

◆ **EPPO Standards** ◆

DIAGNOSTIC PROTOCOLS FOR REGULATED PESTS

CERATOCYSTIS FAGACEARUM

PM 7/1(1) English



European and Mediterranean Plant Protection Organization
1, rue Le Nôtre, 75016 Paris, France

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.

REFERENCES

EPPO/CABI (1996) *Quarantine Pests for Europe*, 2nd edn. CAB International, Wallingford (GB).

IPPC (1993) *Principles of Plant Quarantine as Related to International Trade*. ISPM no. 1. IPPC Secretariat, FAO, Rome (IT).

IPPC (1999) *Glossary of Phytosanitary Terms*. ISPM no. 5. IPPC Secretariat, FAO, Rome (IT).

FAO (1997) *International Plant Protection Convention* (new revised text). FAO, Rome (IT).

OEPP/EPPO (1999) EPPO Standards PM 1/2(8) EPPO A1 and A2 lists of quarantine pests. In: *EPPO Standards PM1 General phytosanitary measures*, pp. 5-17. OEPP/EPPO, Paris (FR).

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.

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 a general expert (i.e. an entomologist, mycologist, virologist, bacteriologist etc.) who is not necessarily a specialist on the organism or its taxonomic group. 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

This is a new series.

Diagnostic protocols for regulated pests

CERATOCYSTIS FAGACEARUM

Specific scope

This standard describes a diagnostic protocol for *Ceratocystis fagacearum*. First approved in 2000-09.

Specific approval and amendment

Introduction

Ceratocystis fagacearum is the cause of oak wilt in North America and has not yet spread to other continents. It is a classic vascular wilt pathogen, with the fungus confined to the vessels of the outermost xylem ring until the trees become moribund. The most important means of dispersal is through natural root grafts. Above-ground spread may occur through beetles such as *Colopterus truncatus* or *C. sayi* (Coleoptera: Nitidulidae) or, in some areas, *Pseudopityophthorus minutissimus* and *P. pruinosus* (Coleoptera: Scolytidae).

Identity

Name: *Ceratocystis fagacearum* (Bretz) Hunt

Anamorph: *Chalara quercina* Henry

Synonym: *Endoconidiophora fagacearum* Bretz

Taxonomic position: Fungi: Ascomycota: Microascales

Bayer computer code: CERAFA

Quarantine pest: EPPO A1 list no. 6, EU Annex I/A1

Detection

Disease symptoms

In summer, infected trees show sudden wilting and death of the foliage, often over the entire tree. Occasionally, leaves may become brown from the apex, while their base remains green. There may be blackened, longitudinal streaks in the outermost sapwood ring. These symptoms are similar to those caused by other wilting organisms. In oak wilt, however, unlike other wilt diseases, sporulating mats may form below the bark within a few months after tree death. These take the form of a central 'pressure cushion' surrounded by a greyish mat of mycelium and spore-bearing structures. The mats have a strong, fruity smell. Oak wood carrying sporulating mats of the fungus is the most likely pathway for international spread of the fungus. The expression of symptoms may vary depending on the *Quercus* species involved. Moreover, although the presence of a sporulating mat is a characteristic feature of *C. fagacearum*, mats are often not formed on diseased trees. Isolation and identification of the fungus *in vitro*, therefore, remains necessary.

Isolation

Samples are best taken from affected branches 2 cm in diameter, while the inner bark is still fresh and green. Several branches should be taken because the fungus may not be isolated from all branches. Small chips of wood (1 mm³ to 1 cm³) are cut through the two or three outer annual rings and sterilized by dipping in 70% alcohol and flaming. The chips are then pushed down into 2% Difco malt agar medium (although the type of medium is not critical) in 9-cm sealed Petri dishes (fig 1.). After 8-10 days, in either light or dark, at 20-25°C, the isolates can be examined.

Identification

Growth characteristics in culture

Mycelium usually appears around the chips after 3-5 days. The conidiophores are usually formed in loose clusters and are frequently produced only on certain portions of the mycelium. Fine mycelial branches can be observed, which are frequently curved or curled. On malt agar, the culture is greyish with a characteristic sweet, fruity smell (if it can be determined subject to the containment and safety rules of the laboratory concerned). Endoconidiophores and endospores of the anamorph are produced. Comparison with a known reference isolate is recommended.

If the two mating types of the fungus are present, perithecia appear after 7-10 days, exuding a sticky creamy-white mass of ascospores. However, in practice, isolates from the xylem are almost exclusively of one mating type only. In order to see the perithecia, it will be necessary to culture the fungus being identified in contact with known reference isolates of the two mating types of *C. fagacearum*.

Morphology in culture

Hyphae: subhyaline to brown, septate, branched, 2.5-6 µm in diameter

Conidiophores: subhyaline to brown, septate, simple or branched, 2.5-5 µm in diameter, and often slightly tapered towards the apex.

Conidia: hyaline, continuous, cylindrical, truncate at each end, 2-4.5 × 4-22 µm (mean 3 × 6.5 µm), endogenous and catenulate.

Sclerotia: sometimes present, tan to black, loosely knit, irregular in shape, up to 2.5 cm in diameter.

Perithecia: flask-shaped, black, spheroidal base, 240-380 µm in diameter, and with an erect beak 250-450 µm long.

Ascospores: hyaline, one-celled, elliptical, 2-3 × 5-10 µm, exuded in a sticky creamy-white mass.

Molecular biological methods

A PCR-based system for identification of *Ceratocystis* spp. has been described by Witthuhn *et al.* (1999), but has not yet been critically assessed for the identification of *C. fagacearum*.

Comparison with similar species

Several similar fungi have been reported from oak. The *Ophiostoma* species can be distinguished from *C. fagacearum* because they have a *Leptographium*, *Graphium* or *Sporotrix* anamorph. The *Ceratocystis* species are more similar to *C. fagacearum* but can be distinguished, among other things, by the size and shape of the ascospores.

Other ophiostomatoid fungi found on oak:

- *Ceratocystis coerulescens* (Münch) Bakshi
- *Ceratocystis fimbriata* Ellis & Halstead
- *Ceratocystis moniliformis* (Hedgecock) Moreau
- *Ophiostoma leptographioides* (Davidson) von Arx
- *Ophiostoma megalobrunneum* (Davidson & Toole) de Hoog & Scheffer
- *Ophiostoma quercus* (Georgevitch) Nannfeldt
- *Ophiostoma pluriannulata* (Hedgecock) H. & P. Sydow
- *Ophiostoma rostrocylicum* (Davidson) von Arx
- *Ophiostoma stenoceras* (Robak) Melin & Nannfeldt

Reference cultures

ATCC, 12301 Parklane Drive, Rockville, MD 20852-1776, USA. Fax: +1 301 231 5826.

CBS, Oosterstraat 1, P.O. Box 273, 3740 AG Baarn (Netherlands). Fax: +31 35 541 6142.

Requirements for a positive identification

The procedures for detection and identification described in this protocol should have been followed. The fungus should have been isolated in pure culture. The growth characteristics of the fungus and the morphology of fungal organs in culture should be those of *C. quercina*, as described in this protocol and as established by comparison with a known

reference isolate. If perithecia are formed (by culturing with reference isolates of *C. fagacearum*), they should correspond to the description given in this protocol. The presence of a sweet fruity smell, characteristic of the reference isolate, is a valuable confirmation of the identification (if it can be determined subject to the containment and safety rules of the laboratory concerned).

Report on the diagnosis

A report on the execution of the protocol should include:

- information on the origin of the infected material;
- a description of the disease symptoms (including photographs);
- a description of the growth characteristics of the fungus *in vitro*;
- measurements and drawings or photographs of fungal organs;
- an indication of the magnitude of infection on the commodity;
- comments on certainty or uncertainty of the identification.

A pure culture of the isolated pathogen should be preserved.

Further information

Further information on this organism can be obtained from:

J.N. Gibbs, Forest Research, Alice Holt Lodge, Farnham, Surrey GU10 4LH (UK)

J. Pinon, INRA, Centre de Recherche de Nancy, 54280 Champenoux (France)

W.L. MacDonald, Division of Plant & Soil Science, West Virginia University, Morgantown, WV 26506-6057 (USA)

F.H. Tainter, Department of Forest Resources, Clemson University, Clemson, SC 29634 (USA)

D.N. Appel, Department of Plant Pathology and Microbiology, Texas A & M University, College Station, TX 77843-2132 (USA).

Acknowledgements

This protocol was originally drafted by:

R. Pieters, Plantenziektenkundige Dienst, P.O. Box 9102, 6700 HC Wageningen (Netherlands).

J.N. Gibbs, Forest Research, Alice Holt Lodge, Farnham, Surrey GU10 4LH (UK).

References

- Barnett HL (1953) Isolation and identification of the oak wilt fungus. *Bulletin of the West Virginia University Agricultural Experiment Station* no. 359, 5-15.
- EPPO/CABI (1997) *Ceratocystis fagacearum* and its vectors. In *Quarantine Pests for Europe*, 2nd edn, pp. 668-673. CAB International, Wallingford (GB).
- Henry BW (1944) *Chalara quercina* n.sp., the cause of oak wilt. *Phytopathology* **34**, 631-635.
- Hunt J (1956) Taxonomy of the genus *Ceratocystis*. *Lloydia* **19**, 1-58.
- Wingfield MJ, Seifert KA & Webber JF (1992) *Ceratocystis and Ophiostoma, Taxonomy, Ecology and Pathogenicity*. APS Press, St Paul (US).
- Witthuhn RC, Wingfield BD, Wingfield MJ & Harrington TC (1999) PCR-based identification and phylogeny of species of *Ceratocystis sensu stricto*. *Mycological Research* **103**, 743-749.

Fig. 1 Cultures of *Ceratocystis fagacearum* growing from chips of oak xylem onto 2% Difco malt extract agar containing 100 mg L⁻¹ streptomycin.

