UPDATE ON ACTIVITIES OF THE TECHNICAL PANEL ON DIAGNOSTIC PROTOCOLS (TPDP) FROM MAY 2015 TO APRIL 2016

(Prepared by the IPPC Secretariat)

1. Background

[1] The IPPC Secretariat support for the Technical Panel on Diagnostic Protocols (TPDP) are:

- Ms Adriana G. Moreira (lead)
- Mr Martin Farren (support)

[2] The TPDP membership and contact information can be found on IPP[1]. In table 1 there is a simplified version of the TPDP membership as of April 2016.

Table 1. TPDP membership (as of April 2016) and expertise of its members

Participant role	Name (country)	Expertise	Term expires
Steward	Ms Jane CHARD (United Kingdom)		
Assistant steward	Mr Guillermo S. CHINCHILLA (Costa Rica)		
Member	Mr Robert TAYLOR (New Zealand)	Bacteriology	May 2021 (2nd term)
Member	Ms Liping YIN (China)	Botany	April 2018 (2nd term)
Member	Mr Norman B. BARR (United States)	Entomology	July 2017 (1st term)
Member	Ms Juliet GOLDSMITH (Jamaica)	Entomology	November 2019 (1st term)
Member	Mr Johannes DE GRUYTER (the Netherlands)	Mycology	April 2018 (2nd term)
Member	Ms Géraldine ANTHOINE (France)	Nematology	April 2019 (2nd term)
Member	Mr Delano JAMES (Canada)	Virology	November 2020 (2nd term)
Member	Mr Brendan RODONI (Australia)	Virology (and back up for bacteriology)	July 2017 (1st term)

[3] It is to be noted that Ms Ana Lia TERRA (Uruguay) left the TPDP in August 2015.

[4] Considering the term of Mr Norman Barr will expire in July 2017, it is proposed to extend his term for a further five years period, due to his valuable contributions to the panel.

2. TPDP volume of work

[5] The TPDP work programme currently comprises 21 diagnostic protocols (DPs) under six disciplines in various stages of development (figure 1). Most of the diagnostic protocols are drafted. However, one DP "Tephritidae: Identification of immature stages of fruit flies of economic importance by molecular techniques (2006-028)" is with "pending status"[2] due the lack of validated and verified data on molecular methods for identification of fruit fly larvae of all genera.

[1] TPDP main page on IPP: https://www.ippc.int/en/core-activities/standards-setting/expert-drafting-groups/technical-panels/technical-panel-diagnostic-protocols/

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

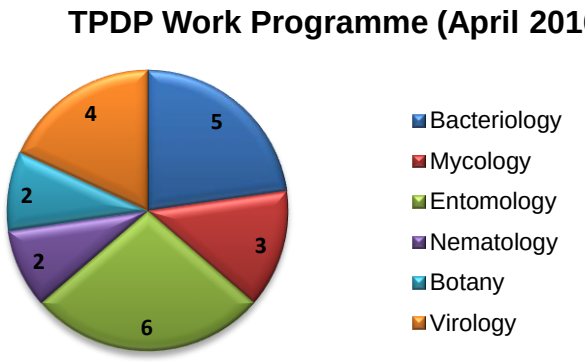

Figure 1. Number of diagnostic protocols per discipline under the TPDP work programme.

[6] A total of 18 draft diagnostic protocols were processed through the standard setting system in 2015 which is 1.3 times more compared to 2014. In 2016, a total of 20 draft diagnostic protocols are projected to flow through the standard setting process (figure 2) representing the management of over 100 DP authors.

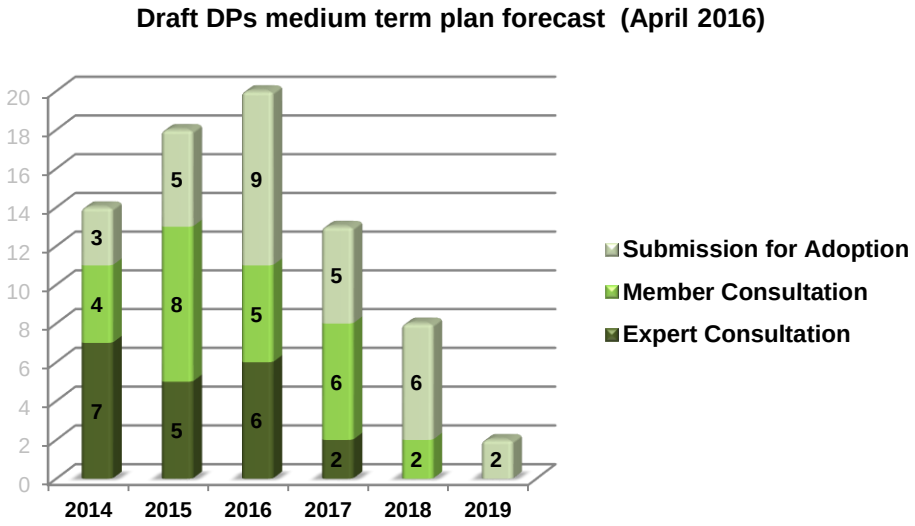

Figure 2. Medium term plan forecast for draft diagnostic protocols (annexes to ISPM 27).

[7] The TPDP work programme is being delivered through several activities. Since May 2015, the activities were as follow:

- three member consultations[3] (30 January – 30 June 2015, 01 July – 30 November 2015, 01 February – 30 June 2016): 10 draft diagnostic protocols
- two DP notification periods[4] (01 July – 15 August 2015 and 15 December 2015 – 30 January 2016): six draft diagnostic protocols

[3] Member consultation (MC) on draft ISPMs on IPP: https://www.ippc.int/en/core-activities/standards-setting/member-consultation-draft-ispms/

[4] Notification Period for draft DPs on IPP: https://www.ippc.int/en/core-activities/standards-setting/draft-ispms/notification-period-dps/

- three Expert Consultations on draft diagnostic protocols (ECDP)[5] (10 March – 24 April 2015, 24 March – 24 April 2015 and 02 October – 30 November): five draft Diagnostic protocols
- 13 TPDP e-decisions: 13 draft diagnostic protocols

Highlights on the work

[8] The TPDP continued to work on its work programme during the May 2015 to April 2016 period, managing more than 100 DP authors from various countries. In 2015 six diagnostic protocols were adopted as annexes to ISPM 27 (*Diagnostic protocols for regulated pests*). This means an enormous workload for all involved. The panel engaged in several discussions on horizontal issues that may affect diagnosis, such as quality assurance, best practices for sequences molecular methods and the detection of viable organisms, especially in seeds and in wood material.

[9] To date, there are 12 adopted diagnostic protocols, annexes to ISPM 27. The panel engaged in discussions on the ongoing need to develop diagnostic protocols and their usefulness, highlighting that the diagnostic protocols are an essential parts of surveillance programme and pest reporting. Diagnostic protocols also support pest eradication programmes, export certification, import inspections and the application of appropriate phytosanitary treatments.

[10] The TPDP also reviewed its working procedures related to information to be provided to the DP drafting groups by revising the Instruction to Authors and a brochure targeted on communicating how to engage DP authors in a more meaningful way and to explain how the standard setting process works, in particular for the development of diagnostic protocols[6].

[11] The panel was informed and consulted on the drafting of ISO standard 13484 (Horizontal methods for molecular biomarker analysis), an ISO draft standard related to plant health issues and provided technical input to ensure this standard considered appropriate phytosanitary issues. The panel also liaised with the Convention on Biological Diversity (CBD) Secretariat on diagnostic issues related to the Global Taxonomy Initiative (GTI) in an effort to try to obtain more information about the GTI work and how a synergistic relationship can be established between the TPDP and GTI, in particular for the development of better diagnostic protocols.

3. TPDP Meetings

[12] The TPDP has held the following four meetings since May 2015, reports are posted on the IPP[7]:

- 2015 TPDP June (face-to-face meeting): 22 – 26 June 2015, Shanghai (P.R. China)
- 2015 TPDP September virtual meeting
- 2015 TPDP November virtual meeting
- 2016 TPDP March virtual meeting

[13] A summary of the discussions and outcomes of each meeting are detailed below, as well as intersession activities.

[5] Expert consultation on draft DPs on IPP: https://www.ippc.int/en/expert-consultation-on-draft-diagnostic-protocols-ecdp/

[6] An Introduction for Authors of IPPC Diagnostic Protocols: https://www.ippc.int/largefiles/IPPC_IntroToAuthors_e_W.pdf

[7] Reports of the TPDP meetings: https://www.ippc.int/en/core-activities/standards-setting/expert-drafting-groups/technical-panels/technical-panel-diagnostic-protocols/

[14] Detailed information on the draft diagnostic protocols submitted to the several steps in the standard setting process can be found in the Commission on Phytosanitary Measures document CPM 2016/19 (Report on the activities of the Standards Committee in 2015)[8].

2015 June Meeting (Shanghai, P.R. China)

[15] The Secretariat presented the status of the TPDP work programme, highlighting the dates when it is expected the 27 draft diagnostic protocols will reach the various steps in the standard setting process noting that, in an optimistic scenario, all draft diagnostic protocols are expected to be submitted for adoption by 2019[9].

[16] The TPDP conducted detailed revisions of the following five draft diagnostic protocols:

1. *Liberibacter solanacearum* (2013-001)
2. *Fusarium moniliformis / moniforme* syn. *F. circinatum* (2006-021)
3. *Phytophthora ramorum* (2004-013)
4. *Dendroctonus ponderosae* syn. *Scolytus scolytus* (2006-019)
5. *Anguina* spp. (2013-003)

[17] It was noted that four draft diagnostic protocols had been submitted to the Expert Consultation on draft DPs[10] in 2015. The draft DP for *Liberibacter solanacearum* (2013-001) was submitted in the fourth quarter of 2015.

[18] One general issue was identified regarding the detection of viable organisms by molecular methods and tests in several protocols (e.g. *Bursaphelenchus xylophilus* (2004-016)[11], *Liberibacter solanacearum* (2013-001), *Fusarium moniliformis / moniliforme* syn. *F. circinatum* (2006-021), *Phytophthora ramorum* (2004-013)). The panel noted this is a horizontal issue and needed further discussion. Another concern raised was the consistency on the use of the words "assay", "method" and "test". However, further discussions and agreement were postponed to the next face to face meeting of the TPDP.

[19] For the five draft diagnostic protocols discussed at the meeting, the TPDP invited the diagnostic protocol drafting groups to consider its recommendations and consequently adjust the draft diagnostic protocols, noting that some would require some TPDP e-decisions before submitting them to the Standards Committee (SC) with the recommendation for approval for member consultation.

[20] While reviewing of its work programme, the TPDP discussed the issue of the diagnostic protocol drafting group for the draft diagnostic protocol for *Anoplophora* spp. (2004-020) which has a "pending status". The TPDP asked the Secretariat to try to contact and engage the current diagnostic protocol drafting group and possibly to open a call for authors for *Anoplophora* spp. (2004-020), as a last resort.

[21] The TPDP was informed that ISO is developing the standard *General requirements for molecular biology analysis for detection and identification of destructive organisms in plants and derived products* now, which overlaps with some of the work of the IPPC in relation to diagnostic protocols.

[22] The TPDP reviewed their work plan for 2015-2016[12]. The TPDP briefly discussed the challenges and the importance of the TPDP work. It was felt that a review of the panel's work would be beneficial to prepare a better medium term plan. The panel highlighted some challenges to the production of diagnostic protocols including: nomination of experts; length of time taken to get agreement between

[8] CPM 2016/19: Report on the activities of the Standards Committee in 2015 - https://www.ippc.int/en/publications/82090/

[9] TPDP June 2015 meeting report: https://www.ippc.int/en/publications/81330/

[10] Expert consultation on draft DPs: https://www.ippc.int/core-activities/expert-consultation-draft-diagnostic-protocols

[11] *Bursaphelenchus xylophilus* (2004-016) draft DP was submitted for member consultation on February 2015: https://www.ippc.int/en/publications/2736/

[12] TPDP work plan 2015-2016 can be found in Appendix 4 of the 2015 June TPDP meeting at https://www.ippc.int/en/publications/81330/

experts; diagnostic protocols which cover several aspects (surveillance, testing of imports and confirmation of new pests in a country); cost of development of a DP; the need for continual updating. Regarding benefits, the major example was the IPPC diagnostic protocols as global standards, i.e. scrutiny by all IPPC contracting parties, so consensus on reliable methods (sensitivity, specificity and reproducibility globally harmonized) should help to minimize disputes. Another aspect of importance of a harmonized DP is to aid the development of expertise and technical cooperation among contracting parties. The panel felt that more discussion on the challenges and importance of the TPDP would be beneficial, and proposed to discuss this in future.

2015 TPDP September virtual meeting

[23] The Secretariat updated the panel on the 27 draft diagnostic protocols under the TPDP work porgramme[13].

[24] Regarding the draft DP for *Anoplophora* spp. (2004-020) the Secretariat informed the panel that the Standards Committee (SC) had been approached and requested to help identify additional authors for this diagnostic protocol drafting group. It was noted that up to now only one author confirmed his willingness to continue on the diagnostic protocol drafting group. It was pointed out that this subject will be discussed again at the SC meeting in November 2015. Given the lack of commitment of the current diagnostic protocol drafting group to work on this draft and the unsuccessful attempts to identify other authors to form the diagnostic protocol drafting group, the SC removed the draft DP for *Anoplophora* spp. (2004-020) from the *List of topics for IPPC standards*.

[25] The TPDP briefly discussed the formal objection received on the draft diagnostic protocols for Phytoplasma (2004-018)[14] on the last diagnostic protocols notification period, which closed on 15 August 2015. The panel acknowledged the issue raised in the formal objection, which pointed out that the negative extraction control can be both a buffer or a nucleic acid. It was felt that it needed more discussion and the panel agreed to work virtually to address this formal objection. It was also noted that extracting material from uninfected host tissue is a horizontal issue and should be addressed for all draft diagnostic protocols. The TPDP approved the responses in relation to this formal objection and revised the draft diagnostic protocol via e-decision (2015_eTPDP_Oct_03). The response to the formal objection and the revised draft DP were presented to the SC for approval for the DP Notification Period via e-forum (2015_eSC_Nov_10) was adopted by the SC on behalf of CPM in January 2016.

[26] Quality assurance issues and best practices for sequencing were briefly discussed. It was stressed that this theme is very important and may have several implications for the development of diagnostic protocols and should be discussed at the next face to face meeting.

2015 TPDP November virtual meeting

[27] The TPDP discussed the challenges and the importance of the TPDP work[15]. Most of the diagnostic protocols on the list of topics for IPPC standards will be adopted in the next three years and therefore the panel needs to consider their medium term plan. The steward acknowledged the work and commitment by panel members, as well as the authors in charge of the development of diagnostic protocols. During the discussion, it was noted that the Secretariat's human resources to coordinate the panel's work might face constraints in dealing with the high volume of diagnostic protocols being processed.

[28] It was recalled that the IPPC Implementation Review and Support System (IRSS) published the results of a survey[16] conducted in 2014 on the implementation of ISPM 17 (*Pest reporting*) and ISPM 19

[13] TPDP September 2015 virtual meeting report: https://www.ippc.int/en/publications/81612/

[14] Link to formal objection received for Phytoplasma (20014-018): https://www.ippc.int/en/publications/81394/

[15] TPDP November 2015 virtual meeting report: https://www.ippc.int/en/publications/81861/

[16] IRSS survey report (ISPM 17 and ISPM 19): https://www.ippc.int/largefiles/2014/Survey-Analysis-NPPOs-17-19.pdf

(*Guidelines on lists of regulated pests*). The panel had previously discussed these results when developing the TPDP's strengths, weaknesses, opportunities and threats ("SWOT analysis") table in 2014[17]. In this IRSS survey, countries were asked to list the five pests of most concern. It was noted that there are inherent issues in creating such lists, for example: not all IPPC contracting parties responded to the questionnaire; importance may change rapidly as new pests occur; there is no information on the status of these pests; and IPPC contracting parties were not asked to provide the five pests for which diagnostic protocols are required. It was pointed out that over 50% of the diagnostic protocols that the TPDP have developed or are working on were also the pests listed in this survey.

[29] The panel discussed the ongoing need to develop diagnostic protocols and their usefulness. It was highlighted that the TPDP and the scientific community play an essential role in assisting countries dealing with various pests and of course, with the development of diagnostic protocols. Harmonized diagnostic protocols are highly beneficial and help harmonize diagnostic activities and meet the needs and demands of the IPPC community. It was noted that a questionnaire to seek views on the utility of diagnostic protocols and the needs of contracting parties was developed but put on hold in 2014 until more diagnostic protocols had been adopted. The panel agreed that a major priority will be to review adopted diagnostic protocols, as this is a task (task 5) in the TPDP Specification[18]. The panel stressed that there was also a need to update publications (literature references) and modernize the diagnostic protocols with the latest technology applicable as it is imperative that diagnostic protocols do not become outdated.

[30] Regarding the draft DP for Tephritidae: Identification of immature stages of fruit flies of economic importance by molecular techniques (2006-028), the lead author and TPDP member introduced a paper**[19]** highlighting that the lack of validated and verified data on molecular methods for identification of fruit fly larvae is hampering the progress on this DP. The panel agreed to recommend that the SC change the scope of this DP (see "Attachment 1" of this document).

2016 TPDP March virtual meeting

[31] The TPDP discussed a technical revision on the adopted DP 07: *Potato spindle tuber viroid*[20]. The panel was informed that a scientist contacted the lead author with regards to a discrepancy in the sequence of the internal primer COX F. This discrepancy was on a three base pair sequence according to the referenced literature, Weller et al. 2000[21]. However, it was acknowledged by the scientist that both sequences do work, but the results using the sequence as described by Weller et al. 2000 is more efficient and it also matches the COX sequences in Genebank.

[32] It was recalled that the SC in May 2013 established criteria for revising diagnostic protocols such as a technical revision that should be carried out by the TPDP, noting that other revisions would need to be subject to the normal DP adoption process (i.e. member consultation, redrafting, SC approval, formal objection period, SC adoption). The TPDP agreed to invite the SC to approve this technical revision (via SC e-decision: refer to 2016_eSC_May_15 in document 12_SC_2016_May), stressing that it was a typographical error in the primer sequence and thus falls under the jurisdiction of the TPDP. It was suggested issuing an erratum, as per in scientific publications. It was pointed out that, despite encountering errors in adopted diagnostic protocols, this was a good example that contracting parties are implementing IPPC diagnostic protocols.

[33] The panel discussed the title of the draft diagnostic protocol for "Liberibacter spp. / Liberobacter spp. on *Citrus* spp. (2004-010)" which is planned to be submitted to the expert consultation soon. The panel

[17] 2014 July TPDP Meeting Report: https://www.ippc.int/en/publications/2579/

[18] Specification TP 1 - Technical Panel on Diagnostic Protocols: https://www.ippc.int/en/publications/1297/

[19] 04_TPDP_2015_Nov

[20] DP 07: Potato spindle tuber viroid: https://www.ippc.int/en/publications/8073/

[21] Weller, S.A., Elphinstone, J.G., Smith, N.C., Boonham, N. & Stead, D.E. 2000. Detection of *Ralstonia solanacearum* strains with a quantitative multiplex, real-time, fluorogenic PCR (TaqMan) assay. *Applied and Environmental Microbiology*, 66: 2853–2858. (http://aem.asm.org/content/66/7/2853.full.pdf+html)

agreed to change its title to "*Candidatus* Liberibacter spp. on *Citrus* spp. (2004-010)" to reflect the current taxonomy classification.

[34] The TPDP briefly reviewed their work plan, now comprising of 21 draft diagnostic protocols. The Secretariat provided updates on the upcoming CPM-11 and SC May meeting.

4. Participation of TPDP representatives in other relevant meetings

[35] The discipline lead for Viruses and Phytoplasmas, Mr Delano JAMES, provided a lecture on "*International perspective – International Plant Protection Convention (IPPC)*" at the 2015 Barcode of Life Conference held in 18 – 21 August 2015 at Guelph, Canada.

[36] The IPPC Secretariat lead for the TPDP, Ms Adriana G. MOREIRA, provided a lecture on "*International Plant Protection Convention (IPPC): Use of reference material in international diagnostic protocols*" at the Q-Collect Workshop held in 08 – 09 September 2015 at Rome, Italy.

[37] The IPPC Secretariat lead for the TPDP, Ms Adriana G. MOREIRA, provided a lecture on "*International standard on pest surveillance – ISPM 6*" at the FAO-IPPC-CIHEAM International Workshop on *Xylella fastidiosa* & the Olive Quick Decline Syndrome (OQDS) held in 19-22 April 2016 at CIHEAM/Istituto Agronomico Mediterraneo of Bari, Italy. The importance of diagnosis for surveillance was highlighted. The discipline lead for Bacteriology, Mr Robert TAYLOR, attended the workshop as the TPDP lead for the draft DP for *Xylella fastidiosa.*

5. Tentative work plan for the period May 2016-April 2017

[38] The next face to face meeting will be convened in Montego Bay, Jamaica, on 11 – 15 July 2016. This meeting will be hosted Ministry of Agriculture and Fisheries and organized by the Plant Quarantine Produce Inspection Branch, Jamaica's NPPO. The tentative agenda is a deep discussion of four draft diagnostic protocols and at least eight discussion papers on horizontal issues of pest diagnosis and TPDP mid-term work plan.

[39] The TPDP will continue to work on the 21 draft diagnostic protocols. It is hoped that the current diagnostic protocols will all be submitted for adoption by 2019.

[40] The TPDP tentative work plan for May 2016 – April 2017 is summarized in figure 2.

5.1 Expert Consultations on draft Diagnostic protocols:

[41] Two additional expert consultations are planned to take place in the 2nd and 4th quarter of 2016 for at least six draft diagnostic protocols (further information on the IPP calendar).

ATTACHMENT 1:

PROPOSALS TO REDEFINE SCOPE AND NAME OF DP ENTITLED "TEPHRITIDAE: IDENTIFICATION OF IMMATURE STAGES OF FRUIT FLIES OF ECONOMIC IMPORTANCE BY MOLECULAR TECHNIQUES" (2006-028)

(Prepared by Norman Barr and reviewed by Juliet Goldsmith. Agreed by the TPDP in November 2015)

[42] The development of a Diagnostic Protocol (DP) entitled "Tephritidae: Identification of immature stages of fruit flies of economic importance by molecular techniques (2006-028)" was accepted as a topic for the Technical Panel on Diagnostic Protocols (TPDP)[22]. The TPDP selected a team of authors (DP drafting group)[23]: Norman Barr (lead), Beatriz Sabater Munoz, Isabel Frioni Barboza, and Deuk-Soo Choi. However, after consideration of the goals and possible options for draft development, the lead author concluded that additional refinement would be needed in order to proceed. The DP is currently in "pending status". The issues needing consideration by the TPDP prior to restarting drafting of this protocol concern its scope, practicality, and format.

[43] The family Tephiritdae includes near 5,000 species of which 100-200 are classified as pests or of economic significance. Excluding all morphological methods from the DP should significantly reduce the final size of the document and the work required in drafting the document. Unfortunately, focusing on molecular methods alone would still result in a lengthy draft that would be hard to draft for the team of authors. Each species requires description in an adopted DP (as prescribed in the guidelines/instructions and preceding adopted protocols). For example, the *Anastrepha* DP includes seven species and requires an entire page of text for taxonomic details alone. This requirement should need to be removed or modified for a DP that included many species such as the family Tephiritidae. This inclusion of many species would also impact other sections such as the background of pests. Not all fruit flies have the same bionomic and pest information and the number of references needed to explain the potential diagnostic tests, geographic spread, and host use could be substantial. This is not in line with existing DP to date that focus on one or a few pests of interest within a genus.

[44] The request for molecular methods of identification is also unusual in comparison to other DP topics by requesting the diagnosis be limited to molecular methods. There are currently two accepted/active topics for fruit flies that target groups within the family: the adopted *Anastrepha* protocol and the *Bactrocera dorsalis* complex DP (currently in draft). These protocols include all diagnostic methods for the pests and are not limited to morphology. The request for a molecular diagnostic protocol for all fruit flies could introduce redundant information to these existing protocols once they are adopted. If the original topic is to be pursued then this potential introduction of redundancy should be considered. It should be resolved prior to initiating additional diagnostic protocols.

[45] There are many molecular studies that examine diagnostic capabilities for fruit flies but these are not all verified or used routinely. Many protocols also investigate population level variation of specific species. Inclusion of all of these methods or even discussion of these tests would result in a long document given the current scope. It is possible to focus on a few methods such as DNA barcoding and Ribosomal DNA Restriction Fragment Length Polymorphism but this would still require extensive method details because of variation in analysis of DNA results. That is because there is not a single interpretation rule for all fly species given the evidence of cryptic species and distinct evolutionary histories for the group. In addition, the use of host records for immature fly identification is often included in the interpretation of results and will require documentation. Host lists require verification

[22] List of topics for IPPC standards: https://www.ippc.int/en/publications/8016/

[23] IPPC Diagnostic Protocols (DPs) drafting groups: https://www.ippc.int/en/publications/2582/

and this can become a difficult task for a team to compile and teams that must verify or review proposed documents. Similarly, test methods proposed for identification by some Plant Protection Organizations are dependent on pathway information for identification. If the potential geographic sources of the material is not known (as is true for domestic captures of invasive species or fruit collected from hand carried baggage) or includes a country where fruit flies are known to be diverse (e.g. *Anastrepha* in Brazil) the reported methods may not provide reliable identifications. Focusing on a more limited taxonomic group of flies will make it easier to address these limitations of methods.

[46] The recommendation is that the scope (and name) of the protocol be modified if it is to continue as an IPPC topic. To be consistent with existing methods for for the adopted DP 9 on Genus *Anastrepha* and the draft DP on *Bactrocera dorsalis* complex (2006-026), the new scope should not be limited to one method of identification, i.e. molecular methods. This is because there are many molecular studies that examine diagnostic capabilities for fruit flies but these are not all validated or verified, or used routinely.

[47] Revising the protocol to address pests in the genus *Ceratitis* would provide a DP that is consistent with other DP topics. This group includes the worldwide pest *Ceratitis capitata* (the Mediterranean Fruit Fly) and additional pests of concern to Africa and Europe (e.g., *C. cosyra, C. anonae*). There are at least 10 species of economic significance as pests within the genus. It is possible that the authors could limit this to highest impact pests once the drafting is reinitiated.

[48] The TPDP agreed with the following:

(1) To reduce the scope of the target pest from fruit flies in the Tephritidae family to fruit flies in the Genus *Ceratitis* (*Ceratitis* spp.) because this genus includes many fruit flies of economic significance and are pests of concern (and also appeared in the IRSS survey mentioned above);

(2) To include all life stages (from immature to adult) to be consistent with existing methods and adopted or draft diagnostic protocols, and to provide more options for identification of immature stages when difficulties in using certain methodologies are encountered;

(3) To include several types of methods, not only molecular, because up to now, not all molecular methods available were verified or are used routinely, and morphological methods are commonly used;

(4) To change the scope and the title from "Tephritidae: Identification of immature stages of fruit flies of economic importance by molecular techniques (2006-028)" to "Genus *Ceratitis* (2006-028)".