Phytophthora quercina

Scientific Name
Phytophthora quercina Jung, Cooke, Blaschke, Duncan, and Oswald

Synonyms:
none known

Common Names
Oak decline, *Phytophthora* root rot (a common name applicable to many *Phytophthora* spp.)

Type of Pest
Fungus-like

Taxonomic Position
Kingdom: Chromista, **Phylum:** Oomycota, **Order:** Pythiales, **Family:** Pythiaceae

Reason for inclusion in manual
CAPS Priority Pest (FY 2006 – FY 2013)

Pest Description
Distinctive morphological features of *P. quercina* are not visible with the unaided eye. Even when examined under a microscope, the size, shape, and appearance of hyphae, sporangiophores, sporangia, oogonia, oospores, antheridia, and chlamydospores vary considerably for *P. quercina* (Jung et al. 1999).

Sporangia – Sporangia bear a lump or swelling and are egg shaped (ovoid), round (globose), nearly round (sub-globose), pear-shaped, with broad end proximal (obpyriform), flask like (ampulliform), or "banana- or peanut-like" (Fig. 1) (Jung et al. 1999). Dimensions: 19-112 µm long, 14-47 µm wide.

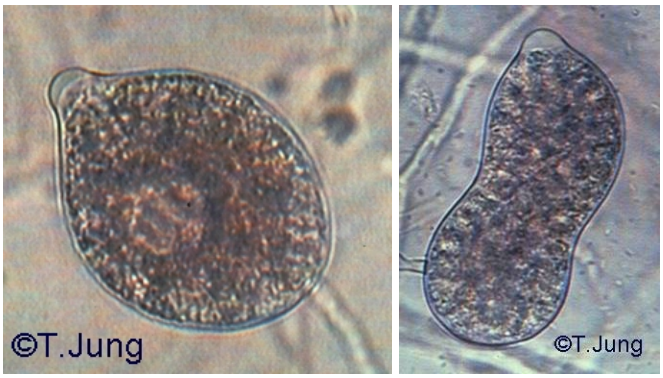

Fig. 1. Sporangia of *Phytophthora quercina*
[Image from T. Jung, 2000.
http://www.forst.uni-muenchen.de/EXT/LST/BOTAN/LEHRE/PATHO/QUERCUS/oakdec.htm]

Chlamydospores – Chlamydospores may not be observed because they are not produced consistently. When grown on malt extract agar, chlamydospores are spherical, 17-35 µm diameter (Jung et al. 1999).
Oospores – Oospores are globose, 18- 38 µm diameter (Jung et al. 1999).
Oogonia – Oogonia are irregularly shaped, spherical to ovoid, 19-45 µm diameter and up to 52 µm long (on malt extract agar) (Jung et al. 1999). Isolates from France had a slightly different appearance, with oogonia sometimes enveloped in a sheath (Hansen and Delatour 1999).

Phytophthora quercina is in Group I of the Waterhouse (1970) key to *Phytophthora* and is distinguishable from species in Group III by the thickness and shape of the papilla (Jung et al. 1999), a nipple-like projection at the apical end of the sporangium. Group I species have conspicuous papillae that are ~3.4 µm thick, while papillae of Group III species are less conspicuous, only ~2.1 µm thick. Unlike Group IV species, *P. quercina* has paragynous antheridia and sporangia that are easily dislodged from sporangiophores (Jung et al. 1999). Group IV species also have lower optimum and maximum temperatures for growth compared to Group I species. The sporangia of *P. quercina* look a bit like *P. nicotianae* from Group II, however, *P. nicotianae* has amphigynous antheridia and other characters which are distinguishable from *P. quercina* (Jung et al. 1999).

Biology and Ecology

The biology of *P. quercina* remains poorly described (Cree 2005), partly because the species is still relatively new to science (Jung et al. 1999). Mycelia will grow at a wide range of temperatures (between 5-27.5°C [41-81.5°F]); however, optimal growth occurs between 22.5-25°C [72.5-77°F] (Jung et al. 1999, Barzanti et al. 2001).

A single isolate can produce numerous oospores, which can survive in a state of dormancy for several years (Jung et al. 1999). *Phytophthora quercina* has reportedly survived for 2 years at 18°C [64°F] in dry oak forest soil (Cooke et al. 2005). The pathogen is homothallic, so oospores can be produced if only one mating type is present.

Though this pathogen can grow under a wide variety of conditions, drier sites with nutrient-rich clayey and loamy soils and soil pH ranging from 3.5-7.0 appear to be most favorable (Jung et al. 2000, Hartmann and Blank 2002, Balci and Halmschlager 2003a, Jonsson et al. 2005). Higher concentrations of calcium and magnesium may favor the development of the pathogen and indirectly facilitate the infection process (Balci and Halmschlager 2003a).

See ‘Known Hosts’ for a list of plants that can be infected by *P. quercina* and ‘Pest Importance’ for a discussion of the impact the pathogen is having in Europe.

Damage

Symptoms of infection by *P. quercina* are similar to those caused by other pathogens associated with oak decline: leaf clusters; twig abscission; epicormic shoots; crown thinning; branch and crown dieback; reduced growth; yellowing leaves; wilted leaves;

leaf and trunk necroses; “bleeding” stipe cankers at root collar; tyloses formation; loosened bark; and sapwood discoloration (reviewed in Jung et al. 1996, Balci and Halmschlager 2002). Tree mortality is gradual (Jung et al. 1996). Reliable diagnosis of oak infection by *P. quercina* in the field is not possible.

Pest Importance

Phytophthora quercina has been isolated from declining European oak stands (Hansen and Delatour 1999, Schubert et al. 1999, Balci and Halmschlager 2002, Hartmann and Blank 2002, Vettraino et al. 2002). In Germany, annual oak mortality [attributed in part to oak decline] is estimated at 2-5 mature oaks/ha [~ 1-2 oaks/acre] (Heiser et al. 1999). *Phytophthora quercina* is predominantly a soilborne root pathogen (Jung et al. 1999, Balci and Halmschlager 2003a, Jonsson et al. 2003). In greenhouse inoculation studies, the pathogen was able to kill 35-50% of the fine roots of year-old oak (*Quercus robur*) seedlings in <4 months (Jung et al. 1996). Minor lesions (10-23 mm after 3 months) developed on *Q. robur* when stems were injected with the pathogen (Jung et al. 1996). However, another isolate of the pathogen was unable to infect the stem or collar of *Q. petraea* seedlings in a separate greenhouse inoculation test (Balci and Halmschlager 2003a).

In previous pest risk assessments, the pathogen was considered moderately likely to invade the United States and cause economic harm (Cree 2005); the pathogen was considered relatively unlikely to cause environmental harm that could be distinguished from other causes of oak decline in the United States. Even in Europe, the relationship between oak decline and *P. quercina* is not absolute. Although the pathogen is frequently isolated from stands with oak decline (Hansen and Delatour 1999, Schubert et al. 1999, Jung et al. 2000, Balci and Halmschlager 2002, Hartmann and Blank 2002, Vettraino et al. 2002), it is also isolated from apparently healthy stands (Hansen and Delatour 1999, Jung et al. 2000, Balci and Halmschlager 2003b). Pathogens other than *P. quercina* may be isolated from stands with oak decline (Jung et al. 1996, Vettraino et al. 2002, Balci and Halmschlager 2003b, Jonsson et al. 2003). The pathogenicity of *P. quercina* may depend on other predisposing factors, such as drought, flooding, defoliation, and tree species composition (Hansen and Delatour 1999, Schubert et al. 1999, Balci and Halmschlager 2002, Hartmann and Blank 2002, Vettraino et al. 2002). Alternatively, *P. quercina* may be an inciting factor that leaves a tree vulnerable to other types of environmental stress that alone would be inadequate to kill a tree (Vettraino et al. 2002).

Known Hosts

Like other Group I *Phytophthoras*, *P. quercina* appears to have a fairly restricted host range, as only infections of *Quercus* spp. have been reported (Jung et al. 1999).

Hosts	**References**
Quercus cerris (European turkey oak)	(Cree 2005)
Quercus frainetto (Italian oak)	(Balci and Halmschlager 2002)
Quercus hartwissiana (Hartwissiana oak)	(Balci and Halmschlager 2002)

Hosts	References
Quercus ilex (holly oak)	(Cree 2005)
Quercus petraea (sessile oak)	(Balci and Halmschlager 2003b)
Quercus pubescens (downy oak)	(Balci and Halmschlager 2003b, Cree 2005)
Quercus robur (common, pedunculate oak)	(Balci and Halmschlager 2003b, Cree 2005)
Quercus vulcanica (Kasnak oak)	(Balci and Halmschlager 2002)
Quercus sp. (oak)	(Cree 2005)

Known Distribution

The pathogen has only been reported from Europe and western Asia.

Asia: Turkey; **Europe:** Austria, Belgium, France, Germany, Hungary, Italy, Luxembourg, Netherlands, Serbia, Sweden, and United Kingdom

(Jung et al. 2000, Balci and Halmschlager 2002, Hartmann and Blank 2002, Vettraino et al. 2002, Balci and Halmschlager 2003c, Jonsson et al. 2003, Cree 2005, Jonsson et al. 2005, CABI 2010)

Pathway

This species can spread naturally through movement of zoospores in soil and surface water. Long-distance dispersal can occur when infested soil is moved on material (including machinery and trecking boots). This species has been found transported with nursery stock (this includes oak seedlings as well as non-host material) (EPPO n.d.).

Potential Distribution within the United States

The presence of *P. quercina* has not been confirmed in the United States, however, a *P. quercina* 'like' organism has been identified from oak forests in Minnesota, Wisconsin (Balci et al. 2005) and Missouri (Juzwik, pers. comm.).

A coarse climatic analysis based on reported occurrences of *P. quercina* in the field suggests the pathogen may do particularly well in Mediterranean shrubland (California) and temperate broadleaf forests (northeastern United States). Collectively, these two biomes account for approximately 29% of the area within the contiguous United States.

A recent risk analysis by USDA-APHIS-PPQ-CPHST shows that most of the southeastern portion of the United States is at the greatest risk of *P. quercina* establishment based on host density, climate, and pathway.

Survey

CAPS-Approved Method:

1. In situ water sampling with rhododendron leaf baits (preferred method): Use rhododendron leaves as bait by cutting the leaves in a herringbone pattern. Place 3 to 4 cut leaves into a mesh bag. Place the mesh bag into the water source for a

minimum of 48 hours to 1 week (preferable).

Oak leaflets have also been used as baits for *P. quercina*.

2. Collect soil from symptomatic trees.

3. For visual survey, collect root samples from trees in various stages of decline.

Symptoms include twig abscission, epicormic shoots, crown thinning, branch and crown dieback, reduced growth and yellowing leaves.

Literature-Based Methods:
Soil and/or root samples are typically needed to isolate and identify *P. quercina*. Two general approaches are common: direct isolation or baiting techniques (Erwin and Ribeiro 1996). For direct isolation, diseased tissue (cankered bark or necrotic roots) is thoroughly cleaned (rinsed) and surfaced sterilized (e.g., dipped in 95% ethanol). Small pieces are placed on semi-selective media (e.g., PARPNH, an agar medium containing pimaricin, ampicillin, rifampicin, pentachloronitrobenzene, nystatin, and hymexazol). Alternatively, a bait (i.e., a piece of attractive vegetation such as a young oak leaf) is floated on the surface of soil flooded with water. Motile zoospores are attracted to the leaves, cause infection, and cause the bait to decay. Once a bait appears infected, it is rinsed, surface sterilized, and plated just as with the direct isolation method. Occasionally, bacteria or species of *Pythium* may also grow on PARPNH and a number of techniques are available to purify contaminated cultures. Pure cultures are necessary to confirm species identity, based upon morphological or molecular methods.

Jung (1996) collected two to three "soil-root monoliths 20 x 20 x 30cm [9 x 9 x 12 inches]" per tree with a spade. Samples were collected 80 to 100 cm [31 to 39 inches] from the base of the tree between root buttresses. Smaller samples were hand dug 50 to 200 cm [20 to 80 inches] along main roots. All soil collected from a tree was bulk mixed. Roots were <u>not</u> removed. A 250 to 500 ml subsample of soil was divided into 30 ml aliquants which were flooded with water to ca. 1-inch depth in 12-cm Petri dishes. Leaflets from *Q. robur* seedling were floated on the water surface. Flooded soils with baits were held at 20°C [68°F]. When a leaf turned blackish brown, the bait was rinsed in demineralized water and dipped in 95% ethanol. Pieces of infected tissue were excised under sterile conditions and placed on PARPNH. If no *Phytophthora* were isolated, the flood water was decanted, and the soil was allowed to dry completely at room temperature. Soils were flooded again and re-baited. The same protocol was used by Jung (2000), and Jonsson et al. (2003).

A similar protocol was used by Hansen and Delatour (1999); however, they removed leaf litter from the soil surface before collecting a soil sample and processed slightly different volumes of soil. A total of ~1L of soil was collected from 4 locations around each tree. Soil was bulked, and a 200 ml subsample was flooded with ~ 500ml of water. Ten young (<3 cm [~ 1 inch]) leaflets of *Q. robur* were floated on the water surface and allowed to incubate for 3 days at 18 to 20°C [64 to 68°F]. Vettraino (2002)

followed a protocol similar to Hansen and Delatour (1999), but noted that samples were collected in the spring and fall.

Baits of apple or pear fruit did not attract *P. quercina* (Jung et al. 1996).

Species are traditionally identified by measuring and comparing morphological features (including colony growth and spore dimensions) of pure cultures with reported descriptions of known species. Molecular methods for species identification are being developed. Schubert (1999) developed a species specific primer that produced a unique, 1105 bp amplicon for *P. quercina* through polymerase chain reaction.

Key Diagnostics

CAPS-Approved Method:

Confirmation of P. quercina is by serological and morphological means. An ELISA of dipstick test is available for *Phytophthora* at the genus level for primary screening. A positive does not indicate *quercina*.

ID must be confirmed by other methods.

Morphological:

1. Baiting technique (soil):
    a. Submerge a rhododendron leaf, oak leaflet in soil sample. Cover with water. Remove baits after 48 hrs. Examine for lesions after 7 days.
    b. Species are identified morphologically (Jung et al. 1999).

2. Direct Isolation (bark/roots):
    a. Place pieces of tissue on semi-selective media.
    b. Species are identified morphologically (Jung et al. 1999).

Literature-Based Methods:

Molecular: PCR has been used to detect *P. quercina* in leaves.

Nested PCR has been used to detect *P. quercina* in soil and water samples (Schubert et al. 1999; Nechwatal et al. 2001).

Real-time PCR: method has been developed that uses two universal primers and a probe to monitor the quality of DNA extracted from environmental samples followed by a multiplex real-time PCR to detect *P. quercina*, *P. ramorum*, *P. kernoviae*, and *P. citricola* from symptomatic leaves (Schena et al. 2006).

Schena et al. 2008 developed a PCR-based molecular tool box that could identify 15 *Phytophthora* species that damage forests and trees.

Single-strand conformation polymorphism (SSCP) analysis of PCR-amplified ribosomal DNA internal transcribed region I has now been used to identify and provide a molecular

fingerprints for 59 *Phytophthora* species, including *P. quercina* (Gallegly and Hong 2008).

Easily Confused Pests

Symptoms of *P. quercina* are not characteristic as they are common for other forest *Phytophthora* species.

References

Balci, Y., and E. Halmschlager. 2002. First confirmation of *Phytophthora quercina* on oaks in Asia. Plant Disease 86: 442.

Balci, Y., and E. Halmschlager. 2003a. *Phytophthora* species in oak ecosystems in Turkey and their association with declining oak trees. Plant Pathology 52: 694-702.

Balci, Y., and E. Halmschlager. 2003b. Incidence of *Phytophthora* species in oak forests in Austria and their possible involvement in oak decline. Forest Pathology 33: 157-174.

Balci, Y., and E. Halmschlager. 2003c. First report of *Phytophthora quercina* from oak forests in Austria. Plant Pathology 52: 403.

Balci, Y., S. Balci, J. Eggers, W. MacDonald, J. Juzwik, R. Long, and K. Gottschalk. 2005. Occurrence of *Phytophthora* species in central and eastern U.S. oak ecosystems. Phytopathology 95: 960.

Barzanti, G. P., P. Capretti, and A. Ragazzi. 2001. Characteristics of some *Phytophthora* species isolated from oak forest soils in central and northern Italy. Phytopathologia Mediterranea 40: 149-156.

CABI. 2010. *Phytophthora quercina*. Crop Protection Compendium. Accessed January 30 2013 from: http://www.cabi.org/cpc.

Cooke, D. E. L., T. Jung, N. A. Williams, R. Schubert, W. Osswald, and J. M. Duncan. 2005. Genetic diversity of European populations of the oak fine-root pathogen *Phytophthora quercina*. Forest Pathology 35: 57-70.

Cree, L. 2005. *Phytophthora quercina*. North American Forest Commission Exotic Forest Pest Information System (NAFC-ExFor).

EPPO. No date. Draft data sheets on quarantine pests: *Phytophthora quercina*. European and Mediterranean Plant Protection Organization. 12 pp.

Erwin, D. C. and O. K. Ribeiro. 1996. *Phytophthora* Diseases Worldwide. APS Press, St. Paul.

Gallegly, M.E., and C. Hong. 2008. *Phytophthora*: Identifying species by morphology and DNA fingerprints. The American Phytopathological Society. APS Press.

Hansen, E. and C. Delatour. 1999. *Phytophthora* species in oak forests of north-east France. Annals of Forest Science 56: 539-547.

Hartmann, G. and R. Blank. 2002. Occurrence and site relations of *Phytophthora* spp. in declining oak stands in North-Western Germany. Forst und Holz 57: 539-545.

Heiser, I., J. Fromm, M. Giefing, J. Koehl, T. Jung, and W. Osswald. 1999. Investigations on the action of *Phytophthora quercina*, *P. citricola* and *P. gonapodyides* toxins on tobacco plants. Plant Physiology & Biochemistry 37: 73-81.

Jonsson, U., T. Jung, K. Sonesson, and U. Rosengren. 2005. Relationships between health of *Quercus robur*, occurrence of *Phytophthora* species and site conditions in southern Sweden. Plant Pathology 54: 502-511.

Jonsson, U., T. Jung, U. Rosengren, B. Nihlgard, and K. Sonesson. 2003. Pathogenicity of Swedish isolates of *Phytophthora quercina* to *Quercus robur* in two different soils. New Phytologist 158: 355-364.

Jung, T., H. Blaschke, and P. Neumann. 1996. Isolation, identification and pathogenicity of *Phytophthora* species from declining oak stands. European Journal of Forest Pathology 26: 253-272.

Jung, T., H. Blaschke, and W. Osswald. 2000. Involvement of soilborne *Phytophthora* species in Central European oak decline and the effect of site factors on the disease. Plant Pathology 49: 706-718.

Jung, T., D. E. L. Cooke, H. Blaschke, J. M. Duncan, and W. Osswald. 1999. *Phytophthora quercina* sp. nov., causing root rot of European oaks. Mycological Research 103: 785-798.

Nechwatal, J., A. Schlenzig., T. Jung, D. E. L. Cooke, J. M. Duncan, and W. F. Osswald. 2001. A combination of baiting and PCR techniques for the detection of *Phytophthora quercina* and *P. citricola* in soil samples from oak stands. Forest Pathology 31: 85-97.

Schena, L., K. J. D. Hughes, and D. E. L. Cooke. 2006. Detection and quantification of *Phytophthora ramorum*, *P. kernoviae*, *P. citricola*, and *P. quercina* in symptomatic leaves by multiplex real-time PCR.

Schena, L., J. M. Duncan, and D. E. L. Cooke. 2008. Development and application of a PCR-based 'molecular tool box' for the identification of *Phytophthora* species damaging forests and natural ecosystems. Plant Pathology 57: 64-75.

Schubert, R., G. Bahnweg, J. Nechwatal, T. Jung, D. E. L. Cooke, J. M. Duncan, G. Mueller-Starck, C. Langebartels, H. Sandermann, Jr., and W. Osswald. 1999. Detection and quantification of *Phytophthora* species which are associated with root-rot diseases in European deciduous forests by species-specific polymerase chain reaction. European Journal of Forest Pathology 29: 169-188.

Vettraino, A. M., G. P. Barzanti, M. C. Bianco, A. Ragazzi, P. Capretti, E. Paoletti, N. Luisi, N. Anselmi, and A. Vannini. 2002. Occurrence of *Phytophthora* species in oak stands in Italy and their association with declining oak trees. Forest Pathology 32: 19-28.

Waterhouse, G. M. 1970. The genus *Phytophthora* de Bary. Mycological Papers 122: 101 pp.