that appropriate guidance should be added to the Instructions to authors in this respect.
- [136] The TPDP agreed that the use of NGS could be an excellent subject for the special topics session at CPM-13 (2018) to help build awareness among NPPOs. The Secretariat would propose the idea to the CPM Bureau and the SC, and if approved, the TPDP offered to champion this event by organizing it, contacting appropriate experts and selecting and editing presentations.
- [137] The Secretariat also suggested that the TPDP could suggest to their NPPOs or RPPOs that a CPM recommendation should be produced on the use of NGS as a diagnostic tool, as this is a means of providing guidance to countries.
- [138] One TPDP member noted that the European and Mediterranean Plant Protection Organization (EPPO) would be holding a number of events relating to NGS, including workshops, and that some EPPO countries were very active and interested in using these technologies.
- [139] A TPDP member informed the TPDP that ISO had set up an expert group to work on NGS technologies as an ISO horizontal committee. It was noted that initially this ISO working group would work on NGS for identification of food (e.g. meat and rice), but not for pests.
- [140] The TPDP revised the paper drafted by the small group and agreed to recommend it to the SC for consideration at their May 2017 meeting.
- [141] The TPDP:
- (21) *agreed* to append the paper “Use of Next generation sequencing (NGS) technologies as a diagnostic tool for phytosanitary purposes” to the report (Appendix 06).
  - (22) *agreed* to recommend the paper “Use of Next generation sequencing (NGS) technologies as a diagnostic tool for phytosanitary purposes” to the SC for their consideration and to inform the CPM on the challenges associated with these new technologies.

## 8. Identify the need for DPs to be developed

- [142] The Secretariat recalled that during the TPDP July 2016 meeting the TPDP identified eight important pests and agreed to discuss justifications for possible new DPs for those pests at a future meeting. The Secretariat stressed that a recommendation for the inclusion of a DP into the IPPC work programme should be submitted by an NPPO or a RPPO during a call for topics.
- [143] The TPDP discussed the identified pests and possible justifications. The priorities for the possible development of diagnostic protocols were determined using the *Criteria for the prioritization of diagnostic protocols*, developed by the TPDP and supported by the SC<sup>35</sup>.

### - *Agrillus plannipennis* (“Emerald Ash Borer”) and *A. anxius* (“Bronze birch borer”)

- [144] Mr Norman BARR introduced the document<sup>36</sup>. He noted that there have been hardly any disputes on the identification of *A. plannipennis* (EAB). Detection of the pests seems to be more important as they are usually only detected once they have killed trees. He noted that the identification would possibly be

<sup>35</sup> Approved by the TPDP 2007-09, modified and approved by the SC 2007-11, minor editorial by the TPDP in 2010 (Annex 8 of the report), submitted to, modified and supported by the SC 2011-11 (see IPPC Standard Setting Procedure Manual section “8.4 Technical Panel on Diagnostic Protocols (TPDP)”).

<sup>36</sup> 18\_TPDP\_2017\_Feb

more relevant for Asia because there are more species present in that region. However, Europe has great concerns as to preventing the introduction of EAB. In conclusion, he noted that it should be relatively easy to develop an international DP for this pest.

[145] One TPDP member noted that EAB is a quarantine pest in some countries and that China has developed a diagnostic protocol for it.

[146] The TPDP agreed that it did not seem there was a global need for a DP for this pest and therefore did not recommend that a topic be submitted for an IPPC DP on *Agrillus plannipennis* and *A. anxius*

**- *Citrus leprosis virus* (“Citrus leprosis”)**

[147] Mr Delano JAMES introduced the document<sup>37</sup> and stressed that this virus (actually a complex of two viruses) is economically very important, especially as it attacks citrus fruits. Currently, the virus is distributed only in Central and South America. The region accounts for nearly 30% of the global production of sweet oranges, with Brazil being the largest producer in the world. The virus is transmitted by a mite vector (*Brevipalpus* spp.), which makes it difficult to stop its potential spread as the vector may spread the virus in many ways. It is possible to detect the virus in the vector and the identification methods are available, but they have not been harmonized.

[148] One TPDP member suggested that the development of a DP for the vector could be an option. Other TPDP members stressed that the transmission mechanisms are complicated, vectors are widely distributed and that there are at least two strains of the virus.

[149] The TPDP agreed that the development of an IPPC DP for this pest would be a valuable tool in preventing spread and facilitating safe trade. Also it would address a concern specific to countries in the Caribbean and North, Central and South Americas. The TPDP recommended that an IPPC DP for *Citrus leprosis virus* should be developed with high priority.

**- *Pyricularia oryzae* (syn. *Magnaporthe oryzae*) on *Triticum* spp. (“wheat blast”)**

[150] The Secretariat introduced the document<sup>38</sup> prepared by Mr Brendan RODONI. This pest is of high importance for some countries in Asia, has as hosts wheat and rice. The pest is spread via seeds and outbreaks are difficult to predict. It was noted that there are limited resistance varieties available and fungicides are not very effective.

[151] One TPDP member noted that this pest is difficult to detect in rice and in few years it causes severe damage on this crop. It would be useful to have an international DP to distinguish *P. oryzae* infecting *Triticum* from rice- and ryegrass-infecting populations. The information on detection and identification methods does not seem to be sufficiently available. One TPDP member noted that recently a paper was published on the identification of pathotypes of the pest.

[152] Noting that wheat is one of the most important crops worldwide, and according to the “Wheat Initiative”<sup>39</sup>, providing 20% of the world’s protein and calorie consumption, the TPDP recommended that an IPPC DP for *Pyricularia oryzae* (syn. *Magnaporthe oryzae*) on *Triticum* spp should be developed with high priority.

**- *Microcyclus ulei* (“South American leaf blight”)**

[153] Ms G eraldine ANTHOINE and Ms Jane CHARD introduced the paper<sup>40</sup>. They pointed out that this pest has high importance for rubber (*Hevea brasiliensis*). It affects rubber production in Latin America and

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<sup>37</sup> 19\_TPDP\_2017\_Feb

<sup>38</sup> 27\_TPDP\_2017\_Feb

<sup>39</sup> Wheat Initiative:

[http://www.wheatinitiative.org/sites/default/files/attached\\_file/wheatinitiative\\_visiondocument.pdf](http://www.wheatinitiative.org/sites/default/files/attached_file/wheatinitiative_visiondocument.pdf)

<sup>40</sup> 20\_TPDP\_2017\_Feb

has resulted in the complete loss of rubber production in some areas. The potential introduction of the pathogen is a major threat to rubber production in Asia.

- [154] They mentioned that there are reliable diagnostic methods for *M. ulei* based on morphological identification. The International Rubber Research Development Board (IRRDB) regularly organizes training for morphological identification, especially for technicians working on rubber crops. There are different physiological races of the fungus and molecular markers have been developed to determine genetic variability of isolates.
- [155] There have been calls, particularly in the Asia-Pacific region, for the standardization of a diagnostic protocol for *M. ulei*. The 2011 FAO Report entitled *Protection against South American leaf blight of rubber in Asia and the Pacific region*<sup>41</sup> includes an Appendix 1 for a standardized diagnostic protocol, but no agreed protocol was included. A workshop, organized by the Asia and Pacific Plant Protection Commission, was held in October 2016 on *Mitigation of South American Leaf Blight (SALB) of rubber in the Asia-Pacific Region*. One of the recommendations from the workshop was to develop and improve a protocol for pest diagnosis, particularly on identification using molecular techniques. There appears to be a need, at least in the Asian region, for a harmonized diagnostic protocol for *M. ulei*. Nevertheless, one of the difficulties facing the development of such a protocol is the access to biological material where the pest is alive in a non-infested area, such as in the Asian region.
- [156] It was noted that the FAO report from 2011 contains a list of laboratories in the Asia-Pacific region that have capacity to undertake diagnosis for this pest. Laboratories in other regions should also be able to undertake the diagnosis, thus implementation of an IPPC DP for this pest should be feasible.
- [157] *M. ulei* is a major pest of concern, as also identified in the IRSS general survey, and it is listed as a pathogen of relevance on the Biological Weapons Convention. Accurate identification would be essential to which end an international DP would be useful. The TPDP recommended that an IPPC DP for *M. ulei* should be developed with medium to high priority.

#### - *Moniliophthora roreri* (“frosty pod rot of cocoa”)

- [158] Mr Robert TAYLOR introduced the document<sup>42</sup> mentioning that most countries that are free of this pest prohibit the import of cocoa pods (and seeds). Testing of pods could be useful to facilitate the movement of germplasm (for research or development purposes). An infestation may cause reduction of cocoa yields by up to 80%.
- [159] Although *M. roreri* has a narrow host range and its combination of symptoms may be sufficient to diagnose the disease in the field, it may be confused with other pests. A number of accepted diagnostic methods are available for this pest, however, there are no published PCR tests. The diagnostic capacity exists and description of a confirmatory test would be useful.
- [160] The TPDP recommended the development of an IPPC DP for *M. roreri* with low priority, as the pest is regulated by most of the cocoa producing countries, and there are diagnostic methods available and currently some laboratories know how to handle the diagnosis of this pest. An IPPC DP could be useful for countries to maintain the pest free status or provide guarantees for pest freedom of exported consignments of cocoa.

#### - *Mononychellus tanajoa* (“cassava green mite”)

- [161] Ms Juliet GOLDSMITH introduced the document<sup>43</sup>. She noted that there is a lot of confusion on the cassava green mite (CGM), as the name might have been used for a number of species, including *M. tanajoa*, *M. caribeannae* and *M. progresivus*. She mentioned that *Mononychellus* was introduced into Africa from South America in the early 1970s and that it was identified as *M. tanajoa*. This designation

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<sup>41</sup> See <http://www.fao.org/3/a-i2157e.pdf>

<sup>42</sup> 21\_TPDP\_2017\_Feb

<sup>43</sup> 22\_TPDP\_2017\_Feb

was given to all CGM collected in Africa for several years until the discovery of *M. progresivus* in Nigeria. Some confusions ensued with some authors referring to the *Mononychellus* complex. This issue was further compounded by the realization that characters used as a criterion for distinguishing the species varied considerably from one strain to another and even between specimens of the same strain. Currently experts are not clear about which species are present in which geographical areas (Africa, Caribbean, South America). The TPDP agreed that an international DP could help resolve the issue.

[162] *M. tanajoa* is regulated in many countries, but the pest cannot be clearly identified because of the different opinions on the taxonomy of the genus, including disputes on the number of species. Most pest risks comes from fresh plant material being moved between countries.

[163] The main host for the CGM, cassava (*Manihot esculenta*), is important for food security as it is the third most important source of calories in the tropics, after rice and maize. Millions of people depend on cassava in Africa, Asia and Latin America.

[164] The TPDP recommended the development of an IPPC DP for *M. tanajoa* with high priority, but waiting for availability of molecular identification for identification.

#### - *Puccinia graminis* f. sp. *tritici* UG 99 (“wheat stem rust”)

[165] Mr Robert TAYLOR introduced the document<sup>44</sup>. He noted that *P. graminis* is common around the world and its most resistant strain UG 99 is present in some African countries and spreading towards Middle East. Early detection is important to take action against the pest, but distinguishing UG 99 from other strains is difficult. Some research on the identification using molecular methods was carried out, but a diagnostic protocol is not available. It was highlighted that, and UG 99 is considered an emerging threat to world wheat production. Detection and identification is difficult to distinguish between other *P. graminis* strains that are present in most wheat growing countries. There is a need to develop a consistent approach for the early detection of this novel virulent strain type. Development of a globally agreed DP could be helpful in the struggle to stop the spread of the pest.

[166] The Secretariat mentioned that international surveillance and monitoring efforts for this pest are being coordinated by FAO as part of the Borlaug Global Rust Initiative - an international coalition working to mitigate the threat of cereal rust diseases. According to the information from the FAO website, there is lack of consistency between differential sets used by laboratories to distinguish the races of the pathogen, even though some diagnostic methods are available.

[167] It was noted that, wheat is grown on approximately 215 million hectares worldwide and provides about 20% of the food calories for 4.5 billion people in 94 developing countries (Singh *et al.* 2011). Demand for wheat in developing countries is projected to increase 60% by 2050. Approximately, 80% of the wheat varieties grown are susceptible to UG 99 and its variants.

[168] The TPDP agreed that the development of a DP for this pest should be recommended with high priority.

#### - *Thecaphora solani* (“potato smut”)

[169] Mr Hans de GRUYTER introduced the document<sup>45</sup>. He noted that this pest is present only in the Andean region of South America, where it is a causal agent of the potato smut. The fungus infects plants belonging to a number of *Solanaceae* including *Solanum tuberosum*, *Datura* spp. and *S. lycopersicon*. He mentioned that data for the detection and identification of *T. solani* are limited. The most recent paper was published in 2004. There are no new publication known on *T. solani* and no known DP has been developed. The pest may spread by movement of seed potatoes or soil and the risk is usually managed by import bans.

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<sup>44</sup> 23\_TPDP\_2017\_Feb

<sup>45</sup> 24\_TPDP\_2017\_Feb

- [170] There are no reports of this fungi in the last 13 years, indicating that the risk of spread is probably low, due to strict quarantine regulations in other parts of the world.
- [171] The TPDP recommended that, as *T. solani* does not meet many of the criteria for the development of an IPPC DP, an IPPC DP should not be developed.

## 9. Liaison

### European and Mediterranean Plant Protection Organization (EPPO) update on diagnostic protocols

- [172] The invited expert, Ms Françoise PETTER (EPPO), presented an overview of EPPO's recent activities in the area of diagnostic protocols. She noted that recently three EPPO standards were adopted: on DNA barcoding as an identification tool for selected regulated pests, on guidelines for the authorization of laboratories, and the revision of the standard on the use of the EPPO DPs. A draft EPPO standard on national reference laboratories was sent for EPPO country consultations.
- [173] The expert informed the TPDP about the projects that EPPO is taking part: a project on validation of diagnostic methods for regulated pests and on the concept of flexible scope for accreditation in plant health in order to be able to react to the variability of matrixes that need to be tested. She also noted that in 2017 EPPO is going to organize some workshops, e.g. on the use of NGS, on the flexible scope of accreditation, and on nematode collections.
- [174] EPPO is working to align their diagnostic protocols with the IPPC DPs. Till now, the following EPPO DPs were aligned: *Aphelenchoides besseyi*, *Ditylenchus dipsaci* and *D. destructor*, *Xiphinema americanum sensu lato*, *Tilletia indica* and *Bursaphelenchus xylophilus*. The following EPPO DPs are currently being aligned: *Thrips palmi*, *Phyllosticta citricarpa* and *Plum pox virus*.
- [175] EPPO also adopted a number of revisions to the following EPPO DPs: *Xylella fastidiosa*, *Epitrix* sp., *Diabrotica virgifera*, *Phytoplasma mali* and *Tobacco ringspot virus*. New DPs were adopted for *Acidovorax citrulli* and *Xanthomonas axonopodis* pv. *allii*. Other draft EPPO DPs at the advanced stage of revision include *Synchytrium endobioticum*, *Ralstonia solanacearum*, *Globodera rostochiensis* and *G. pallida*, *Heterodera glycines* and *Dacus ciliatus*.
- [176] Other issues that were discussed included the development of the standard ISO/CD 13484 *Foodstuffs – General requirements for molecular biology analysis for detection and identification of plant pests* and update on Euphresco – a phytosanitary research coordination network hosted by EPPO. Recently Australia, Canada, Mexico and USA joined Euphresco.
- [177] The TPDP:
- (23) noted the update activities on EPPO diagnostic protocols.

### International Organization for Standardization (ISO)

- [178] Mr Delano JAMES updated the TPDP on the development of the ISO standard ISO/TC 34/SC 16/ 13484: *Molecular Biomarker Analysis: General requirements for molecular biology analysis for detection and identification of plant pests*. He mentioned that this draft standard is now under the voting process which will close on 23 February 2017. This is the final stage of the ISO adoption process. This ISO standard, if adopted, may be an alternative to ISO 17025 (i.e. complementary and not conflicting with it) as it is less stringent and focused only on the analysis of plants pests. The ISO standard does not cover morphological methods. He noted that overlaps with ISO 17025 were reported to be a concern for some European countries.
- [179] The TPDP:
- (24) noted the update on ISO activities related to the development of the ISO standard ISO/TC 34/SC 16/ 13484: *Molecular Biomarker Analysis: General requirements for molecular biology analysis for detection and identification of plant pests*.

## Global Taxonomy Initiative (GTI) of the Convention on Biological Diversity (CBD)

[180] Mr Norman BARR updated the TPDP on the recent activities under the GTI. He mentioned that GTI continues its focus on capacity building for experts, on taxonomy based on DNA sequence identification, with some focus on biodiversity.

[181] The TPDP:

(25) noted the updates on the GTI.

## 10. TPDP work plans

[182] The TPDP reviewed their tentative work plan for 2017-18 and modified it according to decisions taken during this meeting (Appendix 07).

[183] The Secretariat informed the TPDP that Mr Robert TAYLOR would be the TPDP lead for mycology and Mr Johannes DE GRUYTER would support him to finalize the draft DP for *Phytophthora ramorum* (2004-013) and in the preparation of responses to consultation comments on draft DPs for *Puccinia psidii* (2006-018).

[184] For ease of reference, a list of action points arising from the meeting is attached as Appendix 08.

## 11. Other business

### Objection received during DP notification period (15 December 2016 – 30 January 2017): *Tomato spotted wilt virus, Impatiens necrotic spot virus and Watermelon silver mottle virus (2004-019)*

[185] The EU submitted an objection<sup>46</sup> during the DP notification period that closed on 30 January 2017. It was noted that the EU had previously submitted an objection on an earlier version of the draft DP<sup>47</sup> and that the SC had provided responses to it<sup>48</sup>.

[186] In the objection submitted it was also noted that the primers described in the paper of Hassani-Mehraban *et al.* (2016)<sup>49</sup> had been included, as a result of adjustment to the draft DP based on the first objection received. However, no data is available on the performance (e.g. sensitivity, specificity, etc.) of these primers included in the draft DP, as the primers included are only used for confirmation of the identity of the isolates. It was suggested that the generic primer sets from Hassani-Mehraban *et al.* (2016) should be included instead of the specific primer sets.

[187] The EU presented additional information not only related to the objection but also on other sections of the draft DP, (although not part of the objection but rather a suggestion). The TPDP acknowledged that the EU suggestions may be useful in the future when the DP is revised. The TPDP stressed that one of the issues with internationally agreed DPs is to try ensure they contain the relevant current research findings, but that it is impossible, due to the lengthy approval and adoption process, to continuously include all new findings in the draft DP. At some point, the draft DP proposed for adoption should be considered final, to allow it to be adopted in a timeframe to be used by all IPPC contracting parties.

[188] For this reason, the TPDP agreed that only elements relating strictly to the objection would be considered at this time.

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<sup>46</sup> 2017-01 objection received: <https://www.ippc.int/en/publications/83990/>

<sup>47</sup> 2016-07 objection received: <https://www.ippc.int/en/publications/82787/>

<sup>48</sup> SC responses to the 2016-07 objection received: <https://www.ippc.int/en/publications/83852/>

<sup>49</sup> Hassani-Mehraban, A., Westenberg, M., Verhoeven, J.T.J., van de Vossen, B.T.L.H., Kormelink, R. & Roenhorst, J.W. 2016. Generic RT-PCR tests for detection and identification of tospoviruses. *Journal of Virological methods* 233: 89-96.

[189] The discipline lead, together with the DP drafting group, would prepare the revision of the draft DP with the responses to the objection. The final version of the revised draft should be sent to the Secretariat by 3 April 2017 and the final approval by the TPDP would be made via a e-decision.

## 12. Recommendations to the SC

[190] The SC is invited to:

- (1) *consider* the paper “Use of Next generation sequencing (NGS) technologies as a diagnostic tool for phytosanitary purposes” to the SC for their consideration and to *inform* the CPM on the challenges associated with these new technologies (Appendix 6);
- (2) *note* the TPDP recommendations on the need to develop new diagnostic protocols (section 8);
- (3) *note* the TPDP tentative work plan for 2017 – 2018 (Appendix 7).

## 13. Date and location of next meeting

[191] The next TPDP face-to-face meeting was tentatively scheduled for 5 – 9 February 2018. The venue for the meeting will be the headquarters of EPPO in Paris, France.

[192] The TPDP agreed that since two adopted DPs (DP 1: *Thrips palmi*, and DP 3: *Trogoderma granarium*) will have passed the five-year period from adoption, they should be considered for review. Thus, if needed, their review should be added to the 2018 meeting agenda. One member noted that it would be appropriate to review the adopted DPs each year and provide information to the SC.

[193] For entomology there are two discipline leads, the TPDP assigned the following leads for the revisions of the adopted DPs (completing five years of adoption or not):

- Ms Juliet GOLDSMITH – for DP1: *Thrips palmi*, DP 3: *Trogoderma granarium* and DP 16: Genus *Liriomyza*
- Mr Norman BARR – for DP 9: Genus *Anastrepha*.

[194] The Chairperson informed the TPDP that a link to the electronic evaluation of this meeting would be sent to the participants and that they were encouraged to provide their feedback before 6 March 2017.

## 14. Closing of the meeting

[195] The TPDP thanked the Standard Setting Secretariat staff for their professional support and dedication to the work.

[196] The Secretariat thanked the participants for their active participation, especially Mr Johannes DE GRUYTER, as this was his last meeting, and Mr Robert TAYLOR for taking over the role as the TPDP discipline lead for mycology from Mr DE GRUYTER. The Secretariat also requested the discipline leads to pass on thanks to all the members of the DP drafting groups. The Chairperson closed the meeting.

## Appendix 1

**2017 MEETING OF THE  
TECHNICAL PANEL ON DIAGNOSTIC PROTOCOLS**

**13-17 February 2017  
FAO headquarters, Rome, Italy**

Opening: Monday 13 February at 10:00  
Monday schedule: 10:00 – 13:00 and 14:00 – 17:00  
Daily Schedule (Tuesday – Friday): 09:00-12:00 and 13:00-17:00

**AGENDA**

<b>AGENDA ITEM</b>	<b>DOCUMENT NO.</b>	<b>PRESENTER</b>
<b>1. Opening of the meeting</b>		LARSON
1.1 Welcome		LARSON
1.2 Selection of the Chairperson		LARSON
1.3 Selection of the Rapporteur		CHAIRPERSON
1.3 Review and adoption of the agenda	01_TPDP_2017_Feb	CHAIRPERSON
<b>2. Administrative Matters</b>		CHAIRPERSON
- Documents list - Local information - Participants list (and membership)	02_TPDP_2017_Feb 03_TPDP_2017_Feb 04_TPDP_2017_Feb <a href="#">Link to TPDP membership list</a>	FARREN
<b>3. Scrutiny of draft diagnostic protocols</b>		CHAIRPERSON
3.1 Revision of DP 2: <i>Plum pox virus</i> (2016-007) (Priority 1) - Checklist for discipline leads and referees	2016-007 25_TPDP_2017_Feb	JAMES
3.2 <i>Bactrocera dorsalis</i> complex (2006-026) (Priority 2) - Summary of comments from expert consultation - Checklist for discipline leads and referees	2006-026 2006-026_Figures 05_TPDP_2017_Feb 06_TPDP_2017_Feb	BARR
3.3 <i>Conotrachelus nenuphar</i> (2013-002) (Priority 2) - Summary of comments from expert consultation - Checklist for discipline leads and referees	2013-002 2013-002_Figures 07_TPDP_2017_Feb 08_TPDP_2017_Feb	BARR
3.4 <i>Ips</i> spp. (2006-020) (Priority 4) - Summary of comments from expert consultation - Checklist for discipline leads and referees	2006-020 2006-020_Figures 09_TPDP_2017_Feb 10_TPDP_2017_Feb	BARR
<b>4. Updates from relevant IPPC bodies</b>		CHAIRPERSON

AGENDA ITEM	DOCUMENT NO.	PRESENTER
4.1 Relevant updates from other IPPC meetings - Standards Committee (SC) Nov 2016	<a href="#">2016-10 Bureau report</a> <a href="#">2016-11 SC report</a>	Steward (CHARD) / MOREIRA
<b>5. Overview of the TPDP work programme</b>		CHAIRPERSON
5.1 General overview of DPs and next steps	(presentation)	MOREIRA
5.2 General overview of status of protocols - Reports on individual DPs status by discipline leads (scope and status of protocols)  - Review of DP drafting groups associated with the work programme  - Survey results: An introduction for authors of IPPC DPs	26_TPDP_2017_Feb <a href="#">Link to List of topics for IPPC Standards</a>  <a href="#">Link to IPPC DPs drafting groups list</a>  11_TPDP_2017_Feb <a href="#">Link to IPPC brochure: An introduction for authors of IPPC DPs</a>	ALL / IPPC Secretariat
<b>6. Considerations for updating TPDP procedures and guidance</b>		CHAIRPERSON
6.1 Proposed changes based on the review of DPs	<a href="#">TPDP Working procedures</a> <a href="#">TPDP Instruction to authors</a> <a href="#">Checklist for discipline leads and referees</a> (work area page)	IPPC Secretariat / Steward (CHARD)
<b>7. Follow-up on actions from the TPDP previous meetings</b>		CHAIRPERSON
7.1 ELISA controls and interpretation of results	12_TPDP_2017_Feb	ANTHOINE/TAYLOR
7.2 Control options for molecular tests for pest group categories - Comments from DP notification period (15 December 2016 – 30 January 2017): controls for molecular tests	13_TPDP_2017_Feb 14_TPDP_2017_Feb	ANTHOINE
7.3 Best practices for sequencing	15_TPDP_2017_Feb	BARR
7.4 Quality Assurance for diagnostic protocols	16_TPDP_2017_Feb	BARR
7.5 Next generation sequencing as a diagnostic tool	CRP_01_TPDP_2017_Feb	TAYLOR/BARR/CHARD/PETTER
<b>8. Identify the need for DPs to be developed</b>		
- <i>Agrillus plannipennis</i> ("Emerald Ash Borer") and <i>A. anxius</i> ("bronze birch borer")	18_TPDP_2017_Feb	BARR
- <i>Citrus leprosis virus</i> ("citrus leprosis")	19_TPDP_2017_Feb	JAMES
- <i>Magnaporthe oryzae</i> on <i>Triticum</i> spp. ("wheat blast")	27_TPDP_2017_Feb	MOREIRA

AGENDA ITEM	DOCUMENT NO.	PRESENTER
- <i>Microcyclus ulei</i> ("South American leaf blight")	20_TPDP_2017_Feb	CHARD / ANTHOINE
- <i>Moniliophthora roreri</i> ("frosty pod rot of cocoa")	21_TPDP_2017_Feb	TAYLOR
- <i>Mononychellus tanajoa</i> ("cassava green mite")	22_TPDP_2017_Feb	GOLDSMITH
- <i>Puccinia graminis</i> f. sp. <i>tritici</i> UG 99 ("wheat stem rust")	23_TPDP_2017_Feb	TAYLOR
- <i>Thecaphora solani</i> ("potato smut")	24_TPDP_2017_Feb	DE GRUYTER
<b>9. Liaison</b>		
<ul style="list-style-type: none"> <li>European and Mediterranean Plant Protection Organization (EPPO) update on diagnostic protocols</li> </ul>	-	PETTER
<ul style="list-style-type: none"> <li>International Organization for Standardization (ISO)</li> </ul>	-	JAMES / MOREIRA
<ul style="list-style-type: none"> <li>Global Taxonomy Initiative (GTI) of the Convention on Biological Diversity (CBD)</li> </ul>	-	BARR / MOREIRA
<b>10. TPDP work plans</b>		
- TPDP 2017-2018 work plan	(To be prepared during the meeting)	IPPC Secretariat (MOREIRA / WLODARCZYK)
<b>11. Other business</b>		
- Objection received during DP notification period (15 December 2016 – 30 January 2017): <i>Tomato spotted wilt virus</i> , <i>Impatiens necrotic spot virus</i> and <i>Watermelon silver mottle virus</i> (2004-019)	17_TPDP_2017_Feb 2004-019	CHAIRPERSON / JAMES / CHARD / MOREIRA
<b>12. Recommendations to the SC</b>		CHAIRPERSON
<b>13. Date and location of next meeting</b>		CHAIRPERSON
<b>14. Closing of the meeting</b>		
- Evaluation of the meeting		IPPC Secretariat
- Close		CHAIRPERSON

## Appendix 2

**DOCUMENTS LIST***(Documents are presented in the order of the document numbers)*

DOCUMENT NO.	AGENDA ITEM	DOCUMENT TITLE	POSTED
<b>Draft Diagnostic Protocols</b>			
2004-019	11	Draft DP for <i>Tomato spotted wilt virus</i> (TSWV), <i>Impatiens necrotic spot virus</i> (INSV) and <i>Watermelon silver mottle virus</i> (WSMoV) (2004-019) – EU comments	2017-01-26
2006-020	3.4	<i>Ips</i> spp. (2006-020)	2017-01-25
2006-020	3.4	Draft DP for <i>Ips</i> spp. (2006-020) Figures	2017-01-26
2006-026	3.2	<i>Bactrocera dorsalis</i> complex (2006-026)	2017-01-25
2006-026	3.2	<i>Bactrocera dorsalis</i> complex (2006-026) Figures	2017-01-26
2013-002	3.3	<i>Conotrachelus nenuphar</i> (2013-002)	2017-01-25
2013-002	3.3	<i>Conotrachelus nenuphar</i> (2013-002) Figures	2017-01-25
2016-007	3.1	Revision of DP 2: <i>Plum pox virus</i> (2016-007)	2017-01-25
<b>Other documents</b>			
01_TPDP_2017_Feb	1.3	Agenda	2016-12-21 (Updated version posted on: 2017-01-27)
02_TPDP_2017_Feb	2	Documents list	2017-01-27
03_TPDP_2017_Feb	2	Local information	2016-12-16
04_TPDP_2017_Feb	2	Participants list	2017-01-27
05_TPDP_2017_Feb	3.2	Summary of comments from expert consultation - <i>Bactrocera dorsalis</i> complex (2006-026)	2017-01-26
06_TPDP_2017_Feb	3.2	Checklist for discipline leads and referees - <i>Bactrocera dorsalis</i> complex (2006-026)	2017-01-26
07_TPDP_2017_Feb	3.3	Summary of comments from expert consultation - <i>Conotrachelus nenuphar</i> (2013-002)	2017-01-26
08_TPDP_2017_Feb	3.3	Checklist for discipline leads and referees - <i>Conotrachelus nenuphar</i> (2013-002)	2017-01-26
09_TPDP_2017_Feb	3.4	Summary of comments from expert consultation - <i>Ips</i> spp. (2006-020)	2017-01-26

DOCUMENT NO.	AGENDA ITEM	DOCUMENT TITLE	POSTED
10_TPDP_2017_Feb	3.4	Checklist for discipline leads and referees - <i>Ips</i> spp. (2006-020)	2017-01-26
11_TPDP_2017_Feb	5.2	Survey results: An introduction for authors of IPPC DPs	2017-01-26
12_TPDP_2017_Feb	7.1	ELISA controls and interpretation of results	2017-01-26
13_TPDP_2017_Feb	7.2	Control options for pest group categories	2017-01-26
14_TPDP_2017_Feb	7.2	Comments from DP notification period (15 December 2016 – 30 January 2017): Controls for molecular tests	2017-01-26
15_TPDP_2017_Feb	7.3	Best practices for sequencing	2017-01-26
16_TPDP_2017_Feb	7.4	Quality Assurance for diagnostic protocols	2017-01-26
17_TPDP_2017_Feb	11	Objection received during DP notification period (15 December 2016 – 30 January 2017): <i>Tomato spotted wilt virus</i> , <i>Impatiens necrotic spot virus</i> and <i>Watermelon silver mottle virus</i> (2004-019)	2017-01-26
18_TPDP_2017_Feb	8	Consideration of a proposal for a DP for <i>Agrilus plannipennis</i> ("Emerald Ash Borer") and <i>A. anxius</i> ("bronze birch borer")	2017-01-26
19_TPDP_2017_Feb	8	Consideration of a proposal for a DP for <i>Citrus leprosis virus</i> ("citrus leprosis")	2017-01-26
20_TPDP_2017_Feb	8	Consideration of a proposal for a DP for <i>Microcyclus ulei</i> ("South American leaf blight")	2017-01-26
21_TPDP_2017_Feb	8	Consideration of a proposal for a DP for <i>Moniliophthora roreri</i> ("frosty pod rot of cocoa")	2017-01-26
22_TPDP_2017_Feb	8	Consideration of a proposal for a DP for <i>Mononychellus tanajoa</i> ("cassava green mite")	2017-01-26
23_TPDP_2017_Feb	8	Consideration of a proposal for a DP for <i>Puccinia graminis</i> f. sp. <i>tritici</i> UG 99 ("wheat stem rust")	2017-01-26
24_TPDP_2017_Feb	8	Consideration of a proposal for a DP for <i>Thecaphora solani</i> ("potato smut")	2017-01-26
25_TPDP_2017_Feb	3.1	Checklist for discipline leads and referees - Revision of DP 2: <i>Plum pox virus</i> (2016-007)	2017-01-27
26_TPDP_2017_Feb	5.2	General overview of status of protocols	2017-01-27
27_TPDP_2017_Feb	8	Consideration of a proposal for a DP for <i>Magnaporthe oryzae</i> on <i>Triticum</i> spp. ("wheat blast")	2017-02-06
CRP_01_TPDP_2017_Feb	7.5	Next generation sequencing as a diagnostic tool	2017-02-17

**Documents links** (presented in the order of the agenda items)

<b>DOCUMENT NO.</b>	<b>AGENDA ITEM</b>	<b>DOCUMENT LINK</b>
TPDP Membership list	2	<a href="#">Link to TPDP Membership list</a>
Updates from other relevant IPPC meetings – CPM Bureau meeting (October 2016)	4.1	<a href="#">Link to the October 2016 CPM Bureau meeting</a>
Updates from other relevant IPPC meetings - Standards Committee (SC) November 2016	4.1	<a href="#">Link to the SC November 2016 Report</a>
DP Drafting groups list	5.2	<a href="#">DP Drafting groups list</a>
IPPC brochure: An introduction for the authors of IPPC DPs	5.2	<a href="#">An Introduction for the authors of IPPC DPs</a>
List of Topics for IPPC Standards	5.2	<a href="#">List of topics for IPPC Standards</a>
Checklist for discipline leads and referees	6.1	<a href="#">Checklist for discipline leads and referees</a>
TPDP Instructions to authors	6.1	<a href="#">TPDP Instruction to authors</a>
TPDP Working procedures	6.1	<a href="#">TPDP Working procedures</a>
IPPC Standard Setting Procedure Manual	-	<a href="#">IPPC Standard Setting Procedure Manual</a>
IPPC Style Guide	-	<a href="#">IPPC Style Guide</a>

## Appendix 3

**PARTICIPANTS LIST**

A check (✓) in column 1 indicates attendance at the meeting.

	Participant role	Name, mailing, address, telephone	Email address	Term begins	Term ends
<b>TPDP members</b>					
✓	Steward	<b>Ms Jane CHARD</b> Head of Plant Biosecurity and Inspections SASA, Scottish Government Roddinglaw Road Edinburgh EH12 9FJ <b>United Kingdom</b> Tel: (+44) 131 2448863 Fax: +44 131 2448940	<a href="mailto:jane.chard@sasa.gsi.gov.uk">jane.chard@sasa.gsi.gov.uk</a> ; <a href="mailto:janemchard@yahoo.co.uk">janemchard@yahoo.co.uk</a> ;		
✓	Bacteriology	<b>Mr Robert TAYLOR</b> Plant health and Environment laboratory, Ministry for Primary Industries 231 Morrin Road St Johns PO Box 2095 Auckland 1140 <b>New Zealand</b> Tel: (+64) 9 909 3548 Fax: (+64) 9 909 5739	<a href="mailto:Robert.Taylor@mpi.govt.nz">Robert.Taylor@mpi.govt.nz</a> ;	May 2011	2021 (2 <sup>nd</sup> term 2016- 2021)
✓	Botany	<b>Ms Liping YIN</b> Plant Quarantine Laboratory Animal and Plant Inspection and Quarantine Technology Center Shanghai Entry-Exit Inspection and Quarantine Bureau 1208 Minsheng Road Shanghai, 200135 <b>China</b> Tel: (+86) 21 6854 0577 Fax: (+86) 21 6854 6481	<a href="mailto:yinlp@shciq.gov.cn">yinlp@shciq.gov.cn</a> ; <a href="mailto:yinlp2013@hotmail.com">yinlp2013@hotmail.com</a>	April 2008	2018 (2 <sup>nd</sup> term 2013- 2018)
✓	Entomology	<b>Mr Norman B. BARR</b> Assistant Director Mission Laboratory 22675 N. Moorefield Rd. Moore Air Base Bldg. S-6414 Edinburg, TX 78541 <b>USA</b> Tel. (+1) 956 205 7658 Fax: (+1) 956 205 7680	<a href="mailto:Norman.B.Barr@aphis.usda.gov">Norman.B.Barr@aphis.usda.gov</a>	July 2012	2022 (2 <sup>nd</sup> term 2017- 2022)

	<b>Participant role</b>	<b>Name, mailing, address, telephone</b>	<b>Email address</b>	<b>Term begins</b>	<b>Term ends</b>
✓	Entomology	<b>Ms Juliet GOLDSMITH</b> Plant health specialist Caribbean Agricultural Health and food safety Agency (CAFSA) Paramaribo, Jamaica Tel: 1876-9777160 Fax: 1876-9776992	<a href="mailto:julietgoldsmith@gmail.com">julietgoldsmith@gmail.com</a>	November 2014	2019
✓	Mycology	<b>Mr Johannes DE GRUYTER</b> Head, Mycology Department Plant Protection Service (NPPO) 15 Geertjesweg P.O. Box 9102 6706 HC Wageningen <b>Netherlands</b> Tel: (+31) 317 496 831 Fax: (+31) 317 421 701	<a href="mailto:j.degruyter@nvwa.nl">j.degruyter@nvwa.nl</a>	April 2008	2018 (2 <sup>nd</sup> term 2013- 2018)
✓	Nematology	<b>Ms Géraldine ANTHOINE</b> Directrice adjointe / Deputy head Chef d'unité coordination de la référence / Head of unit "coordination of reference activities" 7 rue Jean Dixméras 49044 ANGERS cedex 01 <b>France</b> Tel: (33) 241207431 Fax: (33) 240207430	<a href="mailto:geraldine.anthoine@anses.fr">geraldine.anthoine@anses.fr</a>	April 2009	2019 2 <sup>nd</sup> term 2014- 2019)
✓	Virology	<b>Mr Delano JAMES</b> Head, Research Section, Canadian Food Inspection Agency Sidney Laboratory 8801 East Saanich Road Sidney, BC, V8L 1H3 <b>Canada</b> Tel: (+1) 250 363 6650 ext 235 Fax: (+1) 250 363 6661	<a href="mailto:Delano.James@inspection.gc.ca">Delano.James@inspection.gc.ca</a>	November 2010	2020 (2 <sup>nd</sup> term 2015- 2020)
	Virology, and backup bacteriology	<b>Mr Brendan RODONI</b> Biosciences Research Division AgriBio Centre Ring Road La Trobe University Bundoora 3083 <b>Australia</b> Tel: (+61) 3 9032 7319 Fax: (+61) 3 9800 3521	<a href="mailto:brendan.rodoni@ecodev.vic.gov.au">brendan.rodoni@ecodev.vic.gov.au</a>	July 2012	2022 (2 <sup>nd</sup> term 2017- 2022)

<b>Other participants</b>			
✓	Invited Expert	<p><b>Ms Françoise PETER</b>  Assistant Director  European and Mediterranean Plant Protection  Organization (<b>EPPO</b>)  21 Boulevard Richard Lenoir  75011 Paris  <b>France</b>  Tel: +33 1 45 20 77 94 / Fax: +33 1 70 76 65 47</p>	<a href="mailto:petter@epo.int">petter@epo.int</a>
✓	IPPC Secretariat	<p><b>Mr Brent LARSON</b>  Agricultural Officer / Standard Setting Senior  Officer  International Plant Protection Convention  Secretariat (IPPC)  Food and Agriculture Organization of the United  Nations (FAO/UN)  Viale delle Terme di Caracalla  00153 Rome, Italy  Phone: + 39 06 570 54915</p>	<a href="mailto:Brent.Larson@fao.org">Brent.Larson@fao.org</a>
✓	IPPC Secretariat	<p><b>Ms Adriana G. MOREIRA</b>  Agricultural Officer / Standard Setting  Programme Specialist  International Plant Protection Convention  Secretariat (IPPC)  Food and Agriculture Organization of the United  Nations (FAO/UN)  Viale delle Terme di Caracalla  00153 Rome, Italy  Phone: + 39 06 570 55 809</p>	<a href="mailto:Adriana.Moreira@fao.org">Adriana.Moreira@fao.org</a>
✓	IPPC Secretariat	<p><b>Mr Martin FARREN</b>  Standard Setting Consultant  International Plant Protection Convention  Secretariat (IPPC)  Food and Agriculture Organization of the United  Nations (FAO/UN)  Viale delle Terme di Caracalla  00153 Rome, Italy  Phone: + 39 06 570 55020</p>	<a href="mailto:Martin.Farren@fao.org">Martin.Farren@fao.org</a>
✓	IPPC Secretariat	<p><b>Mr Piotr WLODARCZYK</b>  Standard Setting Consultant  International Plant Protection Convention  Secretariat (IPPC)  Food and Agriculture Organization of the United  Nations (FAO/UN)  Viale delle Terme di Caracalla  00153 Rome, Italy  Phone: + 39 06 570 54468</p>	<a href="mailto:Piotr.Wlodarczyk@fao.org">Piotr.Wlodarczyk@fao.org</a>

## Appendix 4

**ELISA CONTROLS AND INTERPRETATION OF RESULTS****Positive and negative controls for ELISA test<sup>50</sup>**

- [1] When using a commercial ELISA kit, the following controls should be added in addition to the positive and negative controls provided in the kit:
- a positive control ideally of the same matrix, inoculated or spiked with the target bacterium/virus for tests used for detection in plant material. For identification of bacterial cultures positive controls can consist of a suspension of the target bacterium.
  - a negative control from a healthy host plant for tests used for detection in plant material. For testing bacterial cultures negative controls can consist of suspension buffer only or a suspension of a non-target bacterial species.
- [2] These positive and negative controls should be checked (preferably in advance) with the same antibodies following the appropriate ELISA procedure.

**In house controls****Positive controls**

- [3] For bacteria, positive controls of the reference strain of the target organism should be suspended in healthy host plant extract or in an appropriate buffer. It is recommended that reference strains are used as positive controls to avoid misinterpretations due to cross-reactions. Reference strains are available from a number of international culture collections for example, National Collection of Plant Pathogenic Bacteria (NCPPB), FERA, York, UK; Culture Collection of the National Plant Protection Organization (NPPO NL), Wageningen, the Netherlands; or Colléction Française de Bacteries Phytopathogenes (CFBP), INRA Station Phytobacteriologie, Angers, France, International Collection of Microorganisms from Plants (ICMP), New Zealand.
- [4] Naturally infected tissue (maintained by lyophilization or freezing at below -16°C) should be used whenever possible.
- [5] For viruses, naturally infected tissue or extracts (maintained by lyophilization or freezing at below -20°C) should be used whenever possible. Aliquots of positive controls should be prepared to prevent repeated freezing and thawing.
- [6] Two wells or tissue prints should be prepared per positive controls.

**Negative controls**

- [7] Healthy plant extract (for detection in plant material) or a suspension of a non-target bacterial species (for identification of bacteria) should be used as negative controls. The healthy plant should whenever possible be the same species/variety and the same plant part at the same growth stage to allow for comparison with tested samples. Aliquots / extracts of the same host plant which previously tested negative for the target bacterium/virus can also be used as negative controls. For Tissue print-ELISA, healthy controls previously immobilized on membranes can be used.
- [8] At least two wells or tissue prints should be prepared per negative control.

**Blank or buffer controls**

- [9] A further negative control consisting of extraction or suspension buffer only in place of sample extract can be included. These wells do not receive any sample and these blank wells control for any variation (or contamination) due to the plate and test reagents to the measured OD. Reference:

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<sup>50</sup> Adapted from EPPO PM7/101 and PM7/125

## Interpretation of ELISA tests results

[10] Verification of the controls:

[11] Negative ELISA readings in positive control wells / print or dot indicate that the test has not been performed correctly or that it has been inhibited. Positive ELISA readings in negative control wells / print or dot indicate that cross-contamination or non-specific antibody binding has occurred. In such cases, the test should be performed again with the appropriate modifications.

### Interpretation of ELISA tests results for detection of bacteria (plant material)

[12] For detection in plant material the interpretation of the optical density (OD) value of the negative sample extract well should be the basis for determining the thresholds of detection (background) minus the OD of the substrate well. The positive result is determined on a case-by-case basis depending on the pest and the matrix. It is recommended that the test be repeated for samples just below the limit of the threshold.

### Interpretation of ELISA tests results for identification (pure cultures of bacteria)

For commercial kits, it is recommended to follow the instructions of the supplier.

[13] The ELISA test is considered negative if:

- the average absorbance or OD reading from duplicate sample wells is  $< 2 \times$  OD of that in the negative sample control well,
- and the OD for the positive controls are all above 1.0 (after 120 min incubation with the substrate) and are greater than twice the average OD obtained for negative sample extracts.

[14] The ELISA test is considered positive if:

- the average OD reading from each of the duplicate sample wells is  $\geq 2 \cdot$  OD in the negative sample extract well,
- and the OD readings in all negative control wells are  $< 2 \times$  those in the positive control wells.

[15] It is recommended that the test be repeated for samples that give a reaction just below the limit of the threshold.

### Interpretation of ELISA tests results for detection / identification of viruses

For commercial kits, it is recommended to follow the instructions of the supplier.

[16] There are different options for interpreting ELISA test results and in particular to establish a threshold. Further information is provided in Sutula *et al.* (1986). The following procedure is recommended, however it is recognized that in particular when the negative control of healthy plant material is not the same as the plant to be tested, the laboratory should adjust and validate the calculation of the threshold, or confirm positive results by another method.

[17] The ELISA test is considered negative if:

- the average OD value from duplicate sample is less than 0.1 or is  $< 2 \times$  OD of that in the negative control of healthy plant extracts.

[18] Usually the ELISA test is considered positive if the average OD value from each of the duplicate sample wells is  $\geq 2 \times$  OD of that in the negative control of healthy plant extracts.

[19] Note that when using polyclonal antibodies, it is essential that the negative controls are as similar as possible to the matrix (e.g. plant species, cultivar, tissue type) tested in the same plate.

[20] The test should be repeated when duplicate wells differ by more than 50% OD value. In critical cases, for samples that give a reaction close to the threshold of e.g.  $2 \times$  OD of that in the negative control of healthy plant extracts or when matrix effects cannot be excluded, it is recommended that another test (different source of antibody or another method) be used.

- [21] Other procedures for interpretation are in use involving consideration of standard deviations (average of healthy controls + 3× standard deviation).

### **Interpretation for tissue print, squash or dot ELISA tests results**

- [22] The ELISA test is negative if there is no coloured precipitate in the sample print or dot, provided that the positive control is positive and the negative control is negative. The test is positive, if there is purple–violet-coloured precipitate in the sample print or dot, provided that the positive control is positive and the negative control is negative.
- [23] For some viruses restricted to the phloem tissues, the observation of precipitates should occur in the vascular area only.

### **References:**

- [24] **Sutula C.L., Gillett J.M., Morrissey S.M. & Ramsdell D.C.** (1986). Interpreting ELISA data and establishing the positive–negative threshold. *Plant Disease*, 70: 722–726.

## Appendix 5

**BEST PRACTICES FOR SEQUENCING: USING DNA SEQUENCES TO DIAGNOSE A PEST****I. Purpose**

- [1] The purpose of this document is to outline the technical and scientific expectations for a diagnostic method that compares similarity or dissimilarity of DNA sequences generated from relatively short fragments (<2,000 bases) of an organismal genome. This includes the technique of DNA barcoding that is based on fragments of the mitochondrial cytochrome oxidase I gene for animals and combinations of other short gene fragments such as internal transcribe spacer regions of other taxa. The document does not consider diagnostic processes that involve comparison of large genome and transcriptome data sets.

**II. Criteria for ensuring the DNA sequence data to be queried in an analysis is appropriate**

- [2] The protocol should state that controls are needed during generation of DNA sequence from the suspect organism (as detailed in TPDP instructions to authors).
- [3] The protocol should define a measure of data quality using an algorithm such as phred scores or comparison of results from multiple reactions using distinct primers to confirm base calls.
- [4] Translation of coding sequences should be used to detect pseudogenes that would compromise interpretation.

**III. Criteria for ensuring that the DNA sequence library used to diagnose a query is fit for purpose**

- [25] The protocol should identify the DNA sequence resource (the record, bank or library) to be used for the desired comparison and diagnosis. This resource could be:
- (1) A single GenBank accession record but it should be readily accessible and monitored regularly for changes over time.
  - (2) One or more DNA sequence records stored in a static repository or file that cannot be altered without controlled permissions; (this is static because changes to the library effectively creates a new resource that can be verified for being fit for purpose).
  - (3) A dynamic database that is quality controlled to ensure that new records do not alter the outcome of each comparison; (dynamic means that new records are entered and old records are removed over time to a data base).
- [26] The protocol should provide a **published reference** as evidence that the library (or part of it) meets the sampling expectations for generating the expected diagnosis. The reference should provide an explicit recommendation for use of DNA sequence data for diagnosis of a targeted pest or pathogen. This includes:
- (4) States that the DNA sequence library includes the appropriate taxonomic sampling (i.e. sequence records of species/variants other than the targeted species) to enable biologically relevant diagnosis. If too few species are included in the database it might not function as a replacement of the morphological methods.
  - (5) States that the DNA sequence library includes the appropriate intraspecific sampling to enable biologically relevant diagnosis. If too few populations are sampled for a species it is possible that the dissimilarity between species is a sampling artifact.

- (6) States that the DNA records are derived from specimens that are retained as vouchers (when appropriate for the taxonomic group), have been expertly identified, and are traceable for future investigation.

#### **IV. Required information for proper interpretation of the comparison**

- [27] The protocol should indicate the alignment strategy for the method and indicate if it uses a global strategy (i.e. alignment of entire sequence length using clustal), a local alignment method (such as BLAST), or other so that labs can select the appropriate technique.
- [28] The protocol should indicate the method of sequence comparison and include a reference on how to perform that analysis: genetic distance values, character state at set nucleotide sites, perfect match criteria, or phylogenetic analysis
- [29] The protocol should provide clear interpretation rules to identify a suspect sample.
- (7) It should state how similar the query sequence and the reference sequence for the pest must be in order to determine a match.
- a. To confirm the match is not the result of missing information<sup>51</sup> in the edited sequence or rare genotypes<sup>52</sup> in the population, the protocol should indicate how dissimilar the query sequence should be from the next most genetically similar species in the reference library.
- (8) When appropriate, it should state how dissimilar the query sequence and the reference sequence for the pest must be in order to generate a mismatch.
- a. To confirm the mismatch is not the result of pseudogenes or other genetic variants and contaminants<sup>53</sup>, the protocol should indicate how similar the query sequence should be to other species in the reference library.

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<sup>51</sup> If the quality control measures for the protocol are not stringent it is possible for a query sequence to match more than one species in a library.

<sup>52</sup> If a new population or species is sampled it could exhibit affinity to two or more species in the database. For example match species 1 and species 2 by >98%.

<sup>53</sup> If the sequence is of high quality but does not match with any of the taxa in the library it is possible that it is a contaminant during the extraction or PCR steps and should not be used in a diagnosis.

## Appendix 6

**TPDP RECOMMENDATIONS ON NEXT GENERATION SEQUENCING (NGS) TECHNOLOGIES AS A DIAGNOSTIC TOOL FOR PHYTOSANITARY PURPOSES**

*(Developed by the Technical Panel on Diagnostic Protocols, February 2017)*

**Background**

- [1] Next generation sequencing (NGS) technologies allow the sequencing of the whole genome and can be used for all types of organisms. NGS technologies can be used for targeted detection of regulated pests and also allow the detection of unknown organisms. Indeed, application of these technologies has recently resulted in the discovery of previously undetected microorganisms, in particular viruses. Researchers and diagnosticians using NGS technologies will continue to identify and describe new taxa due to the large volume of as yet undiscovered organisms. These technologies therefore enable a new and comprehensive approach to the detection and characterization of pests in a biological sample.
- [2] Research findings based on NGS technologies may have significant implications within a phytosanitary framework. For example, there is a risk that plant material may be restricted in movement due to the perceived presence of a microorganism (i.e. virus) that may not have the potential to be pathogenic to its host. Not all organisms associated with plants are pests; some may be mutualists providing benefit to the host plant or commensal agents. There is also the issue, as with other indirect methods, NGS technologies will detect non-viable organisms.
- [3] Policies for interpreting data resulting from NGS technologies and to enable appropriate regulatory decisions are lacking globally.
- [4] Some fundamental questions relating to the biology of the latent organisms identified using NGS technologies require investigation to inform pest risk analysis and other science-based policy decisions. Although the potential of the technology is recognized, use of NGS technologies as a tool for plant pest diagnostics is still at an early stage.
- [5] For phytosanitary managers that are determining policies based on NGS technologies, the following should be considered:
- Do the newly detected organisms present an economic or trade risk?
  - What is the biological significance (e.g. host range) of the newly detected organism?
  - How to determine the geographic distribution of this organism if the organism is recently discovered and is cryptic or latent in nature?
  - What type of actions would be appropriate following findings based on NGS technologies (e.g. destruction of an imported consignment, further testing using other methodologies)?
- [6] There are a number of initiatives underway to explore the use of NGS technologies as a diagnostic tool for phytosanitary purposes (for example in Australasia, Europe and North America). These include discussions on associated policies that may be developed. NPPOs need harmonized approaches, however, in order to provide diagnosticians and phytosanitary managers with appropriate guidance on how to interpret NGS data sets.
- [7] The Technical Panel on Diagnostic Protocols (TPDP) discussed the issue during its meeting in February 2017 and made a number of conclusions and recommendations. The TPDP is developing guidance for DP authors on criteria for inclusion of an NGS method in IPPC diagnostic protocols.

**Appendix 07: TPDP 2017 – July 2018 work plan (tentative)**

<b>Action 1: 2017 - 2018 Diagnostic Protocols (DPs) overall management</b>	
<b>Goals:</b> a) Track, manage and ensure high quality DPs b) Overall management of 13 draft DPs	
<b>Activities</b>	<b>Responsible</b>
<b>DP drafting groups management:</b> TPDP members to update lead authors and DP drafting groups on the outcomes of the 2017 TPDP meeting and inform the deadlines to the lead authors.	TPDP members
<b>Draft DPs under the TPDP work programme<sup>54</sup></b>	
<ul style="list-style-type: none"> <li>• Tephritidae: Identification of immature stages of fruit flies of economic importance by molecular techniques (2006-028), priority 1</li> <li>• Genus <i>Ceratitis</i> (2016-001), priority 1</li> <li>• <i>Striga</i> spp. (2008-009), priority 1</li> <li>• <i>Tomato spotted wilt virus</i> (TSWV), <i>Impatiens necrotic spot virus</i> (INSV) and <i>Watermelon silver mottle virus</i> (WSMoV) (2004-019), priority 1</li> <li>• Revision of DP 2: <i>Plum pox virus</i> (2016-007), priority 1</li> <li>• <i>Xylella fastidiosa</i> (2004-024), priority 2</li> <li>• <i>Candidatus Liberibacter</i> spp. on <i>Citrus</i> spp. (2004-010), priority 2</li> <li>• <i>Phytophthora ramorum</i> (2004-013), priority 2</li> <li>• <i>Puccinia psidii</i> (2006-018), priority 2</li> <li>• <i>Bactrocera dorsalis</i> complex (2006-026), priority 2</li> <li>• <i>Conotrachelus nenuphar</i> (2013-002), priority 2</li> <li>• Begomoviruses transmitted by <i>Bemisia tabaci</i> (2006-023), priority 2</li> <li>• <i>Ips</i> spp. (2006-020), priority 4</li> </ul>	-

<sup>54</sup> See List of topics for IPPC standards: <https://www.ippc.int/en/core-activities/standards-setting/list-topics-ippc-standards/>

<b>Action 2: Expert Consultation on draft Diagnostic Protocols (ECDPs)</b>				
<b>Goals:</b> a) Ensure improvement on quality for the development of DPs, through inputs and feedback, in a scientific basis, from a wider number of experts worldwide not part of the DP drafting groups				
b) Facilitate the work to submit 4 DPs to the Expert Consultation on draft Diagnostic Protocols				
Activities	Start Date	Due Date	Related Steps	Responsible
<b>First 2017 ECDPs</b> Begomoviruses transmitted by <i>Bemisia tabaci</i> (2006-023)	10 August 2017	10 October 2017	Draft DPs back to the Secretariat: <b>01 August 2017</b>	Respective discipline lead and Secretariat
<b>Second 2017 ECDPs:</b> Tentative: Genus <i>Ceratitis</i> (2016-001) <i>Striga</i> spp. (2008-009) Tephritidae: Identification of immature stages of fruit flies of economic importance by molecular techniques (2006-028)	10 September 2017	10 October 2017	Draft to Secretariat: <b>01 September 2017</b>	Respective discipline lead and Secretariat

<b>Action 3: TPDP meetings</b>				
<b>Goal:</b> Discuss deeply the technical content of draft DPs, as well as challenges and strengthens of the panel and review the TPDP work programme.				
Activities	Start Date	Due Date	Related Steps	Responsible
<b>TPDP face to face meeting 2018</b> Tentative agenda: 1. Genus <i>Ceratitis</i> (2016-001) 2. <i>Striga</i> spp. (2008-009) 3. Tephritidae: Identification of immature stages of fruit flies of economic importance by molecular techniques (2006-028)	10 January 2018	10 January 2018	(Draft DPs going for Expert Consultation – see section above)	TPDP members and Secretariat
<b>TPDP virtual meetings (tentative)</b> • 25 May 2017 • 23 August 2017 • 09 November 2017	-	-		Secretariat and TPDP members

<b>Action 4: Consultation Period on draft ISPMs<sup>55</sup></b>				
<b>Goals: a) To ensure a transparent and inclusive process for the development of high quality DPs</b>				
<b>b) Facilitate the work to submit draft DPs to the consultation period</b>				
<b>Activities</b>	<b>Start Date</b>	<b>Due Date</b>	<b>Related Steps</b>	<b>Responsible</b>
<b>2017 July Consultation Period</b> 1. <i>Xylella fastidiosa</i> (2004-024) 2. <i>Puccinia psidii</i> (2006-018) 3. <i>Candidatus Liberibacter</i> spp. on <i>Citrus</i> spp. (2004-010) 4. <i>Bactrocera dorsalis</i> complex (2006-026) 5. <i>Conotrachelus nenuphar</i> (2013-002) 6. <i>Ips</i> spp. (2006-020)	01 July 2017	30 September 2017	(see above: Diagnostic Protocols (DPs) overall management and Expert consultation)	Respective Discipline lead and Secretariat
<b>2018 July Consultation Period (tentative):</b> 1. Genus <i>Ceratitis</i> (2016-001) 2. <i>Striga</i> spp. (2008-009) 3. Tephritidae: Identification of immature stages of fruit flies of economic importance by molecular techniques (2006-028)	01 July 2018	30 September 2018	(see above: Diagnostic Protocols (DPs) overall management and Expert consultation)	Respective Discipline lead and Secretariat

<sup>55</sup> Pending Standards Committee's approval

<b>Action 5: DP Notification period for draft DPs<sup>56</sup></b>				
<b>Goals:</b> a) To ensure a transparent and inclusive process for the adoption of draft DPs b) Facilitate the work to recommend draft DPs to the Standards Committee for adoption				
<b>Activities</b>	<b>Start Date</b>	<b>Due Date</b>	<b>Related Steps</b>	<b>Responsible</b>
<b>Draft DPs for approval for the July 2017 DP Notification Period (01 July – 15 August 2017)</b> <ol style="list-style-type: none"> <li>1. <i>Tomato spotted wilt virus</i> (TSWV), <i>Impatiens necrotic spot virus</i> (INSV) and <i>Watermelon silver mottle virus</i> (WSMoV) (2004-019)</li> <li>2. <i>Phytophthora ramorum</i> (2004-013)</li> </ol>	01 July 2017	15 August 2017	(see above: Diagnostic Protocols (DPs) overall management and Consultation Period)	Respective Discipline lead and Secretariat
<b>Draft DPs for approval for the December 2017 DP Notification Period (15 December 2017 – 30 January 2018)</b> <ol style="list-style-type: none"> <li>1. <i>Xylella fastidiosa</i> (2004-024)</li> <li>2. <i>Puccinia psidii</i> (2006-018)</li> <li>3. '<i>Candidatus Liberibacter spp.</i>' on <i>Citrus</i> spp. (2004-010)</li> <li>4. <i>Bactrocera dorsalis complex</i> (2006-026)</li> <li>5. <i>Conotrachelus nenuphar</i> (2013-002)</li> <li>6. <i>Ips</i> spp. (2006-020)</li> </ol>	15 December 2017	30 January 2018	(see above: Diagnostic Protocols (DPs) overall management and Consultation Period)	Respective Discipline lead and Secretariat

<sup>56</sup> Pending Standards Committee's approval

**Appendix 8: Action points arising from the February 2017 TPDP meeting (by agenda item)**

	Action	Agenda Item	Responsible	Deadline
1.	The TPDP agreed that guidance on the controls for the immunocapture RT-PCR should be drafted by the discipline lead and the DP drafting group, with the purpose of adding it to the Instructions to Authors.	3.1	Mr Delano JAMES and DP drafting group	Next virtual meeting
2.	The TPDP requested the discipline lead and the DP drafting group to revise the draft DP on Revision of DP 2: <i>Plum pox virus</i> (2016-007) and send it to the Secretariat by 17 March 2017.	3.1	Discipline lead and DP drafting group	17 March 2017
3.	The TPDP requested the discipline lead and the DP drafting group to revise the draft DP on <i>Bactrocera dorsalis</i> complex (2006-026) and send it to the Secretariat by 17 March 2017.	3.2	Discipline lead and DP drafting group	17 March 2017
4.	The TPDP requested the discipline lead and the DP drafting group to revise the draft DP on <i>Conotrachelus nenuphar</i> (2013-002) and send it to the Secretariat by 27 March 2017.	3.3	Discipline lead and DP drafting group	27 March 2017
5.	The TPDP agreed that a TPDP e-decision for final approval of the revised draft DP on <i>Conotrachelus nenuphar</i> (2013-002) to the SC should be made only if information on the use genitalia for the pest identification was included in the draft DP.	3.3	Secretariat	15 May 2017
6.	The TPDP agreed that Ms Juliet GOLDSMITH should be acknowledged as a co-author in the DP drafting group for <i>Conotrachelus nenuphar</i> (2013-002)	3.3	Secretariat (to updated list of DP drafting groups)	No deadline set
7.	The TPDP requested the discipline lead and the DP drafting group to revise the draft DP on <i>Ips</i> spp. (2006-020) and send it to the Secretariat by 23 March 2017.	3.4	Discipline lead and DP drafting group	23 March 2017
8.	The TPDP agreed to have a strategic discussion in their next face-to-face meeting on how the TPDP can to make a proper and strategic transition to a new way of working.	4.1	TPDP members	10 January 2018
9.	Draft DPs to be discussed at the next face to face meeting: <ol style="list-style-type: none"> <li>1. Genus <i>Ceratitis</i> (2016-001)</li> <li>2. <i>Striga</i> spp. (2008-009)</li> <li>3. Tephritidae: Identification of immature stages of fruit flies of economic importance by molecular techniques (2006-028)</li> </ol>	5.2	Discipline leads (and referees) and DP drafting groups	10 January 2018
10.	Secretariat to try contact the DP drafting groups: <ol style="list-style-type: none"> <li>1. <i>Striga</i> spp. (2008-009)</li> <li>2. Begomoviruses transmitted by <i>Bemisia tabaci</i> (2006-023)</li> <li>3. <i>Candidatus Liberibacter</i> spp. on <i>Citrus</i> spp. (2004-010)</li> </ol>	5.2	Secretariat (and Discipline leads and referees)	30 May 2017
11.	Update the DP drafting groups contact information list	5.2	Secretariat	No deadline set
12.	Update the Instructions to Authors.	6.0	Secretariat	No deadline set
13.	The TPDP requested the lead to prepare a paper for the next TPDP face-to-face meeting with a standardized text proposition on "ELISA controls and interpretation of results" for inclusion in the TPDP Instruction to authors.	7.1	Ms Géraldine ANTHOINE	10 January 2018

	Action	Agenda Item	Responsible	Deadline
14.	The TPDP requested the leads to revise the document "Control options for molecular tests for pest group categories" for the next TPDP face-to-face meeting.	7.2	Ms Géraldine ANTHOINE and Mr Norman BARR	10 January 2018
15.	TPDP members to submit additional comments to the document "Control options for molecular tests for pest group categories" to the leads by 30 August 2017.	7.2	TPDP members	30 August 2017
16.	The TPDP requested the lead to prepare a paper for the next TPDP face-to-face meeting with a standardized text proposition on "Best practices for sequencing" for inclusion in the TPDP Instruction to authors.	7.3	Mr Norman BARR	10 January 2018
17.	TPDP members to submit additional comments to the document "Best practices for sequencing" to the lead by 30 August 2017.	7.3	TPDP members	30 August 2017
18.	The TPDP requested the lead to revise the document "Quality Assurance for diagnostic protocols" for the next TPDP face-to-face meeting	7.4	Mr Norman BARR	10 January 2018
19.	TPDP members to submit comments to the document "Quality Assurance for diagnostic protocols" to the lead by 30 August 2017	7.4	TPDP members	30 August 2017
20.	The TPDP agreed to recommend the paper "Use of Next generation sequencing (NGS) technologies as a diagnostic tool for phytosanitary purposes" to the SC for their consideration and to inform the CPM on the challenges associated with these new technologies.	7.5	Secretariat	Next SC meeting
21.	The discipline lead, together with the DP drafting group, would prepare the revision of the draft DP on <i>Tomato spotted wilt virus</i> , <i>Impatiens necrotic spot virus</i> and <i>Watermelon silver mottle virus</i> (2004-019) with the responses to the objection.	11	Discipline lead and DP drafting group	03 April 2017
22.	Analyze the need for a revision for the adopted draft DPs: 1. DP1: <i>Thrips palmi</i> 2. DP 3: <i>Trogoderma granarium</i> 3. DP 16: Genus <i>Liriomyza</i> 4. DP 9: Genus <i>Anastrepha</i>	13	Ms Juliet GOLDSMITH and Mr Norman BARR	10 January 2018