Recovery Plan
for
Phytophthora kernoviae

Cause of Bleeding Trunk Cankers, Leaf Blight and Stem Dieback in Trees and Shrubs

November, 2008

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This recovery plan is one of several disease-specific documents produced as part of the National Plant Disease Recovery System (NPDRS) called for in Homeland Security Presidential Directive Number 9 (HSPD-9). The purpose of the NPDRS is to ensure that the tools, infrastructure, communication networks, and capacity required to mitigate the impact of high consequence plant disease outbreaks are such that a reasonable level of crop production is maintained.

Each disease-specific plan is intended to provide a brief primer on the disease, assess the status of critical recovery components, and identify disease management research, extension and education needs. These documents are not intended to be stand-alone documents that address all of the many and varied aspects of plant disease outbreaks and all of the decisions that must be taken to achieve effective response and recovery. They are, however, documents that will help USDA guide further efforts directed toward plant disease recovery.

Executive Summary

Phytophthora kernoviae, a recently described species of *Phytophthora,* is an invasive pathogen of forest trees and shrubs such as beech *(Fagus sylvatica)* and rhododendron *(Rhododendron ponticum)* that has become established in woodlands and public gardens in Cornwall, United Kingdom. Although the origin of *P. kernoviae* is unknown, the pathogen has been detected in New Zealand where only limited disease has been observed. In the U.K., *P. kernoviae* occurs in some of the same woodlands as the sudden oak death pathogen, *P. ramorum,* that has caused extensive losses to forests in California. *Phytophthora ramorum* quarantines have caused significant economic hardship to the nursery industry in the United States.

Introduction of *P. kernoviae* to the U.S. 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. A U.S. risk map for potential forest hosts 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.

Symptoms of *P. kernoviae* include bleeding trunk cankers on beech that eventually girdle and kill the tree. On foliage hosts, leaf spots, blight and shoot dieback develop. The pathogen sporulates on foliage hosts and is dispersed by rain to bole hosts such as beech *(Fagus sylvatica)* and oak (*Quercus* spp.) where cankers develop. *Phytophthora kernoviae* can be detected in host tissue by direct isolation, ELISA, and real-time TaqMan PCR. However, since *P. kernoviae* also has been associated with asymptomatic tissues, monitoring poses special challenges for inspectors and regulators since roots and asymptomatic tissues may be a possible hidden pathway of movement.

No monitoring program is in place to detect *P. kernoviae* should it be present or introduced to the United States. The National Plant Diagnostic Network might detect the pathogen by chance in clinic samples but diagnosticians rarely identify *Phytophthora* isolations to species due to cost limitations. A stream monitoring system for *P. ramorum* in U.S. forests, which could be adapted for *P. kernoviae,* is in place but has been reduced due to limited funds. Emergency restrictions are in place for *P. kernoviae* for nursery stock imported to the U.S. from countries where the pathogen occurs. USDA-APHIS-PPQ action plans in use for *P. ramorum* could be adapted relatively quickly should *P. kernoviae* be detected in the U.S.

If we are to be prepared for *P. kernoviae,* a number of initiatives are indicated.

Recommendations:

- Available molecular tests should be validated with real samples and used to determine sensitivity of detection methods for the pathogen on different hosts.
- A permanent stream monitoring system for *P. kernoviae* and other invasive *Phytophthora* species should be established along with nursery surveys.
- The susceptibility of native North American species (trees and understory plants) and important ornamental plant species should be determined in inoculation trials.
- Since *P. kernoviae* is a recently described species, information on its biology and epidemiology is needed.
- An effective method for *P. kernoviae* education in North America should be developed by USDA APHIS, ARS, FS, and CSREES in cooperation with Land Grant institutions.

Phytophthora kernoviae
Cause of Bleeding Trunk Cankers, Leaf Blight and Stem Dieback in Trees and Shrubs

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I. Introduction

Phytophthora kernoviae is a recently described invasive pathogen in southwest England, and its name reflects the ancient name for Cornwall 'Kernow', the county in which it was first detected (Brasier et al., 2005). The epidemiology of the disease in the United Kingdom is noteworthy in that *P. kernoviae* is an invasive pathogen that utilizes an invasive plant, *Rhododendron ponticum,* as a platform for sporulation and dispersal in woodlands. *Phytophthora kernoviae* is a fungus-like microorganism in the Oomycota, a group of water molds with diploid hyphae and cellulose in the cell wall. Contributing to the invasive nature of the pathogen, *P. kernoviae* produces sporangia that are easily dislodged from infected tissues during rain events and splash dispersed to nearby hosts. Multiple motile zoospores are liberated from each sporangium to cause new infections (Fig 1). *P. kernoviae* is homothallic, meaning that a single isolate can produce sexual oospores; mating is not necessary. Although oospore production is abundant in culture, the pathogen's ability to produce oospores in naturally-infected plant tissues is as yet unknown (Brasier et al., 2005; Brown and Brasier, 2007). Since chlamydospores have not been reported for this species, oospore production and subsequent survival could be very important epidemiological factors should oospores occur in naturally-infected tissues.

Phytophthora kernoviae was first found during a delimiting survey for *P. ramorum,* causal agent of sudden oak death and ramorum blight, in a Cornwall woodland (southwest England) in November 2003. It was first associated with a bleeding trunk canker on mature European beech *(Fagus sylvatica)* and stem dieback and foliar necrosis on invasive rhododendron *(R. ponticum).* Many additional infested woodland and garden sites in Cornwall have since been identified (Fig. 2). Since morphological characters of the beech and rhododendron isolates did not match known *Phytophthora* species, the

isolates were referred to initially as *Phytophthora* taxon C (Brasier et al., 2004). Subsequently, Brasier et al., (2005) published the species description for *P. kernoviae* based on isolates collected from *F. sylvatica* bark (beech), *R. ponticum* leaves, *Liriodendron tulipifera* (tulip tree) bark, *Quercus robur* bark (English oak), *Magnolia* sp. leaves, *R. catawbiense* leaves/shoots, *Pieris formosa* leaves/shoots, and *Q. ilex* leaves (Holm oak).

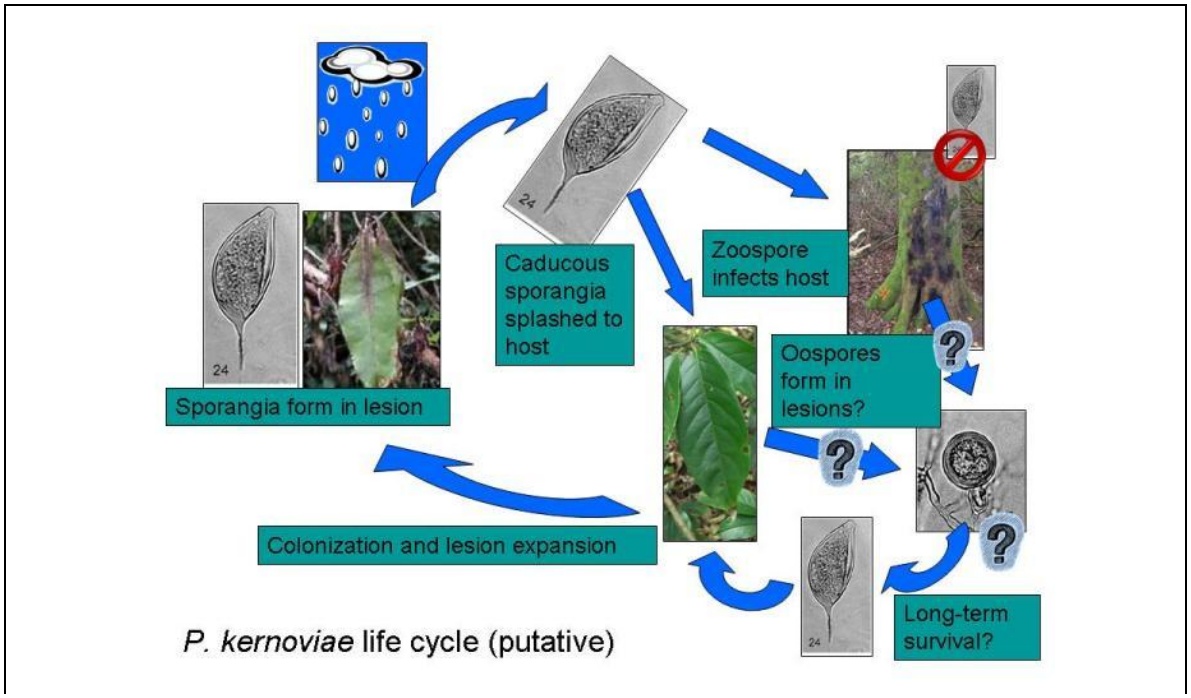


Fig. 1. Putative life cycle of *Phytophthora kernoviae* in a mixed woodland of beech and *Magnolia doltsopa* with the invasive understory host, *Rhododendron ponticum*.

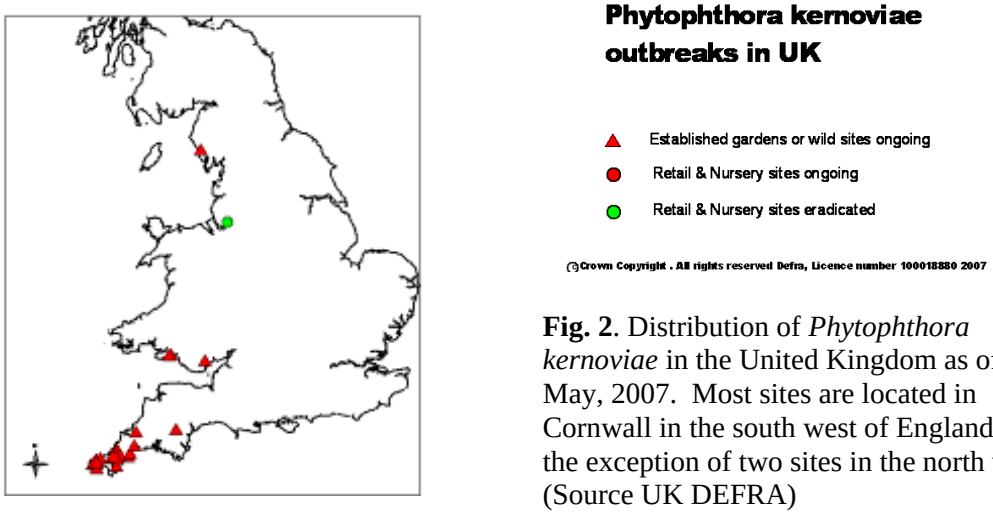


Fig. 2. Distribution of *Phytophthora kernoviae* in the United Kingdom as of May, 2007. Most sites are located in Cornwall in the south west of England with the exception of two sites in the north west. (Source UK DEFRA)

Based on DNA sequence data, plant pathologists in New Zealand recently reclassified *Phytophthora heveae* from *Annona cherimola* (custard apple or cherimoya) as *P. kernoviae*. Similar isolates had been collected as long ago as the 1950s from forest soil

in other parts of New Zealand (Ramsfield et al., 2007). However, in New Zealand, *P. kernoviae* does not appear to be an aggressively invasive pathogen but rather has survived without the occurrence of major epidemics. Thus far *P. kernoviae* is only known to occur in the United Kingdom and New Zealand. The origin of *P. kernoviae* is unknown.

In addition to the hosts above from which initial isolates were collected, *P. kernoviae* has been isolated in nature from the following shrubs/herbaceous plants: *Hedera helix* (English ivy), *Lomatia myricoides,* 16 *Magnolia* spp., *Pieris japonica, Vaccinium myrtillus* (bilberry) and the following trees: *Drimys winterii* (Winter's bark), *Gevuina avellana* (Chilean hazelnut), *Magnolia doltsopa* (syn. *Michelia doltsopa,* Chinese magnolia*), Ilex aquifolium, Podocarpus salignan,* and *Prunus laurocerasus* as of May, 2008 (see DEFRA 'host list' in 'Web resources' section). 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.

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. However, to date *P. kernoviae* has only been detected in four nurseries in the U.K. so it is not clear if movement of infected nursery stock will be as effective in spread as it was in the case of *P. ramorum* where this pathogen has been detected in more than 600 U. K. nurseries. Given the potential for a devastating epidemic if *P. kernoviae* were introduced to the U.S. where many potential hosts occur in both east and west coast forests, the USDA Office of Pest Management Policy requested development of this recovery plan.

II. Symptoms

Phytophthora kernoviae causes a range of symptoms. Bleeding cankers develop on beech, tulip tree, and oak in woodlands and established gardens. Bleeding cankers may be sunken or demarcated by black lines as the pathogen colonizes phloem and xylem tissue resulting in tissue discoloration (Brown and Brasier, 2007) (Fig. 3). In some cases, "lagoon" cavities develop in the xylem as a result of cambial necrosis and subsequent callus formation and tissue breakdown. As with *P. ramorum, P. kernoviae* does not form sporangia on the surface of bark cankers; therefore these canker hosts are not considered important for subsequent pathogen dispersal but may be for pathogen survival (Fig. 3). Since the pathogen is homothallic, the possibility exists for the production of oospores on mycelium colonizing phloem and xylem tissue in trunk canker lesions. Oospore production might provide a potential pathway for long-term survival when trees die and bark is shed; however, oospore production has yet to be documented in nature.

Foliar necrosis and stem dieback occur on hosts such as rhododendron, *Magnolia* spp., *Pieris formosa, Gevuina avellana*, and *Magnolia doltsopa*, and leaf and shoot dieback is

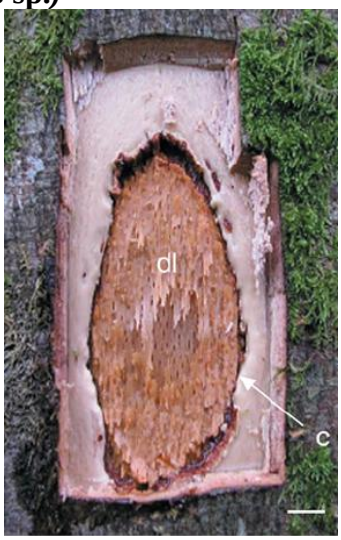
observed on Holm oak *(Q. ilex)* (Brasier et al., 2005). Excised, intact leaves of *Rhododendron* 'Cunningham's White' developed lesions within 3 to 6 days after dipping in a zoospore suspension of *P. kernoviae* (Brasier et al., 2005). The pattern of foliar necrosis is variable even on the same host depending on the infection court. For instance on mature rhododendron leaves leaf tips may become necrotic where rain water accumulates during runoff. If zoospores infect along the leaf margin, necrosis may spread from the margin inward on the leaf blade. If a stem is infected, the pathogen may colonize the leaf through the petiole resulting in a 'V-shaped' pattern of necrosis on the leaf. On other hosts such as *M. doltsopa*, necrotic spots develop at the tip of leaves where inoculum accumulated in water droplets hanging on the leaf tip. On magnolia, leaf spots usually remain fairly restricted and random across the leaf (Fig. 3).

Numerous sporangia develop on the surface of leaf lesions during periods of leaf wetness (Fig. 1). During periods of rain, sporangia are splashed and blown by wind to other hosts where motile zoospores are liberated. Zoospores presumably infect through the bark of beech and other tree hosts in a manner similar to those of *P. ramorum*, to cause bleeding cankers. Additionally, *P. kernoviae* has been found to produce sporangia on asymptomatic foliage (Denman et al., 2007), rendering it difficult for inspectors to easily identify potentially-infected plants and increasing the sample quantities necessary to detect the pathogen.

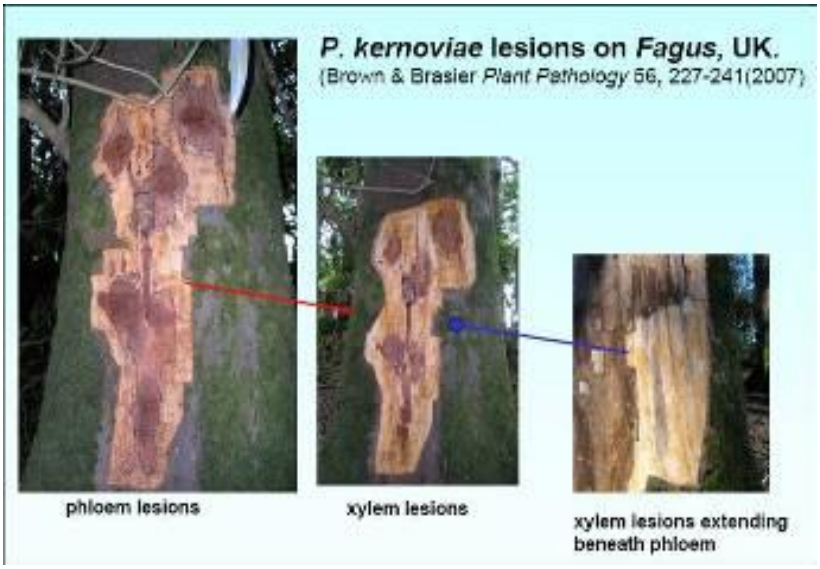
Fig. 3. Symptoms of *Phytophthora kernoviae* on beech *(Fagus* sp.*)*

Bleeding canker on beech
(Forest Research, Great Britain)

Bleeding canker on beech-close up
(Clive Brasier, Forest Research, Great Britain)



Lagoon cavity (dl) in beech stem with callusing (c) at margin. White scale bar: 1 cm
(Brown and Brasier, 2007)



Symptoms of *Phytophthora kernoviae* other hosts

Shoot dieback on rhododendron
(Forestry Research. Great Britain)

Individual shoot dieback on rhododendron
(Forestry Research. Great Britain)

Leafspots on magnolia
(Forest Research, Great Britain)

Leafspots on magnolia - close-up
(Forest Research, Great Britain)

Leafspots on *Magnolia doltsopa*
(DEFRA, Great Britain)

Shoot dieback on cherimoya
(M. Braithwaite, MAF Biosecurity New Zealand)

Stem infections with resulting stem dieback develop in hosts such as rhododendron, *Pieris formosa*, and cherimoya (Fig. 3). In many cases, *Phytophthora* spp. gain entry to stems through petioles of infected leaves. Rapid colonization of young stems results in tissue collapse and shoot dieback. In older stems the rate of colonization is slower but eventually stem dieback occurs.

The possibility of root infection by *P. kernoviae* in hosts that develop foliar necrosis and shoot dieback is unknown at present. Preliminary studies suggest that *P. kernoviae* may be associated with asymptomatic roots of *R. ponticum* in woodlands invaded by *P. kernoviae* in Cornwall (Fichtner et al., 2007). In the case of *P. ramorum*, roots of hosts such as rhododendron can be infected. Under some circumstances the pathogen grows internally through root and stem tissue, subsequently inducing foliar infections (Parke and Lewis, 2007, Shishkoff, 2007).

III. Spread

Local spread in the U.K. 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 (Fig. 1). 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 associated with the rain event. With time, extensive areas can be impacted by this type of dispersal. In the case of tanoak forests of coastal California, *P. ramorum* was spread 100-200 m in one season in low wind environments and 1-3 km in high wind environments (Mascheretti et al., 2008). These dispersal gradients would be less for a shorter rhododendron canopy (Tjosvold et. al., 2005). In Oregon tanoak forests, splash dispersal is confined to adjacent tree canopies and susceptible plants growing beneath infested tanoak crowns. Longer

distance transport occurs via other mechanisms, perhaps turbulent dispersal of dehiscent sporangia in the absence of rain (Hansen et al., 2008).

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 that enters streams. In fact, stream sampling has proven effective in detecting *P. ramorum* and other *Phytophthora* spp. in a number of forest watersheds in California and Oregon as well as in the Appalachian forests of the eastern U.S. For *P. ramorum* at least, use of stream water contaminated with the pathogen to irrigate container-grown rhododendron resulted in foliage infections (Tjosvold et. al., 2005).

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. For instance, boots of hikers exiting California state parks were found to contain inoculum of *P. ramorum* in adhering soil (Tjosvold et al., 2002, Cushman et al. 2007). Similarly, over 30% of soil samples from walkers' boots in U.K. infested areas were found positive for *Phytophthora* spp, including *P. kernoviae* (Webber and Rose, 2007). However, the establishment of new infection centers in the environment from introduced sources of soil containing inoculum of *P. ramorum* or *P. kernoviae* has not been demonstrated.

Potential long-distance dispersal of *P. kernoviae* on logs, wood products, and ornamental nursery stock should be of regulatory concern as a potential pathway. Infection of xylem tissues by *P. kernoviae* could result in introduction on imported logs or untreated wood products even when the bark is removed. Any nursery stock imported into the U.S. must be certified free of *P. kernoviae* and *P. ramorum*. For *P. kernoviae* the following genera of propagative material (except seeds) were named in 2005 as regulated: *Fagus* spp., *Gevuina* spp., *Liriodendron* spp., *Magnolia* spp., *Pieris* spp., *Quercus* spp., *Rhododendron* spp. However, even though regulatory inspections are in place, certified plants may still harbor *P. kernoviae* because *i)* foliar symptoms may not be expressed despite the presence of root infections, *ii)* potting mix may be infested with oospores or sporangia if plants are not imported bare-rooted, *iii)* latent foliar infections may not be detected until after entry, and *iv*) the pathogen could potentially sporulate on leaf tissues without apparent necrosis (Denman et al., 2007). Although a regulatory program is in place for commercial importation, amateur gardeners must be educated about the danger of moving this pathogen to avoid potential introductions into the U.S. Many of the historic gardens in the U.K. infested by *P. ramorum* and *P. kernoviae* receive thousands of visitors each year. Visitors need to be reminded that they should not take plant samples from these gardens because of the risk of transporting the inoculum of these pathogens outside infested areas.

IV. Monitoring, Detection and Identification

Isolation from plant tissue and morphological identification

Because *Phytophthora kernoviae* has been associated with both symptomatic and asymptomatic plant tissues, and with both aboveground and belowground plant parts (Denman et al., 2007; Fichtner et al., 2007), detection of the pathogen in either natural

systems or in nursery settings poses a distinct challenge for inspectors and regulators. The known host range is likely to continue expanding, with hosts exhibiting aboveground symptoms having a higher probability of interception. Consequently, root infections and asymptomatic infections represent hidden pathways for dissemination of the pathogen.

Symptomatic tissues: 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*, whereas the efficacy of other selective media has not been determined. Because *P. kernoviae* does not form diagnostic structures on SMA+MRP, colonies must be transferred to carrot agar for oospore production. Selective media such as PARP-H and PARP-V8 should also be evaluated for their potential use. PARP-V8 is known to induce sporangial production and chlamydospore production of *P. ramorum* and is used in the regulatory diagnostic protocols for that pathogen.

Asymptomatic tissues: 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 (Elizabeth Fichtner, pers. comm.). Leaf disks are then blotted dry and placed on SMA+MRP medium for isolation and subcultured onto carrot agar for identification. The efficacy of other *Rhododendron* spp. and cultivars for baiting of *P. kernoviae* is unknown. Defra Plant Health and Seed Inspectorate (PHSI) apparently routinely collect batches of asymptomatic leaves (30 per batch) from high risk nurseries. Each batch of leaves is then water baited for the detection of *P. ramorum* and/or *P. kernoviae*. This type of testing has yielded both pathogens (mainly *P. ramorum*).

Root tissue: Because *P. kernoviae* has been associated with asymptomatic roots of *R. ponticum* in the U.K. (Fichtner et al. 2007), the possibility and probability of the pathogen infecting roots of native *Rhododendron* spp. in the United States and other known and unknown hosts must be considered. The pathogen has been associated with roots of *R. ponticum* seedlings lacking aboveground symptoms; therefore, detection of the pathogen in roots requires random sampling from asymptomatic plants in areas know 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'.

Diagnostics in culture

Morphology: *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 (Gallegly and Hong, 2008). 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). Other species with similar morphology include *P. botryosa, P. heveae, P. hibernalis, P. ilicis, P. katsurae, P. meadii, P. megakarya,* and *P. nemorosa*, a recently described species (Hansen et al., 2003), but they all have distinct

morphological characters from *P. kernoviae* (Gallegly and Hong, 2008). Specifically, *P. heveae* and *P. katsurae* are homothallic with tapered oogonial stalks and amphigynous antheridia; their sporangia are papillate but they are non-caducous. Similarly, *P. ilicis*, *P. hibernalis*, and *P. nemorosa* are homothallic, produce amphigynous antheridia and caducous sporangia with medium-length pedicels, but they all are semi-papillate (papillae flattened). In addition, *P. botryosa*, *P. meadii*, and *P. megakarya* produce amphigynous antheridia and caducous papillate sporangia, but they are heterothallic.

Temperature: The optimal temperature for growth of *P. kernoviae* on carrot agar is approximately 18°C, with the upper growth limit around 26°C (Brasier et al., 2005). This temperature maximum is slightly higher than those of *P. hibernalis* and *P. illicis* (25°C), and *P. nemorosa* (<25°C) but significantly lower than those of *P. botryosa*, *P. heveae* and *P. katsurae* (32°C), *P. meadii* (33°C) and *P. megakarya* (30°C) (Gallegly and Hong, 2008). These temperature differences also could be used as an additional character to separate *P. kernoviae* from other species with similar morphology.

Serological detection

The ELISA procedure used in the APHIS approved procedures as a first step in the diagnosis of *P. ramorum* will also work as a first step to detect *P. kernoviae*. However, while ELISA will detect *Phytophthora* spp., it is not species or genus specific so additional procedures are needed. In investigations with *P. ramorum* comparing the frequency of isolation by ELISA and several molecular detection methods, the ELISA method was the most consistent across laboratories and type of host tissue (M. Garbelotto, pers. comm.); similar results would be expected for *P. kernoviae*. A lateral flow device developed by Pocket Diagnostics (Central Science Lab, York, U.K.) will also detect the *Phytophthora* genus (Lane et al., 2007). Both an ELISA and lateral flow format is in development to detect *P. ramorum* and *P. kernoviae*, although the test will not separate these two species (Agdia, Inc. pers. comm.).

Molecular diagnostics

Presently there are two real time TaqMan diagnostic procedures for detection of *P. kernoviae* that have been reported in the literature, one relying on the ITS region (developed at the CSL, York, U.K.; Defra SID5 Research Final Project Report) and the other using the spacer region in the ras-related protein *Ypt*1 (Schena et al., 2006; Schena et al., 2008). See appendix for details of real-time TaqMan methodology.

Molecular identification

There are a variety of molecular methods that can be used to confirm the identification of *P. kernoviae* isolates. Sequence analysis of the ITS region of the rDNA is commonly used to identify isolates in the genus *Phytophthora* and recent research has identified six nuclear and five mitochondrial genes that in some cases exhibit a greater level of sequence divergence than the ITS region among *P. kernoviae* and closely related species (www.phytophthoradb.org). Digestion of a PCR amplified *cox1* and *2* gene cluster can identify a number of *Phytophthora* spp. including *P. kernoviae* by restriction fragment length polymorphism (RFLP) analysis (Martin and Tooley, 2004; F. Martin, unpublished). A single-strand conformation polymorphism (SSCP) analysis of ribosomal DNA developed by Kong et al. (2003) can separate a number of *Phytophthora* species including *P. ramorum*, and *P. gonapodyides* (Kong, et al., 2004) as well as *P. kernoviae*

(Hong et al., unpublished). SSCP analysis using an automated sequencer for data collection can also identify *P. kernoviae* (T. Kubisiak, unpublished, in Martin et al., 2008). Check the USDA APHIS PPQ National Plant Germplasm and Biotechnology Laboratory (NPGBL) website (http://www.aphis.usda.gov/plant_health/identification/mdl.shtml) for the latest available approved molecular diagnostic for *P. kernoviae*.

Molecular markers to differentiate isolates of *P. kernoviae*

Thus far *P. kernoviae* has been recovered from only two countries, the U.K. and New Zealand. Sequence analysis of a mitochondrial gene from isolates recovered from these locations revealed 2 single nucleotide polymorphisms (SNPs) (F. Martin, unpublished). Two restriction enzymes have been identified that will differentiate isolates from these locations by RFLP analysis. While primers that would amplify the appropriate region from infected plant tissue have not been developed, given the sequence database for this region and the levels of intraspecific polymorphisms exhibited in sequence alignments it should be possible to develop the appropriate primers for RFLP template amplification or for development of isolate specific primers. Differences between UK and New Zealand isolates have not been identified in AFLP studies but only a small number of isolates have been studied (K. Hughes, pers. comm.).

V. Response

The response to all plant health emergencies in the United States is under USDA-APHIS-Plant Protection and Quarantine's authority delegated by the Secretary of Agriculture under the Plant Protection Act of 2000.

After a detection of *P. kernoviae* has been confirmed by a USDA-APHIS-PPQ recognized authority, APHIS, in cooperation with the appropriate State department(s) of agriculture, would be in charge of the response. The response would begin with an initial assessment. For a nursery site, the Rapid Assessment Team (RAT) consisting of state and federal *Phytophthora* experts and regulatory personnel would be deployed on-site to take additional plant, soil, and water samples in order to delimit (if possible) the infestation. A hold would be placed on the site to prevent the movement of regulated articles, conduct epidemiological investigations, and initiate environmental delimiting surveys outside the nursery grounds. Possible actions include quarantines of infested or potentially infested production areas, prohibiting movement of infected or potentially infected articles in commerce, host removal and destruction, requiring adherence to sanitary practices and the application of registered fungicides and disinfestants. Trace forward and trace back surveys would be needed at locations sending or receiving potentially infected nursery stock to/from the confirmed nursery. APHIS imposes quarantines and regulatory requirements to control and prevent the interstate movement of quarantine-significant pathogens or regulated articles (high risk host material), and works in conjunction with states which impose actions parallel to APHIS regulatory actions to restrict intrastate movement. The rapid assessment team will also attempt to ascertain if the introduction was intentional or accidental. If the organism in question is a select agent covered under the Agricultural Bioterrorism Act of 2002, federal and local

law enforcement may be involved in the initial assessment to determine if a bioterrorism event or biocrime event has occurred.

The USDA-APHIS-PPQ response will also depend on where *P. kernoviae* is found (forest, plantation, orchard vs. nursery) and how widespread it is based on the initial assessment by the rapid assessment team and associated delimitation surveys. If *P. kernoviae* is found in a nursery or orchard, attempts will be made to eradicate the pathogen through several measures including plant destruction/eradication, soil/surface disinfestation, trace-forwards, and trace-backs similar to management of *P. ramorum* in the United States. The practicality of eradication in a forest or plantation setting will be assessed by the rapid assessment team and a technical working group of *Phytophthora* experts.

VI. USDA Pathogen Permits

PPQ permit and registration requirements for plant diseases and laboratories fall under two authorities, the Plant Protection Act (7 CFR Part 330) and the Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331). Laboratories receiving suspect infected plant material or cultures are required to have PPQ permits. Laboratories possessing, using, or transferring select agents are required to be registered as select agent laboratories. However, diagnostic laboratories that identify select agents are exempt from this requirement if they complete an APHIS/CDC Form 4 and destroy or transfer infected material to a laboratory registered with the APHIS Select Agent Program within seven calendar days of a positive confirmation.

- The Plant Protection Act permit requirements apply to all plant pests and infected plant material, including diagnostic samples, regardless of their quarantine status. If any material is shipped interstate, it is a requirement that the receiving laboratory has a permit. For further guidance on permitting of plant pest material, consult the PPQ permit website at: http://www.aphis.usda.gov/ppq/permits/ or contact PPQ Permit Services at (301) 734-8758.

- Federal regulation by the Agricultural Bioterrorism Protection Act of 2002 (7 CFR Part 331) specifies requirements for possession, use, and transfer of organisms listed as select agents and toxins. Once a diagnostic laboratory identifies a select agent, it must immediately notify the APHIS Agriculture Select Agent Program, complete an APHIS/CDC Form 4 and submit within 24 hours, and either destroy or transfer the agent to a registered entity within 7 calendar days. In compliance with this Act, if a diagnostic laboratory held back part of a screened sample for voucher purposes and the sample that was forwarded to the USDA Beltsville Laboratory came back as positive for a select agent, the diagnostic laboratory is required to notify the APHIS Select Agent Program immediately. If the determination of the laboratory is to destroy the sample, this must take place within seven (7) calendar days of results notification and a PPQ Officer must witness the destruction of the sample on or before the 7-day period expires. Clarification of this and other information related to adherence to the select agent regulations is available on the following APHIS website: http://www.selectagents.gov/ , or call (301) 734-5960.

VII. Economic Impact and Compensation

The potential for *P. kernoviae* to become established in U.S. hardwood forests is considered high, as is the likelihood of it causing extensive mortality, therefore the potential economic and ecological impact to U.S. natural resources due to pathogen establishment is potentially very high. In a worst case scenario, *P. kernoviae* infection of oaks combined with oak decline, a disease complex, could forever change the dominant tree species in the eastern U. S. forests just like chestnut blight pathogen did over 100 hundred years ago by removing chestnut trees. Oak/hickory forests are estimated to cover 150 million acres in the eastern U.S. Oak is a dominant genus where it occurs. Loss of a significant portion of the U. S. oak distribution to *P. kernoviae* would be a series upheaval for the nation's forests.

In addition to potential economic, ecological, and aesthetic impacts to rural and urban forests, several different types of crops are potentially impacted by the introduction of *P. kernoviae:* nursery crops, orchards and forest products.

Forest ecosystems. It is not possible to estimate the ecological impact that an epidemic of *P. kernoviae* might have on U.S. forest ecosystems other than to say catastrophic. Not only plant diversity but also wildlife diversity would be at risk because the flora and fauna of forest ecosystems are highly interdependent. A major effort is needed by government and stakeholders to protect our U.S. forests by preventing the introduction of *P. kernoviae* or containing it should it be accidentally introduced.

Forest products. The threat of economic damage from *P. kernoviae* is considered significant in the United Kingdom's pest risk assessment. The full potential socio-economic and environmental impact is unknown but could be substantial, especially to forests or natural ecosystems.

Based on the reported host range of *P. kernoviae* in the U.K., we assume beech/maple/birch forests and oak/hickory forests would be at risk in the U.S. Beech and oak timber value as of 2007 are estimated to range between $22 million to $94 million dollars, annually. Although beech is not a major timber product, beech wood is used for flooring, furniture, novelties, railroad ties, baskets, pulp, and firewood. More importantly, beech nuts are a major component of the forest ecosystem as a crucial source of mast for a wide variety of birds and mammals. Ohio alone is estimated to have 90 billion beech trees. The maple/beech/birch forest type is estimated to cover 55 million acres in the eastern U.S. Currently, beech bark disease, a disease complex involving the beech scale insect *Cryptococcus fagi* and the canker-causing fungus *Nectria coccinea* var. *faginata*, has been expanding its geographic range from the original introduction in New England in the 1920s to beech in the southern Appalachians by the 1990s.

Nursery crops. The detection of *P. kernoviae* in nursery plants could cause significant economic loss and disruption of the nation's nursery industry with the anticipated restrictions of intra- and interstate plant movement and associated surveys and other

regulatory actions. Major producing states include California, Florida, Oregon, New Jersey, Pennsylvania, North Carolina, Tennessee and Washington.

Many nurseries may have already incurred costs associated with investment in preventative pest management practices to reduce the risk of *P. ramorum*. For instance in Washington State, 32 retail nurseries had over 17,000 plants destroyed worth an estimated $420,000 due to Emergency Action Notifications (Dart and Chastagner, 2007). In addition, the indirect costs at one Washington State nursery where only 109 plants were destroyed amounted to $30,000 for labor, disposal fees and mitigation measures. The additional investments by nurseries in inventory and labor practices and by applying fungicide to limit exposure to *P. ramorum* were estimated to be less than three percent of all production expenses for the average nursery (Zwane and Gilless, 2005).

Based on the 2006 USDA NASS Nursery Crops Summary, the U.S. value of wholesale nursery plants including broadleaf evergreens (pieris, magnolia rhododendron, and holly), deciduous flower and shade trees (magnolia and oak), and other ornamentals (ivy) that are hosts of *P. kernoviae* totaled $2.1 billion. As the host range of *P. kernoviae* increases this segment of the $4.6 billion nursery industry at risk will increase.

U.S. risk map. A subgroup of contributors at USDA APHIS PPQ CPHST and North Carolina State University generated a U.S. risk map for *Phytophthora kernoviae* that was based on four variables: climate, overstory host density, host sporulation potential, and introduction pathways.

Climate risk was based on the pathogen's moisture and temperature requirements. We generated the climate risk map using an infection model in the NAPPFAST modeling system. Based on climate the entire eastern U.S. and west coast are at severe risk for *P. kernoviae* establishment. (Appendix, Fig. 5)

Overstory host density was based on the basal area of economically important species that have the potential to be injured or killed by the pathogen. One included species, tulip tree (*Liriodendron tulipifera*), is a known host for the pathogen. White oaks (*Quercus* section *Quercus*) and American beech (*Fagus grandifolia*) were assumed to be suitable overstory hosts because related species (*Quercus robur* and *Fagus sylvatica*) have been shown to be susceptible in the U.K. Host density is greatest in the eastern U.S. The central Appalachians are at particularly high risk whereas forests on the west coast are at moderate risk. (Appendix, Fig. 6)

The host sporulation potential described the likelihood that the sporulating hosts would be available for rapid epidemic development. The weighted risk index was created for sporulating hosts that incorporated, in increasing importance, (1) deciduous shrubs, (2) evergreen shrubs, (3) deciduous or sparsely distributed evergreen mid-story species, and (4) tall, densely growing evergreen mid-story species. The two species comprising the latter group were *Umbellularia californica*, which has been shown to be a sporulating host for *P. ramorum*, and *Rhododendron maximum*, which is closely related to *R. catawbiense*, another demonstrated sporulator. The host sporulation potential indicates high risk in the eastern U.S. particularly the central Appalachians as well as in coastal areas along the Oregon-California border. (Appendix, Fig. 7)

We quantified the effects of introduction pathways using a ranked classification of wildland-urban interface (WUI). This pathways map estimated the potential for the pathogen to spread into the forest environment if it were present in adjacent or nearby developed landscapes. Numerous regions of the U.S. are at risk as introduction pathways include the northeast, Appalachia extending into Georgia, upper Michigan, the Rockies west of Denver, the Sierra Nevada of California and the Puget Sound area of Washington. (Appendix, Fig. 8)

Our composite risk map was created by multiplying the four variable risk maps and dividing by the resulting maximum value. Our results estimated that the east coast was more at risk than the west (Fig. 4). The eastern slope of the Appalachian Mountains was at greatest risk due to the confluence of human development (primarily residential activities associated with landscaping home grounds) and hosts. The limiting factors appeared to be the distribution of overstory and sporulating hosts. We note that this is a preliminary risk map and future iterations will incorporate additional parameters, e.g. human population, to increase the precision of the risk estimate.

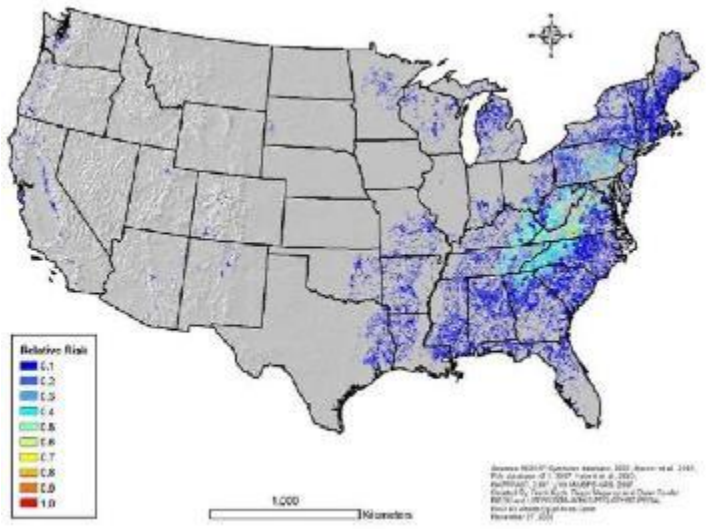


Figure 4. Risk map for *Phytophthora kernoviae* based on climate, overstory host density, host sporulation potential and introduction pathways. Red is highest risk.

Sources: Brasier et al., 2005, Huberli et al., 2003. NAPPFAST 2007. UWI-M/USFS-NRS 2007.

Created by Frank Koch, Roger Magarey, and Glenn Fowler. North Carolina State University, and USDA-APHIS-PPQ-CPHST-PEARL. NAD 83 Albers Equal Area Conic. November 27, 2007.

Detailed information on the parameters used to construct the various risk maps is included in the appendix.

Compensation

The Plant Protection Act of 2000 under which APHIS operates does not allow for compensation to growers except under a declaration of Extraordinary Emergency by the Secretary of Agriculture. Because the manner in which *P. kernoviae* spreads is not predictable, the eradication strategy necessarily calls for the destruction of trees and nursery stock that are asymptomatic. Growers and landowners, on their own, would not have the incentive to destroy nursery stock or cut down trees that appear uninfected as would be necessary in an eradication program.

For nurseries, the APHIS Confirmed Nursery Protocol for *P. ramorum* dictates the eradication of asymptomatic nursery stock that is commingled with confirmed infected

plants. Thus, in the effort to eradicate the pathogen at the nursery site, growers are mandated to destroy nursery stock that is uninfected in blocks of plants where the pathogen is confirmed.

VIII. Mitigation and Disease Management

Currently the USDA APHIS PPQ Emergency and Domestic Programs Emergency Planning and Preparedness group is developing a New Pest Response Guideline for Exotic *Phytophthora* species in Forest and Nursery systems. This set of guidelines will be utilized in the event of a detection of an exotic *Phytophthora* species to respond to the emergency.

Prevention, early detection and rapid response are key elements of this *P. kernoviae* recovery plan. Regulatory exclusion is our primary defense, but integrated pest management practices for nurseries that reduce the likelihood of establishment and increase the likelihood of early detection are also important.

Exclusion. USDA APHIS should immediately consider promulgating regulations requiring testing and certification of nursery stock from areas where *P. kernoviae* is known to occur (i.e., the U.K. and New Zealand). In the interim, imports from these areas should be closely examined by inspectors upon arrival, kept at least 7 feet away from other plants in the nursery, not sprayed with fungicides for 3 to 6 months to allow any latent symptoms to develop, and then be examined by state regulatory personnel before incorporating into the rest of the nursery.

Logs of known hosts of *P. kernoviae* can carry the organism in the bark and outer wood (Brown and Brasier, 2007). Logs from infested areas should be treated according to established guidelines in the Universal Import Option for logs, chips, raw lumber, and other unmanufactured wood products.

Eradication. Any first detection of *P. kernoviae* in a nursery or in the environment should be eradicated if possible. APHIS has a Confirmed Nursery Protocol in place for eradication of *P. ramorum* from nurseries that should be used as a model for *P. kernoviae.* Their Forest Wildland Protocol, modeled on that used by state and federal regulators in Oregon, could be used as a guide for a *P. kernoviae* introduction in a U.S. forest. It should be noted however, that the first detection of a new pathogen might justify more stringent regulatory actions to eradicate the organism, as opposed to those implemented to contain an existing pathogen population, as was the case for *P. ramorum*. For example, because so little is known about the host range of *P. kernoviae*, all plant movement from the infested site should be restricted for 90 days, rather than just known hosts and associated plants. Access to the contaminated area should be limited, and protective clothing worn. Shoe wash stations should be required, and water runoff should be contained and treated.

Best Management Practices (see Suslow, 2008 for complete nursery operations list)

Nursery operations:

Exclusion of the pathogen. Develop best management practices (BMPs) to prevent *P. kernoviae* from entering the nursery.

- Know source location of plants: Make sure that nursery stock is propagated from plants on-site or is purchased from a nursery that is practicing good sanitation.
- Meet trucks carrying incoming nursery stock at the loading dock. Inspect all plants (buy-ins, transfers, and returns), regardless of origin, for symptoms of *Phytophthora* infection.
- Off-load known high risk hosts to an area that can be cleaned of leafy debris. Sweep the plant debris from the receiving area and the delivery truck. Collect debris and dispose of by bagging, burning, or burying off site.
- Avoid product returns to your nursery.
- Make sure that trucks used for shipping high-risk plants are cleaned between shipments to remove plant debris and mud. Provide a way to wash the truck undercarriage and tires.

Water management

- Irrigate in a manner to avoid prolonged leaf wetness. Avoid overhead irrigation of known host plants. Properly time irrigation to reduce conditions favorable for disease development.
- Prevent standing water in the nursery.

Sanitation

- Remove and dispose of leaf debris from known hosts by bagging, burning, or burying off-site.
- After every crop rotation, disinfect propagation mist beds, sorting area, cutting benches, machines and tools to minimize the spread or introduction of pathogens.
- Ensure that runoff from all cull piles is directed away from soil components, soil mixing area, and growing beds to prevent contamination. Ensure cull pile is clearly separated from soil mix components.
- Take cuttings from apparently healthy stock plants and dip cuttings in an approved disinfectant solution before sticking.
- Put a barrier between the native soil and nursery stock to prevent splash dispersal of pathogens from the potentially infested ground.
- Use clean soil and prevent contamination from potentially infested compost, bark, or other organic components of the substrate.
- Adequately control weeds on the nursery site as they can potentially harbor the pathogen.

Training and inspection by nursery personnel

- Each nursery should designate certain individuals to be responsible for training and inspection.
- Train all nursery personnel to recognize and report all insect and disease problems.

Forest managers:

Forest managers have best management practices in place for the different types of forest tree stands they manage. The biggest threat to forest introduction of *P. kernoviae* is from infected nursery crops inadvertently planted in the landscape. Spread of

inoculum from infected landscape plants through soil and watercourses into forested areas could result in establishment and disease foci.

Epidemiological/risk models. The U.K. Central Sciences Laboratory maintains a host list (see Web Resources), but the full host range potential of *P. kernoviae* is unknown. No risk maps or decision models have been developed to date for the U.K., but contributors to this recovery plan developed a risk map for the U.S. (see above).

Education. CSREES and States should produce educational materials to inform growers about the threat and symptoms of *P. kernoviae*. (see section X)

Germplasm. The pathogen is known to affect hosts in nine plant families. No resistance has been detected, but little research has been accomplished to date.

Chemical management. A number of fungicides including several with new chemistry are available that prevent infection of plants by *Phytophthora* spp. However, no information is available about the specific sensitivity of *P. kernoviae* to available products. Fungicides used to manage *Phytophthora* spp. generally do not kill the organism. They can prevent it from becoming established, and stop growth within the plant temporarily. After 2 to 3 months without additional fungicide treatments, the organism can resume growth.

IX. Infrastructure and Experts

A minimal infrastructure exists to investigate diseases caused by *Phytophthora* species, consisting of experts at universities and in government, websites, and the World Phytophthora Collection. A Phytophthora database (www.phytophthoradb.org) project exists with cultural and molecular data on most or all described species. The World Phytophthora Collection at the University of California-Riverside currently contains about 6500 isolates representing at least 90 distinct taxonomic species or distinct as yet undescribed taxa. The isolates are from worldwide sources and a wide range of hosts. USDA-ARS facilities at Ft. Detrick, MD are available for containment studies for *Phytophthora* species not currently in the U.S. and there is an active *P. kernoviae* project there.

In terms of industry's role, there is a current active industry group led by Karen Suslow, Hines Horticulture, Vacaville, CA involved in setting research priorities for work with the sudden oak death pathogen, *Phytophthora ramorum*. Perhaps the focus of her group could be expanded to include *P. kernoviae*.

In the forestry and forest products area, the International Union of Forest Research Organizations Working Party 7.02.09, Phytophthoras in Forests and Natural Ecosystems with Everett Hansen, Oregon State University, as U.S. coordinator, brings together Phytophthora researchers with expertise in forests and includes researchers in the U.K. studying *P. kernoviae*. Strategies developed against *P. ramorum* could be tried on *P. kernoviae*. For example, the USDA Forest Service, Pacific Southwest Research Station, national research program developed to address *Phytophthora ramorum* is a model of research response to a new exotic invasive forest *Phytophthora* species. The California

Oak Mortality Task Force, www.suddenoakdeath.org, a non-profit organization, coordinates over 75 organizations and agencies working on *P. ramorum.*

The forest health committees of the National Association of State Foresters and the Society of American Foresters; the USDA Forest Service, State and Private Forestry, Forest Health Protection network of forest health specialists; the National Plant Board; the Nature Conservancy; National Invasive Species Council; National Plant Diagnostic Network; American Phytopathology Society, North American Forestry Commission, North American Plant Protection Organization and many others would be key partners for communication, monitoring, management and outreach.

We have searched the USDA/CSREES Current Research Information System website at http://cris.csrees.usda.gov/ which holds a concise description of nearly all the public research on plant disease in the U.S. Only one project on *P. kernoviae* dealing with oospore germination and viability was found (CRIS 1920-22000-036-00D**)**. However, there are a number of efforts to clarify taxonomy and develop identifying markers for most species of the *Phytophthora* genus, including *P. kernoviae*. The Sudden Oak Death/*Phytophthora ramorum* research program, USDA Forest Service, Pacific Southwest Research Station funds a few studies that compare biology of *P. ramorum* and *P. kernoviae* in the U.K.

The following experts on *Phytophthora* sp. have been identified:

Gloria Abad - knowledge of *Phytophthora* identification, Molecular Diagnostics Lab (MDL), USDA-APHIS-PPQ-PHP-PSPI-MDL, BLDG 580, BARC-E, Powder Mill Road, Beltsville, MD 20705, 301-504-5700 (ext# 323), fax 301-504-6124, Gloria.Abad@aphis.usda.gov

Kerry Britton – expert on invasive species research, management and policy, USDA Forest Service, Research & Development, 1601 N. Kent Street, RPC-4, Arlington, VA 22209, 703-605-4170, kbritton01@fs.fed.us

Russ Bulluck – expert on biology and response to *Phytophthora* diseases. USDA, APHIS, PPQ, Center for Plant Health Science Technology, 1730 Varsity Drive, Raleigh, NC 27607, 919-855-7646, fax 919-855-7480, russ.bulluck@aphis.usda.gov

Michael D. Coffey - expert on general aspects of *Phytophthora*, morphological identification, and curator of World Phytophthora Collection, Department of Plant Pathology, University of California, Riverside, CA 92521, 951-827-4764, fax 951-827-4764/4294, coffey@ucr.edu, m_d_coffey@yahoo.com

Susan J. Frankel – expert on research, management, and policy for *P. ramorum* and other forest Phytophthoras, 800 Buchanan Street, West Annex Building, Albany, CA 94710-0011, 510-559-6472, sfrankel@fs.fed.us

Mannon Gallegly - expert on morphological identification of *Phytophthora* species, West Virginia University (retired), Morgantown, WV 26506, 304-293-3911, fax 304-293-2872, megall@wvu.edu

Matteo Garbelotto - expert on *P. ramorum* particularly molecular and forest aspects. University of California, Berkeley, ESPM Ecosystem Sciences,137 Mulford Hall #311, Berkeley, CA 94720, 510-643-4282 , fax**:** 510-643-5436, matteo@nature.berkeley.edu

Nik Grunwald - expert on *P. ramorum* and *P. infestans*, knowledgeable about *P. kernoviae*, USDA-ARS, Horticultural Crops Research Lab, 3420 N. W. Orchard Ave., Corvallis, OR 97330, 541-738-4049, grunwaln@onid.orst.edu

Everett Hansen - expert on diseases caused by *Phytophthora*, Oregon State University, Department of Botany and Plant Pathology, Cordley Hall 2082, Corvallis, OR 97331, 541-737-3451, fax 541-737-3573, hansene@science.oregonstate.edu

Chuanxue Hong - expert on aquatic ecology of *Phytophthora* species and irrigation water decontamination technology, molecular pathogen identification and detection, Virginia Polytechnic Institute and State University, Hampton Roads Agricultural Research and Extension Center, 1444 Diamond Springs Road, Virginia Beach, VA 23455, 757-363-3908, fax 757-363-3950, chhong2@vt.edu

Kelly Ivors - expert on molecular aspects of Phytophthora pathogens esp. *P. ramorum*, NC State University, Mountain Hort. Crops Research & Extension Center, 455 Research Drive, Fletcher, NC 28732, 828-684-3562 x 143, fax 828-684-8715, kelly_ivors@ncsu.edu

Steve Jeffers - expert on epidemiology and detection of *Phytophthora* species from soil and streams, Diseases of Ornamental Crops & Trees, Department of Entomology, Soils, & Plant Sciences, 120 Long Hall, Box 340377, Clemson University, Clemson, SC 29634, 864-656-7157, sjffrs@clemson.edu

Jonathan Jones - expert on regulatory aspects of *Phytophthora* diseases, USDA-APHIS, National Phytophthora ramorum Program Manager, USDA-APHIS-PPQ Invasive Species and Pest Management, 4700 River Rd. Unit 160 Suite 5A04.4, Riverdale, MD 20737, 301-734-5038, fax 301-734-8584, jmjones@aphis.usda.gov

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Steve Oak – expert on forest surveys and watercourse detection of *Phytophthora*, USDA FS, Forest Health Protection, 200 W.T. Weaver Blvd, Asheville, NC 28804, 828- 257-4322 fax 828-257-4856, soak@fs.fed.us

Jennifer Parke – expert on *Phytophthora* management in horticultural nurseries, extension and education, Oregon State University, Department of Crop and Soil Sciences, ALS 3017, Oregon State University, Corvallis, OR 97331, (541) 737-8170, jennifer.parke@orst.edu.

David Rizzo – expert on *Phytophthora* ecology and management in forests, Department of Plant Pathology, University of California, One Shields Ave., Davis, CA 95616, 530-754-9255, dmrizzo@ucdavis.edu.

Paul Tooley - expert on *Phytophthora* diseases, USDA-ARS, Foreign Disease-Weed Science Research Unit, 1301 Ditto Ave., Ft. Detrick, MD 21702, 301-619-7338, fax 301-619-2880 paul.tooley@ars.usda.gov

Tim Widmer - expert on *Phytophthora* diseases, currently working on biology of *P. kernoviae*, USDA-ARS, Foreign Disease-Weed Science Research Unit, 1301 Ditto Ave., Ft. Detrick, MD 21702, 301-619-7338, fax 301-619-2880 tim.widmer@ars.usda.gov

X. Research, Extension, and Education Priorities

Research Priorities

The following lines of research are needed to enhance understanding of the biology, detection, and treatment of *P. kernoviae*. The research priorities listed below are broken down into three groups based on relative importance. Priorities within groups are considered equal. The top group is listed as 'Most Important', the second group is 'Highly Important,' and the last group is 'Needs Evaluation' – important but less so than the first two groups. Currently, there is very little current research being performed on *P. kernoviae* in the U.S.

Most Important

- Develop/adapt culture and molecular detection methods to distinguish *P. kernoviae* from other *Phytophthora* spp. Test molecular diagnostic techniques with DNA extracted in the U.K. (cooperative program) from environmental samples. Validate molecular tests with real samples and determine sensitivity of methods for pathogen detection on different hosts (cooperative program). Stress the importance of detecting multiple targets and flexibility of adding new targets when evaluating options. It would also be desirable to have a multiplexed amplification including a plant DNA marker to serve as an internal control of DNA quality.

- Determine susceptibility of key native North American species (trees and understory plants) and important ornamental plant species.

- Expand *Phytophthora* surveys in U.S. watercourses and nurseries to monitor for *P. kernoviae* and other invasive *Phytophthora* species.

Highly Important

- Investigate the role of oospores in *P. kernoviae* life cycle. Determine incidence of oospores in naturally infected host tissue and their survivability in host tissue and soil, and control measures to mitigate.

- Research on wildland treatment, eradication and management for forest Phytophthoras is needed to develop effective response strategies. For example, determine effectiveness of removing sporulation hosts (i.e., to what distance) in stopping the spread of *P. kernoviae*.

- Investigate sensitivity of *P. kernoviae* to fungicides used to control other oomycetes.

- Determine factors that affect infection including zoospore biology, inoculum production, symptom expression and symptoms on various host species. Knowledge of temperature and moisture requirements for sporulation and infection on different host species is needed to make predictions about conditions under which disease will occur and for risk analysis.

- Determine geographic population diversity of *P. kernoviae* using molecular tools such as SNPs and SSRs. This will help in determining the exotic nature of this pathogen in infested areas, as well as allowing for the detection of new introductions in infested areas.

Needs Evaluation

- Determine origin and pathways for introduction of *P. kernoviae.* In addition to providing evolutionarily significant knowledge, determining the origin of the pathogen could provide us with some ideas on how to coexist with this pathogen were it to become established in the U.S. and insight into how to prevent introductions.

- *P. kernoviae* is related to the very poorly understood "Gondwanaland" clade of *Phytophthora* species. So, basic phylogenetics and evolutionary biology investigations may yield a better understanding of the nature of this pathogen.

- The recent discovery of *P. pinifolia*, on pine needles in Chile, underscores the potential threat from foliar Phytophthoras. Pine needles of U.S. trees need to be sampled and tested for *Phytophthora* species.

- Reassess and invigorate biological control approaches for containment of *P. kernoviae* and other exotic pathogens after accidental introduction.

Extension Priorities

- Identify *Phytophthora* spp. similar in morphology and DNA sequence to *P. kernoviae* and compare host range.

- Morphological and DNA sequence information for *P. kernoviae* made available for federal and state investigators (first responders) conducting SOD nursery and forest surveys to expand detection for this pathogen.

- Develop standardized survey and monitoring protocols and disseminate to first detectors.

- Determine *P. kernoviae* symptoms on different host species and how to differentiate from symptoms caused by other *Phytophthora* species.

- Compile and develop digital images of disease symptoms and pathogen morphology on relevant hosts and distribute via the NPDN.

- Describe measures necessary to report and confirm potential *P. kernoviae* detections.

- Train and certify accredited NPDN labs and conduct diagnostic ring testing as soon as standard operating procedures and diagnostic assays are available.

- Develop fact sheets and briefing papers on the threats from *Phytophthora* spp. Compile and disseminate all survey information on detection of *Phytophthora* spp. in horticultural nurseries with a focus on new species detections.

- Work with growers to improve management of *Phytophthora* spp. in nurseries, forest, orchards, and agricultural systems. The 'Grower Assisted Inspection Program' under consideration in Oregon is an example.

- Investigate incidence of *Phytophthora* spp. on imported nursery stock to better define risk from the nursery stock pathway.

- Develop a national Phytophthora diagnostic database.

- Continue to refine and promote the best management practices (BMP) program in nursery and floral industry.

Education Priorities

- Develop an effective method for *P. kernoviae* education in North America that would include websites for *P. kernoviae* information like that currently available for sudden oak death at http://www.suddenoakdeath.org/ and at APHIS http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/index.shtml .

- Provide speakers on biosecurity threats from *P. kernoviae* and other *Phytophthora* spp. for the National Plant Board, Interagency Forum on Invasive Species Research and other venues where key stakeholders are assembled.

- Develop database of *P. kernoviae* citations and distribute via the National Plant Diagnostic Network website and researchers.

- Prepare picture field guides and educational materials for the extension and crop advisor community, and garden centers and educators to increase likelihood that potential introductions will be noticed by the general public. Advise how and where to take samples from suspicious material.

- Provide pictures of symptoms of *P. kernoviae* on different host species to garden centers, botanical gardens, and educators to increase likelihood that potential introductions will be noticed by the general public.

- Provide training to Master Gardeners to recognize symptoms and move materials to the NPDN lab nearest them.

- Strive to reach out to the public and raise the awareness of increasing risk of unintentional introduction to this country of *P. kernoviae* and other exotic pathogens as the agricultural economy further globalizes and the world gets smaller (more international travel).

References

Brasier, C.M., Denman, S., Brown, A. and Webber, J.F. 2004. Sudden oak death *(Phytophthora ramorum)* discovered on trees in Europe. Mycol. Res. 108:1107–1110.

Brasier C.M., Beales P.A., Kirk S.A., Denman S., and Rose J. 2005. *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. Mycol. Res. 109:853-859.

Brown, A.V. and Brasier, C.M. 2007. Colonization of tree xylem by *Phytophthora ramorum, P. kernoviae* and other *Phytophthora* species. Plant Pathol. 56:227–241.

Cushman, J. H., Cooper, M.,. Meentemeyer, R. K, and Benson, S. 2008. Human activity and the spread of *Phytophthora ramorum*. In Frankel, Susan J.; Kliejunas, John T.; Palmieri, Katharine M., tech. coords. Proceedings of the sudden oak death third science symposium. Gen. Tech. Rep. PSW-GTR-214. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 491 p.

Dart, N. L., and Chastagner, G. A. 2007. Estimated economic losses associated with the destruction of plants due to *Phytophthora ramorum* quarantine efforts in Washington State. Online. Plant Health Progress doi:10.1094/PHP-2007-0508-02-RS.

Denman, S., Moralejo, E., Kirk, S.A., Orton, E., and Whybrow, A. 2008. Sporulation of *Phytophthora ramorum* and *Phytophthora kernoviae* on asymptomatic foliage and fruit. pp. 201-207. In Frankel, Susan J.; Kliejunas, John T.; Palmieri, Katharine M., tech. coords. Proceedings of the sudden oak death third science symposium. Gen. Tech. Rep. PSW-GTR-214. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 491 p.

Elliott, C. G., Hendrie, M. R. & Knights, B. A. 1966. The sterol requirement of *Phytophthora cactorum*. Journal of General Microbiology 42: 425–435.

Erwin, D. and Ribero, O. 1995. *Phytophthora* Diseases Worldwide. APS Press.

Fichtner, E.J., Rizzo, D.M., Kirk, S.A., Whybrow, A., and Webber, J.F 2007. Root associations of *Phytophthora ramorum* and *Phytophthora kernoviae* in UK woodlands. IUFRO Phytophthoras in Forests & Natural Ecosystems. (Abstr.).

Gallegely, M. E., and Hong, C. X. 2008. *Phytophthora*: Identifying Species by Morphology and DNA Fingerprints. APS Press, St. Paul, MN 168 pp.

Hansen, E.M., Reeser, P.W., Davidson, J.M., Garbelotto, M., Ivors, K.L., Douhan, L. and Rizzo, D.M. 2003. *Phytophthora nemorosa*, a new species causing cankers and leaf blight of forest trees in California and Oregon, USA. Mycotaxon 88: 129–138.

Hansen, E. M., Kanaskie, A., Prospero, S., McWilliams, M., Goheen, E. M., Osterbauer, N., Reeser, P., and Sutton, W. 2008. Epidemiology of *Phytophthora ramorum* in Oregon tanoak forests. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 38:1133-1143.

Hüberli, D., Van Sant, W., Swain, S., Davidson, J. and Garbelotto, M. 2003. Susceptibility of *Umbellularia californica* to *Phytophthora ramorum*. In Eighth International Congress of Plant Pathology. Christchurch, New Zealand.

Kong, P., Hong, C. X., Richardson, P. A., and Gallegly, M. E. 2003. Single-strand conformation polymorphism of the ribosomal DNA for rapid species differentiation in genus *Phytophthora*. Fungal Genetics and Biology 39:238-249.

Kong, P., Hong, C. X., Tooley, P. W., Ivors, K., Garbelotto, M., and Richardson, P. A. 2004. Rapid identification of *Phytophthora ramorum* using PCR-SSCP analysis of ribosomal DNA ITS-1. Letters in Applied Microbiology 38:433-439.

Lane C.R., Beales, P.A., Hughes, K.J.D., and Giltrap, P.M. 2007. Overview of diagnostic strategies for *Phytophthora ramorum* and *P. kernoviae*. Aspects of Applied Biology 83:39-42.

Mascheretti, P.J.P., Croucher, A. Vettraino, S., Prospero,ý and Garbelotto, M. 2008. Reconstruction of the Sudden Oak Death epidemic in California through microsatellite analysis of the pathogen *Phytophthora ramorum.* Molecular Ecology 17:2755-2768.

Martin, F.N., and Tooley, P.W. 2004. Identification of *Phytophthora* isolates to species level using RFLP analysis of a PCR amplified region of mitochondrial DNA. Phytopathology 94: 983-991

Martin, F.N., Tooley, P.W. and Blomquist, C. 2004. Molecular detection of *Phytophthora ramorum,* the causal agent of sudden oak death in California, and two additional species commonly recovered from diseased plant material. Phytopathology 94:621-631.

Martin, F.N., Coffey, M.D., Zeller, K., Hamelin, R.C., Tooley, P., Garbelotto, M., Hughes, K.J.D., Kubisiak, T., Bilodeau, G.J., Levy, L., Blomquist, C. and Berger, P.H. 2008. Evaluation of molecular markers for *Phytophthora ramorum* detection and identification; testing for specificity using a standardized library of isolates. Phytopathology : In review.

Parke, J.L. and Lewis, C. 2007. Root and stem infection of rhododendron from potting medium infested with *Phytophthora ramorum*. Plant Dis. 91:1265-1270.

Parke, J.L., Oh, E., Voelker, S., Hansen, E.M., Buckles, G., and Lachenbruch, B. 2007. *Phytophthora ramorum* colonizes tanoak xylem and is associated with reduced stem water transport. Phytopathology 97:1558-1567.

Ramsfield, T.D., Dick, M. A. Ross E. Beever, R.E. and Horner, I.J. 2007. *Phytophthora kernoviae* – of southern hemisphere origin? 4th IUFRO Phytophthoras in Forests & Natural Ecosystems, August, 2007

Sansford, Claire, Brasier, Clive, and Inman, Alan. 2005. Central Sciences Laboratory Pest Risk Assessment. www.defra.gov.uk/planth/pra/forest.pdf

Schena, L., and Cooke, D.E.L. 2006. Assessing the potential of regions of the nuclear and mitochondrial genome to develop a "molecular tool box" for the detection and characterization of *Phytophthora* species. Journal of Microbiological Methods 67:70-85.

Schena, L., Hughes, K.J.D and Cooke, D.E.L. 2006. Detection and quantification of *Phytophthora ramorum, P. kernoviae, P. citricola,* and *P. quercina* in symptomatic leaves by multiplex real-time PCR. Molecular Plant Pathology 7:365-379.

Schena, L.; Duncan, J.M.; and Cooke, D. E. L. 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.

Suslow, K. 2008. Recommended industry best management practices for the prevention of *Phytophthora ramorum* introduction in nursery operations. In Frankel, Susan J.; Kliejunas, John T.; Palmieri, Katharine M., tech. coords. Proceedings of the sudden oak death third science symposium. Gen. Tech. Rep. PSW-GTR-214. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 491 p.

Tjosvold, S. A., Chambers, D. L., Davidson, J. M. and Rizzo, D. M. 2002. Incidence of *Phytophthora ramorum* inoculum found in soil collected from a hiking trail and hikers' shoes in a California park in: Sudden Oak Death, a Science Symposium: The State of Our Knowledge Monterey, CA., December 17-18, 2002.

Tjosvold, S. A., D.L. Chambers, and E. Fitchner. 2005. Epidemiology of *Phytophthora ramorum* infecting rhododendrons under simulated nursery conditions. Pages 459-461 in: Proceedings of the sudden oak death

second symposium: the state of our knowledge Gen. Tech. Rep. PSW-GTR-196. Albany, CA: Pacific Southwest Research Station, Forest Service, U.S. Department of Agriculture; Monterey, CA

Shishkoff, N. 2007. Persistence of *Phytophthora ramorum* in soil mix and roots of nursery ornamentals. Plant Dis. 91:1245-1249.

Tooley, P.W., Martin, F.N., Carras, M.M. and Frederick, R.D. 2006. Real-time Fluorescent PCR Detection of *Phytophthora ramorum* and *Phytophthora pseudosyringae* Using Mitochondrial Gene Regions. Phytopathology 96: 336-345.

Webber, J., and Rose, J. 2008. Dispersal of aerial and soilborne Phytophthoras by human vectors. In Frankel, Susan J.; Kliejunas, John T.; Palmieri, Katharine M., tech. coords. 2008. Proceedings of the sudden oak death third science symposium. Gen. Tech. Rep. PSW-GTR-214. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station. 491 p.

Zwane, A. P. and J. K. Gilles. 2005. The Economic Impact of Sudden Oak Death on the California Nursery Sector. *Unpublished report* prepared for the USDA-Forest Service, Pacific Southwest Research Station.

Web Resources

Investigation of alternative eradication control methods for *P. ramorum* and *P. kernoviae* on/in plants – Central Science Laboratories http://www.defra.gov.uk/planth/ramorum/phe2122reports.pdf

Phytophthora kernoviae found on *Vaccinium*—Information Bulletin, 14 January2008
UK Department for Environment, Food, and Rural Affairs
http://www.defra.gov.uk/news/2008/080114b.htm

P kernoviae -- NAPPO Phytosanitary pest alert
http://www.pestalert.org/viewNewsAlert.cfm?naid=16

P kernoviae -- UK Department for Environment, Food, and Rural Affairs (Main page)
http://www.defra.gov.uk/planth/pkernovii.htm

P. kernoviae – UK DEFRA, Host list
http://www.defra.gov.uk/planth/kernovii/kernhost.pdf

P. kernoviae – UK DEFRA, Threat to trees and woodlands
http://www.defra.gov.uk/planth/pestnote/kern.pdf

P. kernoviae – UK DEFRA, Research Project Final Report, 2005. (PCR diagnostics)
http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=12762&FromSearch=Y&Publisher=1&SearchText=kernoviae&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

P. kernoviae – UK DEFRA, Research Project Final Report, 2006. (Control strategies)
http://www.defra.gov.uk/science/Project_Data/DocumentLibrary/PH0318/PH0318_6105_FRP.pdf

P. kernoviae -- Great Britain, Forestry Commission, (Background & status)
http://www.forestry.gov.uk/forestry/infd-66jlgb

Appendix
IV. Monitoring Detection and Identification

Molecular detection methods

Presently there are two real time TaqMan diagnostic procedures for detection of *P. kernoviae* that have been reported in the literature, one relying on the ITS region (developed at the CSL, York, UK; Defra SID5 Research Final Project Report) and the other using the spacer region in the ras-related protein *Ypt*1 (Schena and Cooke, 2006).

ITS Procedure – three different primer pairs have been developed, one based on the ITS1 region and two designed from sequences in the ITS2 region (the primers are essentially the same for both of the ITS2 markers except for a G/T substitution in the third base from the 3' end for the forward primer to enhance specificity of the third primer pair). The ITS1 marker was more sensitive than the ITS2 with a calculated detection limit of 1fg, 1000 fg, and 100 fg, respectively. All three marker systems identified multiple isolates of *P. kernoviae* and were specific when tested against culture extracted DNA from 29 *Phytophthora* spp. at a concentration of 100 ng, however, this level of sensitivity and specificity was not fully supported when field samples were tested. The ITS 1 marker system was evaluated with DNA extracted from 225 culture grown fungal pathogens recovered from diseased plant tissue. Of these cultures, 93 were *P. kernoviae* and had a $C_t < 30$ but there were some false positives; 2 cultures had a $C_t < 30$ and 62 cultures (some of these were *P. ramorum*) had a $C_t < 40$. DNA extracted from infected plant tissue was also analyzed, out of 76 infected leaf samples the 14 that were isolation positive for *P. kernoviae* were PCR positive (all but one had a $C_t < 30$) but 27 other samples infected with other pathogens (most commonly *P. ramorum*) had a C_t between 34 and 40. Based on these observations a positive diagnosis is made at the CSL in the UK if the final reading has a $C_t < 30$ with a retest/reisolation for C_t between 30 and 36. Similar results were observed with the ITS2 marker with the modified forward primer but the threshold C_t was increased to 36. One advantage of these markers is they can be multiplexed with a positive control for plant DNA (based on the cox gene) but the technique has not been worked out for multiplexing with the CSL *P. ramorum* marker system. The marker system has been tested with a 96 well ABI Prism 7700 and the Cepheid SmatCycler II at the CSL Laboratory in York, UK and is the standard detection method used in the UK.

Ras-related protein *Ypt*1 – This nuclear encoded gene contains introns that are conserved within a species but divergent between species. Sequences from introns 3, 4, and 5 were used to develop a multiplexed TaqMan real-time PCR capable of simultaneously detecting *P. ramorum, P. kernoviae, P. citricola,* and *P. quercina* with a sensitivity of 100 fg target DNA (Schena et al. 2006). When tested with 77 DNA samples extracted from naturally infected plant material at the CSL the *P. kernoviae* marker correctly identified 14 of the 15 positive samples. More recently, the same group developed a "molecular tool box" for detecting of fifteen species of *Phytophthora* attacking forests and natural ecosystems (Schena et al., 2008). A universal TaqMan marker pair based on a region of the large subunit of the rDNA was also developed to detect a wide range of organisms so it could be used as a positive control to assess the quality of the extracted DNA. Multiplexing this universal marker with the *P. kernoviae*

marker so a second amplification is not needed to assess DNA quality has not been done but should be possible (D. Cooke, pers. comm.).

Marker systems yet to be published – A TaqMan real-time PCR detection technique based on spacer sequences between the mitochondrially encoded *cox1* and *2* genes is in the final stages of validation with field samples (P. Uribe and F. Martin, unpublished). This is the same approach that has been used for developing diagnostic markers for *P. ramorum* (Martin et al. 2004; Tooley et al. 2006) and tests with 71 *Phytophthora* spp. have shown the marker to be specific to *P. kernoviae*. The amplification is multiplexed with an internal control marker for plant DNA to evaluate the quality of the extracted DNA at the same time as pathogen detection.

VII. Economic Impact and Compensation

Methods used for risk mapping.

Climate risk (fig. 5)

Our climate risk map for *P. kernoviae* was constructed using the NAPPFAST pest mapping system (Magarey et al., 2007). The climate map was based on an infection model and 10-year daily climatic data (1997 to 2006). We used a generic 'fill-in-the-blanks' template for creating models 'on the fly' to construct the model. It used the daily combination of average temperature and total leaf wetness hours per day to estimate the number of days in each year suitable for infection.

We assumed that *P. kernoviae* would climatically be limited by temperature for growth and moisture requirements for zoosporic infection. Our temperature thresholds (minimum, optimum, maximum) for *P. kernoviae* infection were 3, 18, and 26°C (Brasier et al., 2005). We estimated a moisture requirement of at least 12 hours using data describing zoosporic infection caused by *P. ramorum* on *Umbellularia californica* leaves (Huberli et al., 2003).

We used the infection model of Magarey et al. (2005) to estimate the number of days favorable for infection. We considered a climatic frequency of ≥ 60 favorable days for infection to be an indication of climatic risk (Smith, 2002). For each 10 km^2 pixel, each day was assigned a value between 0 (unfavorable for infection) and 1 (favorable for infection) and these values were accumulated over the year. The resulting layer represented the frequency of years where ≥ 60 accumulated favorable days occurred over the whole year.

We are unaware of any data on cold survival of *P. kernoviae*. Since *P. kernoviae* forms resistant oospores (Brasier et al., 2005), unlike *P. ramorum*, it is likely that its cold survival threshold is lower. Consequently, we did not use a cold survival threshold.

Overstory host density (fig. 6)

We constructed a host layer for the conterminous U.S. to depict the relative abundance of tree species that were:

- likely to develop stem lesions due to infection by *P. kernoviae*
- common in the overstory of forested areas.

We chose our set of susceptible species based either on direct observation of infection in the United Kingdom or by selecting close relatives of confirmed hosts. Tulip tree (*Liriodendron tulipifera*), which is native to North America, has exhibited symptoms of *P. kernoviae* infection on foliage and shoots as well as on the trunk (DEFRA, 2005b), with at least one tree planted ornamentally in the United Kingdom killed by a bleeding lesion (DEFRA, 2005a). Apparently lethal stem lesions have also occurred on European beech (*Fagus sylvatica*) (Brasier et al., 2005; DEFRA, 2005a), and we robustly included American beech (*Fagus grandifolia*), the only beech species native to North America. English oak (*Quercus robur*) exhibits bleeding lesions similar to those on beech (Brasier et al., 2005; DEFRA, 2005b). Phylogenetically, English oak is a member of the white oak group (*Quercus* sect. *Quercus*), of which there are numerous species in North America (Manos et al. 1999). We included all North American white oaks documented in the USDA Forest Service Forest Inventory and Analysis (FIA) database in the overstory host layer (USFS, 2006) (Table 1). It should be noted that neither American beech nor any North American white oak species have yet been tested for susceptibility to *P. kernoviae*.

Table 1. White oak (*Quercus* sect. *Quercus*) species included in the overstory host layer.

Species	Common Name
Q. alba	White oak
Q. arizonica	Arizona white oak
Q. bicolor	Swamp white oak
Q. douglasii	Blue oak
Q. engelmannii	Engelmann oak
Q. gambelii	Gambel oak
Q. garryana	Oregon white oak
Q. lobata	California white oak
Q. lyrata	Overcup oak
Q. macrocarpa	Bur oak
Q. margarettiae	Dwarf post oak
Q. michauxii	Swamp chestnut oak
Q. minima	Dwarf live oak
Q. montana	Chestnut oak
Q. muehlenbergii	Chinkapin oak
Q. oblongifolia	Mexican blue oak
Q. oglethorpensis	Oglethorpe oak
Q. prinoides	Dwarf chinkapin oak
Q. rugosa	Netleaf oak
Q. similis	Delta post oak
Q. sinuata var. sinuata	Durand oak
Q. stellata	Post oak
Q. virginiana	Live oak

We generated separate surfaces of tulip tree, American beech and white oak basal area (in square feet per acre) through ordinary kriging of FIA plot data. For these three surfaces, we first identified all USDA Forest Service ecoregion sections (McNab et al., 2005) containing plots where the species of interest were present (48 sections for tulip tree, 64 sections for American beech, and 138 sections for the white oaks). We then assembled all FIA plots that fell within these sections into geographically referenced samples of basal area values (N=53,136 for tulip tree, N=68,618 for American beech, and N=97,040 for the white oaks). We fit spherical semivariogram models (Cressie, 1993) to each sample using weighted least squares. The semivariograms determined kriging weights during the interpolation process. To predict basal area values for unknown locations, we used the 30 nearest neighboring FIA plots or, if fewer plots were available within a 60-km radius of the unknown location, we

included all plots within this distance threshold. To ensure that only ecologically similar plots were used in predicting values for unknown locations, we performed separate interpolations for each ecoregion section, and then mosaiced the results into a single surface with a 1 km2 spatial resolution. We used a forest cover map, developed from MODIS satellite data by the USDA Forest Service Remote Sensing Applications Center, to mask out non-forested areas from each of the three host surfaces.

We created a single overstory layer using map algebra, first adding the tulip tree, American beech, and white oak basal area surfaces together and then dividing by the maximum sum value (111.57 ft^2 /ac). This yielded a surface with values scaled between 0 and 1.

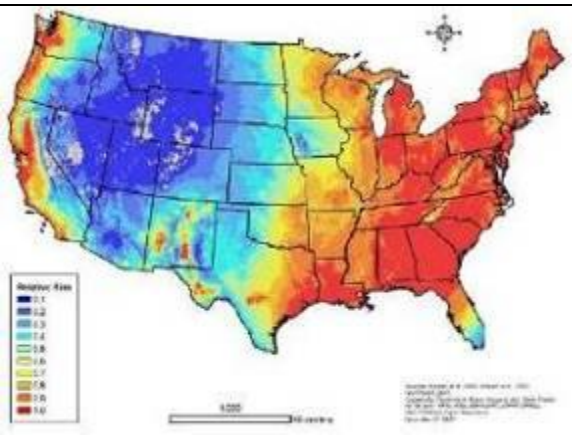

Figure 5. Risk map for *Phytophthora kernoviae* based on climate. Red indicates highest risk.

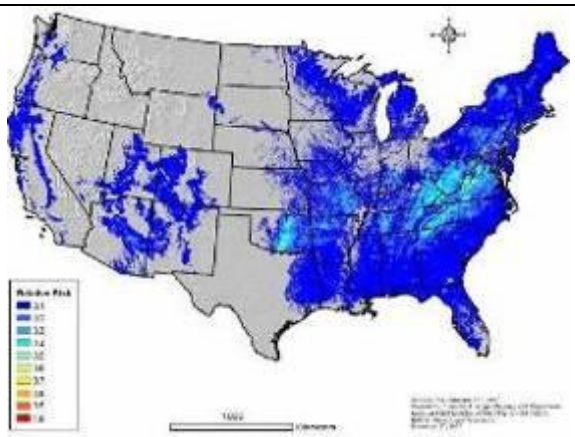

Figure 6. Risk map for *Phytophthora kernoviae* based on overstory host density. Red indicates highest risk.

Host sporulation potential (fig. 7)

We constructed a list of sporulating host species for North America (Table 2) based on current information available from the United Kingdom (Brasier et al., 2005; DEFRA, 2005b; DEFRA, 2006; Denman et al., 2006). Some of these species (e.g., *L. tulipifera, U. californica*, *Rhodendron catawbiense*, *Vaccinium myrtillus*, *V. vitis-idaea*) have been demonstrated as sporulators; we also selected species related to these known sporulating hosts. For example, *R. maximum* has not been confirmed as a host, but it has a close phylogenetic relationship with *R. ponticum* and *R. catawbiense* (Milne, 2004). These hosts vary greatly in their potential to act as inoculum sources. Therefore, we developed a four-group ranking of host sporulation potential based upon many factors, including height, evergreenness, leaf size, plant density and sporulation density. For an aerial disperser like *P. kernoviae*, tall trees may spread inoculum much further than small shrubs, especially to sporulating hosts lower in the canopy. Evergreen species provide a year-round source of inoculum. Although *P. kernoviae* forms oospores and is highly likely to persist in the soil, inoculum present in the canopy will initiate epidemics more efficiently. Species with large leaves can generate more inoculum than those with small ones. We assume species that grow in dense stands have a greater inoculum potential than those that grow sparsely. *Magnolia grandiflora*, although it is tall and evergreen is placed in group III because it tends to grow sparsely unlike those in group IV, which form dense stands.

Table 2. Risk ratings for host sporulation potential.

Group	Group definition and species	Risk rating	Reference
I	Deciduous shrubs with small leaves and sparse sporulation: *R. alabamense, R. albiflorum, R. arborescens, R. atlanticum, R. austrinum, R. calendulaceum, R. canadense, R. cumberlandense, R. eastmanii, R. flammeum, R. oblongifolium, R. occidentale, R. periclymenoides, R. prinophyllum, R. prunifolium, R. vaseyi, R. viscosum, R. ×bakeri, R. ×pennsylvanicum, V. alaskaense, V. angustifolium, V. arboreum, V. boreale, V. caesariense, V. caespitosum, V. corymbosum, V. deliciosum, V. erythrocarpum, V. formosum, V. fuscatum, V. hirsutum, V. membranaceum, V. myrtilloides, V. myrtillus, V. ovalifolium, V. pallidum, V. parvifolium, V. scoparium, V. simulatum, V. stamineum, V. tenellum, V. uliginosum, V. virgatum, V. ×atlanticum, V. ×margarettiae, V. ×marianum*	0.1	BONAP*
II	Evergreen shrubs: *R. carolinianum, R. catawbiense, R. chapmanii, R. lapponicum, R. macrophyllum, R. minus, R. ×welleslyanum, V. crassifolium, V. darrowii, V. macrocarpon, V. myrsinites, V. ovatum, V. oxycoccos, V. vitis-idaea*	0.2	BONAP
III	Mid-story, deciduous or sparsely distributed evergreen: *Liriodendron tulipifera* *Magnolia sp.*	0.5	FIA**
IV	Mid-story, dominant evergreen with large leaves and generally dense sporulation: *Umbellularia californica* *Rhododendron maximum*	1.0	FIA BONAP

* BONAP – Biota of North America Program, Synthesis database (Kartesz, 2007)
** FIA – Forest Inventory Assessment database, version 2.1 (USFS, 2006)

For each species group a measure of density was developed using available data. For groups I and II, the number of species in each group was totaled for each grid cell. For group III the number of trees per acre was totaled for all species in the group for each grid cell. For all three groups, a relative density value for each cell was calculated as a proportion of the maximum observed value, yielding values scaled between 0 and 1. With respect to group IV, only presence-absence distribution data were available for *R. maximum*, although trees per acre data were available for the other species in the group, *U. californica*. To ensure equal weight of these species in the risk rating, we also converted *U. californica* to presence-absence, such that the "relative density" value for group IV was 0 (absence) or 1(presence). The relative density for each group was multiplied by the risk rating (Table 2) and then the four weighted scores were added together. The total weighted score for each cell was divided by the maximum observed value to scale the scores between 0 and 1.

Introduction Pathways (fig. 8)

Introduction pathways were measured by classification of urban/forest interface. The urban/forest interface is a measure of the potential for the pathogen to be introduced into the forest environment if it is present. It was assumed that the urban/forest interface was a better indicator of long-term introduction risk than nurseries where plants may remain for only a short period.

We adapted wildland-urban interface (WUI) data, developed by the University of Wisconsin-Madison and the USDA Forest Service Northern Research Station, to represent areas facing risk of *P. kernoviae* spread were the pathogen to be introduced. In brief, these data depict where houses and natural vegetation meet (Radeloff et al., 2005). Epidemiologically, the riskiest locations for spread of *P. kernoviae* are those where potentially infected nursery plants, such as rhododendrons or other sporulating host species, may be planted on residential

land in close proximity to natural forest stands that also contain sporulating hosts. For our analysis, we started with WUI coverages for each state in the conterminous United States. The primary map unit of these polygon coverages is the U.S. census block. Each census block was assigned a housing density value based on the 2000 Census. Then, National Land Cover Data (NLCD) from 1992 was integrated by summing land cover percentages by census block. Census block polygons were then assigned to one of 14 classes (Table 2) based on these measures (Radeloff et al., 2005).

We developed a new ranking of the original WUI classes to emphasize those categories we believe face the greatest risk of *P. kernoviae* spread (Table 3). In particular, we assigned Low and Medium Density Intermix the highest risk ranking (3) because each census block in these categories can be expected to contain many large inclusions of natural vegetation throughout. We assigned Low and Medium Density Interface the next highest ranking (2) because they generally have fewer inclusions, although there are areas dominated by natural vegetation within 2.414 km. We assigned High Density Intermix and High Density Interface a risk ranking of 1 to reflect the presence of numerous home lots distributed throughout the census block, likely meaning smaller (but not necessarily fewer) inclusions of natural vegetation. The negative values for non-vegetated areas and water served as temporary placeholders; after performing the "edge zone" analysis described below, these categories were set to zero in the final pathways layer.

Table 3. WUI coverage classes and assigned ranking with respect to *P. kernoviae* spread risk.

WUI Class	Description	Ranking
Low Density Interface	Housing density >= 6.177635 and < 49.42108 units/km^2, Vegetation <= 50%, within 2.414 km of area with >= 75% Vegetation	2
Medium Density Interface	Housing density >= 49.42108 and < 741.3162 units/km^2, Vegetation <= 50%, within 2.414 km of area with >= 75% Vegetation	2
High Density Interface	Housing density >=741.3162 units/km^2, Vegetation <= 50%, within 2.414 km of area with >= 75% Vegetation	1
Low Density Intermix	Housing density >= 6.177635 and < 49.42108 units/km^2, Vegetation > 50%	3
Medium Density Intermix	Housing density >= 49.42108 and < 741.3162 units/km^2, Vegetation > 50%	3
High Density Intermix	Housing density >= 741.3162 units/km^2, Vegetation > 50%	1
Uninhabited, No Veg.	Housing density = 0, Vegetation <= 50%	-1*
Very Low Density, No Veg.	Housing density = 0, Vegetation > 50%	-1
Low Density, No Veg.	Housing density > 0 and < 6.177635 units/km^2, Vegetation <= 50%	-1
Medium Density, No Veg.	Housing density > 0 and < 6.177635 units/km^2, Vegetation > 50%	-1
High Density, No Veg.	Housing density >= 6.177635 and < 49.42108 units/km^2, Vegetation <= 50%	-1
Uninhabited, Veg.	Housing density >= 49.42108 and < 741.3162 units/km^2, Vegetation <= 50%	0
Very Low Density, Veg.	Housing density >= 741.3162 units/km^2, Vegetation <= 50%	0
Water	Water	-2

* Negative values disappear from the analysis (see text for details).

The WUI coverages, originally in vector format, were joined into a single nationwide coverage, rasterized at 0.625 km^2 spatial resolution, and then resampled to 1 km^2 using a block majority filtering approach. In a final step, we extracted an "edge zone" where pixels labeled as natural vegetation (risk ranking = 0) or high-risk intermix (risk ranking = 3) were adjacent

to one or more pixels in the opposing category using an eight-neighbor rule. Pixels in this edge zone were assigned a very high risk ranking, yielding a new risk scale of 0 to 4. We then divided the ranking by four to constrain scores between 0 and 1 for our final pathways layer.

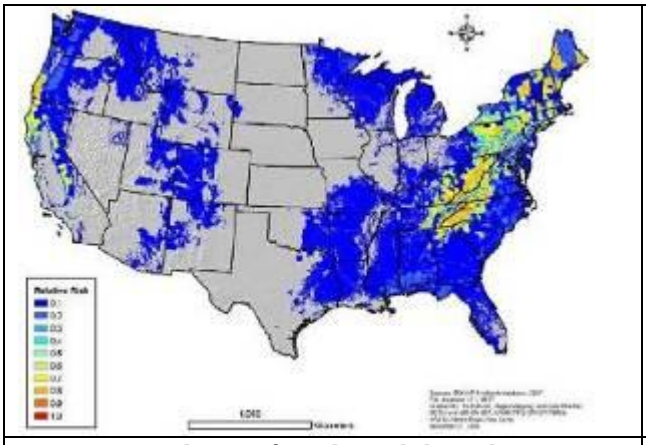

Figure 7. Risk map for *Phytophthora kernoviae* based on host sporulation potential. Red indicates highest risk.

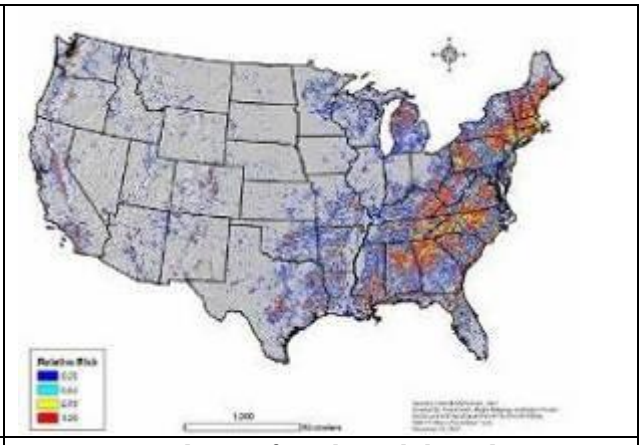

Figure 8. Risk map for *Phytophthora kernoviae* based on introduction pathways. Red indicates highest risk.

Composite risk map (fig. 4)

The composite risk map was created by multiplying the risk maps for climate, overstory host, sporulation potential and urban/forest interface and dividing by the maximum calculated value.

References

Brasier, C.M., Beales, P.A., Kirk, S.A., Denman, S. and Rose, J. 2005. *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. Mycological Research. 109: 853-859.

Cressie, N.A.C. 1993. Statistics for spatial data. John Wiley & Sons, New York. 900 p.

Denman, S., Kirk, S., Whybrow, A., Orton, E., and Webber, J.F. 2006. *Phytophthora kernoviae* and *P. ramorum*: host susceptibility and sporulation potential on foliage of susceptible trees. EPPO Bulletin 36: 373–376.

Department for Environment, Food, and Rural Affairs (DEFRA). 2005a. Pest risk analysis for a new *Phytophthora* species informally named as *Phytophthora kernoviae* (also known as *P. kernovii* – formally *Phytophthora* taxon C). DEFRA Plant Health Division, UK. 15 p.

Department for Environment, Food, and Rural Affairs (DEFRA). 2005b. *Phytophthora kernoviae*: a new threat to our trees and woodlands. DEFRA Plant Health Division, UK. 6 p.

Department for Environment, Food, and Rural Affairs (DEFRA). 2006. Final summary report: determining the susceptibility of key / dominant UK heathland species to *Phytophthora kernoviae.* DEFRA Plant Health Division, UK. 14 p.

Hüberli, D., Van Sant, W., Swain, S., Davidson, J. and Garbelotto, M. 2003. Susceptibility of *Umbellularia californica* to *Phytophthora ramorum*. In Eighth International Congress of Plant Pathology. Christchurch, New Zealand.

Kartesz, J.T. 2007. Synthesis of the North American Flora, Version 2.0. [Computer program]. Biota of North America Program, North Carolina Botanical Garden, University of North Carolina at Chapel Hill.

Magarey, R.D., Sutton, T.B. and Thayer, C.L. 2005. A Simple Generic Infection Model for Foliar Fungal Plant Pathogens. Phytopathology 95:92-100.

Magarey, R.D., Borchert, D.M., Fowler, G.A., Sutton, T.G., Colunga-Garcia, M. and Simpson, J.A. 2007. NAPPFAST, an internet system for the weather-based mapping of plant pathogens. Plant Disease 91(4): 336-345.

Manos, P.S., Doyle, J.J. and Nixon, K.C. 1999. Phylogeny, biogeography, and processes of molecular differentiation in *Quercus* subgenus *Quercus* (Fagaceae). Molecular Phylogenetics and Evolution 12(3): 333-349.

McNab, W.H., Cleland, D.T., Freeouf, J.A., Keys Jr., J.E., Nowacki, G.J. and Carpenter, C.A. (comps.). 2005. Description of ecological subregions: sections of the conterminous United States. First approximation. USDA Forest Service, Washington, DC. 80 p.

Milne, R.I. 2004. Phylogeny and biogeography of *Rhododendron* subsection *Pontica*, a group with a tertiary relict distribution. Molecular Phylogenetics and Evolution 33: 389-401.

Radeloff, V.C., Hammer, R.B., Stewart, S.I., Fried, J.S., Holcomb, S.S. and McKeefry, J.F. 2005. The wildland-urban interface in the United States. Ecological Applications 15(3): 799-805.

Smith, W. 2002. Sudden Oak Death National Detection Survey For Forests and Risk/Hazard Map. USDA Forest Service, Forest Health Monitoring Program.

USFS. 2006. The Forest Inventory and Analysis Database: Database Description and Users Guide Version 2.1. USDA Forest Service, National Forest Inventory and Analysis Program. 211 p.