

Monitoring *Phytophthora ramorum* and *P. kernoviae* in Soil and Rainwater Samples Collected at Two Sites on a Cornish Estate¹

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Abstract

Soil samples were collected from quadrats marked out below the canopies of two rhododendrons, one infected by *Phytophthora ramorum* (Site 1) and the other (Site 2) infected by *Phytophthora kernoviae* and *P. ramorum*. Rainwater was collected in high-level and low-level traps. Soil and rainwater were sampled at roughly monthly intervals from November 2003 until April 2006, and tested for the presence of *P. ramorum* and *P. kernoviae* at the Central Science Laboratory. The infected rhododendrons were removed in May 2004 (Site 1) and July 2004 (Site 2) after which the quadrats and rain traps were reinstated. *P. ramorum* was initially detected in soil from less than a third of the quadrats at Site 1 and declined to very low levels after removal of the host. All rainwater samples at Site 1 were negative for *P. ramorum*. At Site 2, both *P. ramorum* and *P. kernoviae* were initially readily found in soil samples. After removal of the host, levels of *P. kernoviae* in the soil declined rapidly, but *P. ramorum* was still detected in a relatively high proportion of soil samples for up to 21 months. *P. ramorum* was found in rain traps at Site 2 over the winter of 2006, indicating an influx of wind blown spores. Rhododendron leaf baits buried in the soil at Site 2 frequently became infected by *P. ramorum* for up to 21 months after removal of the infected host.

Key words: *Phytophthora ramorum*, *Phytophthora kernoviae*, soil, rainwater, rhododendron.

Introduction

An extensive outbreak of *Phytophthora ramorum* was discovered in 2003 at a large estate in Cornwall containing one of the world's great collections of magnolias, camellias and rhododendrons, with many specimens originating from China and planted a hundred years ago. Further investigations revealed the presence of a new *Phytophthora* species, later named *P. kernoviae*.

Materials and Methods

Two sites with infected rhododendrons, one with *P. ramorum* (Site 1) and the other with both pathogens (Site 2), were selected in November 2003 for monitoring work. Nineteen 1m² quadrats were marked out on the ground below the rhododendron

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canopies at each site and leaf litter and/or soil was sampled from each quadrat at roughly monthly intervals. Rain traps were also set up and sampled monthly. High-level rain traps consisted of a sterile plastic bottle fitted with a 15cm diameter funnel, secured to a post 1m above ground level. Low-level rain traps were introduced later and consisted of a plastic bowl placed on the ground. Four or five clean rhododendron leaves were clamped between two plastic discs and placed in the bowl, which was then covered with fine netting to prevent leaves being blown into the trap.

Samples were tested for the presence of *P. ramorum* and *P. kernoviae* at the Central Science Laboratory, York. Soil samples were placed in a plastic sandwich box and Petris mineral solution added until the soil was just covered. Pieces of fresh, clean rhododendron and magnolia (evergreen) leaves were then floated on the solution. After 7 days the rhododendron and magnolia pieces were removed and rinsed twice in tap water before being plated onto *Phytophthora* selective media (PARPH). After a further 7 days the plate was visually assessed for the growth of any *Phytophthora*. Positive visual assessment was then validated using TaqMan real time PCR. Water samples from high-level rain traps were processed in a similar way to the soil samples, with rhododendron and magnolia leaf pieces floated directly on the water sample.

The rhododendron at Site 1 was removed in May 2004 and the plant at Site 2 in July 2004 after which the quadrats were reinstated and monitoring continued until April 2006. In order to determine whether the recovery of *P. ramorum* from soil samples indicated a risk to susceptible plants growing on the site, soil baits were buried every two or three months after removal of the infected rhododendron at Site 2. The baits, which consisted of cut pieces of rhododendron leaves enclosed in small muslin bags, were buried just beneath the soil surface in a quadrat which had previously yielded positive soil results. The baits were retrieved after a week and the leaf pieces soaked and then rinsed twice before being plated on PARPH and processed as above.

Results and Discussion

Soil contamination by *P. ramorum* was initially relatively low at Site 1 with only 31 percent of quadrats positive in December 2003. After the removal of the infected host, *P. ramorum* was detected in 0 to 10 percent quadrats over the following winter and spring, and then only occasionally in 5 percent samples. *P. ramorum* was never detected in any of the rain traps at Site 1, although *P. kernoviae* was recovered from a low-level trap in November 2005.

P. ramorum and *P. kernoviae* were readily detected in soil from quadrats at Site 2 prior to removal of the host. After removal, *P. ramorum* was still found in a high proportion (up to 63 percent) of quadrats for 21 months, after which time, monitoring was concluded. Recovery of *P. ramorum* was greatest in the winter and spring when soils were wetter, than in the summer. *P. kernoviae* however, declined and was rarely recovered in soil samples after removal of the infected host. No *P. ramorum* was detected in rain water samples from high-level traps before removal of the host, but it was detected in rain collected over the winter of 2006 in both high- and low-level traps, suggesting an influx of wind blown inoculum. Surprisingly, despite

considerable initial foliar infection by *P. kernoviae* at Site 2, the pathogen was not recovered from any of the rain traps.

Soil baits at Site 2 frequently became infected by *P. ramorum*, but never by *P. kernoviae*. Infection was lower in the summer 2005 and greater in the winter and spring 2006. At the final baiting in April 2006, all baits became infected by *P. ramorum*. The presence of *P. ramorum* in soil sampled in 2006 may be due to new inoculum deposited by wind and rain or long term survival of chlamydospores originating from infected leaf litter.

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