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

Normes OEPP
EPPO Standards

Diagnostics
Diagnostic

PM 7/45

Organisation Européenne et Méditerranéenne pour la Protection des Plantes
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 Standards on Diagnostics are intended to be used by NPPOs in their capacity as bodies responsible for the application of phytosanitary measures. Standards on diagnostic protocols are concerned with the diagnosis of individual pests and describe different methods which can be used to detect and identify pests of phytosanitary concern for the EPPO region. General Standards on diagnostics are in preparation on: (1) the purpose of diagnostic protocols (which may differ according to the circumstances of their use); and (2) reporting and documentation of diagnoses.

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 EPPO Standards on Diagnostics:

- 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 (FR).

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 Standards on Diagnostics 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

Forty-one 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

PM 7/20 (1) *Erwinia amylovora*. *Bulletin OEPP/EPPO Bulletin* **34**, 159–172

PM 7/21 (1) *Ralstonia solanacearum*. *Bulletin OEPP/EPPO Bulletin* **34**, 173–178

PM 7/22 (1) *Xanthomonas arboricola* pv. *corylina*. *Bulletin OEPP/EPPO Bulletin* **34**, 179–182

PM 7/23 (1) *Xanthomonas axonopodis* pv. *dieffenbachiae*. *Bulletin OEPP/EPPO Bulletin* **34**, 183–186

PM 7/24 (1) *Xylella fastidiosa*. *Bulletin OEPP/EPPO Bulletin* **34**, 187–192

PM 7/25 (1) *Glomerella acutata*. *Bulletin OEPP/EPPO Bulletin* **34**, 193–200

PM 7/26 (1) *Phytophthora cinnamomi*. *Bulletin OEPP/EPPO Bulletin* **34**, 201–208

PM 7/27 (1) *Puccinia horiana*. *Bulletin OEPP/EPPO Bulletin* **34**, 209–212

PM 7/28 (1) *Synchytrium endobioticum*. *Bulletin OEPP/EPPO Bulletin* **34**, 213–218

PM 7/29 (1) *Tilletia indica*. *Bulletin OEPP/EPPO Bulletin* **34**, 219–228

PM 7/30 (1) *Beet necrotic yellow vein benyvirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 229–238

PM 7/31 (1) *Citrus tristeza closterovirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 239–246

PM 7/32 (1) *Plum pox potyvirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 247–256

PM 7/33 (1) *Potato spindle tuber pospiviroid*. *Bulletin OEPP/EPPO Bulletin* **34**, 257–270

PM 7/34 (1) *Tomato spotted wilt tospovirus*. *Bulletin OEPP/EPPO Bulletin* **34**, 271–280

PM 7/35 (1) *Bemisia tabaci*. *Bulletin OEPP/EPPO Bulletin* **34**, 281–288

PM 7/36 (1) *Diabrotica virgifera*. *Bulletin OEPP/EPPO Bulletin* **34**, 289–294

PM 7/37 (1) *Thaumetopoea pityocampa*. *Bulletin OEPP/EPPO Bulletin* **34**, 295–298

PM 7/38 (1) *Unaspis citri*. *Bulletin OEPP/EPPO Bulletin* **34**, 299–302

PM 7/39 (1) *Aphelenchoides besseyi*. *Bulletin OEPP/EPPO Bulletin* **34**, 303–308

PM 7/40 (1) *Globodera rostochiensis* and *Globodera pallida*. *Bulletin OEPP/EPPO Bulletin* **34**, 309–314

PM 7/41 (1) *Meloidogyne chitwoodi* and *Meloidogyne fallax*. *Bulletin OEPP/EPPO Bulletin* **34**, 315–320

Some 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 'inter-comparison' 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 PM 7. They will in future be subject to review by EPPO procedures, on the same terms as other members of the series.

Diagnostics[1]
Diagnostic

Cryphonectria parasitica

Specific scope

This standard describes a diagnostic protocol for *Cryphonectria parasitica*.

Specific approval and amendment

Approved in 2004-09.

Introduction

Cryphonectria parasitica is a bark-inhabiting fungus causing blight of chestnut (*Castanea* spp.) and other susceptible tree genera and species (mostly *Quercus* spp.). At the end of the nineteenth century, the disease spread from the Far East (probably from China or Japan) to North America and, during the late 1930s, to Europe. There is variation in susceptibility between host tree species. The most susceptible are the American chestnut (*Castanea dentata*) and the European chestnut (*Castanea sativa*). The virulent form of the disease develops quickly in these species causing necrosis of bark and mortality of the distal part of the tree (Heiniger & Rigling, 1994). Hypovirulence due to infection of the fungus by the double-stranded RNA virus, *Cryphonectria* hypovirus 1 (CHV 1), has however, enabled the regrowth of chestnut trees and stands in many regions of Europe. Virulent and hypovirulent strains of the fungus give rise to different types of cankers and this may, in some cases, make detection and identification difficult. In more tolerant hosts (in Europe, mostly *Quercus petraea* and less often *Quercus robur*, *Quercus ilex* and other oaks) or in its hypovirulent form, chestnut blight appears as perennial 'healing' cankers or superficial infections of the bark that rarely causes the death of branches, stump, sprouts or the whole tree. Further information on biology and geographical distribution can be found in EPPO/CABI (1997). See also Fulbright (1999), Heiniger & Rigling (1994), Roane *et al*. (1986).

Identity

Name: *Cryphonectria parasitica* (Murrill) M.E. Barr
Synonyms: *Endothia parasitica* (Murrill) P.J. Anderson & H.W. Anderson

Anamorph: *Endothiella parasitica* Roane
Taxonomic position: *Fungi*: *Ascomycota*: *Diaporthales*: *Valsaceae*
EPPO computer code: ENDOPA
Phytosanitary categorization: EPPO A2 list: no. 69, EU Annex designation: II/A2

Detection

Host plants may carry the fungus in the bark or wood.

Symptoms caused by virulent strains

The disease in its typical virulent form is easily recognized as it is always associated with extensive necrosis of the bark.

In *Castanea sativa* (seedlings, young trees and recently grafted trees) grown for fruit production, bark symptoms (necroses) are variable and their colour ranges from bright brown through yellowish-brown or orange to red-brown compared with the natural, olive-green colour of non-infected bark. On grafted trees, infections are most frequently found in the region of the graft, where callusing occurs. In coppices or orchards, infections are often located at the base of the stem (collars or insertion points). The cambium under the infected bark is killed and the bark appears sunken or swollen. Cracks can also form on the infected surface. Characteristic buff-coloured, flat mycelial fans are normally present throughout the bark. At some distance from the advancing edge of the necrosis, numerous yellow to yellow-orange stromata develop, each containing one, rarely more, conidiomata (locular pycnidia). In moist conditions, these may extrude twisted yellow tendrils consisting of sticky conidia. Tendrils can be more than 1 cm long and their production can be triggered artificially by incubating infected bark in moist chambers. Stromata are semi-immersed in the bark. After abundant production of conidia, ascomata are formed in the same stromata. Old stromata are brick-red to red-brown and may contain numerous flask-shaped perithecia

[1]The Figures in this Standard marked 'Web Fig.' are published on the EPPO website www.eppo.org.

with long necks, deeply embedded in the stroma. Ostioles of perithecia can be observed using a hand-magnifying lens and appear as black dots on papillate protuberances on the surface of the stroma. Old infections are characterized by longitudinal cracks and bark sloughing and exposing the underlying wood. Remnants of dried, light-brown mycelial mats are often seen on the exposed wood.

Quercus spp. (seedlings and young trees) are rarely infected. Symptoms are localized and consist of a slow-developing canker that can eventually kill individual branches.

Poles of *C. sativa* and *Quercus* spp. in trade show symptoms as described above. The poles often show remnants of epicormic shoots, which frequently develop just below an infection that has girdled the stem. Infected parts of the poles are often hypertrophic. In logs, mature thick, cracked bark may lack the symptoms of bark discoloration. Mycelial fans may be found throughout the inner and/or outer layers of loose/cracked bark and/or cambium layer. Some annual rings of sapwood can be also infected, although mycelial fans do not form there. Stromata with conidiomata and ascomata are formed in bark cracks.

Debarked wood of *C. sativa* can harbour mycelium in (remnants of) the cambium layer and in the sapwood. Light brown, usually dry, dead mycelial fans may be visible on the wood surface. Stromata or bare conidiomata can develop on the sapwood, most commonly at the cut end but also on the sides. Bark fragments and chips of wood can contain mycelium and stromata.

Symptoms caused by hypovirulent strains or by virulent strains on resistant hosts

Hypovirulence in *C. parasitica* is defined as the inability of the fungus to cause the disease in its virulent form. The pathogenicity of the fungus is reduced and infection of the bark has less explicit symptoms. Hypovirulence is the basis for natural disease control.

Stems of young *C. sativa* infected with hypovirulent strains may exhibit early symptoms similar to virulent forms. Older infections are limited to superficial bark layers and show callus production. Small plants, or parts of them, may be killed. Seedlings, young and grafted trees of *C. sativa* are seldom infected and hypovirulent strains rarely causes dieback of branches. Infections are localized and have a typical canker-like appearance.

Poles of *C. sativa* and *Quercus* spp. in trade are the most probable pathway for the long-distance spread of hypovirulent strains of *C. parasitica*. There are two distinctive types of infections: healing cankers and healed cankers. Healing cankers are superficial in their early stages and are characterized by a reddish-orange surface with more or less pronounced swelling. The infected part of the stem is slightly swollen. Later, the bark is divided into small angular scales of uniform size that originate from the bark cracks. This part of the stem differs markedly from the smooth appearance of adjacent uninfected bark. The cambium under the infected bark is alive. There may be extensive sloughing off of colonized tissues and the exposed underlying bark may appear reddish-brown or black. These areas may be small, only a few centimetres in diameter and oval in shape; alternatively the whole circumference of the stem may be extensively affected. Stromata are rarely formed in the cracks of the bark: conidiomata are usually produced; ascomata are almost never formed. Mycelial fans are not easily found and are smaller, paler and thinner than in the virulent form of the disease. They are located only in certain dead bark tissues but can sometimes be revealed by peeling off the bark in thin slices. Healed cankers are callused and swollen. Exposed wood is present in the centre of infection and is surrounded by a prominent callus ridge. The symptoms of the hypovirulent form of the disease are principally seen on the callus, although nearby bark can also be colonized extensively. This colonization is characterized by the sloughing away of the outermost bark tissues and by the formation of cracks in the remaining bark. The characteristics of hypovirulent colonization of bark are as above. Logs of *Castanea* and *Quercus* spp. with thick bark seldom show symptoms of colonization by hypovirulent strains of the fungus.

Symptoms caused by intermediate strains

Symptoms can occur on infected trees that are not typical of either the virulent or the hypovirulent strains of *C. parasitica*. In such cases, cankers usually start as a normal virulent infection, killing the inner bark and covering the sapwood with abundant mycelial mats. Pycnidia of the fungus also develop. However, in the reactive swollen area encircling the dead regions, wound cork barriers develop. Epicormic shoots normally develop below the cankers. In general, this type of canker does not kill the infected branches or stems and develops into healing canker.

In summary, the following symptoms are characteristic of *C. parasitica* infections:

- extensive necrosis and discoloration of the bark in *Castanea* spp. and *Quercus* spp. seedlings, young trees and grafted trees. Cutting the bark with a sharp knife into thin layers reveals typical mycelial fans in and/or under the bark
- presence of mycelial fans and stromata on any commodity (samples for microscopic examination should be taken)
- presence of cankers, swollen parts on the wood, dead bark peeling off logs (samples for isolation should be taken)
- presence of symptoms of the hypovirulent form of the disease (samples for isolation should be taken).

Identification

The fungus can be identified either from its characteristic fruiting structures formed in situ, after incubation under damp conditions, or by isolation in culture.

Incubation in damp chamber

Pieces of bark, if possible with typical stromata, are placed in a glass Petri dish (or, if large, in a larger plastic transparent box) on several sheets of blotting paper. After damping with distilled water, this damp chamber is held at room temperature under fluorescent light with an alternate cycle of 12 h daylight and

12 h darkness (or in a light place in the laboratory, not directly exposed to the sun). After 4 days of incubation, the sample is observed under the binocular microscope and fruit bodies are removed for microscopic examination. If no ripe fruit bodies are found, the sample is returned to the damp chamber and observed again at intervals for up to 3 weeks.

Isolation

Samples are taken with an axe, knife, leather borer (few cm in diameter) or cork borer from affected bark or wood (sapwood only; sampling of wood is only necessary when the sample is debarked wood). The advancing edge of the necroses is the best area to sample, although isolates of the fungus are also readily obtained from any visible mycelial mat. When the hypovirulent form of the disease is suspected, samples should be taken from the dead bark tissues. In the laboratory, small chips (not exceeding a few millimetres) are removed from the inner portion of the infected tissue with a scalpel, or a borer is used to extract a core of bark, including outer bark. The sample is surface-sterilized for up to 2 min in a solution of sodium hypochlorite (commercial bleach, 2–6% active chlorine) or 70% ethanol for a few seconds, and rinsed in sterile distilled water. It is then aseptically transferred onto potato dextrose agar (PDA) and incubated at room temperature for 3–5 days. Alternatively, the chips may be dipped in 70% ethanol (1 s), flamed for 1 s and then plated on water agar with streptomycin (1.5% water agar with 100 µg mL^{-1} streptomycin sulphate). Any sparse mycelium growing from the edge of the chips is subcultured to PDA. Cultures are incubated on the laboratory bench at room temperature (an alternating cycle of 12 h fluorescent light and 12 h darkness may be used) to induce colony pigmentation and pycnidia formation.

Microscope examination of samples

C. parasitica is positively identified when the reproductive organs conform to the following description (Ellis & Ellis, 1985): stromata yellow to dark orange, 0.5–3–4 mm in diameter and 2.5 mm high (smaller, if dry), embedded in the bark with only the upper parts protruding from the surface (Web Fig. 1); conidiomata (pycnidia) 100–300 µm in diameter (but compound stromata are sometimes found where several pycnidia have coalesced to form large labyrinthiform conidioma more than 1 mm in diameter), formed as irregular locules within stromatic tissue; in moist conditions, extruding twisted yellow tendrils of sticky conidia (Web Fig. 1); conidiophores branched, with lateral and terminal branches bearing conidiogenous cells; conidia 3–5 × 1.5–2 µm (mean 3.6 × 1.8 µm), ellipsoidal to bacilliform, occasionally slightly curved, hyaline and aseptate (Web Fig. 4); ascomata (perithecia), 300–400 µm in diameter, globose and deeply immersed in the stromatic tissue (Web Fig. 3); each perithecium with a long cylindrical periphysate neck (up to 300–600 µm long and 200 µm in diameter) converging through the external stromatic disc to form a small black papillate structure; asci 32–55 × 7–8.5 µm, clavate to cylindric clavate, short-stalked, thin-walled, and 8-spored; ascospores arranged in two rows, 7–12 × 3.5–5.0 µm (mean 8.6 × 4.5 µm), ellipsoidal, two-celled, smooth; sometimes with minor constriction at the septum (Web Fig. 4).

Growth characteristics in culture

Mycelial growth rate can be up to 5 mm per day at room temperature (20°C) although much lower growth rates can be observed in hypovirulent isolates. Mycelium is white when young, becoming light yellow and then orange-yellow, then finally, after some months, turning red-orange to violet. In hypovirulent isolates, it can remain white. Intermediate isolates can exhibit any of the transitional colours from white to yellow to orange. Yellow to orange tints in such isolates are confined to the central part of the colony (Web Fig. 5). About 5 days after subculturing, the primordial conidiomata are seen in culture initially appearing as a dense, globose mat. Only conidiomata are formed in culture; ascomata are not produced (Web Fig. 2). In virulent isolates, conidiomata are produced abundantly in diurnal concentric rings. Hypovirulent isolates are characterized by a decreased ability to form conidiomata and by irregular distribution over the surface of the medium (Web Fig. 5).

If there are no signs of the fungus, but other typical symptoms of *C. parasitica* are present, isolation of the fungus should be performed. The growth characteristics of the fungus and the morphology of fungal organs in culture should correspond to *Endothiella parasitica*, as described in this protocol.

Other fungi on chestnut bark

On wood and bark of *C. sativa*, less pathogenic and saprophytic fungi may also be present. *Valsa* spp. (mostly *Valsa ceratophora* Tulasne & C. Tulasne but also *Valsa intermedia* Nitsche) in their *Cytospora* conidial states macroscopically resemble *C. parasitica*, but differ in their microscopic characters (conidia 4–5 × 1 µm) (fruiting bodies not orange). The black acervuli of the *Coryneum* state of *Melanconis modonia* Tulasne & C. Tulasne may occur. *Cryptodiaporthe castanea* (Tulasne) Wehmeyer, in its *Diplodina* state, has larger conidia (8–12 × 2–3 µm) but also produces stem cankers (fruiting bodies not orange, beige in culture). *Nectria cinnabarina* (Fries) Fries forms bright or dark red perithecia superficially on stromata and in its *Tubercularia* state produces red to white sporodochial conidiomata. *Nectria coccinea* (Fries) Fries produces bright or dark red perithecia superficially on stromata or on bare wood. The *Cylindrocarpon* state has 1–5-septate, curved conidia. *Libertella* sp. has curved hyaline conidia, larger than *Endothiella*. *Diatrype stigma* (Hoffmann) Fries, in its *Naemospora* state has bright orange spore tendrils which contain allantoid conidia 4–6 × 1–1.5 µm.

On other commodities, some relatives in the genus *Endothia* and *Cryphonectria* can be confused with *C. parasitica* (Roane *et al.*, 1986). *Endothia gyrosa* (Schweinitz) Fries has one-celled allantoid ascospores. The *Endothiella* state has hyaline, one-celled cylindric to allantoid conidia 3.4 × 1.5–2 µm. The

production of a perilla-purple exudate in culture distinguishes this fungus from *C. parasitica*. *Cryphonectria cubensis* (Bruner) Hodges causes cankers on *Eucalyptus*. Cryphonectria radicalis (Fries) Barr has two-celled hyaline ascospores 6–10(–12) × 3–4 µm; on PDA, purple droplets are formed in the mycelium; cornmeal medium turns purple (Hoegger *et al*., 2002).

Reporting and documentation

Guidelines on reporting and documentation are given in EPPO Standard PM7/– (in preparation).

Further information

Further information on this organism can be obtained from:

Università degli Studi della Tuscia, Dipartimento di Protezione delle Piante, v.s. Camillo de Lellis, I-01100 Viterbo (Italy); E-mail: vannini@unitus.it

Istituto per la Patologia degli Alberi Forestali del C.N.R., Piazzale delle Cascine 28, 50144 Firenze (Italy)

INRA, Station de Pathologie Végétale, Domaine de la Grande Ferrade BP 81, F-33883 Villenave d'Ornon (France); E-mail: robin@bordeaux.inra.fr

Swiss Federal Research Institute WSL, CH-8903 Birmensdorf (Switzerland); E-mail: heiniger@wsl.ch or daniel.rigling@wsl.ch

Slovenian Forestry Institute (SFI), Večna pot 2, 1000 Ljubljana (Slovenia); E-mail: dusan.jurc@gozdis.si.

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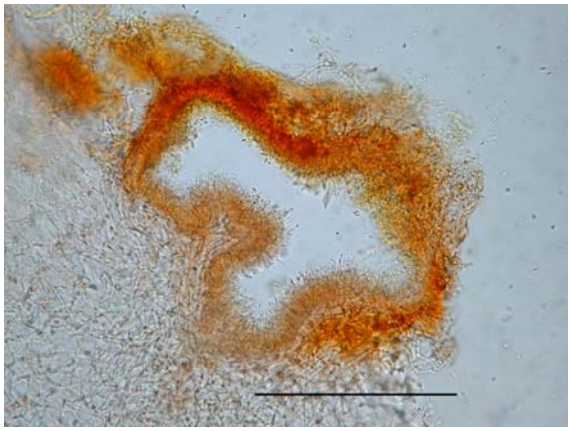
Heiniger U & Rigling D (1994) Biological control of chestnut blight in Europe. *Annual Review of Phytopathology* **32**, 581–599.

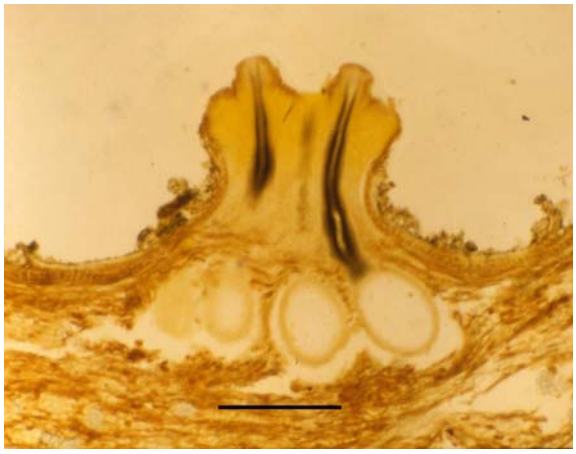
Hoegger PJ, Rigling D, Holdenrieder O & Heiniger U (2002) *Cryphonectria radicalis*: rediscovery of a vanished fungus. *Mycologia* **94**, 105–115.

Roane MK, Griffin GJ & Elkins JR (1986) Chestnut Blight. Other Endothia Diseases, and the Genus *Endothia*. APS Press, St Paul (US).

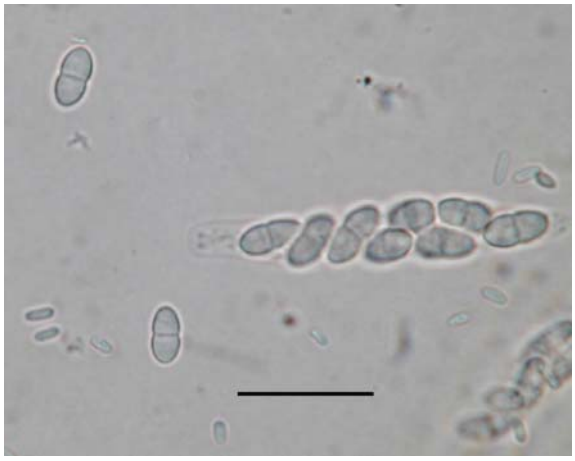
Web Fig 1: Pycnidial stromata in the bark, conidial tendrils are excreted (LNPV, Nancy, FR)



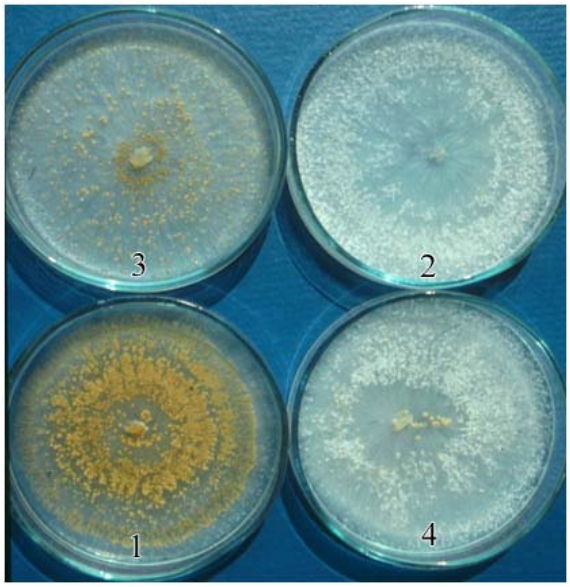
Web Fig 2: Pycnidium from culture, inner wall is covered with conidiogenous cells (bar = 50 µm) (SFI, Ljubljana, SI)



Web Fig 3: Perithecia in a stroma, necks and ostioles in papillate protuberances of the stroma (bar = 500 µm) (SFI, Ljubljana, SI)



Web Fig 4: Ascus, ascospores and conidia (bar = 20 µm) (SFI, Ljubljana, SI)



Web Fig 5: Cultures of *C. parasitica* isolates on PDA (1 – virulent, 2 – hypovirulent, 3 and 4 – intermediate virulence) (SFI, Ljubljana, SI)