

International Plant Protection Convention (IPPC): Use of reference material in international diagnostic protocols

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Protecting the world's plant resources from pests



How to secure cooperation among nations



protect global plant resources

prevent spread
introduction
of pests

preserve food security
biodiversity

facilitate international trade ?





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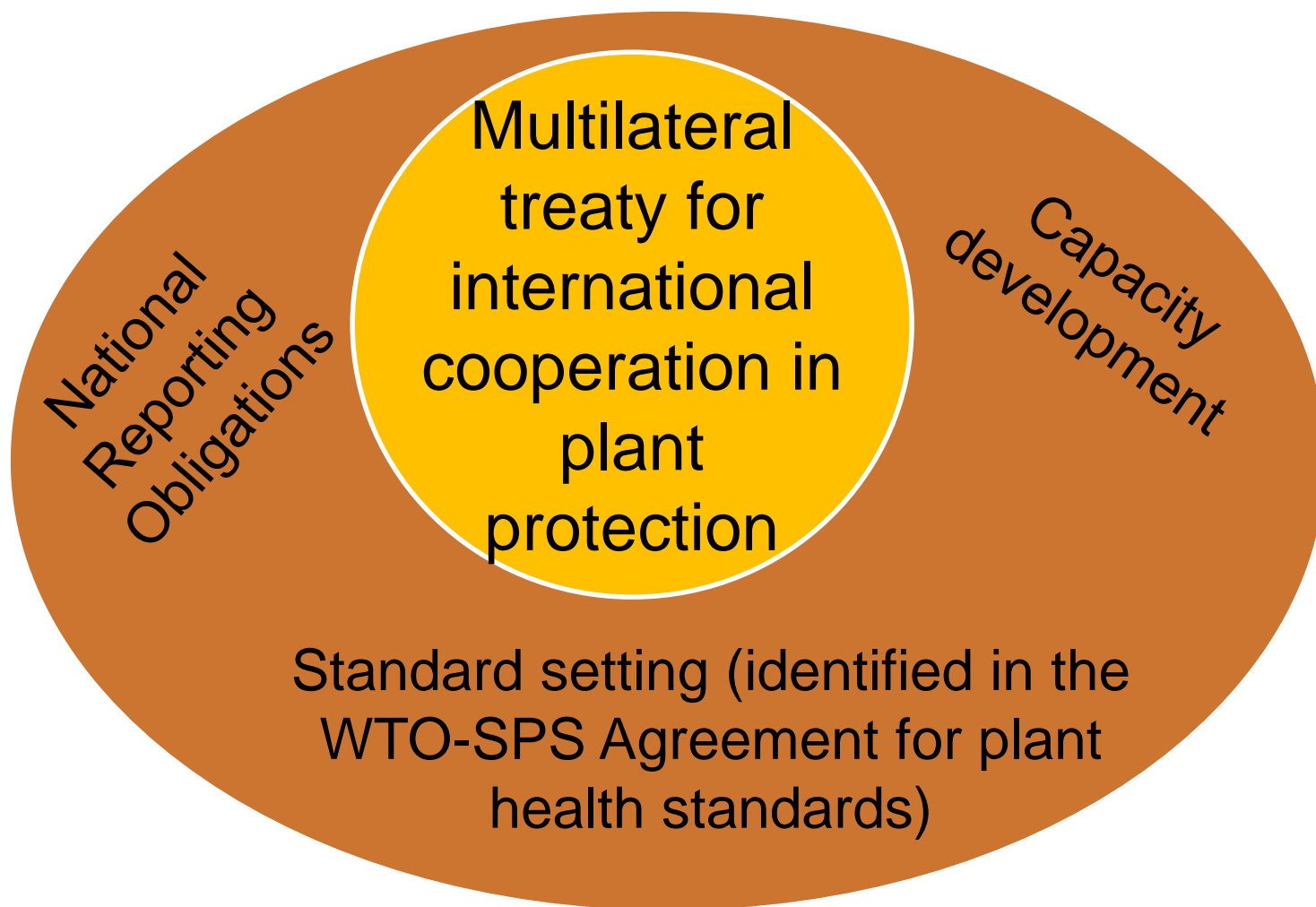


IPPC

- **International treaty** for international cooperation in **plant protection**
- The global instrument for the **harmonization of phytosanitary measures** for trade and environment
- The **standard setting organization** for plant health recognized by **WTO-SPS**

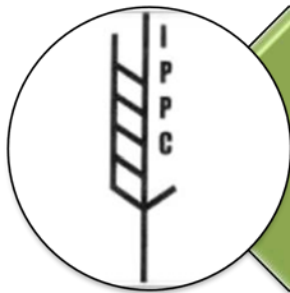


IPPC

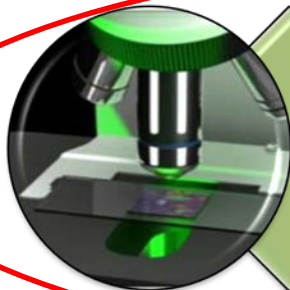


International Standards for Phytosanitary Measures (ISPMs)





> 58 ISPMs



9 DPs



19 PTs



DIAGNOSTIC PROTOCOLS FOR REGULATED PESTS (ISPM 27)

Minimum requirements for reliable diagnosis

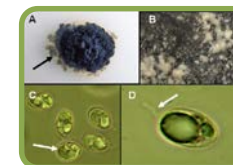
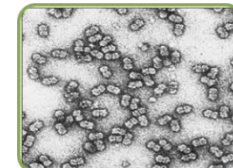
Sensitivity

Specificity

Reproducibility

9 adopted diagnostic protocols

- DP 1: *Thrips palmi*
- DP 2: *Plum pox virus*
- DP 3: *Trogoderma granarium*
- DP 4: *Tilletia indica* Mitra
- DP 5: *Phyllosticta citricarpa* (McAlpine) Aa on fruit
- DP 6: *Xanthomonas citri* subsp. *citri*
- DP 7: *Potato spindle tuber viroid*
- DP 8: *Ditylenchus destructor* and *Ditylenchus dipsaci*
- DP 9: Genus *Anastrepha*



Technical Panel on Diagnostic Protocols (TPDP)



Technical Panel on Diagnostic Protocols (TPDP)

9 members
> 100 authors

Many other collaborators ...

TPDP work programme:

26 DPs / 6 topics

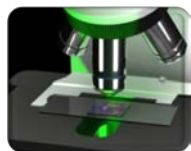




Diagnostic protocols and the use of reference material

ISPM 27. Diagnostic Protocols For Regulated Pests

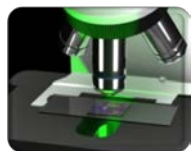
- The aspects of quality assurance and in particular the **reference materials** that are required by diagnostic protocols (such as inclusion of positive and negative controls or collection of specimens) are specifically indicated in the corresponding section of the protocol. (Section 1. General Requirements for Diagnostic Protocols)
- Where appropriate, guidance is provided on positive and negative controls and **reference material** to be included in tests. (Section Detection)



Diagnostic protocols and the use of reference material

ISPM 27. Diagnostic Protocols For Regulated Pests

- Where appropriate, guidance is provided on positive and negative controls and **reference material** to be included in tests. (Section Identification)
- Those cases where the use of appropriate controls for a specific technique, including where relevant **reference material**, is essential are clearly indicated in the protocol. When appropriate controls are not available, other tests, preferably based on different identification principles, may increase the certainty of the identification. Alternatively, a sample, specimen or, where appropriate, an image should be sent to another laboratory with experience in diagnosis of the suspected pest and possessing the required control or **reference materials**. Specimen(s) or **material for reference purposes** should be properly preserved. (Section Identification)



DP 4: *Tilletia indica* Mitra

2. Taxonomic Information

Name: *Tilletia indica* Mitra, 1931

Synonyms: *Neovossia indica* (Mitra) Mundkur, 1941

Taxonomic position: Eukaryota, Fungi, Basidiomycota, Ustilaginomycotina, Exobasidiomycetes, Exobasidiomycetidae, Tilletiales, Tilletiaceae

Common name: Karnal bunt or partial bunt

Reference: MycoBank 267835

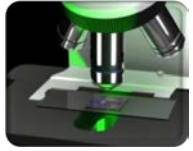
DP 4: *Tilletia indica* Mitra

Table 3. Sequences and modifications of the primers and probes used in the five-plex fluorescent PCR diagnostic assay for *T. indica* and other related *Tilletia* spp.

Primer pairs (sequence 5'-3')	Probes (modifications 5', 3')	Channel	Target
KB-DL-For: CTTCGGAAGAGTCTCCTT (nt. 64–81 ^a) KB-DL- Rev: CCGGACAGGTACTIONCAG (nt. 127–142)	ACGGAAGGAACGAGGC (nt. 105–120) (6-FAM, BHQ1)	Green	<i>T. indica</i>
	ACGGAAGGAACAAGGC (nt. 67–82 ^b) (JOE, BHQ1)	Yellow	<i>T. walkeri</i>
Hor-DL-For: GGCCAATCTTCTACTATC (nt. 40–59 ^c) Hor-DL-Rev: CCGGACAGGATCACTA (nt. 87–102)	CAACCCAGACTACGGAGGGTGA (nt. 60–81) (CAL Fluor Red 610, BHQ2)	Orange	<i>T. horrida</i> (some strains are not detected)
Tri-DL-For: ATTGCCGTACTTCTCTTC (nt. 56–73 ^d) Tri-DL-Rev: GTAGTCTTGTGTTGGATAATAG (nt. 99–112)	AGAGGTCGGCTCTAATCCCATC A (nt. 75–97) (Quasar 670, BHQ2)	Red	Broad range*
Ehr-DL-For: CGCATTCTTATGCTTCTTG (nt. 72–90 ^e) Ehr-DL-Rev: GTTAGGAACCAAAGCCATC (nt. 128–146)	CAGAGTCATTGGTTCTTCGGAG C (nt. 104–126) (Quasar 705, BHQ2)	Crimson	<i>T. ehrhartae</i>

Notes: GenBank accession numbers are ^aAF398434, ^bAF310180, ^cAF310171, ^dAF398447 and ^eAY770433. The list of the reference material used and place of origin is in Tan *et al.* (2009), and material is held at Elizabeth Macarthur Agricultural Institute (EMAI), NSW Dept. of Primary Industries in Australia (See section 6, contact points. nt., nucleotide.

*Includes *T. caries*, *T. laevis*, *T. controversa*, *T. fusca*, *T. bromi*, *T. goloskokovii*.



DP 5: *Phyllosticta citricarpa* (McAlpine) Aa on fruit

2. Taxonomic Information

Name: *Phyllosticta citricarpa* (McAlpine) Aa, 1973

Synonyms: *Phoma citricarpa* McAlpine, 1899

Guignardia citricarpa Kiely, 1948

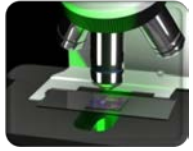
Phyllostictina citricarpa (McAlpine) Petr., 1953

Leptodothiorella sp. (spermatial state)

Taxonomic position: Eukaryota, Fungi, Ascomycota, Pezizomycotina, Dothideomycetes, Botryosphaeriales, Botryosphaeriaceae

Common names: Citrus black spot (for common names in other languages, see CABI (2011))

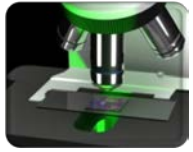
Reference: MycoBank 320327



DP 5: *Phyllosticta citricarpa* (McAlpine) Aa on fruit

4.2.1.3 Essential procedural information

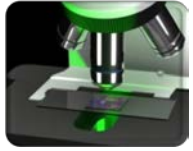
- DNA from a **reference strain** of *P. citricarpa* (positive control) must be included as an additional sample to ensure that amplification has been successful.



DP 5: *Phyllosticta citricarpa* (McAlpine) Aa on fruit

4.2.2 Identification of *P. citricarpa* by real-time PCR

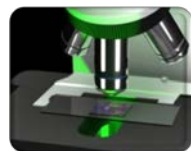
- *Specificity (analytical specificity) was assessed with the P. citricarpa reference strain CBS 111.20 (representative for 10 P. citricarpa isolates ITS sequence group I; Baayen et al., 2002), the P. capitalensis reference strain GC14 (representative for 22 P. capitalensis isolates ITS sequence group II; Baayen et al., 2002), 12 other citrus pests (Alternaria spp., Penicillium spp., Colletotrichum spp.), Phyllosticta artocarpina and Guignardia bidwellii. Only P. citricarpa gave a positive reaction. The sensitivity (analytical sensitivity; detection limit) is 10 fg DNA per reaction and the diagnostic sensitivity is 100% (Gent-Pelzer et al., 2007).*



DP 5: *Phyllosticta citricarpa* (McAlpine) Aa on fruit

4.2.2.3 Essential procedural information

- *DNA from a reference strain of P. citricarpa (positive control) must be included as an additional sample to ensure that amplification has been successful.*

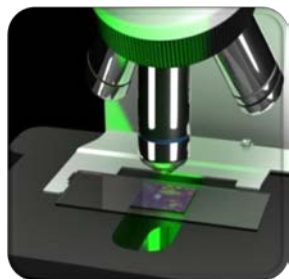


DP 6: *Xanthomonas citri* subsp. *citri*

4. Identification

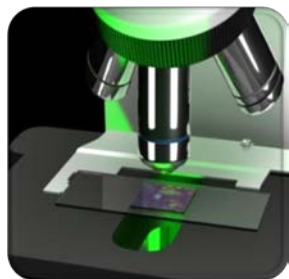
The following collections, among others, can provide X. citri subsp. citri reference strains (the X. citri subsp. citri isolates recommended for use as positive controls are given):

- *NCPPB 3234 from National Collection of Plant Pathogenic Bacteria, Central Science Laboratory, York, United Kingdom*
- *CFPB 2911 from Collection Française de Bactéries Phytopathogènes, INRA Station Phytobactériologie, Angers, France (this is a X. citri subsp. citri A* strain)*
- *ICMP 24 from International Collection of Microorganisms from Plants, Landcare Research (Manaaki Whenua) New Zealand Ltd, Auckland, New Zealand*
- *ATTC 49118 from American Type Culture Collection, Manassas, VA, United States*
- *IBSBF 1594 from Biological Institute Culture Collection of Phytopathogenic Bacteria, Centro Experimental Central do Instituto Biológico - Laboratório de Bacteriologia Vegetal, Campinas, Brazil.*



Some draft DPs under the work programme

- *Phytophthora ramorum* (2004-013)
- *Dendroctonus ponderosae* (2006-019)
- *Fusarium moniliformis* / *moniforme* syn. *F. circinatum* (2006-021)
- Genus *Liriomyza* (2006-017)
- *Xanthomonas fragariae* (2004-012)



Diagnostic protocols and the use of reference material

- IPPC DPs: refer to reference material when available, especially for molecular methods (e.g. barcoding).
- IPPC is willing to include relevant information or reference to this material, as soon as reference material is available.
- IPPC visualizes the free access of reference material.

To secure cooperation **among nations**



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prevent spread
introduction
of pests
preserve food security
biodiversity
facilitate
international trade

