Dissemination of Aerial and Root Infecting *Phytophthoras* by Human Vectors[1]

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Abstract

Two new *Phytophthora* pathogens, *Phytophthora kernoviae* and *P. ramorum*, have recently established in parts of the U.K. They are most prevalent in the south west of England where they cause intense episodes of foliar blight and dieback on both ornamental and naturalised rhododendron such as *Rhododendron ponticum*, but both also cause lethal stem cankers on a range of broadleaved trees. Patterns of disease spread suggest that both pathogens could be spread over longer distances by vertebrate movement. People and animals frequently walk through these contaminated areas and may pick up infested soil or litter on their feet and transfer it to new sites. A study was therefore set up to analyse how frequently *Phytophthora* could be isolated from the soil or litter attached to people's boots, particularly those walking in woodlands and gardens known to be infested with *P. kernoviae* and/or *P. ramorum*. The study, which started in July 2004, has shown that in total more than 30 percent of samples collected from walker's boots were contaminated with *Phytophthora*. The most commonly occurring species was *P. citricola*, but 10 to 15 percent of the *Phytophthora* positive samples contained either *P. ramorum* or *P. kernoviae.* The source of inoculum could be fragments of the infected leaves which are shed from infected *Rhododendron* and rapidly break down as they incorporate into the litter layer in affected woodlands. Tests have shown that *P. kernoviae* can survive in both air-exposed and litter-embedded infected leaves for more than a year, although there is a decline in the amount of inoculum that survives, indicated by the success of isolation. In air-exposed leaves isolation success dropped from 60 percent to 15 percent over 12 months, and from 78 percent to 18 percent for litter embedded leaves over the same time.

Key words: *Phytophthora kernoviae*, *P. ramorum*, sudden oak death, dissemination, rhododendron, infected foliage.

Introduction

Two invasive *Phytophthora* pathogens, *Phytophthora kernoviae* and *P. ramorum*, appear to be recent introductions in the U.K. (Brasier and others 2004). Since the first finding of *P. ramorum* in southern England in 2002 (Lane and others 2003) around 580 *P. ramorum* outbreaks in nurseries or plant retail outlets have been confirmed, with a further 160 found at outdoor sites (http://www.defra.gov.uk/planth/pramorum.htm) such as gardens, parks and woodlands. In contrast, *P. kernoviae* has been found at only two nurseries, with around 40 outbreaks in woodlands and gardens

[1] A version of this paper was presented at the Sudden Oak Death Third Science Symposium, March 5–9, 2007, Santa Rosa, California.

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(http://www.defra.gov.uk/planth/pkernovii2.htm). Many of the *P. ramorum* non-nursery outbreaks are in the south-west of England and, in the case of *P. kernoviae*, almost all the outbreaks are in this region. Here the two species have been found to cause foliar and shoot necrosis of species and cultivars of *Rhododendron*; on some of the most heavily infested rhododendron sites the two pathogens have also infected various tree species (mainly *Fagus sylvatica* and *Quercus* spp.) causing bleeding stem lesions. As both these *Phytophthoras* are aerial pathogens their deciduous sporangia, produced on foliage of infected rhododendron, can be dispersed in mists and rain splash on a local basis. However, patterns of disease spread suggest that vertebrate vectors may also aid the dissemination of these pathogens over longer distances. Infected rhododendron leaves are shed and incorporated into the dense litter layer and the inoculum they contain could potentially persist for months, if not years, in the litter layer. People and animals frequently walk through these contaminated areas and may pick up infested soil or litter on their feet and transfer it to new sites. A study was therefore set up to assess (1) how frequently *Phytophthora* could be isolated from the soil or litter attached to people's boots, particularly those walking in woodlands and gardens known to be infested with *P. kernoviae* and/or *P. ramorum;* and (2) how long foliage naturally infected with *P. kernoviae* remained a source of viable pathogen inoculum.

Materials and Methods

Most experimental work was undertaken in the *Phytophthora kernoviae* Management Zone (PkMZ), an area of about 12.95 square km (5.5 square miles) in Cornwall in the south west of England (Anonymous 2004).

Soil and litter from boots

To assess the potential for movement by people, surveyors and scientists working mainly in the PkMZ scraped any leaf litter and soil from their boots just before leaving *P. kernoviae* and *P. ramorum* infested areas. The samples were put into zip-lock bags and sent back to the laboratory for analysis. To determine which pathogen (if any) each sample contained, it was inserted into a fresh green apple to 'bait' for any *Phytophthora*. The apples were incubated at 18 to 20^{o}C and isolations made from any lesions that developed using a modification of Synthetic Mucor Agar which is a *Phytophthora* selective agar medium (Brasier and Kirk 2004). Typically *Phytophthora* lesions on the apples were firm and often visible within 4 to 5 days of inoculation.

Survival in infected foliage

To assess the survival of *P. kernoviae* in naturally infected rhododendron leaves, symptomatic leaves were collected in September 2005 and placed in net bags. Replicated series of these bags were then either placed in the litter layer or suspended 15 to 20 cm above the litter layer in the air; five bags of each were removed at set intervals over a 12 month period. To check for survival of *P. kernoviae*, the leaves were removed from each bag, and then each individual leaf was subject to both direct isolation and water baiting to detect any *Phytophthora* present.

Results

Dissemination via people

The study to assess dissemination of *Phytophthora* by people started in July 2004 and ran over 3 years with about. 400 samples collected from walkers' boots. Overall, more than 30 percent of samples contained *Phytophthora* and within the subset of positive samples the most commonly occurring species was *P. citricola*, but 10 to 15 percent of the samples contained either *P. ramorum* or *P. kernoviae.* Several other aerial and root infecting *Phytophthora* species were also found. These included *P. ilicis, P. citricola* and *P. cambivora*.

Over the year, a seasonal pattern emerged of the time when *Phytophthoras* were most likely to be isolated from the boots of walkers. Positive samples were most frequent in June to July and then again in October to November. The greatest number of negative samples occurred over the summer during August to September. There also appeared to be an increased likelihood of *Phytophthora* occurring in the 'boot' samples if they contained fragments of leaf litter and foliage.

Survival in infected foliage

In 2005, surveys of *Rhododendron ponticum* in the PkMZ *Phytophthora kernoviae* Management Zone identified thousands of infected plants, many with visibly affected foliage which was frequently shed prematurely. When individual rhododendron leaves were selected with a range of symptoms indicative of *P. kernoviae*, around 60 percent of the isolations yielded *P. kernoviae*. When these leaves became part of the litter layer, the frequency of *P. kernoviae* isolation increased even further. After three months, positive isolations for litter-embedded and air-suspended leaf samples were 78 percent and 54 percent respectively. However, after 6 months positive isolations had decreased to 20 percent and 11 percent (litter compared with air); while after a year the percent survival had fallen to 18 percent and 15 percent (litter compared with air). Continuing the experiment beyond this time proved impossible because the litter embedded leaves had become so fragmented they could not be retrieved intact from the bags, although the fragments readily formed part of the soil layer which adhered to boots of people working in the Management Zone.

Conclusions

Overall, these results indicate that human vectors could provide a significant pathway for the spread of quarantine pathogens such as *P. ramorum* and *P. kernoviae* as well as for other *Phytophthora* species. The basis of the inoculum probably consists of fragments of infected leaf litter, and certainly *P. kernoviae* survives in this type of material over many months, presumably as oospores. However, it has yet to be established if *Phytophthora* inoculum carried on boots can initiate a new infection focus in an area remote from the source of *Phytophthora* inoculum, or indeed how often this is likely to happen. In addition, the ability of *P. kernoviae* to persist for many months in air-dried leaves or comminuted leaves in the litter layer suggests eradicating this pathogen from infected sites is likely to be a long term process.

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