Raffaelea quercivora

Scientific Name
Raffaelea quercivora Kubono and Shin. Ito, 2002

Synonyms:
none known

Common Names
Japanese oak wilt
JOW
Japanese oak disease fungus

Type of Pest
Fungus

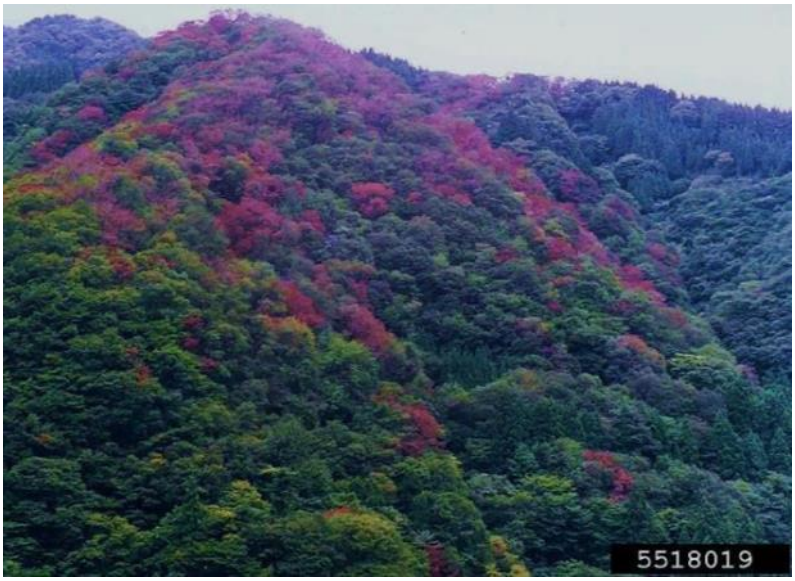
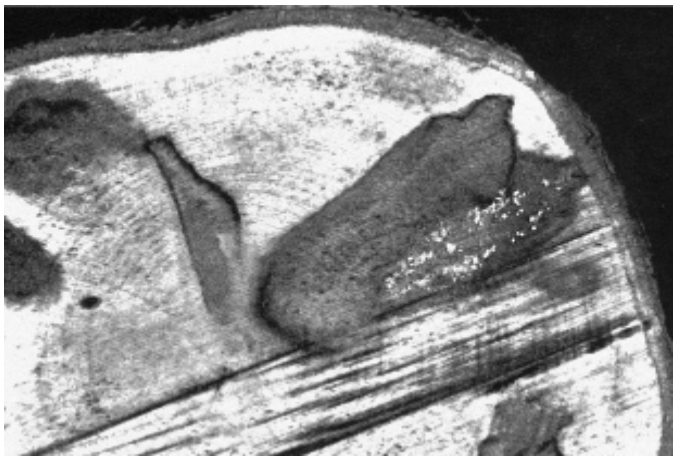


Figure 1. *Raffaelea quercivora* infestation in an oak forest. Troy Kimoto, CFIA, Bugwood.org.

Taxonomic Position
Class: Sordariomycetes **Order:** Ophiostomatales **Family:** Ophiostomataceae

Reason for inclusion in manual
CAPS Pest of Economic and Environmental Importance
CAPS Oak commodity survey list

Pest Description
On the outside of host trees, there is little visible sign of *Raffaelea quercivora*. Symptoms of leaf discoloration and wilting are visible on infected hosts (Kuroda, 2001), but these may be caused by other wilt pathogens. *Raffaelea quercivora* can be seen in cross-sections of symptomatic trees as a brown to black discoloration and small white pustules originating by the tunnels formed by its vector, *Platypus quercivorus* (Fig. 2) (Batra, 1967a).



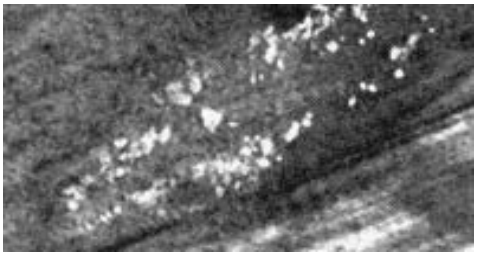


Figure 2. Sporodochia (small white pustules) growing on a cross section of a maple. Reproduced from Batra (1967a) with permission from Mycologia. ©The Mycological Society of America.

Identification of *R. quercivora* requires isolation of the pathogen from host material and growth in culture for morphological identification. Colonies produced on potato dextrose agar (PDA) at 20-25°C (68-77°F) spread rapidly and can reach 80 mm (~3 1/8 in) diameter in five days. The colony itself initially has a white, water-soaked appearance that shifts to a pale olive to brown-olive color after two weeks, taking on a fragrant odor reminiscent of ethyl alcohol (Kubono and Ito, 2002b). Sporodochia or masses of hyphae form and are visible under a light microscope. Within these structures are conidiophores bearing clusters of short, translucent conidia.

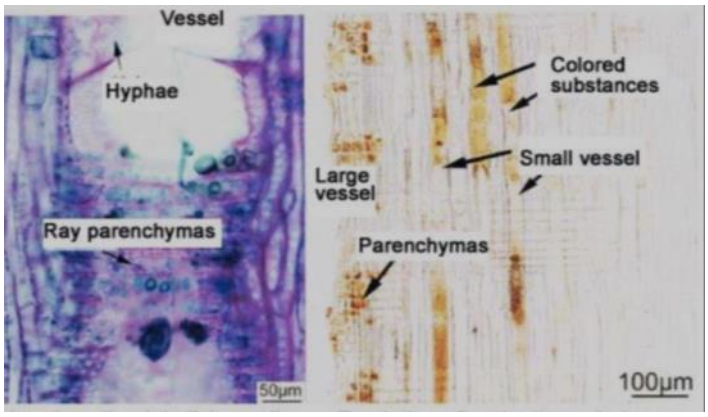


Figure 3: Left: *Raffaelea quercivora* hyphae invading living host cells (radial section). Right: Exudation of protective substances (tyloses) from parenchymas to vessels. Courtesy of Keiko Kuroda.

For photographs of this pathogen in culture, microscopic images showing detailed morphology, and a formal description to aid in identification see Kubono and Ito (2002).

Biology and Ecology

Raffaelea quercivora is one of many species of ambrosia fungi, so-called because of their symbiotic relationships with some species of wood boring Scolytidae and Platypodidae. Most often associated with recently logged timber and trees of low vitality, ambrosia fungi line the tunnels and galleries created in the sapwood and heartwood by their associated insects with a continuous layer of hyphae and conidia-bearing conidiophores (Batra, 1967b). The insect relies on the fungus for nourishment during at least some of its life stages, meanwhile providing the fungus with protection and means of dispersion and inoculation (Baker, 1963; Kinuura, 2002).

Similar to other ambrosia fungi, *R. quercivora* has not been found apart from its insect vector, *Platypus quercivorus* (Coleoptera: Platypodidae). Thus, the life cycle of the fungus in nature is intimately related to the life cycle of this insect. The reciprocal is not necessarily true. Conidia of *R. quercivora* are carried by *P. quercivorus* and contact the plant host when the insect bores into the sapwood and heartwood of the tree (Kinuura, 2002). Conidia may germinate directly, or produce sprout cells which germinate and elongate into septate hyphae (Kubono and Ito, 2002a). Hyphae grow in the tunnels and galleries excavated by the beetle (Fig. 3), and eventually line the entire surface (Kinuura, 2002). Hyphae may aggregate into bundles, called fascicles, which may further aggregate into sporodochia (see ‘Pest Description’) from which conidiophores develop and produce conidia. While providing nourishment for the insect, the fungus continues to grow, and hyphae extend into the wood adjacent to the tunnel.

The creation of insect galleries and the presence of the fungus stimulate defense responses from the tree, which include the production of tyloses (parenchyma cell overgrowths) that extend into the xylem and prevent the ascent of water (Fig. 3) (Yamada and Ichihara, 2003). In the meantime, the female insect lays eggs, which hatch in about a week. Larvae feed on the fungus until they pupate. Young adults may emerge in autumn (October-November), allowing for the possibility of a second generation that year, or remain in the tree over the winter and emerge in the spring (Sone et al., 1998). Young adult beetles consume and acquire the fungus in their mycangia. When they emerge from the tree, they carry the fungus. The insects then move on to another tree and bring the pathogen with them. The fungus may go through many life cycles from the time it is introduced into the tree until the time the next generation of insects emerges with the fungus.

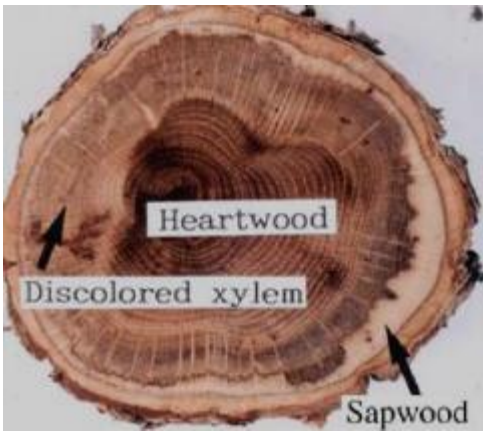


Figure 4. Discoloration of sapwood following the beetle invasion and infection of *R. quercivora*. Courtesy of Keiko Kuroda.

Infection by the fungus is presumed to occur shortly after initial attack by *P. quercivorus,* when the fungus is first introduced into the host. Attacks start in May or June and may extend through August (Mori et al., 1995; Saito et al., 2001). Following attack by *P. quercivorus*, trees discolor and wilt within 2-3 months and die that first season or by the following spring (Fig. 1, 5) (Kobayashi and Ueda, 2003; Kubono and Ito, 2002a). Insect infestations of standing trees and logs were highest at the beginning of the season (June - early July) (Mori et al., 1995; Sone et al., 1998).

While there is a tendency to attribute oak mortality to *R. quercivora* when *P. quercivorus* is present, in many cases the fungus was not actually isolated and identified. Spread of the disease within a stand appears to be a function of vector behaviors and patterns. Disease initially occurs at the edge of a gap or forest and on upper slopes; spread among trees occurs outward and downward from an infection epicenter (Esaki et al., 2004; Kamata et al., 2002). Symptoms spread faster in stands that have a higher percentage of susceptible hosts (Kamata et al., 2002).

Raffaelea quercivora will grow in culture on potato dextrose agar (PDA) media at 20-25°C (68-77°F), and produce all of its life stages (Kubono and Ito, 2002a). In logs the water content of the wood impacts the growth of *R. quercivora*, with low water content having a negative effect (Kobayashi et al., 2004). Hyphae occur in the tunnels and galleries created by *P. quercivorus*, and will grow into the ray and parenchyma cells of the heartwood. In the process, the fungus stains the wood (Fig. 4). Fragments of hyphae also likely occur in the mycangia and on the outer body surface of the insect vector. On PDA, *R. quercivora* grew to an 80 mm (~3 $^{1}/_{8}$ in) diameter colony in five days at 20-25°C (68-77°F) (Kubono and Ito, 2002a). The fungus has an odor and color on

PDA that are distinct from other fungi and yeasts found in *P. quercivorus* mycangia (Kinuura, 2002).

Raffaelea quercivora has been found in several countries in Asia (Kusumoto et al., 2014), but damage to oak hosts has only been reported in Japan. Isolates of *R. quercivora* from other Asian countries were found to be as pathogenic to Japanese oak trees as Japanese isolates of the pathogen (Kusumoto et al., 2014). Therefore, it is likely that oak trees in other countries are less susceptible to the pathogen or possibly the vector.

Symptoms/Signs

In the early stages of disease, oak infected with *R. quercivora* will have curled or withered leaves (Fig. 5). Within a few weeks or less, the leaves become discolored and begin to die. By the end of the season or the beginning of the next season, host mortality can occur (Kubono and Ito, 2002a). Wilting results from disruption of water flow at sites of infection (Kuroda et al., 2002; Yamada et al., 2002; Kuroda et al., 2004;). Young hyphae of *R. quercivora* invade the living ray parenchyma cells, and tyloses form in the vessels around the hyphae (Kuroda, 2001). Tyloses prevent the flow of water and may also form in response to mechanical wounding Yamada et al., 2002.

Figure 5: Top: Tree discoloration and leaf wilting in *Raffaelea quercivora* infected oak (*Quercus* spp.). Bottom: Entrance holes (left) and frass (right) caused by *Platypus quercivorus*. Courtesy of Troy Kimoto, CFIA, Bugwood.org.

Figure 6. Left: Female *P. quercivorus* carries conidia in specialized cavities called mycangia (arrow). Right: male and female specimens of *P. quercivorus*. Courtesy of Keiko Kuroda.

Trees with significant blockage die, and variation in the amount of blockage may account for differences in mortality rates among and within species (Kamata et al., 2002).

Signs of *P. quercivorus* include entrance holes and the presence of frass (Fig. 5). Cross sections of trees infected with *R. quercivora* also show galleries of *P. quercivorus* and discoloration that extends beyond the galleries into the surrounding sapwood (Kobayashi et al., 2001; Kuroda, 1998).

Pest Importance

Raffaelea is among the many genera of ambrosia fungi occurring in symbiotic relationships with a group of wood boring ambrosia beetles, but *R. quercivora* is the first ambrosia fungus that has been reported to kill healthy trees (Ito et al., 2003a; Kamata et al., 2002). Oak trees 20-50 cm (~8-20 in) diameter at breast height (dbh) and 20-30 m (~66-98 ft) tall wilt within 2-3 months after attack by the beetle carrying the fungus (Kubono and Ito, 2002a). The pathogen, which has only recently been described (Kubono and Ito, 2002a), does not occur in the United States at this time and is of concern. The fungus is also a concern in Europe and appeared on the European and Mediterranean Plant Protection Organization (EPPO) Alert List before it was removed from the list in 2008 due to insufficient data on the susceptibility of European oak species to infection (EPPO, 2015).

The complex of *R. quercivora* and *P. quercivorus* has been associated with the mortality of large numbers of oak trees in Japan. Oaks (*Quercus* spp.), mainly *Quercus serrata* and *Q. mongolica* var. *grosseserrata*, are particularly susceptible (Ito et al., 2003a). Since 1980, 100,000 - 200,000 fagaceous trees have been killed annually, and 40% mortality has been reported in susceptible hosts (Ito et al., 2003a; Ito et al., 2003b). The distribution range of *R. quercivora* in Japan has expanded significantly in the last 20 years (Shoda-Kagaya et al., 2010). The extensive oak mortality in Japan may have impacted habitat for Asian black bears, causing them to move into more populated areas (Yamazaki, 2004).

Oak is an important environmental and ornamental host in the United States. Oak is widespread throughout the country particularly in the eastern states (Smith et al., 2004; Kartesz, 2015). In the last 20 years, the sudden oak death epidemic caused by *Phytophthora ramorum* has devastated coastal woodlands in California and southwestern Oregon, killing millions of trees (Grünwald et al., 2012). *Raffaelea quercivora*, if introduced into the United States, has the potential to exacerbate the already extensive damage to oak forests caused by *P. ramorum*.

Raffaelea quercivora is listed as a harmful organism in South Korea (USDA-PCIT, 2018). There may be trade implications with this country of the pathogen becomes established in the United States.

Known Hosts

Major hosts: *Quercus mongolica* (syn. *Quercus crispula*, Mongolian oak), and *Quercus serrata* (konara oak).

Minor hosts: *Castanea crenata* (Japanese chestnut), *Castanopsis sieboldii* (Japanese chinquapin), *Quercus coccinea* (scarlet oak), *Quercus palustris (pin oak), Quercus phillyraeoides* (black ridge oak).

Experimental hosts: *Quercus glauca* (ring-cup oak) and *Quercus rubra* (red oak).

(Kubono and Ito, 2002a; Matsuda et al., 2010; Murata et al., 2009; Torii et al., 2014)

Pathogens or Associated Organisms Vectored

The pathogen is vectored by the ambrosia beetle *Platypus quercivorus* (Fig. 6) (Kinuura and Kobayashi, 2006).

Known Distribution

Asia: Indonesia, Japan (Honshu, Kyushu), Taiwan, Thailand, Vietnam (CABI, 2017; Kusumoto et al., 2014; Shoda-Kagaya et al., 2010; USDA-APHIS-PPQ, 2014).

Pathway

Raffaelea quercivora may spread on infected host plant material or be carried by an insect vector. The cryptic nature of ambrosia beetles makes them difficult to detect, such that they may travel unnoticed in shipments of trees or wood products (Rabaglia et al., 2006). Between 1985-2005, ten species of ambrosia beetles were identified for the first time in the United States (Haack, 2006). Oak products, such as pallets, crates, or dunnage, could spread all life stages of *P. quercivorus* and thus potentially spread *R. quercivora.* Transportation of firewood and logs could establish localized dispersal of the insect and pathogen (USDA-APHIS-PPQ, 2014).

The ambrosia beetle *P. quercivorus* has not been intercepted at U.S. ports of entry, but there have been 73 interceptions of *Platypus* spp. at U.S. ports of entry since 1984 (AQAS, 2018). *Platypus* spp. have been intercepted on plant cuttings, plant parts (like leaves, seeds and fruit), as well as wood products, stoneware, tiles, and cargo. Most

beetles were intercepted on general and permit cargo, but three were intercepted in baggage (AQAS, 2018).

The import of *Quercus* spp. propagative material is currently regulated by a Federal Order effective May 20, 2013 as host material for the Asian longhorned beetle (ALB) and Chinese longhorned beetle (CLB) (USDA, 2018). Under this Federal Order, import of all *Quercus* spp. propagules from Japan is prohibited. However, there may still be a risk of *R. quercivora* spreading on *Quercus* spp. plant material from host countries in passenger cargo or through the mail.

Potential Distribution within the United States

The currently reported distribution of *R. quercivora* in Japan suggests that the pathogen may be closely associated with the temperate-broadleaf-and-mixed-forest biome (USDA-APHIS-PPQ, 2014). This biome is present throughout North America, including much of the eastern United States (ESRI, 2005). In addition, *Quercus* spp. are found throughout the continental United States, making possible the establishment of *R. quercivora* based on host presence (Kartesz, 2015; USDA-APHIS-PPQ, 2014). *Quercus coccinea* is susceptible to *R. quercivora* (Torii et al., 2014) and widespread east of the Mississippi river (Kartesz, 2015). *Quercus palustris* and *Q. rubra* are also susceptible to the pathogen and common in the eastern United States (Kartesz, 2015).

Survey

Approved Methods for Pest Surveillance*:

The Approved Method for Pest Surveillance (AMPS) is visual inspection of host material. Tree crowns should be visually inspected for wilting leaves beginning in June through early September; leaves will appear curled or withered, then become discolored (reddish). A survey for *R. quercivora* should target standing oak trees, oak logs, and the vector, *Platypus quercivorus*. Wilted or dead oaks should be examined for evidence of attack by *P. quercivorus*: entrance holes, most dense within 1 m (~ 3 ft) of the ground, and an accumulation of boring dust and frass at the base. The approved survey method for *P. quercivorus* is a trap-and-lure combination using a multi-funnel trap the *Platypus quercivorus* Lure. Traps baited with a pheromone lure should be placed near fallen or injured hosts. See the CAPS datasheet for *Platypus quercivorus* for additional information.

A suspect tree or log should be cut in the field, and one or more cross sections should be examined for galleries of *P. quercivorus* and discoloration that extends beyond the galleries into the surrounding sapwood.

*For the most up-to-date methods for survey and identification, see Approved Methods for Pest Surveillance on the CAPS Resource and Collaboration Site, at https://caps.ceris.purdue.edu/approved-methods.

Literature-Based Methods:

Methods for monitoring *P. quercivorus* are described in the companion risk assessment by Davis et al. (2005) as well as the New Pest Response Guidelines for Exotic Wood-

boring and Bark Beetles (USDA, 2011). Because affected hosts and the insect vector may be associated with several species of fungi (Ito et al., 1998; Kinuura, 2002; Masuya et al., 1998), identification of *R. quercivora* requires isolation and identification in a laboratory. Aerial surveys using photographs were used to define areas of oak mortality in Japan with some success (Kamata et al., 2001). Landsat imagery was too coarse to accurately identify these areas (Komura et al., 2003).

Oak species are the known major hosts of *R. quercivora*, so they should be the focus of a survey. Tree crowns should be visually inspected for wilting leaves beginning in June through early September; leaves will appear curled or withered, then become discolored – to a reddish color in Japan (Ito et al., 2003a; Kinuura, 2002; Kobayashi and Ueda, 2003; Saito et al., 2001). Wilted trees may be dead by August or not until the following spring, and may appear in clusters. In Japan, centers of oak mortality are often found on the edge of a gap or stand (Esaki et al., 2004).

Sapwood discoloration in an infected oak tree was observed to a height of 4 m (13 ft) (Kuroda, 2001). In Japan, sections of the trunk 20-30 cm (~8-12 inches) in diameter and 50 cm (20 inches) long were cut from felled trees or logs, and the ends were coated in the field with a silicone paste to prevent the wood from drying (Kinuura, 2002). Sealed logs were taken to a laboratory for further analysis. Logs used as bait for *P. quercivorus* can also be used to detect *R. quercivora* (Kobayashi et al., 2004; Kobayashi and Ueda, 2003).

Pheromone-based traps are available to aid in the detection of *P. quercivorus*. Traps should be placed near fallen or injured hosts in May when adults are mating and under increased light to attract adult individuals (Igeta et al., 2003). The pheromone lure to be used is (1*S*,4*R*)-4-isopropyl-1-methyl-2-cyclohexen-1-ol, which is abbreviated as (-)-IMCH and referred to as (1*S*,4*R*)-*p*-menth-2-en-1-ol or quercivorol, which is effective for 28 days (Kamata et al., 2008).

Key Diagnostics

Approved Methods for Pest Surveillance*:

Morphological. Identification will be based on the size and shape of conidiophores and conidia, and details of conidium production.

The colony is pale olive to brown and has a fragrance. The species is characterized by having small pear-shaped sympodioconidia and slender, long conidiophores that taper to a point.

Raffaelea quercivora can be identified in the laboratory from the sapwood of an infected tree or log and from adult *P. quercivorus*. Surface sterilized pieces of discolored sapwood, insect galleries, adult beetles, and beetle mycangia can be plated on potato dextrose agar (PDA) with 100 ppm of streptomycin sulphate. Plates are incubated in the dark at 20-25°C (68-77 °F) for five days.

For easier isolation, it is recommended to aseptically remove slices of galleries and

place them in a sterile moist chamber to encourage further growth of the ambrosia fungus.

*For the most up-to-date methods for survey and identification, see Approved Methods for Pest Surveillance on the CAPS Resource and Collaboration Site, at https://caps.ceris.purdue.edu/approved-methods.

Literature-Based Methods:
Raffaelea quercivora can be identified in the laboratory from the sapwood of an infected tree or log and from adult *P. quercivorus* individuals. Log samples were stored at 5°C (41°F), sawed into discs 2-4 cm (~1-2 inches) thick, cut into small blocks and split with a hatchet to expose the insect galleries from which small pieces of symptomatic material can be removed (Kinuura, 2002;Kubono and Ito, 2002b). For ambrosia fungi in general, Benjamin 2004 suggests preserving samples of thin slices or chips of galleries for later examination, either by drying or by mounting the samples on slides using a fixative mounting medium. Benjamin (2004) also suggests aseptically removing slices of galleries and putting them in a sterile moist chamber to encourage further growth of the ambrosia fungus for easier isolation.

A sterile scalpel is used to remove small pieces (e.g., cube 2-3 mm on a side) from the discolored sapwood and insect galleries (Kubono and Ito, 2002a). Samples are surface disinfected by washing with 80% ethanol and 0.1% solution of mercuric chloride and rinsed in two changes of sterilized water (Kubono and Ito, 2002a) or by rinsing each cube with 99% ethanol, heating over a flame, and repeating three times (Kinuura, 2002). Once disinfected, the cubes are placed on plates of potato dextrose agar (PDA).

Platypus quercivorus, adult beetles should be surface disinfested by immersion in 80% ethanol for 30 seconds, rinsed in a dilute solution of sodium hypochlorite for 2 minutes, then rinsed in sterile distilled water for 30 seconds before mycangia (Fig. 6) or proventriculi (terminal part of the foregut) are removed and placed on PDA for fungal isolation (Kinuura, 2002). Isolation on PDA and incubation in the dark at 20-25°C (68-77°F) will produce colonies within 5 days (Kubono and Ito, 2002a). Identification will be based on the size and shape of conidiophores and conidia, and details of conidium production. Identification should be confirmed by scanning electron microscopy (Gebhardt and Oberwinkler, 2005; Kubono and Ito, 2002a). *Raffaelea quercivora* will grow rapidly (80 mm (~3 1/8 in) diameter in 5 days at 25°C (77°F), and in 2 weeks will become pale olive to brown olive and have a fragrance (Kubono and Ito, 2002a). Microscopic examination of conidiophores and conidia is required for identification, and scanning electron microscopy is better than light microscopy to observe conidiogenesis on fresh culture material (Gebhardt and Oberwinkler, 2005). Confirmation should be made by an expert.Molecular identification of *R. quercivora* can also be performed using pure fungal cultures. Once samples are obtained in culture they can be used for DNA extraction and identified as *R. quercivora* based on PCR amplification of the nucleotide sequences of the partial 28S large subunit ribosomal DNA (D1/D2) region (Torii et al., 2016).

Easily Confused Species

Two diseases that could be confused with the early foliar symptoms of Japanese oak wilt are oak anthracnose and bacterial leaf scorch (BLS). Neither anthracnose nor BLS will cause rapid mortality like *R. quercivora*. Anthracnose is caused by the fungus *Apiognomonia errabunda* (imperfect state *Discula umbrinella*), and it occurs on many *Quercus* spp. in the United States (Gillman, 1999; Tainter and Baker, 1996). Unlike *R. quercivora* infection, fruiting structures of the fungus may be seen (raised brown flecks) on the underside of lesions (Sinclair et al., 1987). Although unsightly and often recurrent, anthracnose is rarely a serious problem on established trees (Gillman, 1999) and outbreaks usually diminish by mid-summer (Sinclair et al., 1987). Bacterial leaf scorch, caused by the bacterium *Xylella fastidiosa*, is another disease that occurs in oaks across the United States, and for which the foliar symptoms may resemble early symptoms of Japanese oak wilt (Bentz et al., 2005; Lashomb et al., 2003). While symptoms of Japanese oak wilt may be apparent early in the season (June) in Japan, BLS symptoms usually appear first in mid to late summer (Lashomb et al., 2003). Symptoms of BLS may occur on only one or a few branches in a season. Over time (years), branches and eventually whole trees may die. Leaves, twigs and branches can be tested for BLS by enzyme linked immuno-sorbent assay (ELISA) or polymerase chain reaction (PCR) (Bentz et al., 2005).

A third disease, oak wilt caused by the fungus *Ceratocystis fagacearum,* produces symptoms in oaks over time that closely resemble those described for infection of *R. quercivora* (Juzwik et al., 2004). Signs on the bark are different for oaks infected with *C. fagacearum* from oaks infected with *R. quercivora*. Red oaks infected with *C. fagacearum* may show small bark crack. If bark is removed, a gray mat of fungal mycelia may be uncovered. These fungal mats grow between the inner bark and the opposing wood, eventually creating enough pressure for the bark to crack (Juzwik et al., 2004). *Raffaelea quercivora*-infected trees have no such mats. *Ceratocystis fagacearum* is also vectored by beetles that do not make extensive galleries in the wood. In addition, the pattern of discoloration caused by *C. fagacearum* is different and begins as brown streaks that longitudinally follow the vessels in the outer sapwood (Juzwik et al., 2004; Sinclair et al., 1987). Descriptions and images of other diseases and problems that produce symptoms similar to the oak wilt caused by *C. fagacearum* should also be reviewed to avoid confusion with disease caused by *R. quercivora* (Juzwik et al., 2004).

No other species of *Raffaelea* have been identified in association with *P. quercivorus*, but several *Raffaelea* spp. have been reported from the galleries and/or mycangia of other ambrosia beetles infecting *Quercus* spp. These fungi include *R. ambrosiae* in England and the United States (von Arx and Hennebert, 1965), *R. montetyi* in France, (Morelet, 1998), and *R. tritirachium* in the United States (Batra, 1967b). These species can be differentiated from *R. quercivora* based on morphological characteristics. In general, *R. quercivora* has more slender conidiophores and smaller conidia than other *Raffaelea* spp., having conidia most similar in shape and size to *R. hennebertii* (Scott and Du Toit, 1970). However, the conidiophores of *R. quercivora* are more slender than those of *R. hennebertii* (Kubono and Ito, 2002a), and the host and location where *R. hennebertii* occurs are very different.

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