

Organisation Européenne et Méditerranéenne pour la Protection des Plantes  
European and Mediterranean Plant Protection Organization

# **Normes OEPP EPPO Standards**

Diagnostic protocols for regulated pests  
Protocoles de diagnostic pour les  
organismes réglementés

PM 7/27



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## Approval

EPPO Standards are approved by EPPO Council. The date of approval appears in each individual standard. In the terms of Article II of the IPPC, EPPO Standards are Regional Standards for the members of EPPO.

## Review

EPPO Standards are subject to periodic review and amendment. The next review date for this EPPO Standard is decided by the EPPO Working Party on Phytosanitary Regulations

## Amendment record

Amendments will be issued as necessary, numbered and dated. The dates of amendment appear in each individual standard (as appropriate).

## Distribution

EPPO Standards are distributed by the EPPO Secretariat to all EPPO member governments. Copies are available to any interested person under particular conditions upon request to the EPPO Secretariat.

## Scope

EPPO Diagnostic Protocols for Regulated Pests are intended to be used by National Plant Protection Organizations, in their capacity as bodies responsible for the application of phytosanitary measures to detect and identify the regulated pests of the EPPO and/or European Union lists.

In 1998, EPPO started a new programme to prepare diagnostic protocols for the regulated pests of the EPPO region (including the EU). The work is conducted by the EPPO Panel on Diagnostics and other specialist Panels. The objective of the programme is to develop an internationally agreed diagnostic protocol for each regulated pest. The protocols are based on the many years of experience of EPPO experts. The first drafts are prepared by an assigned expert author(s). They are 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. As a general rule, the protocol recommends a particular means of detection or identification which is considered to have advantages (of reliability, ease of use, etc.) over other methods. Other methods may also be mentioned, giving their advantages/disadvantages. If a method not mentioned in the protocol is used, it should be justified.

The following general provisions apply to all diagnostic protocols:

- laboratory tests may involve the use of chemicals or apparatus which present a certain hazard. In all cases, local safety procedures should be strictly followed
- use of names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable

- laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated or that proper positive and negative controls are included.

## References

- EPPO/CABI (1996) *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (GB).
- EU (2000) Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. *Official Journal of the European Communities* L169, 1–112.
- FAO (1997) *International Plant Protection Convention* (new revised text). FAO, Rome (IT).
- IPPC (1993) *Principles of plant quarantine as related to international trade*. ISPM no. 1. IPPC Secretariat, FAO, Rome (IT).
- IPPC (2002) *Glossary of phytosanitary terms*. ISPM no. 5. IPPC Secretariat, FAO, Rome (IT).
- OEPP/EPPO (2003) EPPO Standards PM 1/2 (12): EPPO A1 and A2 lists of quarantine pests. *EPPO Standards PM1 General phytosanitary measures*, 5–17. OEPP/EPPO, Paris.

## Definitions

*Regulated pest*: a quarantine pest or regulated non-quarantine pest.  
*Quarantine pest*: a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled.

## Outline of requirements

EPPO Diagnostic Protocols for Regulated Pests provide all the information necessary for a named pest to be detected and positively identified by an expert (i.e. a specialist in entomologist, mycology, virology, bacteriology, etc.). Each protocol begins with some short general information on the pest (its appearance, relationship with other organisms, host range, effects on host, geographical distribution and its identity) and then gives details on the detection, identification, comparison with similar species, requirements for a positive diagnosis, list of institutes or individuals where further information on that organism can be obtained, references (on the diagnosis, detection/extraction method, test methods).

## Existing EPPO Standards in this series

Nineteen EPPO standards on diagnostic protocols have already been approved and published. Each standard is numbered in the style PM 7/4 (1), meaning an EPPO Standard on Phytosanitary Measures (PM), in series no. 7 (Diagnostic Protocols), in this case standard no. 4, first version. The existing standards are:  
 PM 7/1 (1) *Ceratocystis fagacearum*. *Bulletin OEPP/EPPO Bulletin* **31**, 41–44  
 PM 7/2 (1) *Tobacco ringspot nepovirus*. *Bulletin OEPP/EPPO Bulletin* **31**, 45–51  
 PM 7/3 (1) *Thrips palmi*. *Bulletin OEPP/EPPO Bulletin* **31**, 53–60

PM 7/4 (1) *Bursaphelenchus xylophilus*. *Bulletin OEPP/EPPO Bulletin* **31**, 61–69

PM 7/5 (1) *Nacobbus aberrans*. *Bulletin OEPP/EPPO Bulletin* **31**, 71–77

PM 7/6 (1) *Chrysanthemum stunt pospiviroid*. *Bulletin OEPP/EPPO Bulletin* **32**, 245–253

PM 7/7 (1) *Aleurocanthus spiniferus*. *Bulletin OEPP/EPPO Bulletin* **32**, 255–259

PM 7/8 (1) *Aleurocanthus woglumi*. *Bulletin OEPP/EPPO Bulletin* **32**, 261–265

PM 7/9 (1) *Cacoecimorpha pronubana*. *Bulletin OEPP/EPPO Bulletin* **32**, 267–275

PM 7/10 (1) *Cacyreus marshalli*. *Bulletin OEPP/EPPO Bulletin* **32**, 277–279

PM 7/11 (1) *Frankliniella occidentalis*. *Bulletin OEPP/EPPO Bulletin* **32**, 281–292

PM 7/12 (1) *Parasaissetia nigra*. *Bulletin OEPP/EPPO Bulletin* **32**, 293–298

PM 7/13 (1) *Trogoderma granarium*. *Bulletin OEPP/EPPO Bulletin* **32**, 299–310

PM 7/14 (1) *Ceratocystis fimbriata* f. sp. *platani*. *Bulletin OEPP/EPPO Bulletin* **33**, 249–256

PM 7/15 (1) *Ciborinia camelliae*. *Bulletin OEPP/EPPO Bulletin* **33**, 257–264

PM 7/16 (1) *Fusarium oxysporum* f. sp. *albedinis*. *Bulletin OEPP/EPPO Bulletin* **33**, 265–270

PM 7/17 (1) *Guignardia citricarpa*. *Bulletin OEPP/EPPO Bulletin* **33**, 271–280

PM 7/18 (1) *Monilinia fructicola*. *Bulletin OEPP/EPPO Bulletin* **33**, 281–288

PM 7/19 (1) *Helicoverpa armigera*. *Bulletin OEPP/EPPO Bulletin* **33**, 289–296

Several of the Standards of the present set result from a different drafting and consultation procedure. They are the output of the DIAGPRO Project of the Commission of the European Union (no. SMT 4-CT98-2252). This project involved four ‘contractor’ diagnostic laboratories (in England, Netherlands, Scotland, Spain) and 50 ‘intercomparison’ laboratories in many European countries (within and outside the European Union), which were involved in ring-testing the draft protocols. The DIAGPRO project was set up in full knowledge of the parallel activity of the EPPO Working Party on Phytosanitary Regulations in drafting diagnostic protocols, and covered regulated pests which were for that reason not included in the EPPO programme. The DIAGPRO protocols have been approved by the Council of EPPO as EPPO Standards in series PM7. They will in future be subject to review by EPPO procedures, on the same terms as other members of the series.

**Diagnostic protocols for regulated pests<sup>1</sup>**  
**Protocoles de diagnostic pour les organismes réglementés**

## ***Puccinia horiana***

### **Specific scope**

This standard describes a diagnostic protocol for *Puccinia horiana*.

### **Introduction**

*Puccinia horiana* (EPPO/CABI, 1997) is the causal organism of chrysanthemum white rust, an economically important disease of florist's chrysanthemum (*Dendranthema × grandiflorum*). It is an autoecious microcyclic rust fungus, that only produces teliospores and basidiospores. Teliospores germinate *in situ* and form basidiospores (sporidia) without a period of dormancy. The basidiospores are dispersed by air and, under appropriate conditions, cause new infections. High humidity and a thin film of moisture on the leaf surface are essential for the germination of both the telio- and basidiospores. Typical symptoms of the disease are yellow spots on the upper leaf surface and raised pustules (telia) on the lower leaf surface. These pustules are white, hence the name white rust. This fungus infects fresh tissues of growing chrysanthemum plants although older leaves can also be infected. Chrysanthemum white rust originates in Japan but has now been reported in most countries where florist's chrysanthemums are grown (CMI Map no. 403, 1989). Although *D. grandiflorum* is the main host of *P. horiana*, certain true *Chrysanthemum* species can also be infected. Natural infections have been observed on *Chrysanthemum japonense*, *C. makinoi* var. *wakasaense*, *Chrysanthemum shiwogiku*, *Dendranthema boreale*, *Dendranthema japonicum* (Punithalingam, 1968). By inoculation, *Arctanthemum arcticum*, *Dendranthema pacificum*, *Dendranthema yoshinaganthum* and *Nipponanthemum nipponicum* were found susceptible (Hiratsuka, 1957). Species that did not develop symptoms when inoculated include annual chrysanthemums (*Chrysanthemum carinatum*, *Chrysanthemum coronarium*, *Chrysanthemum segetum*), *Tanacetum cinerariifolium*, *Tanacetum coccineum*, *Argyranthemum frutescens*, *Leucanthemum vulgare*, *Leucanthemum × superbum*.

<sup>1</sup>The Figures in this Standard marked 'Web Fig.' are published on the EPPO website [www.eppo.org](http://www.eppo.org).

### **Specific approval and amendment**

Approved in 2003-09.

### **Identity**

**Name:** *Puccinia horiana* P. Hennings

**Taxonomic position:** Fungi: Basidiomycota: Uredinales

**Bayer computer code:** PUCCHN

**Phytosanitary categorization:** EPPO A2 list No. 80, EU Annex designation II/A2

### **Detection**

#### **Disease symptoms**

The fungus primarily attacks the younger leaves of chrysanthemum causing spotting and in severe cases, rolling, twisting and finally death (Firman & Martin, 1968; Smith *et al.*, 1988). Infected dead leaves remain attached to the stems. During early stages of infection, light green to yellow spots can be observed on the upper leaf surface. These spots gradually enlarge up to 5 mm and become light brown (Web Fig. 2). As the infection progresses the spots become sunken, raised white, often faintly pinkish white and waxy pustules are formed on the corresponding lower leaf surface. Maturing lesions as seen on the upper leaf surface can increase in size up to 2 cm in diameter, turn brown and become necrotic (Web Fig. 3). Mature pustules on the lower surface turn white due to a bloom of sporidia (Web Fig. 4). Old pustules eventually become colonized by saprophytic organisms and turn dirty brown. In severe cases infections of petioles, stems and flowers can occur.

### **Identification**

#### ***In vivo* morphological identification**

The obligate parasite *P. horiana* can only be identified on the plant. Typical pustules on the lower leaf surface should be clearly visible without magnification. A part of the suspected

telium should be mounted (e.g. in water with a drop of Tween) and examined at 400 × magnification under normal microscopic light for the morphological characteristics of the teliospores as described under 'Fungal morphology'.

If suspected spots are visible but typical pustules are not yet formed, the plants should be incubated in a damp chamber at 17–21 °C. After 7–10 days, the symptoms should be clear and the identity of the fungus confirmed by microscopic examination as described above. This procedure is summarized in the decision scheme in Appendix I.

### Fungal morphology

Hyaline teliospores (Web Fig. 5) are easily found in the raised pustules (telia). Hypophyllous (occasionally epiphyllous) telia may be scattered over the whole leaf surface. They are 2–4 mm in diameter, solitary or aggregated. The teliospores are cylindrical, fusiform and oblong to oblong clavate (rarely elliptical) with a smooth pale yellow cell wall (1–2 µm thick, 3–10 µm thick at the apex). Predominantly the teliospores are slightly constricted at a single septum, rarely biseptate, 32–45 × 12–18 µm. The pedicel is hyaline, persistent and up to 45 µm long. From one or both cells, the teliospores germinate *in situ* to produce four round kidney-shaped sporidia (Kapoor & Zadocks, 1973).

### Possible confusion with similar species

Other *Puccinia* species known to infect *Chrysanthemum* spp. and species previously classified as *Chrysanthemum* are listed in Table 1 (Punithalingam, 1968)<sup>2</sup>. *P. horiana* is easily distinguished from other species by its smooth, hyaline teliospores that always germinate *in situ* on the living leaf. All the other species except *P. leucanthemi* have brown and/or verruculose teliospores that do not germinate *in situ*. *P. leucanthemi* is distinguished by its pale yellow teliospores and a nonoverlapping host range; it infects *Leucanthemum vulgare*.

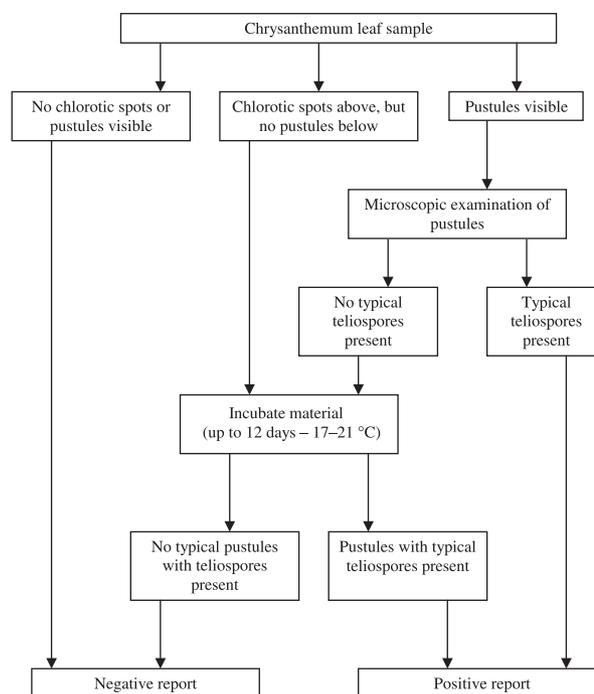


Fig. 1 Decision scheme for the identification of *Puccinia horiana*.

### Requirements for a positive diagnosis

The procedures for detection and identification described in this protocol, and the decision scheme in Fig. 1, should have been followed. Disease symptoms on chrysanthemum plants should accord with those described in this protocol. The fungus should have been identified unambiguously through the presence of pustules and characteristic hyaline teliospores as described in this protocol.

Table 1 *Puccinia* spp. other than *P. horiana* found on *Chrysanthemum* and related species

<i>Puccinia</i> spp.	Host range
<i>P. chrysanthemi</i>	<i>Argyranthemum frutescens</i> , <i>Dendranthema boreale</i> , <i>Dendranthema grandiflorum</i> , <i>Dendranthema lavandulifolium</i>
<i>P. chrysanthemicola</i>	<i>Chrysanthemum coronarium</i>
<i>P. gaeumanni</i>	<i>Tanacetum cinerariifolium</i>
<i>P. heeringiana</i>	<i>Argyranthemum frutescens</i> , <i>Tanacetum parthenium</i>
<i>P. leucanthemi</i>	<i>Leucanthemum vulgare</i>
<i>P. pyrethri</i>	<i>Tanacetum corymbosum</i>
<i>P. tanacetii</i>	<i>Argyranthemum frutescens</i> , <i>Dendranthema boreale</i> , <i>Dendranthema grandiflorum</i> , <i>Tanacetum vulgare</i> and many other <i>Artemisia</i> and <i>Tanacetum</i> spp.

<sup>2</sup>The nomenclature of this group is constantly changing. The names used here were current in the 1990s and are consistent with those used in European phytosanitary regulations.

## Report on the diagnosis

A report on the execution of the protocol should include:

- results obtained by the recommended procedures
- information and documentation on the origin of the infected material
- a description of the disease symptoms (with photographs if possible)
- measurements and drawings or photographs (as relevant) of fungal organs, i.e. teliospores and sporidia
- an indication of the magnitude of the infection
- comments as appropriate on the certainty or uncertainty of the identification (e.g. whether pustules were young and fresh or old and discoloured).

Herbarium specimens of infected chrysanthemum plants (leaves or stems) bearing pustules (telia) of *P. horiana* should be preserved.

## Further information

Further information on this organism can be obtained from: Agricultural Research Centre, Department of Crop Protection, Burg. Van Gansberghelaan 96, B-9820 Merelbeke, Belgium (Tel. ++ 32 9272 24 35, Fax ++ 32 9272 24 29); CSL

Diagnostics, Central Science Laboratory, Sand Hutton, York, YO41 1LZ, UK.

## Acknowledgements

This protocol was originally drafted by: G. Szkuta and J. Butrymowicz, Main Inspectorate of Plant Protection, Torun (PL) and revised by R. Cook (Central Science Laboratory, York, GB) and M. Maes (Agricultural Research Centre, Merelbeke, BE).

## References

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- Firman ID & Martin PH (1968) White rust of chrysanthemums. *Annals of Applied Biology* **62**, 429–442.
- Hiratsuka N (1957) Three species of chrysanthemum rusts in Japan and its neighbouring districts. *Sydowia* Suppl. 1, 34–44.
- Kapooria RG & Zadoks JC (1973) Morphology and cytology of the promycelium and the basidiospore of *Puccinia horiana*. *Netherlands Journal of Plant Pathology* **79**, 236–242.
- Punithalingam E (1968) *Puccinia horiana*. *CMI Descriptions of Pathogenic Fungi and Bacteria* no. 176. CAB International, Wallingford (GB).
- Smith IM *et al.* (1988) *European Handbook of Plant Diseases*, p. 490. Blackwell, Oxford (GB).

**Fig. 1.** Symptoms of *Puccinia horiana* at the start of infection : light brown spots on the upper leaf surface  
(Copyright: Agricultural Research Centre, Dpt. of Crop Protection, Merelbeke, Belgium).



**Fig. 2.** Mature, necrotic lesions on the upper leaf surface of Chrysanthemum due to *Puccinia horiana*  
(Copyright: Agricultural Research Centre, Dpt. of Crop Protection, Merelbeke, Belgium).



**Fig. 3.** Mature pustules on the lower leaf surface of Chrysanthemum due to *Puccinia horiana* (Copyright: Agricultural Research Centre, Dept. of Crop Protection, Merelbeke, Belgium).



**Fig. 4.** Hyaline teliospore of *Puccinia horiana*. (Copyright: Agricultural Research Centre, Dept. of Crop Protection, Merelbeke, Belgium).

