

# Natural Outbreaks of *Phytophthora ramorum* in the U.K.—Current Status and Monitoring Update<sup>1</sup>

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## Abstract

To date (February 2007) there have been 160 outbreaks of *Phytophthora ramorum* in gardens or woodlands in the U.K. Current EU policy requires that appropriate measures be taken to contain *P. ramorum* in such situations. In the U.K., the measures have either been aimed at eradication, through destruction of infected plants, or at containment to minimise the risk of *P. ramorum* being spread from the site to other areas. Of the 160 natural outbreaks recorded, 123 are ongoing, whilst the remainder are considered to have been eradicated as no further plant infections have been recorded, although in some cases the pathogen may still be detected as residual inoculum in soil or water. Monitoring of residual inoculum levels in soil/leaf debris has been carried out monthly for a period of up to three years in several sites in the south of England to investigate the extent of contamination within the gardens or woodlands and to quantify the effect of season on variation in inoculum levels. A number of the gardens were also found to be infected by *P. kernoviae* and in those situations the monitoring was extended to include both pathogens. A range of methods for monitoring levels of *P. ramorum* and *P. kernoviae* has been evaluated for routine use within the project. Initially samples were analysed using rhododendron leaf bait methods followed by isolation and identification on selective agar. All positive identifications were then confirmed using Real-Time Taqman PCR (polymerase chain reaction). More recently, PCR methods have replaced isolation and identification steps and have been used to develop more quantitative methodologies for monitoring seasonal changes in inoculum levels. Results have confirmed that the pathogens can survive and establish in the U.K. environment and that inoculum levels fluctuate in response to seasonal weather factors. During 2006, additional studies have been carried out to monitor inoculum movement from infected plants and has confirmed long distance (>50m) dispersal during wind-driven rain.

Key words: *Phytophthora ramorum*, sudden oak death, *Phytophthora kernoviae*, monitoring, eradication, dispersal.

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## Introduction

Outbreaks of *Phytophthora ramorum* in large managed gardens were first detected in the U.K. in 2003 and a research programme was commissioned by Department for Environment and Rural Affairs (Defra) to monitor residual contamination in affected areas following eradication action. The monitoring was aimed at investigating key questions to inform risk analyses for the pathogen in the U.K. These included seasonal pathogen survival, the levels of pathogen persistence in water, leaf litter and soil, and inoculum dispersal in the presence/absence of rainfall. Originally, the monitoring was focused on *P. ramorum* but was extended to include *P. kernoviae* when it was discovered in late 2003. The project has tested and validated a range of monitoring methods including the use of quantitative PCR for detection of very low levels of inoculum in soil and water.

## Materials and Methods

### Sites

Levels of residual inoculum remaining in soil and water following removal of diseased plants were monitored at a number of sites in southern England between 2003 and 2006. For the purposes of this paper, monitoring data are reported from three sites (A, B, C), which are representative of the findings from the wider monitoring programme. Site A was a large managed garden in the southeast, in which eradication action on all infected plants had been taken following an outbreak of *P. ramorum*. Site B was a large managed garden in southwest England, which was affected by outbreaks of both *P. ramorum* and *P. kernoviae* and where only localised eradication action had been taken. The third site (Site C), a large managed garden in the southwest affected by outbreaks of both *P. ramorum* and *P. kernoviae* and where very limited eradication action had been taken. At all sites the outbreaks occurred primarily on cultivated or wild rhododendron species.

### Monitoring of Residual Inoculum in Soil and Water

Grids composed of 1 m x 1 m quadrats were marked out in selected areas where previously infected plants had either been removed (Site A) or about to be removed (Site B). Soil and leaf litter samples were taken from each quadrat at roughly monthly intervals and examined for the presence of *P. ramorum* and/or *P. kernoviae*. At site A, where an extensive watercourse was present, baits (rhododendron leaves contained in muslin bags) were deployed along streams, and in ponds, for a period of 1 to 3 days and then removed for testing using baiting methods. Monitoring was carried out at three-month intervals between 2004 and 2006.

### Monitoring of Inoculum Dispersal During Rainfall

Two types of rain trap were used, one at ground level to collect splash-borne inoculum and a second attached to a pole at approximately 1 m above ground level to collect inoculum moving above ground during rainfall. The majority of the rain traps were sited near to or under infected plants and sampled every four weeks for presence of spores. However, a few traps were placed at distance from any infected host plants to monitor longer distance dispersal.

## **Diagnostic Methodologies**

All samples were analysed for presence of *P. ramorum* and *P. kernoviae* by leaf baiting, isolation and microscopy methods, with any positives confirmed by Real-Time TaqMan PCR (Hughes and others 2006; Hughes, 2007, personal communication). In 2006, new DNA extraction protocols were validated for use in monitoring trace levels of *P. ramorum* or *P. kernoviae* in soil at Site A and rain water samples from traps at Site C. Firstly, calibration curves were determined using samples of soil or water to which known numbers of sporangia of *P. ramorum* or *P. kernoviae* had been added. DNA was then extracted from these samples and tested for the presence of the target pathogen DNA using TaqMan PCR. The resultant Ct values were plotted against the original number of spores added to each sample to examine and calibrate levels of detection. Results indicated a strong relationship between number of spores present and the Ct value using PCR.

## **Results and Discussion**

### ***Persistence of Inoculum in Soil (Site A)***

Monitoring using traditional baiting methods, undertaken monthly between 2003 and 2005, indicated sparse but persistent levels of *P. ramorum*, with the number of positive grids fluctuating seasonally. Inoculum was more widespread between October and March compared with the summer months. In 2007, the site was revisited and samples taken for testing using both traditional and quantitative diagnostic tests. Despite consistently negative baiting results, quantitative PCR detected inoculum of *P. ramorum* at some sites but also demonstrated that inoculum was absent from some previously contaminated areas. The locations that were free from inoculum were either areas where no run-off was occurring from other parts of the gardens or where root material from the original infected plant had also been removed.

### ***Persistence of Inoculum in Soil (Site B)***

Monitoring at site B was initiated in December 2003, six months prior to the removal of the infected plants (between June and August 2004). Soil samples were taken monthly and the percentage of grids positive for either *P. ramorum* or *P. kernoviae* determined using baiting methods. Incidence of both pathogens was found to fluctuate seasonally with peaks in inoculum levels occurring between October and March (fig. 1). Incidence of *P. ramorum* has persisted since monitoring began whereas that of *P. kernoviae* has declined over time.

Evidence from both sites indicates that rapid and thorough action involving removal of all infected plants, litter, and preferably the root material as well, can be effective in reducing inoculum levels to below the current thresholds for detection using baiting and isolation methods. Although inoculum distribution fluctuated seasonally, reductions in inoculum presence over time have been demonstrated under these scenarios. A comparison of the data on *P. ramorum* and *P. kernoviae* indicated that *P. kernoviae* is less persistent in soil, possibly due to the fact that it does not produce chlamydospores (there is no evidence of the presence of oospores in the natural environment in the U.K.).

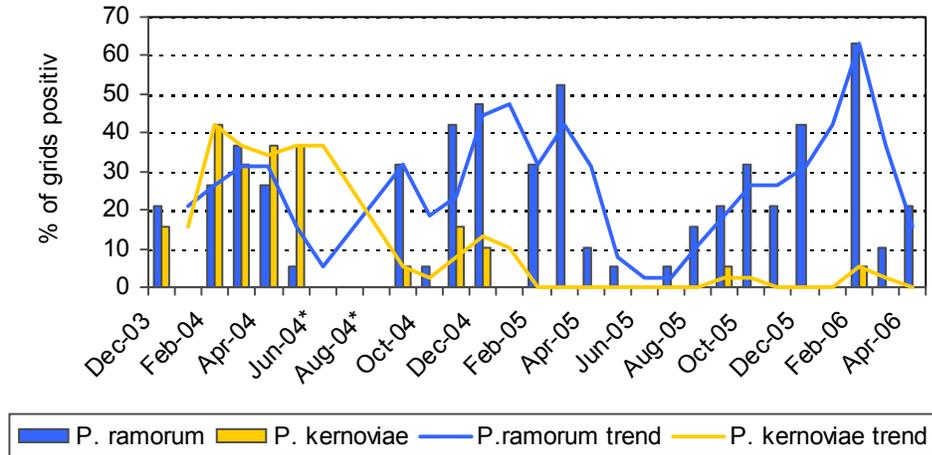


Figure 1—Detection of *P. ramorum* and *P. kernoviae* in soil between December 2003 and April 2006 at Site B. \* indicates period of eradication of the infected plant.

**Persistence of inoculum in water (Site A)**

Inoculum detection frequency in water also showed seasonal patterns with highest frequency generally occurring in winter and spring and lowest in summer (fig. 2). Monitoring of inoculum in watercourses in the U.S. shows similar trends, with reduced detection during the summer months (Tjosvold and others 2002). Although detection frequency at Site A was shown to fluctuate seasonally and persist in water over a period of three years post-eradication of the outbreak, frequency did decline over time and no new plant infections occurred during the period of monitoring. The significance of detection frequency in water and level of risk posed remains unknown, but a positive correlation between detection frequency and inoculum density is assumed.

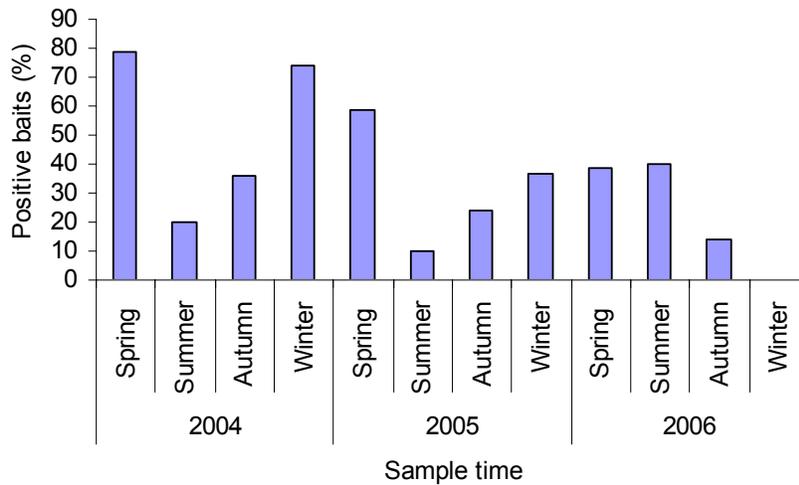


Figure 2—Detection of *P. ramorum* in water courses at Site A.

### Spore Dispersal During Rainfall (Site C)

Analyses of water samples from rain traps placed at ground level at Site C indicated that splash-borne inoculum of both *P. ramorum* and *P. kernoviae* was detectable throughout the monitoring period between March and December 2006. Comparison of data on the two pathogens from all monitoring sites indicates that *P. ramorum* was more frequently detected than *P. kernoviae* as splash-borne inoculum whereas *P. kernoviae* was more frequently detected than *P. ramorum* in wind-driven rain.

Analyses of the samples from the high level traps showed that, whereas *P. ramorum* was detected in wind-driven rainfall during December only, inoculum of *P. kernoviae* was detected in May and June, absent from July to September and then detected again between October and December.

Between October 2006 and February 2007, quantitative PCR methodologies were used in conjunction with bait tests to analyse samples from high-level rain traps. The more sensitive technique showed that very low numbers of spores could be detected in rainwater samples that had tested negative using the bait tests. Quantitative monitoring at five locations within Site C showed that *P. ramorum* inoculum density peaked in December whilst density of *P. kernoviae* peaked in either November or December depending on location. It was estimated that a maximum of 40 spores of *P. ramorum* and 8000 spores of *P. kernoviae* per litre of rainwater were detected during peak months. Quantitative analysis of samples from a rain-trap located at a distance of more than 50m from an infected host also detected inoculum of both pathogens at very low levels (fig. 3). Though Brasier and Jung (2006) have observed that some infections in the U.K. could only be explained by inoculum dispersing to distances of over 50 m, this is the first report of long distance dispersal of spores of *P. ramorum* and *P. kernoviae* in the U.K. The significance of these inoculum densities in terms of disease risk in the U.K. is being investigated.

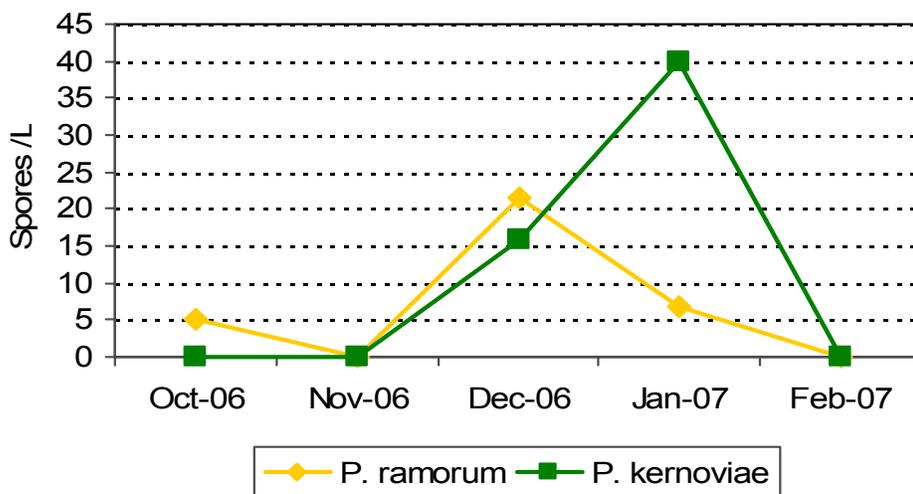


Figure 3—Detection of spore dispersal in wind driven rain in a rain trap located at a 50 m distance from infected plants at Site C.

Monitoring data from this project continue to be used to support U.K. risk analyses and development of policy. Important evidence on the benefits of quick and thorough action has assisted in advising landowners on appropriate courses of action. Data indicate that quantitative methodologies offer significant opportunities to investigate pathogen epidemiology, particularly in situations like the U.K. where the inoculum densities appear currently to be relatively low.

## Acknowledgments

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