Phytophthora kernoviae Brasier, Beales & S.A. Kirk 2005

Synonyms
Phytophthora kernovii

Common Name(s)
Disease: Beech bleeding canker, rhododendron dieback

Pathogen: Phytophthora Taxon C, P. Taxon C, PTC, Pk

Type of Pest
Fungal-like organism

Taxonomic Position
Class: Oomycetes, **Order:** Pythiales, **Family:** Pythiaceae

Reason for Inclusion in Manual
Suggested by CAPS community, OPIS A listing, national threat

Figure 1. Necrotic bark and bleeding lesions on a European Beech (*Fagus sylvatica*) infected with *P. kernoviae*. Crown Copyright, Forestry Commission.

Background Information
Phytophthora kernoviae was discovered in late 2003 during surveys of woodlands in Cornwall, south-west England, United Kingdom (UK) for the presence of another invasive pathogen, *P. ramorum* (Brasier et al., 2005). The species name is derived from the old name of modern Cornwall - Kernow, where the pathogen was first found. Molecular analysis shows that this species is distinct from other *Phytophthora* spp., with *P. boehmeriae* phylogenetically being the closest relative (Aleksandrov and Arbuzova, 2012). A possible origin in the southern hemisphere is hypothesized for *P. kernoviae*, and although present in New Zealand since at least 1953, it is not known if it is an endemic species (Ramsfield et al. 2009). In 2014, *P. kernoviae* was reported for the first time in South America (Chile) (Sanfuentes et al., 2014).

Pest Description
(As described in Brasier et al. (2005), and Dick and Parke (2012)).
P. kernoviae is placed in Clade 9 with *P. boehmeriae* as its closest relative (Blair et al., 2008). Internal transcribed spacer (ITS) sequences of New Zealand isolates matched 812/813 base pairs with the UK reference isolate AY040661. The UK isolate AY040661 has adenine in position 679, whereas New Zealand isolates have either guanine in that position or are polymorphic for adenine and

guanine. This polymorphism in the ITS sequence is interpreted as evidence for some genetic diversity in the New Zealand population (Ramsfield et al. 2009).

Sporangia (34-52 x 19-31 µm, mean range ca 38.5-45.5 x 22.3 x 27 µm) (Fig. 2) papillate and caducous, formed occasionally on carrot agar (CA) in the light, produced abundantly on CA plugs in nonsterile pond water or soil leachate, ovoid, limoniform to asymmetric or 'mouse-shaped', most with a conspicuous vacuole, pedicel length 5-19 µm, borne on sympodially branched sporangiophores. Hyphae sometimes denticulate or tuberculate. Chlamydospores not observed. Colonies in dark on CA largely submerged with small central area of aerial mycelium, with alternating rings of mycelium in diurnal light (Fig. 3). Homothallic, gametangia abundant on CA after 10 d. Oogonia diameter 21-28 µm (mean 23.5-25.5 µm), often with tapered stalks (Fig. 4). Oospores 19-25 µm (mean 21.1-22.5 µm), plerotic, wall thickness 3.5-5 µm (mean ca. 3.5 µm). Antheridia amphigynous, 10-14 x 9 x 12 µm, commonly 10-14 x 9-12 µm. Compared to UK isolates described above (Brasier et al., 2005), New Zealand isolates are reported to grow somewhat slower at 20°C (68°F) and have a few small differences in the size of oogonia, sporangia, and pedicel length (Ramsfield et al. 2009).

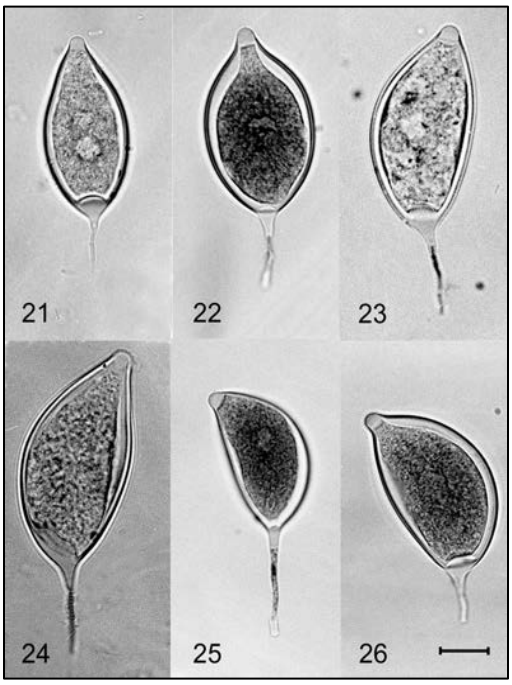

Figure 2. *P. kernoviae* sporangia. Images 21-22. Regular, ovoid limoniform sporangia. Images 23-26. Asymmetrical or 'mouse-shaped' sporangia. Bar=10 µm. Susan Kirk, Forest Research, Bugwood.org

Biology and Ecology

The optimum growth temperature for *P. kernoviae* is 18°C (64.4°F), with little to no growth at temperatures above 26°C (78.8°F). The growth rate in dark at 20°C (68°F) on carrot agar is 3.8-4.6 mm/day (mean 4.2 mm/day) (Brasier et al., 2005). Oospores can survive in moist sand at 30°C (86°F) for at least 1 year (Widmer, 2011). Oospore germination was optimal at 18°C and 20°C, and did not occur at 5°C (41°F) or 25°C (77°F) and higher (Widmer 2010b).

Local spread in the UK results from *P. kernoviae* infection centers that occur when the foliage of understory trees and shrubs in a beech-dominated woodland become infected. The primary platform for dispersal is the invasive rhododendron, *R. ponticum* that can dominate the understory in beech woodlands growing on acid soils. The pathogen is dispersed when sporangia are liberated from the canopy of this host during rain storms to infect beech trunks via motile zoospores. As a splash-dispersed pathogen, the distance

sporangia can travel during rain storms is likely to be proportional to the height of host canopy on which sporangia are produced and the velocity of wind

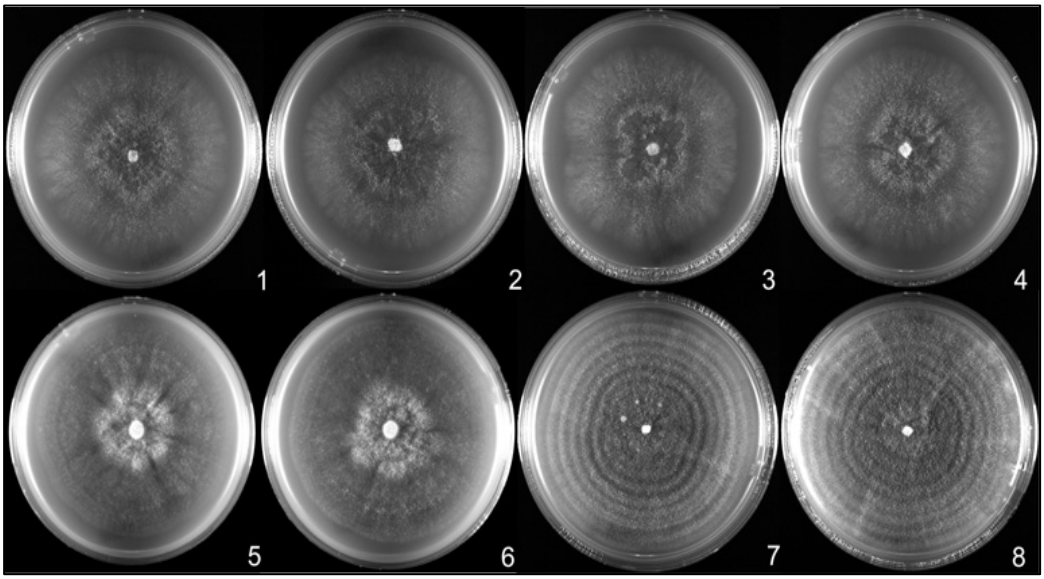

Figure 3: Colony types of *P. kernoviae*. Images1-4: after 10 d at 20°C on Carrot Agar (CA) in complete darkness. Images 5-6: after 10 d at 20°C on CA with some exposure to light at 7 d for colony measurement. Images 7-8: after 10 d on CA at ambient room temperature (23°C) in diurnal light. George Gate, Forest Research, Bugwood.org

associated with the rain event. With time, extensive areas can be impacted by this type of dispersal. Additionally, *P. kernoviae* has been found to produce sporangia on asymptomatic foliage (USDA, 2008). It is postulated that long distance disease spread may occur when coastal winds carry infected, abscised leaves. Local spread may also occur via infected roots and rhizomes (Fichtner et al. 2011). Spread of *P. kernoviae* within a woodland or drainage basin also can occur when propagules such as sporangia, zoospores, or oospores are introduced to runoff water either directly or when associated with host debris enters streams (USDA, 2008). Spread of *P. kernoviae* within an ecosystem is likely to occur by mechanisms similar to those demonstrated for *P. ramorum* including transport in infested soil on animals, and through human activities (USDA, 2008).

Phytophthora kernoviae isolates from the U.K. were shown to be more virulent on *Rhododendron ponticum*, *Magnolia stellata*, and *Annona cherimola* compared to isolates from New Zealand (Widmer 2015).

Symptoms/Signs

P. kernoviae causes various symptoms on infected host plants. These symptoms can be classified into three main types: 1) formation of bleeding lesions (Fig. 1, 5), 2) dieback of branch and shoot tips and various foliage (Fig. 5,

6) and, 3) quite often, shoot necrosis (Fig. 6). Like *P. ramorum*, *P. kernoviae* causes bleeding stem cankers on members of the Fagaceae and foliar blight and shoot dieback on other hosts (Dick and Parke, 2012). As a rule, bleeding lesions are observed on the European beech, tulip tree (*Liriodendron tulipifera*), and English oak (*Quercus robur*) (Aleksandrov and Arbuzova, 2012). The bleeding lesion form of disease is the most dangerous. Due to active lesion development it may lead to dieback and death of the whole tree. Mature trees of the above mentioned species are affected with cankers appearing at the stem bottom. Their size varies from several centimeters to 3 or more meters in length (along the trunk). Lesions can be hollow as lagoons and, sometimes, affected tissues are colored with black stripes separating the healthy part from the discolored affected one. Discoloration of the phloem and xylem tissue is caused by development of *P. kernoviae* mycelium (Aleksandrov and Arbuzova, 2012).

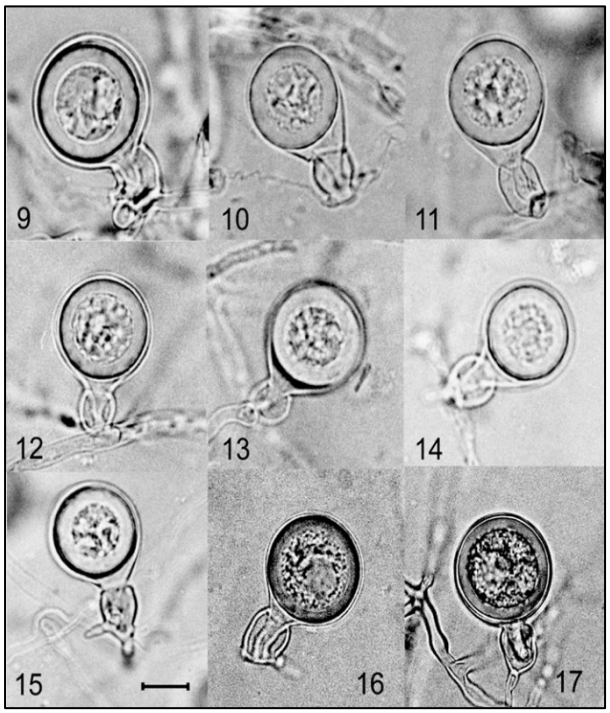

Figure 4. Representative oogonia, antheridia, and thick walled plerotic oospores of *P. kernoviae*. Compare oogonia with tapered bases (Images 10-14, 16) with those without this feature (Images 9, 15, 17). Bar=10 µm. Susan Kirk, Forest Research, Bugwood.org

On foliage hosts, leaf spotting, blight, and shoot dieback develop (Fig. 5, 6). The pathogen sporulates on foliage hosts and

Figure 5. Symptoms of *P. kernoviae* infection in *F. sylvatica* (European Beech) (left), *Rhododendron* spp. (center two photos), and *Magnolia* spp. (right). Crown copyright, Forestry Commission, http://fera.co.uk/copyright.cfm http://www.forestry.gov.uk/forestry/hcou-4ubejz

Figure 6: Symptoms of *P. kernoviae* infection in *Vaccinium myrtillus* (bilberry) (top), and *Rhododendron* spp. (Rhododendron) (bottom). Crown copyright, Forestry Commission, http://fera.co.uk/copyright.cfm
http://www.forestry.gov.uk/forestry/hcou-4ubejz.

is dispersed by rain to bole hosts such as beech (*Fagus sylvatica)* and oak (*Quercus* spp.), where cankers develop.

In New Zealand, the pathogen was associated with diseased leaves, shoots, and fruits of cherimoya trees that were near native shrublands and forest in Northland (Dick and Parke, 2012). Previous isolates from soil in kauri forests 50 km away were not associated with disease, nor were isolates from near Tokoroa, 400 km to the south, from *Pinus radiata* plantations (Ramsfield et al. 2009).

Pest Importance

Comparative inoculations of *P. kernoviae* and *P. ramorum* on beech stems suggest that lesion development and tissue colonization are significantly more rapid for *P. kernoviae* than for *P. ramorum* (Brasier et al., 2005). Thus, the rate of spread and mortality in woodlands is potentially greater for *P. kernoviae* since the more slowly colonizing *P. ramorum* has proven to be a serious threat in invaded forests (USDA, 2008). In addition, *P. kernoviae* produced higher or similar amounts of sporangia and chlamydospores than indigenous *Phytophthora* species, *P. cactorum* and *P. syringae*, on rhododendron, tulip tree, star magnolia, and mountain laurel (Widmer 2015).

Introduction of *P. kernoviae* to the United States could threaten both forests and nursery crops. Because the full potential for establishment of *P. kernoviae* is unknown, all U.S. forests and the U.S. nursery industry (valued at $4.6 billion) could be at risk (USDA, 2008).

Phytophthora kernoviae is listed as a harmful organism in the following countries: Australia, Japan, Nauru, and South Korea (USDA-PCIT, 2015). There may be trade implications with these countries if this pathogen becomes established in the United States.

Known Hosts

Major Hosts: *Fagus sylvatica* (European beech), *Rhododendron ponticum* (rhododendron) (Aleksandrov and Arbuzova, 2012; EPPO, 2014).

Minor Hosts:
Annona cherimola (sugar apple); *Drimys winteri* (winter‘s bark); *Gevuina avellana* (hazelnut); *Hedera helix* (English ivy); *Ilex aquifolium* (English holly); *Liriodendron tulipifera* (tulip tree); *Lomatia myricoides*; *Magnolia* spp. (magnolia); *Michelia doltsopa* (Chinese magnolia); *Pieris* spp. (Pieris); *Podocarpus salignus* (Podocarpus); *Prunus laurocerasus* (cherry laurel); *Quercus ilex* (home oak); *Q. robur* (English oak); *Vaccinium* spp. (bilberry) (USDA, 2008; Aleksandrov and Arbuzova, 2012; EPPO, 2014).

Both oak species mentioned above occur in the United States as introduced species.

Experimental Hosts:
In addition to the hosts listed above, laboratory inoculations have been successful on an additional 45 species in several genera. Based on experience with the expanding host range of *P. ramorum*, it is possible that the host range of *P. kernoviae* will expand with time and more extensive surveys (USDA, 2008). Experimental hosts which have shown high susceptibility to *P. kernoviae* on immature stems include: *Betula pendula* (silver birch); *Castanea sativa* (sweet chestnut); *Pinus contorta* (lodgepole pine); *Pinus nigra* var. *maritima* (Corsican pine); and *Tsuga heterophylla* (western hemlock) (USDA, 2008; Aleksandrov and Arbuzova, 2012).

The following oak species which are present in the United States have shown susceptibility to *P. kernoviae* under experimental conditions. Of these four species, *Q. coccinea* and *Q. rubra* are both widespread in eastern states (BONAP, 2015

Table 1. Oak species susceptible to *P. kernoviae* in experimental conditions (DEFRA, 2008).

Species	Common Name	Susceptibility
Quercus cerris	Turkey oak	Low/Moderate
Quercus coccinea	Scarlet oak	Low
Quercus ilex	Holm oak	Low
Quercus rubra	Red oak	Low

Known Vectors (or associated insects)

Phytophthora kernoviae is not known to be vectored, nor is it known to vector any pathogens.

Known Distribution

Europe: Ireland, United Kingdom (England, Scotland, Wales). **Oceania**: New Zealand (North Island). **South America**: Chile (Brasier et al., 2005; EPPO, 2006, 2008, 2010; Sanfuentes et al., 2014).

Pathway

The most likely pathway into the United States for *P. kernoviae* is by movement of infected host material through the nursery trade. Since *P. kernoviae* has been associated with root infection and asymptomatic infection, monitoring poses special challenges for inspectors and regulators (USDA, 2008).

Currently, the import of many known hosts of *P. kernoviae* are regulated under a federal order that was enacted May 12, 2012 to prevent the spread of *P. ramorum*, and strict phytosanitary measures are taken (USDA, 2015). However, there has still been some import of known host material from Chile, Ireland, New Zealand, and the UK. For example, since 2005, there have been shipments of *Rhododendron* spp. (5) and *Magnolia* spp. (1) plant material from the UK. There have also been shipments of *Magnolia* spp. (53) from New Zealand totaling over 18,800 plant units. There were also shipments of *Vaccinium* spp (20) and *Drimys* spp. (1) plant material totaling over 1,600 plant units from Chile (AQAS, 2015).

The transport of infected host material in cargo and on airline passengers also presents potential pathways into the United States. Since 2005, there have been two interceptions of *Ilex* spp. from the UK, one of which was intended for propagation. There have been 11 interceptions of *Magnolia* spp. plant material from New Zealand, five of which were intended for propagation. There have been 71 interceptions of *Pieris* spp. cut flowers from New Zealand. There have been interceptions of *Vaccinium* spp., mostly fruit intended for consumption, from New Zealand (69) and the UK (1) (AQAS, 2015).

Potential Distribution within the United States

Host plants for *P. kernoviae* are present in every state in the contiguous United States. *Rhododendron* spp. are widespread throughout the eastern United States as well as the great lakes region, west coast, and Pacific Northwest. *Fagus* spp. are also common in the eastern states but are not present in western states. *Quercus* spp. are present throughout the contiguous United States (BONAP, 2015).

An analysis based on climate, overstory host density, host sporulation potential, and introduction pathways estimated that east coast forests were more at risk than those in the west. The eastern slope of the Appalachian Mountains was at greatest risk due to the confluence of human development, climate and hosts (USDA, 2008).

Survey

Approved Methods for Pest Surveillance (AMPS)*:

1. Visual survey: Collect bark lesions, shoot, twigs, or leaves from symptomatic trees.

Trees (trunk/logs): for bleeding cankers, the inner bark in the area directly around the oozing sap is cut until a canker margin is evident. Pieces of phloem and xylem are removed and placed in a sealed container.

Shoots/twigs: a piece of stem including the leading edge (junction between diseased and healthy tissue) is removed and placed in a sealed plastic bag, with a small piece of damp tissue to prevent desiccation.

Leaves: 4–6 leaves showing a suitable range of symptoms are removed and placed in a sealed plastic bag. If possible, all samples of plant material should be sent to the laboratory to arrive by the next day. Overheating or desiccation of samples prior to dispatch should be prevented.

2. Water/Soil sampling with rhododendron leaf baits.

Detecting viable *P. kernoviae* in soil or water can be done by baiting with rhododendron leaves. Soil is placed in a transparent plastic container and covered with Petri's mineral solution or demineralized water. Clean rhododendron (cv. Cunningham's White or *R. ponticum*) whole leaves or leaves cut into pieces (e.g. approximately 1.5 cm- 2.0 cm) are then floated on top. The lid is replaced and the tub incubated for 3–6 days at 18–23°C (64.4 to 73.4°F) in light (12–16 h/day on the laboratory bench). After this time, the leaves are recovered, then surface decontaminated and tested serologically or by real-time PCR.

Water testing can be carried out in a similar manner in the laboratory by simply floating cut leaves on the water to be tested. Alternatively, on-site testing can be

done using cut rhododendron leaves placed in a muslin bag, which is then lowered into the top layers of the water on a string and left for 3 days. Once recovered, the leaves are washed and tested using serological or molecular tests.

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

Literature-Based Methods:

Symptomatic tissues can be sampled from potentially-infected plants and placed on selective medium for isolation. In the U.K., SMA+MRP medium (Elliott et al., 1966) is used for successful isolation of *P. kernoviae*. Because *P. kernoviae* does not form diagnostic structures on SMA+MRP, colonies must be transferred to carrot agar for oospore production (USDA, 2008).

Detection of asymptomatic infections requires collection of a large sample of tissue and the bulking of subsamples for subsequent baiting. Furthermore, to enhance detection by baiting, tissues may be incubated in a moist chamber for 24h after surface disinfestation to stimulate sporangia production. Tissues may then be incubated in shallow deionized water with leaf disks of *Rhododendron* 'Cunningham's White' floated on the surface for 1 week at approximately 20°C. Leaf disks are then blotted dry and placed on SMA+MRP medium for isolation and subcultured onto carrot agar for identification (USDA, 2008).

Note: Oospores can be observed on PARPH agar (Ferguson and Jeffers, 1999) amended with 20 ml/L clarified V8 broth. This would eliminate the need to retransfer to carrot agar from SMA+MRP.

Phytophthora kernoviae has been associated with roots of *Rhododendron ponticum* seedlings lacking aboveground symptoms; therefore, detection of the pathogen in roots requires random sampling from asymptomatic plants in areas known to be infested. Similar to baiting from asymptomatic tissue (above), roots should be incubated in a moist chamber prior to baiting with leaf disks of *R.* 'Cunningham's White' (USDA, 2008).

Key Diagnostics

Approved Methods for Pest Surveillance (AMPS)*

1. Serological: An ELISA test is available for *Phytophthora* at the genus level for primary screening. A positive does not indicate *P. kernoviae*.

ID must be confirmed by other methods.

2. Molecular:

PPQ CPHST has validated work instructions available upon request for a real-time PCR (qPCR) for confirmation of *P. kernoviae*.

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

Literature-Based Methods:

Brasier et al. (2005) describe distinct morphological characteristics of *P. kernoviae*. *P. kernoviae* is homothallic and produces amphigynous antheridia and caducous and conspicuously papillate sporangia (Brasier et al, 2005). *P. boehmeriae* is the only known species that has the same characters. However, *P. kernoviae* can be easily separated from *P. boehmeriae* by shape of oogonial stalks (tapered vs. not tapered) and sporangia (often asymmetric vs. spherical/ovoid) and pedicel length (medium vs. short) (USDA, 2008).

Schlenzig (2011) developed a duplex PCR method, based on the internal transcribed spacer (ITS) regions of the ribosomal DNA, that can simultaneously detect *P. kernoviae* and *P. ramorum*. Hughes et al. (2011) developed a TaqMan real-time PCR assay for detection of *P. kernoviae* that is also based on internal transcribed spacer (ITS) sequence.

Tomlinson et al. (2010) developed a method for nucleic-acid-based detection of *P. kernoviae*. This method involves extraction of DNA on the nitrocellulose membranes of lateral-flow devices, loop-mediated isothermal amplification (LAMP) of target DNA using labeled primers, and detection of the generically labeled amplification products by a sandwich immunoassay in a lateral-flow-device format. This method is suitable for on-site use.

Easily Confused Pests

Symptoms in infected hosts are very similar to those of *P. ramorum*, which infects some of the same hosts. However, *P. kernoviae* has clear morphological distinctions from *P. ramorum* (Aleksandrov and Arbuzova, 2012). Compared to *P. ramorum*, which often occurs in similar habitats in the UK, *P. kernoviae* is homothallic instead of heterothallic, is papillate instead of semi-papillate, does not produce chlamydospores, and has a longer pedicel length (Dick and Parke, 2012).

P. kernoviae may be distinguished from other homothallic *Phytophthora* species with caducous, papillate sporangia with medium-length pedicels by its lower optimal temperature (cfr. *P. botryosa* and *P. hevea*); higher optimum temperature (cfr. *P. nemerosa*), often tapered oogonial stalks (cfr. *P. meadii*, *P. botryosa*, *P. nemerosa*), often asymmetric sporangia (cfr. *P. meadii*, *P. megakarya*, *P.*

nemerosa), and longer pedicels (cfr. *P. boehmeriae*) (Brasier et al., 2005; Dick and Parke, 2012).

Widmer (2010a) developed a diagnostic key to differentiate *P. kernoviae* from other *Phytophthora* species that infect the foliage of rhododendron.

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