

International standards for the diagnosis of regulated pests

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Abstract For the last 10 years, the European and Mediterranean Plant Protection Organization (EPPO) has run a European Panel on diagnostics, which has developed regional standards on diagnostic protocols. Nearly 80 such standards have now been approved, and are in active use in EPPO countries. In 2003, the Commission for Phytosanitary Measures (CPM) of FAO, in reviewing global needs for International Standards for Phytosanitary Measures (ISPMs), recognized that there is a strong interest in developing diagnostic protocols for all contracting parties to the International Plant Protection Convention (IPPC). Such protocols would support the harmonization of detection and identification procedures worldwide, contribute to greater transparency and comparability in the diagnostics for regulated pests, and assist in the resolution of disputes between trading partners. In addition, such protocols would be very useful in technical assistance programmes. In 2004, the CPM adopted a mechanism for rapid development of ISPMs in specific areas, particularly suitable for

diagnostic protocols. A Technical Panel was accordingly established to develop protocols for specific pests and meets on an annual basis. A format for international diagnostic protocols was adopted in 2006 and a list of priority pests was established. In 2003, EPPO initiated a new programme on quality management and accreditation for plant pest laboratories and Standards are now also being developed in this area. In 2006, a survey of existing diagnostic capacities in EPPO member countries was undertaken and a database on diagnostic expertise was created.

Keywords Diagnostic protocols · International Standards for phytosanitary measures · Regional Standards · Accreditation · Diagnostic expertise

Introduction

Global phytosanitary context

During the last century, movement of goods and persons across the world has increased considerably. Natural borders that were once effective barriers to the spread of pests are now under pressure from the increasing volume of international trade. As a consequence, the global community has developed cooperative mechanisms to protect plants and the environment from pests. The International Plant Protection Convention (IPPC) is an international treaty to protect plant health. The Convention,

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adopted in 1951, is deposited with the Director General of the Food and Agriculture Organization of the United Nations (FAO). The purpose of the IPPC is to secure common and effective action to prevent the spread and introduction of pests of plants and plant products and to promote appropriate measures for their control. Since the beginning of the 1990s, the international phytosanitary context has changed considerably. The IPPC was revised in 1997 (FAO 1997) to incorporate principles included in the 1994 SPS agreement (Agreement on the Application of Sanitary and Phytosanitary Measures) of the WTO (World Trade Organization). This agreement states in particular that “Members shall ensure that any sanitary or phytosanitary measure is applied only to the extent necessary to protect human, animal or plant life or health, is based on scientific principles and is not maintained without sufficient scientific evidence” and that “members shall base their SPS measures on international standards, guidelines and recommendations” (WTO 1994). The latter are defined as those ‘developed under the auspices of the Secretariat of the IPPC in cooperation with regional organizations operating within the framework of the IPPC’. The revision of the IPPC in 1997 represented a major updating of the Convention. The changes were primarily intended to strengthen the IPPC, by provision of a mechanism for developing and adopting International Standards for Phytosanitary Measures (ISPMs) and by the creation of a Commission for Phytosanitary Measures (CPM) to promote the full implementation of the objectives of the Convention¹. The New Revised Text of the IPPC came into force on 2005-10-02.

European context

The new revised text of the IPPC also provides for Regional Plant Protection Organizations (RPPOs) to develop internationally agreed standards on phytosanitary measures. In the IPPC context, they are known as ‘regional standards’. The RPPO for Europe is the European and Mediterranean Plant Protection Organization (EPPO). This was created in 1951 to prevent the introduction of dangerous pests from

other parts of the world, and limit their spread within Europe if they were introduced. Today, 48 European and Mediterranean countries (including the 27 members of the European Union) are members of the Organization. Key partners of EPPO are National Plant Protection Organizations (NPPOs), i.e. the official services which are responsible for plant protection in each member country.

How are ISPMs developed by the CPM?

Global level

The process for developing an ISPM includes three stages: the preparation of a draft, a consultation stage and a formal approval stage (Fig. 1). Suggestions for topics for ISPMs can be made by the NPPOs, the IPPC Secretariat or the WTO-SPS Committee. Other organizations, such as the WTO, may also submit proposals for standards through the IPPC Secretariat. A Standards Committee oversees the standard-setting process and assists in the development of ISPMs by agreeing on the specifications for draft standards and checking the drafts before and after the consultation stage. This Committee comprises 25 members drawn from the seven FAO regions. The CPM is in charge, in particular, of establishing standard-setting priorities, and approves the final versions.

Regional level

For the European and Mediterranean region, EPPO has for many years had in place a Working Party on Phytosanitary Regulations which approves regionally harmonized texts on plant quarantine. These texts are developed by Panels of experts (e.g. the EPPO Panel on Diagnostics), are subject to a consultation procedure involving all Member Governments, and are finally approved by the Council of EPPO. When the IPPC introduced the concept of Regional Standards, all such EPPO documents were redefined as EPPO Standards, in a number of different series (for example, series PM 7 on diagnostic protocols). Regional standards are also developed by other RPPOs; in particular, the North American Plant Protection Organization and the ‘Comité de Sanidad Vegetal del Cono Sur’ in South America are also developing standards on diagnostic protocols. These regional initiatives have opened the

¹ Pending the entry into force of the text, an Interim Commission for Phytosanitary Measure was established and worked from 1998 to 2005. To facilitate the reading of the article it is called CPM throughout the text.

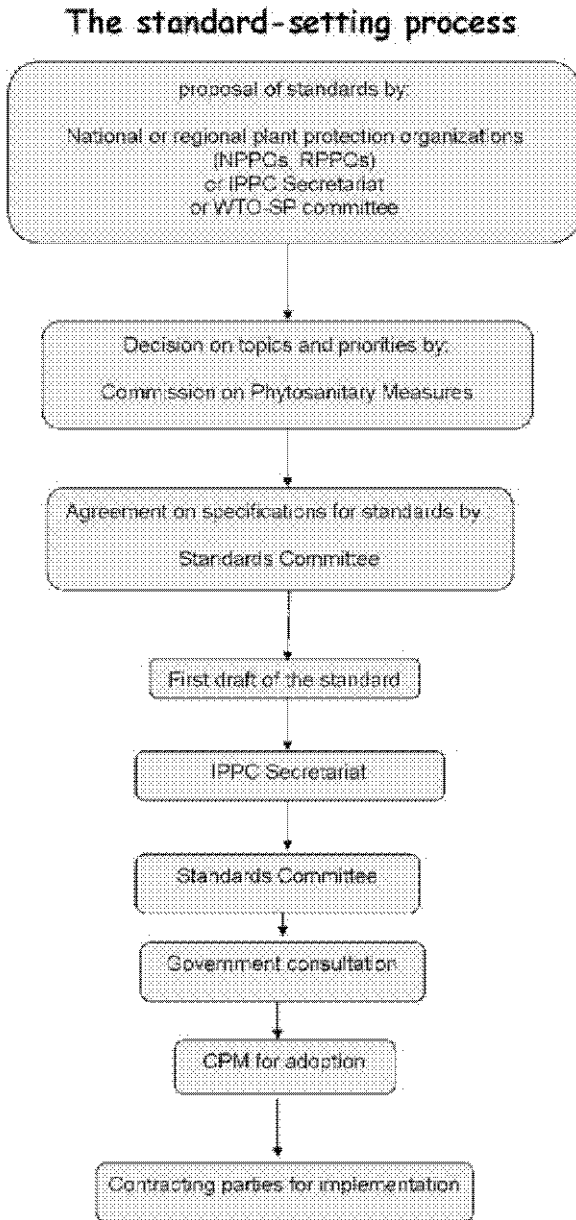


Fig. 1 The process for developing an ISPM. From the Guide to the International Plant Protection Convention (FAO 2002)

way for the development, at global level, of international diagnostic protocols.

Standards on diagnostic protocols

Regional level: EPPO Standards

The first initiatives in developing standards on diagnostic protocols were taken by EPPO. Standards

based on the work of the EPPO Panel on Diagnostics (Zlof et al. 2000) were published in 2001, and nearly 80 diagnostic protocols have now been approved (Table 1). Their preparation involved close collaboration with other EPPO Panels (Bacterial Diseases, Nematodes, Certification of Fruit Crops) and with the European Mycological Network. The EPPO system has also incorporated standards initially developed by the DIAGPRO Project of the Commission of the European Union (no. SMT 4-CT98–2252) (Harju et al. 2000). The objective of the EPPO programme was to develop internationally agreed diagnostic protocols for as many as possible of the regulated pests of the EPPO A1 and A2 lists and of the Annexes of EU Directive 2000/29 (of which there are about 340). The protocols are based on the many years of experience of EPPO experts. Each first draft is prepared by an individual expert, and written according to a ‘common format and content of a diagnostic protocol’ agreed by the Panel on Diagnostics, modified as necessary to fit individual pests. Each protocol is intended to contain all the information necessary to detect and positively identify a particular regulated pest.

Global level: IPPC diagnostic protocols

In reviewing global needs for plant protection in 2003, the CPM recognized that countries had a strong interest in international diagnostic protocols (FAO 2003). It considered in particular that international diagnostic protocols would provide harmonized detection and identification procedures worldwide, and contribute to greater transparency and comparability of diagnostics for regulated pests. Such protocols would be particularly useful in preventing disputes between trading partners. In addition, such protocols would constitute a very good basis for training and technical assistance.

In 2004, the CPM adopted a mechanism to speed up the development of ISPMs in specific areas, in particular diagnostic protocols (FAO 2004). A Technical Panel was accordingly established to develop diagnostic protocols for specific pests, with the following main tasks:

- identification of priorities for specific protocols to be developed and submitted to the Standards Committee. The Technical Panel should identify

Table 1 Published EPPO diagnostic protocols

| Diagnostic protocol |
|--|
| Published Bulletin OEPP/EPPO Bulletin 31(1), 2001 |
| PM 7/1 (1) <i>Ceratocystis fagacearum</i> |
| PM 7/2 (1) <i>Tobacco ringspot nepovirus</i> |
| PM 7/4 (1) <i>Bursaphelenchus xylophilus</i> |
| PM 7/5 (1) <i>Nacobbus aberrans</i> |
| Published Bulletin OEPP/EPPO Bulletin 32(2), 2002 |
| PM 7/6 (1) <i>Chrysanthemum stunt pospiviroid</i> |
| PM 7/7 (1) <i>Aleurocanthus spiniferus</i> |
| PM 7/8 (1) <i>Aleurocanthus woglumi</i> |
| PM 7/9 (1) <i>Cacoecimorpha promubana</i> |
| PM 7/10 (1) <i>Cacyreus marshalli</i> |
| PM 7/11 (1) <i>Frankliniella occidentalis</i> |
| PM 7/12 (1) <i>Parasaissetia nigra</i> |
| PM 7/13 (1) <i>Trogoderma granarium</i> |
| Published Bulletin OEPP/EPPO Bulletin 33(2), 2003 |
| PM 7/14 (1) <i>Ceratocystis fimbriata</i> f. sp. <i>platani</i> |
| PM 7/15 (1) <i>Ciborinia camelliae</i> |
| PM 7/16 (1) <i>Fusarium oxysporum</i> f. sp. <i>albedinis</i> |
| PM 7/17 (1) <i>Guignardia citricarpa</i> |
| PM 7/18 (1) <i>Monilinia fructicola</i> |
| PM 7/19 (1) <i>Helicoverpa armigera</i> |
| Published Bulletin OEPP/EPPO Bulletin 34(2), 2004 |
| PM 7/20 (1) <i>Erwinia amylovora</i> |
| PM 7/21 (1) <i>Ralstonia solanacearum</i> |
| PM 7/22 (1) <i>Xanthomonas arboricola</i> pv. <i>corylina</i> |
| PM 7/23 (1) <i>Xanthomonas axonopodis</i> pv. <i>dieffenbachiae</i> |
| PM 7/24 (1) <i>Xylella fastidiosa</i> |
| PM 7/25 (1) <i>Glomerella acutata</i> |
| PM 7/26 (1) <i>Phytophthora cinnamomi</i> |
| PM 7/27 (1) <i>Puccinia horiana</i> |
| PM 7/28 (1) <i>Synchytrium endobioticum</i> |
| PM 7/29 (1) <i>Tilletia indica</i> |
| PM 7/31 (1) <i>Citrus tristeza closterovirus</i> |
| PM 7/32 (1) <i>Plum pox potyvirus</i> |
| PM 7/33 (1) <i>Potato spindle tuber pospiviroid</i> |
| PM 7/34 (1) <i>Tomato spotted wilt tospovirus</i> |
| PM 7/35 (1) <i>Bemisia tabaci</i> |
| PM 7/36 (1) <i>Diabrotica virgifera</i> |
| PM 7/37 (1) <i>Thaumetopoea pityocamp</i> |
| PM 7/38 (1) <i>Unaspis citri</i> |
| PM 7/39 (1) <i>Aphelenchoides besseyi</i> |
| PM 7/40 (1) <i>Globodera rostochiensis</i> and <i>Globodera pallida</i> |
| PM 7/41 (1) <i>Meloidogyne chitwoodi</i> and <i>Meloidogyne fallax</i> |
| Published Bulletin OEPP/EPPO Bulletin 35(2), 2005 |
| PM 7/42 (1) <i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i> |
| PM 7/43 (1) <i>Pseudomonas syringae</i> pv. <i>persicae</i> |
| PM 7/44 (1) <i>Xanthomonas axonopodis</i> pv. <i>citri</i> |
| PM 7/45 (1) <i>Cryphonectria parasitica</i> |
| PM 7/46 (1) <i>Mycosphaerella dearnessii</i> |

Table 1 (continued)

| Diagnostic protocol |
|---|
| PM 7/47 (1) <i>Mycosphaerella pini</i> |
| PM 7/48 (1) <i>Phoma tracheiphila</i> |
| PM 7/49 (1) <i>Tomato ringspot nepovirus</i> |
| PM 7/50 (1) <i>Tomato yellow leaf curl and Tomato mottle begomoviruses</i> |
| PM 7/51 (1) <i>Aonidiella citrina</i> |
| PM 7/52 (1) <i>Diaphorina citri</i> |
| PM 7/53 (1) <i>Liriomyza</i> spp. |
| PM 7/54 (1) <i>Lopholeucaspis japonica</i> |
| PM 7/55 (1) <i>Rhizoecus hibisci</i> |
| PM 7/56 (1) <i>Scirtothrips aurantii</i> , <i>Scirtothrips citri</i> and <i>Scirtothrips dorsalis</i> |
| PM 7/57 (1) <i>Trioza erytrae</i> |
| Published Bulletin OEPP/EPPO Bulletin 36(1), 2006 |
| PM 7/58 (1) <i>Burkholderia caryophylli</i> , |
| PM 7/58 (1) <i>Clavibacter michiganensis</i> subsp. <i>sepedonicus</i> |
| PM 7/60 (1) <i>Panthoia stewartii</i> subsp. <i>stewartii</i> , |
| PM 7/61 (1) <i>Candidatus Phytoplasma aurantifoliae</i> , |
| PM 7/62 (1) <i>Candidatus Phytoplasma mali</i> |
| PM 7/63 (1) <i>Candidatus Phytoplasma pyri</i> |
| PM 7/64 (1) <i>Xanthomonas arboricola</i> pv. <i>pruni</i> |
| PM 7/65 (1) <i>Xanthomonas fragariae</i> |
| PM 7/66 (1) <i>Phytophthora ramorum</i> |
| PM 7/67 (1) <i>American plum line pattern ilarvirus</i> |
| PM 7/68 (1) <i>Eotetranychus lewisi</i> |
| PM 7/69 (1) <i>Lepidosaphes ussuriensis</i> |
| PM 7/70 (1) <i>Maconellicoccus hirsutus</i> |
| PM 7/71 (1) <i>Opogona sacchari</i> |
| PM 7/72 (1) <i>Tecia solanivora</i> |
| PM 7/73 (2) <i>Thrips palmi</i> |
| Published Bulletin OEPP/EPPO Bulletin 36(3), 2006 |
| PM 7/30 (2) <i>Beet necrotic yellow vein benyvirus</i> |
| PM 7/73 (1) <i>Gymnosporangium</i> spp. (non-European) |
| PM 7/74 (1) <i>Popillia japonica</i> |
| PM 7/75 (1) <i>Toxoptera citricidus</i> |
| PM 7/76 (1) Use of EPPO diagnostic protocols |
| PM 7/77 (1) Documentation and reporting on a diagnosis |

the existing regional standards or protocols used by individual countries and consider suggestions for new protocols (i.e. those put forward by NPPOs, RPPOs, Expert Working Groups or other Technical Panels)

- identification of specialists to draft the Diagnostic Protocols
- production, or supervision of the production, of diagnostic protocols for specific pests

- submission to the Standards Committee of draft diagnostic protocols for specific pests and where necessary revisions of previously adopted protocols.

The Technical Panel is composed of eight diagnostic experts with at least one representing each taxonomic discipline: entomology/acarology, nematology, mycology, plant bacteriology, virology (including viroids and phytoplasmas), botany. An expert on quality assurance will also be included in the group. Between them, participants should have practical expertise in the use of morphological and molecular/biochemical diagnostic techniques, and in phytosanitary procedures. The membership of the Technical Panel is drawn from the seven FAO regions.

The Panel has already met three times since 2004. The Panel has prepared a standard explaining the scope, purpose and content of diagnostic protocols (IPPC 2006), and has recommended that specific protocols are added as annexes to this master standard (which was adopted by the CPM in April 2006 as ISPM no. 27 on *Diagnostic protocols for regulated pests*). The EPPO format was used as a basis for the development of this draft structure for diagnostics standards. The standard provides guidance on the scope and purpose of diagnostic protocols, but it does not address quality assurance issues. The Technical Panel recommended that experts drafting protocols should consider all appropriate methods for diagnosis of pests to ensure flexibility, and that the agreement of all Panel members should be needed for a method to be included in a protocol. Diagnostic protocols should take into account the fact that laboratories have differing capabilities and facilities. They should provide the essential requirements for reliable diagnosis of the pest, with alternative or supplementary methods and procedures to provide flexibility. The sensitivity, specificity and reliability of the methods should be indicated, so that NPPOs can determine the level of confidence given by each method or combination of methods. The Technical Panel also established a list of pests for which diagnostic protocols should individually be prepared (see Table 2). This list of priority pests was assembled on the basis of a list of existing regional diagnostic protocols and on pests for which requests for international diagnostic protocols had been made in 2003.

The IPPC Secretariat has collected names of experts from all parts of the world to take part in

Table 2 List of regulated pests for development of diagnostic protocols agreed in the IPPC work programme in 2007

| Scientific name | Type of pest |
|--|--------------|
| <i>Erwinia amylovora</i> | Bacteria |
| <i>Xylella fastidiosa</i> | Bacteria |
| <i>Liberibacter</i> spp. | Bacteria |
| <i>Xanthomonas fragariae</i> | Bacteria |
| <i>Xanthomonas axonopodis</i> pv. <i>citri</i> | Bacteria |
| <i>Phytophthora ramorum</i> | Fungus-like |
| <i>Tilletia indica</i> / <i>T. controversa</i> | Fungi |
| <i>Guignardia citricarpa</i> | Fungi |
| <i>Gymnosporangium</i> spp. | Fungi |
| <i>Gibberella circinata</i> | Fungi |
| <i>Puccinia psidii</i> | Fungi |
| <i>Anastrepha</i> spp. | Insects |
| <i>Thrips palmi</i> | Insects |
| <i>Anoptophora</i> spp. | Insects |
| <i>Trogoderma granarium</i> | Insects |
| <i>Bactrocera dorsalis</i> complex | Insects |
| <i>Dendroctonus ponderosae</i> | Insects |
| <i>Ips</i> spp. | Insects |
| <i>Liriomyza</i> spp. | Insects |
| <i>Bursaphelenchus xylophilus</i> | Nematodes |
| <i>Ditylenchus destructor</i> / <i>D. dipsaci</i> | Nematodes |
| <i>Xiphinema americanum</i> | Nematodes |
| <i>Aphelenchoides besseyi</i> , <i>A. ritzemabosi</i> , and <i>A. fragariae</i> | Nematodes |
| <i>Plum pox virus</i> | Viruses |
| Tospoviruses (TSWV, INSV, WSMV) | Viruses |
| <i>Citrus tristeza virus</i> | Viruses |
| Potato spindle tuber viroid | Viruses |
| Viruses transmitted by <i>Bemisia tabaci</i> | Viruses |
| Phytoplasmas in general | Phytoplasmas |

Expert Working Groups to prepare these diagnostic protocols. EPPO, acting as an intermediary in the process of selection of European experts, has communicated a list of names to the IPPC Secretariat, consisting mainly of experts who have been involved in the preparation of EPPO Diagnostic Protocols. The Expert Working Groups for drafting specific diagnostic protocols mostly work through e-mail discussions under the supervision of a member of the Technical Panel responsible for the appropriate discipline. The first diagnostic protocols were presented to the Standards Committee in May 2007.

Consequences for EPPO

EPPO with its long experience in coordinating the elaboration of regional diagnostic protocols will be

closely involved in the IPPC process. In particular, appropriate protocols already developed by EPPO may be available as working material for international standards. The work of the EPPO Panel will continue, but with new priorities determined in part by the progress in developing international diagnostic protocols. EPPO experts will concentrate on pests which are present in, and important for Europe, and not necessarily of great significance for other continents. EPPO expects that the international standards will in general fill the needs of EPPO countries for pests on which there is little practical experience.

Other EPPO initiatives in the field of diagnostics

Accreditation and quality management for plant health laboratories

Since 1999, there have been several discussions on the possibility that EPPO should assist national diagnostic laboratories in obtaining accreditation. It was concluded that EPPO can develop a quality assurance standard for diagnostic laboratories but that accreditation can be provided only by an official (national) outside body. In 2003, EPPO created an *ad hoc* Panel on Technical requirements for laboratories and began to develop guidelines on quality assurance. During the EPPO Conference on Diagnostics in Noordwijkerhout (NL) in 2004, accreditation of laboratories, quality assurance systems for laboratories, and EPPO's role in this area were discussed. It was proposed that the *ad hoc* Panel on Technical requirements for laboratories should identify the critical elements in ISO/IEC Standard 17025 on General requirements for the competence of testing and calibration laboratories (ISO, 2005), and develop an EPPO Standard which could serve as an interpretation of this Standard for plant pest identification. This would be particularly useful because national accreditation bodies currently interpret Standard ISO/IEC 17025 differently. An EPPO Standard on *Basic requirements for quality management in plant pest diagnosis laboratories* is being developed and will soon be adopted. Contacts have been made with the European Accreditation (EA) body to develop a standard which could be used as a guidance document for laboratories applying for accreditation, as well as for accreditation bodies.

EPPO database on diagnostic capacity

In 2004, EPPO Council stressed that the implementation of phytosanitary regulations for quarantine pests is being jeopardized by decreasing knowledge in plant protection and by a lack of well maintained collections. The Panel on Diagnostics consequently decided to identify practical actions which could be undertaken by EPPO to improve collaboration on diagnostics in Europe and to provide good scientific support for the diagnostic work of NPPOs. The EPPO Secretariat is accordingly making an inventory of the available expertise on diagnostics in Europe, of training capacities in diagnostics and of collections (including their maintenance), on the basis of a questionnaire to its member countries. Emphasis should be given to regulated pests and not to common pests which are widely distributed in Europe. The aim was to identify experts who could provide diagnosis of regulated species but also those who could help in the identification of new or unusual species for the EPPO region. In May and June 2006, this questionnaire was launched on the EPPO website. All NPPOs of EPPO member countries (and laboratories which were part of their diagnostic network) were invited to participate in this survey. As of May 2007, 27 countries have provided data about their diagnostic expertise (covering 118 diagnostic laboratories and 377 experts). These results are available in the form of a searchable database EPPO website. The database on collections is still under construction.

Conclusion

Activities on diagnostics have been and continue to be of prime importance for EPPO, and have been one of the main areas in which the Organization has made its mark in recent years, in its publications and on its website. A very valuable international resource has been created, and a basis has been laid for active cooperation between European countries in the provision of diagnostic services. This is reflected in the document on EPPO's mission, goals and strategy Period 2006–2009 stating that "*EPPO will expand its role in addressing diagnostic needs by supporting diagnostic laboratories, leading to the introduction of quality assurance systems and/or accreditation*". More than 30 diagnostic protocols and two standards

on quality assurance are in a draft stage. More international cooperation on diagnostics is also expected with the IPPC initiative on Diagnostic protocols.

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