

# The Status of *Phytophthora ramorum* in Ireland<sup>1</sup>

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## Abstract

This paper reports on the first 2 years of data collected to study the ecology of *Phytophthora ramorum* in Ireland. Since spring 2005, sampling has been carried out for the presence of the pathogen in soil and watercourses from 11 susceptible forest sites in Ireland, using a rapid DNA method in conjunction with morphological identification methods. Each site was sampled twice a year, collecting foliage and using rhododendron leaves as baits for water and soil samples. In June 2005, the pathogen was positively identified as the cause of *Rhododendron ponticum* twig and leaf blight in Killarney National Park, in the southwest of Ireland. The pathogen has since been positively identified at a new site, approximately 32.2 km (20 miles) from Killarney National Park, but has not yet been identified on trees. To date, *P. ramorum* is only found at three sites in the southwest and southeast of Ireland. While it is unknown whether trees will become infected, many potential susceptible hosts of the pathogen are widely distributed among these forests. Work is currently underway to study the susceptibility of Irish flora to the pathogen, as well as the sporulation potential, dispersal, infection, latency and survival of the Irish isolates. This is the first report on the molecular identification of *P. ramorum* in Ireland.

Key words: ITS nuclear DNA marker, PCR, *Phytophthora ramorum*.

## Introduction

After the discovery of *Phytophthora ramorum* (Werres and others 2001) in the U.K. in 2002, emergency European Community legislation was implemented to prevent the spread of the pathogen within the European community. Surveys were carried out in nurseries, public and private gardens and susceptible forest sites throughout Ireland by the Department of Agriculture and Food. At nurseries and garden centres the Department of Agriculture and Food inspects the nurseries twice a year and follows all EC legislation when infected material is found. That is, infected material and all susceptible plants within 2 m of the infected plant are destroyed and all susceptible plants within a 10 m radius are retained for at least three months for inspection and testing. From 2003 to 2005, there was a 2 percent reduction in the number of outbreak sites found in nurseries and garden centres in Ireland.

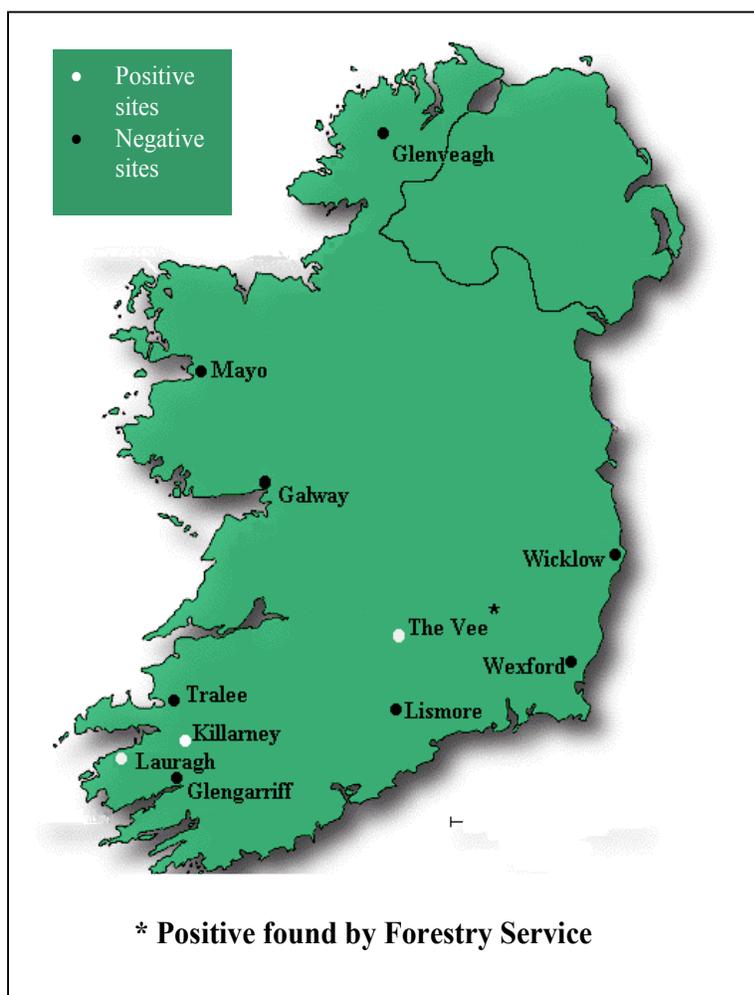
Official surveys carried out since 2003, by the Department of Agriculture and Food, have confirmed the presence of *P. ramorum* in Ireland. This body identifies the pathogen, using only morphological characteristics. The pathogen has only been found at three locations in the wild (fig. 1) and only on *Rhododendron ponticum*.

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Figure 1—Map of 12 sites sampled throughout Ireland during 2005-2006



This is a huge concern as one of the infested sites, Killarney National Park, forms the most extensive semi-natural woodland left in Ireland covering over 1,200 hectares; *R. ponticum* has completely infested upwards of 650 hectares of the park. This park is frequented by thousands of visitors throughout the year, and there are also volunteer groups whose main aim is to clear rhododendron from the park during the summer. The methods of foliage removal and transport are a cause of concern in the spread of the pathogen throughout the park.

One of the aims of our project, which started early in 2004, was to assess the potential of PCR amplification of a molecular marker to identify the pathogen. In addition, the distribution of the pathogen and identification of the possible vectors involved in its movement throughout Ireland would be investigated.

## Materials and Methods

Since March 2005, samples of *R. ponticum*, *Ilex aquifolium* (holly) and *Quercus petraea* (sessile oak) have been analysed twice a year from 11 sites, during the months of March to September. Field sampling was directed towards specific sites with known susceptibility characteristics, in other words, the presence of *Q. petraea*

with an understorey of rhododendron. Sampling was carried out primarily by collecting foliage, but also by using water and soil baits, and lateral flow devices, which were supplied by the Central Science Laboratory in York. Baits were made using 25 cm<sup>2</sup> sections of sterile muslin cloth, into which was placed five pieces of sterile gravel, eight pieces of cut up sterile *R. ponticum* leaves, and two pieces of polystyrene packaging to aid flotation near the water surface. The cloth was tied off, not too tight, with 2.5 m of string (Beales 2006).

Isolation of the pathogen was carried out on P<sub>5</sub>ARP agar and identification was confirmed using morphological characteristics first, and then conventional PCR (Van Leeuwen and others 2003). DNA was extracted, from homogenised samples, using the Nucleospin plant extraction kit (Macherey-Nagel, Germany). Conventional PCR was carried out using Hughes's primers (Van Leeuwen and others 2003), which amplify a 700 base pair fragment from the ITS region of a nuclear ribosomal RNA gene. The amplified products were resolved by electrophoresis on a 1.5 percent agarose gel for 1 hour at 100 volts. The gel was run in 1XTBE buffer containing 15 µl of ethidium bromide and viewed on a UV transilluminator.

## **Results**

Of the 11 sites we investigated, the pathogen was found at two locations in the southwest of Ireland over the course of two years sampling. These were Killarney National Park and Lauragh, which is located 32.2 km (20 miles) from Killarney National Park (fig. 1).

Our first positive was found in May 2005 on *R. ponticum* foliage in Killarney National Park. Shortly after this, the Forestry Service intensively surveyed the entire boundary of the park and signs were erected throughout the park, forbidding the removal of susceptible foliage. In February 2006, we found the first water positive in Ireland, in Killarney National Park. The site was located just off a main road and consisted of a body of still water which had a stream flowing from it into a large lake. Since there are three interconnected lakes in Killarney National Park, 10 additional water baits were placed along an 8.1 km (5 mile) stretch of the watercourse, with no further positives being found. In total we have found 13 positives in Killarney National Park, from soil, water and *R. ponticum* foliage (table 1).

The second positive site we found was in the village of Lauragh and the pathogen was isolated from five samples of *R. ponticum* foliage only (table 1). This site is a privately owned site and is currently under containment in accordance with the EC legal requirements of findings in the wild.

In October 2003, the Forestry Service located the third site in the Vee, in the southeast of the country (fig. 1). Eradication efforts were carried out under quarantine control measures, but in 2006 the pathogen was still found at extremely low levels. Despite several sampling trips, we have not found a positive in this area using the two methods of detection.

**Table 1—Summary of positive sites sampled throughout 2005-2006**

<b>Killarney</b>	2006	2006	<b>Lauragh</b>	2005	2006
No. samples collected	17	30	No. samples collected	11	18
No. +ve samples	5	8	No. +ve samples	0	5

## Conclusions and Future Work

The Department of Agriculture and Food believes that the inoculum level of *P. ramorum* is low in Ireland, being found by both the department and ourselves at a maximum of three sites. This could be the reason why, to date, the pathogen has not been found on trees in Ireland. If efforts to eradicate the pathogen are not increased, it is only a matter of time before an infected tree is found. There is currently no information available to the public on the presence of *P. ramorum* in Ireland.

A GIS model will be developed to determine the epidemiology of the pathogen, which ultimately could be used as a management tool to target threatened forests for early detection, monitoring and protection.

The sporulation potential and infection threshold will also be tested on a range of tree species, focusing primarily on *Q. petraea*, along with *Pseudotsuga menziesii* (Douglas-fir) and *Taxus baccata* (Yew). The Yew forest in Killarney is the most rare habitat type within the Park and the only significant area of Yew woodland left in Ireland. Our investigation will therefore provide important information on the risk posed by the pathogen to native Irish trees. In addition, we will assess the susceptibility of some special flora found in Killarney National Park such as *Trichomanes speciosum* (Killarney Fern), probably the rarest plant species in the Park.

Since the first finding of *Phytophthora kernoviae* in the U.K., in 2005 (Brasier and others 2005), the possible presence of the pathogen in Ireland has become a cause for concern. At present, the Department of Agriculture and Food are surveying for the pathogen using morphological characteristics. Therefore, over the next year our sampling regime will also be targeted at *P. kernoviae* using both morphology and real-time PCR as methods of detection.

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