Root Infections May Challenge Management of Invasive *Phytophthora* spp. in U.K. Woodlands

E. J. Fichtner and **D. M. Rizzo,** University of California, Davis 95616; and **S. A. Kirk** and **J. F. Webber,** Forest Research, Alice Holt Lodge, Farnham, Surrey GU10 4LH, United Kingdom

Abstract

Fichtner, E. J., Rizzo, D. M., Kirk, S. A., and Webber, J. F. 2011. Root infections may challenge management of invasive *Phytophthora* spp. in U.K. woodlands. Plant Dis. 95:13-18.

Because sporulation of *Phytophthora ramorum* and *P. kernoviae* on *Rhododendron ponticum*, an invasive plant, serves as primary inoculum for trunk infections on trees, *R. ponticum* clearance from pathogen-infested woodlands is pivotal to inoculum management. The efficacy of clearance for long-term disease management is unknown, in part due to lack of knowledge of pathogen persistence in roots and emerging seedlings. The main objectives of this work were to (i) investigate whether both pathogens infect *R. ponticum* roots, (ii) determine the potential for residual inoculum of *P. kernoviae* to infect *R. ponticum* seedlings in cleared woodlands, and (iii) assess potential for *R. ponticum* roots to support survival and transmission of *P. kernoviae*. Roots of *R. ponticum* were collected from both unmanaged and cleared woodlands and assessed for pathogen recovery. Both *P. ramorum* and *P. kernoviae* were recovered from asymptomatic roots of *R. ponticum* in unmanaged woodlands, and *P. kernoviae* was recovered from asymptomatic roots from seedlings in cleared woodland. Oospore production of *P. kernoviae* was observed in naturally infected *R. ponticum* foliage and in inoculated roots. Roots also supported *P. kernoviae* sporangia production. The results of this study suggest that post-clearance management of *R. ponticum* regrowth is necessary for long-term inoculum management in invaded woodlands.

Phytophthora kernoviae Brasier, Beales & S.A. Kirk and *P. ramorum* Werres, de Cock & Man in't Veld are exotic pathogens thought to have been introduced to Great Britain within the past 10 to 20 years (9). Both pathogens threaten the composition, health, and sustainability of U.K. mixed-species woodlands, planted woodland gardens, heritage gardens, and national plant collections. *P. kernoviae* is also an emerging threat to U.K. heathland, where it infects *Vaccinium myrtillus*, a key component of heathland habitat (1,26). The geographic origin of each pathogen is unknown; however, *P. kernoviae* was recently recovered from soils in New Zealand forests (19). The risk of *P. ramorum* to forest health has already been realized in the Sudden Oak Death (SOD) epidemic in the western United States (21,22).

P. ramorum was first described as a pathogen on ornamental nursery plants in Europe (27) but its later association with SOD exacted international concern for forest biosecurity. Surveys for *P. ramorum* in Great Britain were initiated in 2001 and the pathogen has since been found at hundreds of sites, in both nursery systems and woodlands (6,17). As a result of the *P. ramorum* surveys, *P. kernoviae*, a previously unknown pathogen, was isolated concurrently from a bleeding lesion on European beech (*Fagus sylvatica*) and foliar lesions of *Rhododendron ponticum* (5), an invasive plant pervading unmanaged woodlands. *P. ramorum* and *P. kernoviae* cause extensive foliar necrosis and shoot dieback on *R. ponticum* and bleeding cankers on *F. sylvatica*, as well as other tree species (6,9). Early surveys of disease incidence elucidated that the majority of infected trees are located within 2 m of infected *R. ponticum*, suggesting a role of *R. ponticum* in disease transmission (3). Bleeding cankers on trees do not support sporulation of either pathogen but infected *R. ponticum* foliage supports sporulation of both pathogens and is the likely source of primary inoculum for new tree infections (9,25).

Because *P. ramorum* and *P. kernoviae* rely on an invasive plant for transmission, removal of *R. ponticum* from infested woodlands is the initial step in implementing a disease management program (11,25) (Fig. 1A and B). *R. ponticum* is generally an undesirable woodland invader, forming an impenetrable barrier which shades other vegetation and has limited value for shelter and game cover (9). The cost of *R. ponticum* clearance has been estimated in the range of millions of GBP (pound sterling) (25). Preliminary data suggest that inoculum levels in leaf litter and soil have dramatically decreased after *R. ponticum* clearance, and incidence of new tree infections in cleared woodlands is low (9,25).

Both the invasive nature of *R. ponticum* and the persistence of both pathogens in litter and soil (25) pose a challenge to pathogen eradication. Even after herbicide treatment, *R. ponticum* regenerates from stumps and seed (25) and can rapidly recolonize a woodland. *Phytophthora* spp. infection of *R. ponticum* regrowth has been observed in cleared woodlands (Fig. 1C and D) (9) but the source of inoculum initiating these infections is unknown. The potential for *P. kernoviae* and *P. ramorum* to infect and persist in *R. ponticum* roots has yet to be explored; however, *P. ramorum* infects rhododendron roots in container culture (18,24), and potting soil infested with chlamydospores can incite root infections (7,8). *P. kernoviae* is homothallic and produces oospores in vitro; however, it is not yet known whether oospores are produced in planta and contribute to pathogen persistence in woodlands.

The development of post-clearance woodland management guidelines relies on a comprehensive understanding of the survival and transmission components of the disease and life cycles of these pathogens. Therefore, this article addresses the potential for (i) both pathogens to infect adventitious roots of *R. ponticum*, (ii) residual inoculum of *P. kernoviae* to infect *R. ponticum* seedlings in cleared woodlands, and (iii) *P. kernoviae* to produce spo-

Corresponding author: J. F. Webber, E-mail: joan.webber@forestry.gsi.gov.uk

Accepted for publication 7 September 2010.

doi:10.1094/PDIS-03-10-0236

rangia on *R. ponticum* roots and oospores in *R. ponticum* roots and foliage.

Materials and Methods

Research locations. All field work was conducted in infested woodlands in Cornwall, southwest England. Three unmanaged woodlands containing dense, established *R. ponticum* were chosen for root excavation. Two of these unmanaged woodlands, located near Truro, were infested with *P. kernoviae*, and the third, near Penzance, was infested with *P. ramorum*. Another Truro-area woodland, cleared in 2004 of *R. ponticum,* was selected for excavation of emergent *R. ponticum* seedlings. All selected woodlands were heavily infested with either *P. ramorum* or *P. kernoviae*, and contained *F. sylvatica* with active infections. All laboratory work was conducted in Forest Research quarantine facilities at Alice Holt Lodge in Farnham, Surrey.

***Phytophthora* spp. recovery from adventitious roots.** In May and November 2007, four sets of *R. ponticum* roots were excavated from each of the two *P. kernoviae*-infested woodlands and the single *P. ramorum*-infested woodland. Adventitious roots formed by layered stems were chosen to ensure that roots sampled were those of *R. ponticum* and not other woodland vegetation. At each point of layerage, the entire root mass was excavated, along with approximately 0.5 liter each of overlying leaf litter and rhizosphere soil, and five symptomatic attached leaves.

To isolate *Phytophthora* spp. from *R. ponticum* foliage, leaves were surface sterilized in 70% ethanol for 30 s (10), then rinsed three times in deionized (DI) water and blotted dry. Samples were excised along lesion margins and submerged in synthetic mucor agar (SMA), a selective medium containing pimaricin (0.01 g/liter), and rifamycin SV sodium salt (3.0 g/liter) (12). Leaf litter and soil samples were baited for *Phytophthora* spp. by saturating samples in DI water and floating 10 1-cm-diameter *R. catawbiense* 'Cunningham's White' leaf disks on the water surface. After 7 days, leaf disks were retrieved, soaked in 30% ethanol for 30 s, rinsed in DI water, blotted dry, and placed on SMA amended with hymexazol (0.05 g/liter), pentachloronitrobenzene (PCNB; 0.025 g/liter), ampicillin (0.2 g/liter), nystatin (50 µl/liter), and rifampicin (0.01 g/liter). All putative *Phytophthora* spp. were then subcultured on carrot agar (CA) for morphological identification. These techniques for pathogen baiting, isolation, and identification were used throughout the study, unless otherwise stated. Isolations from foliage were submerged in SMA, whereas bait materials from soil and leaf litter were submerged in SMA amended with hymexazol, PCNB, ampicillin, nystatin, and rifamycin.

All roots were thoroughly washed with DI water to remove soil, then cut into approximately 3-cm segments. In May 2007, washed roots were incubated in moist chambers for 24 h, then baited for *Phytophthora* spp. The root masses excavated in November 2007 were surface sterilized to ensure that *Phytophthora* spp. recovered by baiting resulted from root infections and not from propagules on the rhizoplane. Because fine roots are only a few cell layers thick and susceptible to disruption during surface sterilization, two surface sterilization techniques were used to enhance detection of root infections (24). Root masses were divided into two portions, with one portion incubated 5 min in 0.025% NaOCl and the other in 30% ethanol for 30 s (23). After surface sterilization, all roots were rinsed three times in DI water, then incubated for 24 h in moist chambers before baiting.

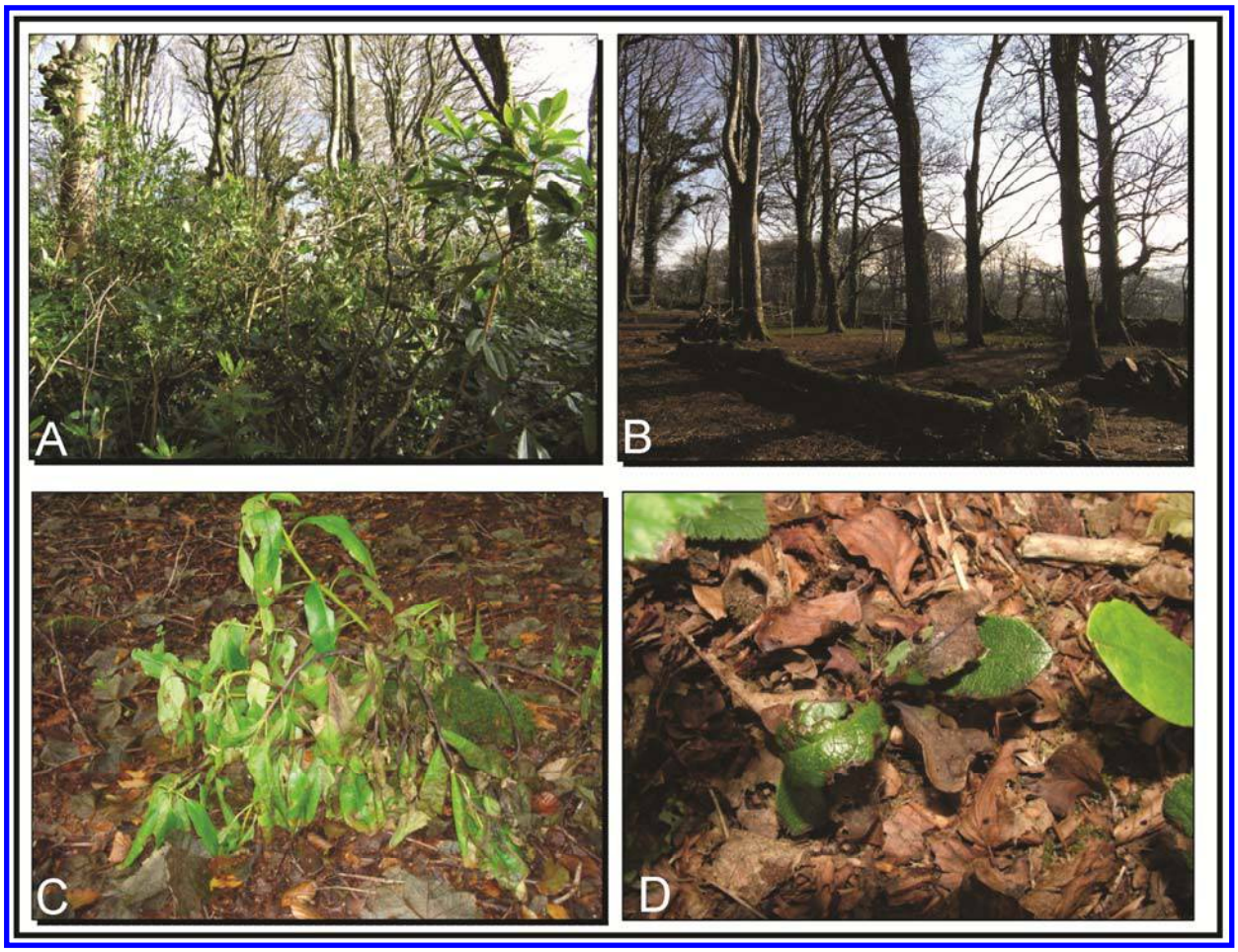

Fig. 1. Woodlands invaded with *Rhododendron ponticum* have been cleared of the invasive plant for management of *Phytophthora kernoviae* and *P. ramorum*. Infested woodland **A,** before and **B,** after clearance of *R. ponticum.* Both images were taken from the same location. (Images courtesy of A. Brown and C. Brasier). Post-clearance infection by *P. kernoviae* of **C,** vegetative shoots and **D,** seedlings.

***Phytophthora* spp. recovery from seedlings.** In May and November 2007, *R. ponticum* seedlings and associated rhizosphere soil were collected from a *P. kernoviae*-infested woodland that had been cleared of *R. ponticum* in 2004. Unlike samples collected in uncleared woodlands, leaf litter was unavailable because it had been removed during *R. ponticum* clearance. In May 2007, 19 *R. ponticum* seedlings were collected and roots were thoroughly washed, incubated in moist chambers for 24 h, and then directly baited for *Phytophthora* spp. In November 2007, 30 seedlings were collected. Roots of 10 seedlings were baited without surface sterilization, roots of 10 seedlings were surface sterilized in 30% ethanol for 30 s, and roots of 10 seedlings were surface sterilized for 5 min in 0.025% NaOCl. All roots were rinsed thoroughly after surface sterilization, then incubated for 24 h in moist chambers before baiting for *Phytophthora* spp.

Determination of sporangia production on roots. After bait leaf disks were removed from samples of both adventitious roots and seedling roots, roots were blotted dry and stored in moist chambers at 18°C. Roots were stored until putative *Phytophthora* isolates grew out of leaf baits, generally 3 to 5 days after placing on selective medium. Roots giving rise to *Phytophthora* colonies from baits were then cleared and stained for microscopic observation. Because of the caducous nature of *P. kernoviae* sporangia (5), observation of sporangia attached to sporangiophores at the root surface was challenging, particularly after disruption during a staining procedure. Consequently, artificially inoculated roots were also used to determine the potential for roots to support sporulation. Roots of micropropagated rhododendron plantlets grown in axenic culture (plantlets donated by R. Smith, Duchy College, Cornwall) were utilized to enable observation of sporangia in the absence of other root-inhabiting fungi. Micropropagated roots of four plants were dipped in a 10^4 zoospore/ml suspension and then incubated for 10 days in moist chambers before clearing and staining for observation. The zoospore suspension was produced by growing two isolates (ROS SD8 and ROS SD9) of *P. kernoviae* on CA for 1 week at 18°C under a continuous lighting regime of 12 h of white light followed by 12 h of black light. Aliquots of DI water (3 ml each) were pipetted onto the surface of each colony and colonies were gently scraped with a glass rod to dislodge the sporangia. The spore suspension, a cocktail of two isolates, was then gathered into a beaker and refrigerated at 4°C for 45 min to allow for zoospore release; then, zoospores were enumerated with a hemacytometer.

A modification of Koske's clearing and staining procedure (16) was used to render roots semitranslucent for visual observation. Roots were soaked in 2.5% KOH for approximately 24 h. Roots were rinsed three times in DI water, then acidified for 10 min in 1% HCl. The HCl was decanted and roots were next placed in 0.05% trypan blue stain and left overnight at room temperature. Trypan blue was then poured off and roots were incubated in a destain solution (500 ml of glycerin, 450 ml of DI water, and 50 ml of 1% HCl) for 2 days before viewing. Fine roots were placed on glass slides and observed under the compound microscope for sporangia. Images were captured using an Olympus BX51 microscope with an Olympus DP70 camera.

Determination of oospore production in *R. ponticum* foliage. A sequential approach was adopted to determine the potential for

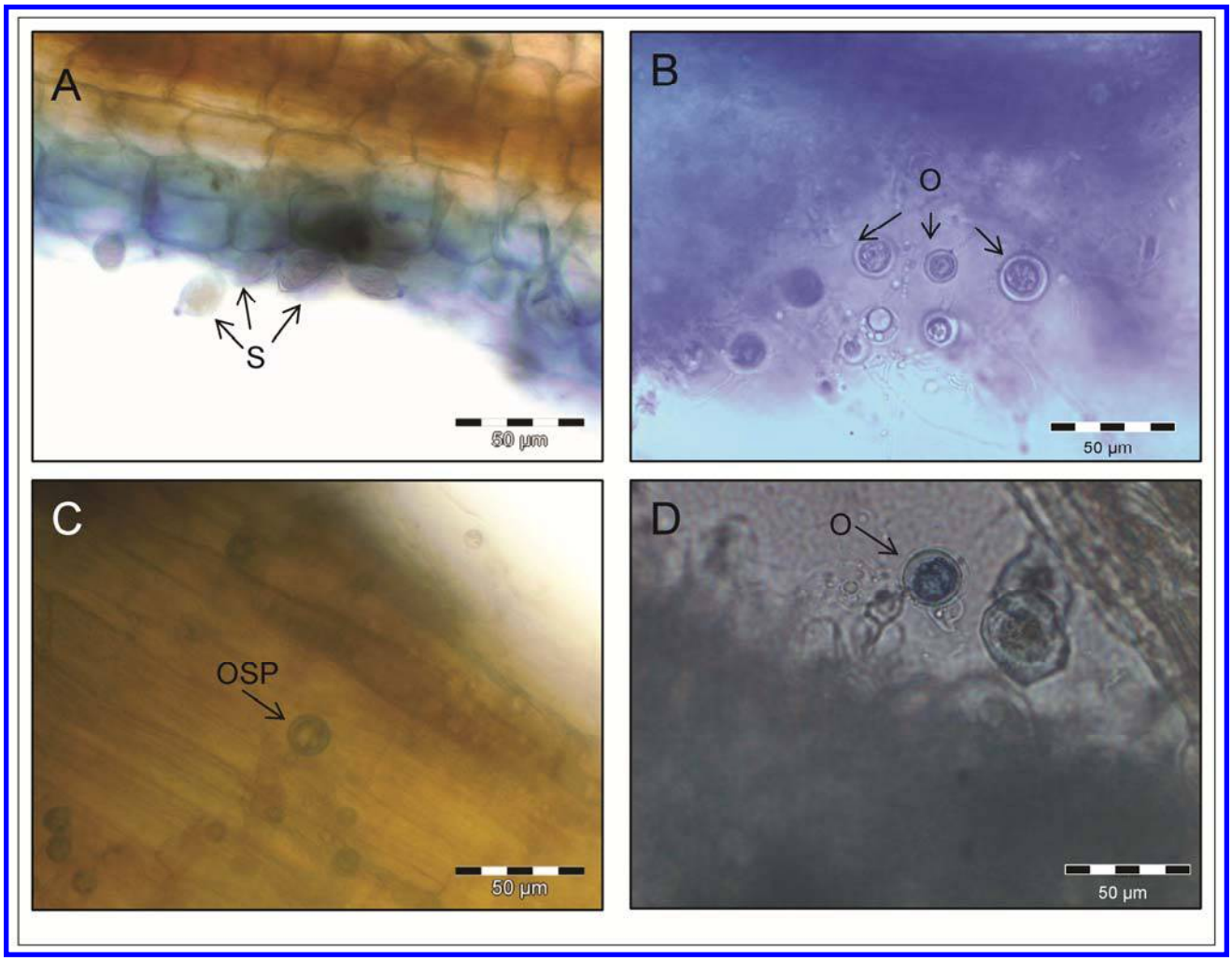

Fig. 2. A, Sporangia (S) of *Phytophthora kernoviae* on the root surface of an inoculated micropropagated rhododendron plant. **B,** Oogonia (O) of *P. kernoviae* on Rhododendron 'Cunningham's White' foliage used as bait material for detection of *Phytophthora* spp. in roots. **C,** Oospores (OSP) of *P. kernoviae* within inoculated roots of *R. ponticum*. **D,** Oogonium (O) of *P. kernoviae* within a naturally-infected *R. ponticum* leaf. Arrows indicate location of propagules within photos.

oospore production in foliage. First, rhododendron leaf baits that yielded *P. kernoviae* in culture were removed from selective medium and then cleared using a modification of the aforementioned protocol (16). In addition to soaking the leaf disks in KOH overnight, disks were further cleared by incubation in KOH solution at 90°C for 20 to 30 min (16). After oospores were observed in bait leaf disks, naturally infected *R. ponticum* leaves were collected and cleared for determination of oospore production. Twenty symptomatic leaves were collected from one of the uncleared sites used in the adventitious roots study. Infection with *P. kernoviae* was confirmed by isolation; then, leaves were incubated at 4°C for approximately 4 weeks. Because conditions required for in planta oospore production are, as yet, unknown, the cool incubation was provided to increase the probability of oospore production and detection. Plant tissue was then cleared and stained using the same technique employed for the leaf disk baits.

Determination of oospore production in *R. ponticum* roots. The potential for roots to support oospore production was determined in artificially inoculated roots. In September 2008, roots of four *R. ponticum* plants were excavated from forest at Alice Holt Lodge (Farnham, Surrey), a site with no history of infestation with either *P. kernoviae* or *P. ramorum.* Root masses from each plant were washed thoroughly and then divided in half, and one half was surface sterilized in 0.025% NaOCl for 5 min, then rinsed. Using the aforementioned baiting procedure, roots were then assessed for natural *Phytophthora* infection. The remaining half of each root mass was divided into 10 4-cm-long segments. Root segments were dipped into a 10^5 zoospore suspension of *P. kernoviae*, produced by the aforementioned technique, then incubated in moist chambers for 6 days. Roots were surface sterilized for 5 min in 0.025% NaOCl, rinsed thoroughly, and incubated in moist chambers for an additional 24 h. Root segments were then cut in half; one half was placed in SMA for quantification of frequency of pathogen recovery and the other half was cleared and stained for observation of oospores. Inoculation of *R. ponticum* root segments with *P. kernoviae* was conducted twice.

Table 1. Recovery of *Phytophthora kernoviae* from litter, soil, foliage, and adventitious roots of *Rhododendron ponticum* in two unmanaged woodlands

	Frequency of *P. kernoviae* recovery[a]	
Site, source	**May 2007**	**October 2007**
Site 1		
Foliage[b]	1/4	4/4
Litter[c]	4/4	3/4
Soil[c]	0/4	0/4
Roots[d]		
Non-surface sterilized	3/4	NA
NaOCl	NA	3/4
EtOH	NA	2/4
Site 2		
Foliage[b]	0/4	4/4
Litter[c]	3/4	3/4
Soil[c]	0/4	0/4
Roots[d]		
Non-surface sterilized	2/4	NA
NaOCl	NA	4/4
EtOH	NA	3/4

[a] N/A = data not available.

[b] Five symptomatic leaves were collected from each of four plants at each site. Leaves were surface sterilized in 70% ethanol and rinsed, and then symptomatic tissues were placed on synthetic mucor agar (SMA), for isolation of *Phytophthora* spp. (10).

[c] Litter overlying adventitious roots and rhizosphere soil were collected from four *R. ponticum* plants at both sites. Litter and soil samples were baited for 7 days with rhododendron leaf disks. Leaf disks were then placed on amended SMA for isolation of *Phytophthora* spp.

[d] Roots sampled in May 2007 were not surface sterilized. Root masses collected in October 2007 were divided in half; one portion was surface sterilized in 30% ethanol (EtOH) for 30 s and the second portion was surface sterilized in 0.025% NaOCl for 5 min (23). All roots were rinsed thoroughly, incubated in moist chambers for 24 h, then baited with rhododendron leaf disks for 7 days. Leaf disks were then placed on amended SMA for isolation of *Phytophthora* spp.

Results

In May 2007, *P. kernoviae* was recovered from both adventitious root masses and leaf litter at sites 1 and 2 (Table 1) and *P. ramorum* was recovered from adventitious root masses and leaf litter at site 3 (Table 2). Similar results were observed in repeated root excavation in October 2007 (Tables 1 and 2); however, in October 2007, all roots were surface sterilized prior to baiting. Roots from sites 1 and 2 yielded *P. kernoviae* after surface sterilization with both the NaOCl and the ethanol (Table 2). At site 3, *P. ramorum* was recovered only in roots surface sterilized with NaOCl (Table 2). None of the excavated roots at any of the three sites showed symptoms of root rot. No *Phytophthora* spp. were baited from soils at the three sites. A shift in the population of *Phytophthora* spp. baited at site 3 was observed between May 2007 and October 2007 (Table 2). In May 2007, only *P. ramorum* was recovered at site 3; however, in October 2007, *P. kernoviae* was baited from two leaf litter samples, *P. hibernalis* was baited from one litter sample, and *P. citricola* was isolated from foliage of one plant (Table 2).

Of the 19 seedlings collected from a cleared woodland in May 2007, only 2 exhibited foliar symptoms of pathogen infection (Fig. 1D). Upon isolation, these foliar lesions were then confirmed to be infected with *P. kernoviae* (Table 3). *P. kernoviae* was baited from two soil samples and from roots of five seedlings (Table 3). The two seedlings containing foliar infections with *P. kernoviae* were also found to have *P. kernoviae* on the roots. All roots were asymptomatic, with no evidence of root rot or general necrosis.

In October 2007, of the 30 seedlings collected, only 3 seedlings contained foliar infection with *P. kernoviae* (Table 3). *P. kernoviae* was not baited from associated soil; however, the pathogen was baited from roots of 11 seedlings, including surface-sterilized roots (Table 3). All roots were asymptomatic and no other *Phytophthora* spp. were detected in the cleared woodland.

Sporangia production of *P. kernoviae* was observed on all inoculated micropropagated rhododendron roots (Fig. 2A). Prolific sporulation was observed on the root surface; however, no oogonia were observed. Sporangia of *P. kernoviae* were also observed on naturally infected roots of two *R. ponticum* seedlings and on ad-

Table 2. Recovery of *Phytophthora ramorum* from litter, soil, foliage, and adventitious roots of *Rhododendron ponticum* in an unmanaged woodland

	Frequency of *P. kernoviae* recovery[a]	
Site 3	**May 2007**	**October 2007**
Foliage[b]	2/4*	3/4†
Litter[c]	4/4	2/4†‡
Soil[c]	0/4	0/4
Roots[d]		
Non-surface sterilized	1/4	NA
NaOCl	NA	2/4
EtOH	NA	0/4

[a] N/A = data not available; * indicates *P. citricola* also isolated from foliage; † indicates *P. kernoviae* also isolated or baited from samples; and ‡ indicates *P. hibernalis* also baited from litter sample.

[b] Five symptomatic leaves were collected from each of four plants. Leaves were surface sterilized in 70% ethanol and rinsed, and then symptomatic tissues were placed on synthetic mucor agar (SMA) for isolation of *Phytophthora* spp. (10).

[c] Litter overlying adventitious roots and rhizosphere soil were collected from four *R. ponticum* plants. Litter and soil samples were baited for 7 days with rhododendron leaf disks. Leaf disks were then placed on amended SMA for isolation of *Phytophthora* spp.

[d] Roots sampled in May 2007 were not surface sterilized. Root masses collected in October 2007 were divided in half, and one portion was surface sterilized in 30% ethanol (EtOH) for 30 s and the second portion was surface sterilized in 0.025% NaOCl for 5 min (23). All roots were rinsed thoroughly, incubated in moist chambers for 24 h, and then baited with rhododendron leaf disks for 7 days. Leaf disks were then placed on amended SMA for isolation of *Phytophthora* spp.

ventitious roots from two plants excavated at site 2. No oogonia or oospores were observed on naturally infected roots.

Oogonia of *P. kernoviae* were observed on all colonized *R. catawbiense* Cunningham's White leaf disks used as baits (Fig. 2B). Oospores were also observed in several artificially inoculated roots of *R. ponticum* (Fig. 2C); however, the number of oospores produced per root segment was not recorded. An oogonium was observed in one naturally infected *R. ponticum* leaf (Fig. 2D). Uninoculated roots of *R. ponticum* yielded no other *Phytophthora* spp., indicating that the observed oospores were the result of *P. kernoviae* infection and colonization. When inoculated root segments were placed on SMA for confirmation of colonization by *P. kernoviae*, the pathogen was recovered from 100% of root segments in both inoculations.

Discussion

The results of this study reveal an underground component of the disease cycles of both *P. ramorum* and *P. kernoviae* in invaded U.K. woodlands. The recovery of both pathogens from surface-sterilized *R. ponticum* roots suggests that the pathogens have the ability to infect and colonize roots. The fact that all root infections were completely asymptomatic is consistent with the observations of Parke and Lewis (18), Tjosvold et al (24), and Riedel (20). This is the first report of root infections of *P. ramorum* and *P. kernoviae* on *R. ponticum*, and the first report of root infections of *P. kernoviae* on any host. Less is known of the life and disease cycles of *P. kernoviae* because it is a more recently described pathogen than *P. ramorum* and is currently limited to causing forest disease in the United Kingdom. The fact that both pathogens cause asymptomatic root infections is of pivotal concern because such infections can escape the visual observation of regulators and inspectors. Asymptomatic root infections offer a hidden pathway of pathogen transmission and persistence in both woodlands undergoing disease management operations and containerized nursery plants being distributed over large geographic areas.

Infected foliage of *R. ponticum* tends to abscise (2), depositing a layer of inoculum on the forest floor. Our results demonstrate the potential for *R. ponticum* leaves to harbor oospores of *P. kernoviae*, and other work has demonstrated that rhododendron leaves harbor chlamydospores of *P. ramorum* (13,14). The deposition of these survival structures on the forest floor does, in part, explain the distribution of inoculum detected in unmanaged woodlands. In unmanaged woodlands, *P. ramorum* and *P. kernoviae* were found in attached leaves, litter, and roots but not in soil. Considering that adventitious roots form at the leaf litter surface and grow through the infested leaf litter into underlying soil, it is likely that the leaf litter serves as primary inoculum for root infections.

The finding of root and foliar infections in *R. ponticum* seedlings in cleared woodland suggests that *P. kernoviae* may persist several years after initial *R. ponticum* clearance, and underscores the import of post-clearance woodland management for disease control. In the absence of *R. ponticum*, the most likely sources of primary inoculum in cleared woodland are residual propagules in soil and plant debris. Though *P. kernoviae* has been known to produce abundant oogonia in vitro, this is the first report of in planta oospore production. Oospores produced in *R. ponticum* roots and foliage may serve as survival structures in soil. Additionally, roots may support polycyclic sporulation, thus producing sporangia near the soil surface which can then be splash dispersed to aboveground plant parts or serve as primary inoculum for new root infections. In support of this hypothesis, 3 years after *R. ponticum* clearance, a new bleeding canker on *F. sylvatica* was observed near the soil line, suggestive of infection by rain-splashed soil inoculum.

The results of this work underscore the need for continued woodland management for years after initial *R. ponticum* clearance. The tenacity of *R. ponticum* regrowth, from both roots and the residual seed bank, serves as a persistent challenge for land managers, while *R. ponticum* vegetation must be continuously removed from woodlands to insure the success of the initial investment in clearance. Though preliminary data suggest that *R. ponticum* clearance reduces woodland populations of *P. ramorum* and *P. kernoviae* (25), it is yet presumptuous to assume that *R. ponticum* clearance may result in pathogen eradication. The observation of asymptomatic root infections on rhododendron opens the possibility that these pathogens may escape notice in cleared woodlands, causing asymptomatic infections on other woodland plants.

While focusing on effective management of these pathogens in U.K. woodlands, we must also consider the potential global impacts of introduction of *P. ramorum* and *P. kernoviae* to other ecosystems. For example, Brasier (4) suggests consideration of the potential impact of *P. ramorum* and *P. kernoviae* on *R. ponticum–Laurus–Quercus* ecosystems in Southern Spain, a system where *R. ponticum* is a native plant. Preliminary studies suggest that *P. kernoviae* may threaten North American native plants, including rhododendron and California bay laurel (15). The key to preservation of natural systems is to prevent pathogen spread; however, the prevalence of asymptomatic root infections may allow pathogens to move undetected, perhaps allowing invasive pathogens to become established in new ecosystems before mitigation strategies can be effectively considered and employed.

Table 3. Recovery of *Phytophthora kernoviae* from roots and foliage of *Rhododendron ponticum* seedlings and associated rhizosphere soil in a managed woodland site[a]

	Frequency of *P. kernoviae* recovery[b]	
	May 2007	**October 2007**
Foliage[c]	2/19	3/30
Soil[d]	2/19	0/30
Roots[e]		
Non-surface sterilized	5/19	5/10
NaOCl	NA	3/10
EtOH	NA	3/10

[a] Woodland was cleared of *R. ponticum* in 2004 for management of *P. kernoviae*.

[b] In May and October 2007, 19 and 30 seedlings, respectively, were excavated from the managed woodland site. N/A = data not available.

[c] Leaves were surface sterilized in 70% ethanol and rinsed, and then symptomatic tissues were placed on synthetic mucor agar (SMA) for isolation of *Phytophthora* spp. (10).

[d] Soil was dislodged from roots, and then samples were individually baited with rhododendron leaf disks for 7 days. Leaf disks were then placed on amended SMA for isolation of *Phytophthora* spp.

[e] Roots collected in May 2007 were not surface sterilized. Of the 30 seedlings collected in October 2007, 10 roots remained non-surface sterilized, 10 were surface sterilized in 0.025% NaOCl for 5 min (23), and 10 were surface sterilized in 30% ethanol (EtOH) for 30 s. After surface sterilization, all roots were rinsed and then incubated in moist chambers for 24 h before baiting. Roots were baited for 7 days with rhododendron leaf disks and leaf disks were placed on amended SMA for detection of *Phytophthora* spp.

Acknowledgments

Funding for this research was provided by The Royal Society, the United States Forest Service, Pacific Southwest Research Station, and the United States Department of Agriculture Animal and Plant Health Inspection Service PPQ Center for Plant Health Science and Technology. We thank R. Smith and Duchy College for donating micropropagated rhododendron plantlets; S. Denman for providing isolates of *P. kernoviae*; D. Beattie for assistance with microscopy; A. Brown and C. Brasier for providing photographs; B. Jones and A. Whybrow for assistance at field sites in Cornwall; and S. Frankel, K. Aram, and two anonymous reviewers for their assistance in revising this manuscript.

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