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Assessment of the eradication measures applied to *Phytophthora ramorum* in Irish *Larix kaempferi* forests

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Running head: Eradicating *P. ramorum* in Irish *Larix* forests

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Abstract

Phytophthora ramorum is the causal agent of the sudden larch death epidemic in Ireland and the UK. Within the EU it is a quarantine pathogen and eradication measures are required if it is detected in horticultural or forest environments. Eradication measures in forests include the clearance of susceptible tree hosts from the infected stand along with all host known to support pathogen sporulation within a 250 m buffer zone of the infected stand. Between 2010 and 2016 these measures have affected over 18,000 ha of *Larix kaempferi* forests in Ireland and the UK but the epidemic continues to spread. An assessment of the efficacy of the eradication measures has not been published to date. Here we provide details of the detection frequency of *P. ramorum* from aerial (rainwater) and terrestrial (soil, watercourses, plant material) sources in three forest locations in Ireland that had significant areas of *L. kaempferi* affected by *P. ramorum* before their removal. Monitoring of six plots with differing infection and eradication management histories was carried out from September 2013 - 2015. Presence of *P. ramorum* was confirmed by plating plant material onto selective media, followed by morphological identification. *Phytophthora ramorum* was detected in 65 of 1283 samples, in

all sample types, and in 17 of the 20 months sampled. Only 5 of the 295 soil samples were positive for *P. ramorum*, with all of these coming from an area under perennial standing water. The most positive samples came from a plot where symptomatic *Larix* trees had not been removed and the findings occurred consistently over the two year study. Plots where infected *Larix* had been removed were rarely positive for *P. ramorum* across all the sample types indicating a level of success from the eradication measures in reducing pathogen levels on the sites.

Introduction

Phytophthora ramorum is an emergent generalist oomycete pathogen that causes sudden larch death in Ireland and the UK (Brasier and Webber 2010; McCracken 2013), sudden oak death (SOD) in the USA (Rizzo et al. 2002), and ramorum blight in both European and North American nurseries (Perez-Sierra and Jung 2013). In the European Union, it is a notifiable organism under EU plant health legislation (2002/757/EC). This means that plants infected with the organism must be notified to the National Plant Protection Organisation, and eradication measures implemented. The wide host range of *P. ramorum* and suitability to the maritime climate make it one of the most threatening pathogens of trees and woody plants in Ireland and the UK (Sansford et al. 2009; Brasier and Webber 2010; McCracken 2013; Jung et al. 2016).

In Ireland, *P. ramorum* has been detected in ornamental horticultural plants since 2002 and on *Rhododendron ponticum* in forests since 2003 (EPPO 2003-2010). Up until 2010 in Ireland, *P. ramorum* infections in the wider environment were only detected on *Rhododendron* spp., while infections on *Vaccinium* spp. and a single infection on the tree species *Quercus phillyraeoides* had been found in managed gardens (EPPO 2003-2010). Following findings of *P. ramorum* on *Larix kaempferi* in Britain in 2009 (Webber et al. 2010a), *P. ramorum* was found infecting non-native commercial plantations of *L. kaempferi* in Ireland and Northern Ireland in 2010 (EPPO 2003-2010; DAERA 2016). Recently it has also been detected infecting *L. kaempferi* in a forest in western France (COMTF 07/2017). It has now been detected on more than 30 hosts in Ireland, including the tree species *Abies alba, Abies procera*, *Castanea sativa*, *Fagus sylvatica*, and *Picea sitchensis* (O'Hanlon et al. 2016).

In Ireland and the UK, the highly invasive woody shrub *Rhododendron ponticum* and commercial forestry tree species *L. kaempferi* are the two hosts of primary concern for spreading the disease epidemic. Both of these hosts are widespread across the Irish landscape, *R. ponticum* is widespread throughout Ireland (NBDC 2016), while *L. kaempferi* accounts for 4.1% (25,980 ha; NFI 2012) and ca. 5% (5500 ha; McCracken et al. 2015) of the tree species composition of the Irish and Northern Irish forest estates, respectively. These hosts are regularly found infected in Ireland (O'Hanlon et al. 2016), and are known to support high levels of *P. ramorum* sporulation (Harris and Webber 2016). Other species of *Larix*, such as hybrid larch (*L.* × *marschlinsii*) and European larch (*Larix decidua*) can also apparently support high levels of sporulation (Harris and Webber 2016) but are likely to be less

important hosts in the sudden larch death epidemic as they have only a limited distribution across Ireland (NFI 2012).

Phytophthora ramorum infection on *Rhododendron* include foliar and stem lesions, along with extensive dieback in some cases (Appiah et al. 2004). *Phytophthora ramorum* infection in *L. kaempferi* causes crown dieback, trunk resinous cankers and foliage death. In some cases the normally deciduous foliage is retained as clusters of dead needles, and this retained foliage has been identified as a possible source of subsequent canopy infections (Webber et al. 2010b). Studies in infected *Rhododendron* sites in Ireland and Britain have indicated that *P. ramorum* is spread in rain splash and in soil and leaf litter, with spread in watercourses being less important (Turner et al. 2005; O'Connor 2009; Elliot 2013). Spread over distances greater than several meters in this habitat is most likely via movement in watercourses (Elliot 2013) and human mediated dispersal mechanisms like infected plant material and contaminated footwear or tyres (Chadfield and Pautasso 2012). The current understanding of the disease epidemiology in *L. kaempferi* forests suggests that spread at the km scale is likely in wind-driven rain and mist (Webber et al. 2010a; Van Poucke et al. 2012; McCracken et al. 2015; King et al. 2015). Monitoring in Douglas fir-tanoak forests in Oregon has indicated putative spread of over 4 km on several occasions (Peterson et al. 2015).

In national surveys in Ireland since 2003, *R. ponticum* has been found infected with *P. ramorum* at 26 forest locations, while infected *L. kaempferi* has been found at 47 locations (DAFM 2015a). In Northern Ireland, *P. ramorum* infected *L. kaempferi* has been confirmed at 92 forest locations (McCracken et al. 2015). While detailed regulations are given for eradicating *P. ramorum* in plant hosts at their places of production, EU Member States have flexibility in their response to detections at places other than places of production (Commission Decision 2002/757/EC). In Ireland, the eradication treatment following detection in forests involves felling of all trees within the infected stand (i.e. compartment) along with all hosts supporting *P. ramorum* sporulation within a 250 m buffer zone. Similar eradication measures are in place in the UK (Anonymous 2014, 2015). Between 2009 and 2016, eradication measures have affected over 18,800 ha of *Larix* forests in Ireland and the UK (COMTF 03/2015; DAERA 2016; DAFM 2015a). The felled material can if suitable, be processed at any of three licensed processing facilities in Ireland and Northern Ireland. Reconstitution grants from government to remove infected *Larix* trees and replace with less susceptible species are available to affected private land owners in Ireland and the UK since 2011 (Anonymous 2013; DAFM 2015b).

Since the description of *P. ramorum* as a species in 2001 (Werres et al. 2001), eradication measures for *P. ramorum* infection in forests have only been applied in Ireland, the UK, and the USA (Oregon and California) as these are the only countries where forest epidemics have occurred. The aim of this work was to assess the efficacy of eradication measures against *P. ramorum* in Ireland by evaluating the persistence of viable *P. ramorum* on sites two years after eradication measures were applied. While there have been several reports detailing the effects of eradication measures in forests in Oregon (Kanaskie 2016),

California (Alexander and Lee 2010), Northern Ireland (McCracken et al. 2015) and England (Webber 2016), there has been no such assessment in Irish forests.

Methods

Site selection

Three *P. ramorum* infected *L. kaempferi* sites were selected in the counties Tipperary (T), Kilkenny (K) and Wicklow (W) in Ireland. All sites are in public ownership, and are managed by the state owned forest management company Coillte. Details about the individual sites are provided in Table 1. The soil type of the site was identified using the soil maps of Fay et al. 2007. No site contained *R. ponticum* but at all three sites substantial dieback and mortality of *L. kaempferi* was recorded during aerial surveys with *P. ramorum* confirmed as the cause after sampling by the regulatory authorities and testing by plant health laboratories in both Ireland and the UK.

All sites had eradication measures applied in either 2011 or 2012 (Table 1). Logging slash (e.g. branches and foliage) was piled in rows and left on-site to decompose. In the T site, a total of 8.6 ha of *L. kaempferi* and 0.05 ha of *F. sylvatica* were felled (P. O'Tuama personal correspondence January 2014). In the K site, a total of 8.7 ha of *L. kaempferi* was felled, along with 21.2 ha of *A. procera* and 2.8 ha of *F. sylvatica* in a 1 km^2 area due to multiple detections at this site. In the W site, the eradication treatment included the felling of ca. 3.5 ha of *L. kaempferi* and 0.05 ha of *F. sylvatica*.

At each site two planting compartments were selected as monitoring plots. The plots varied in size depending on the compartment size, but ranged from 6,175 - 22,100 m2 (Table 1). In all plots the dominant tree species was *L. kaempferi* although some other tree species were also present (Table 1). A tree inventory of each plot was as follows:

- T1 contained 10 *Fagus sylvatica* (40 years old), two isolated *L. kaempferi* (15 years old) and 30 *Sorbus aucuparia* (20 years old); T2 plot contained 10-20 scattered *S. aucuparia* (20 years old).
- K1 plot contained 5-10 trees comprising naturally regenerated *L. kaempferi*, *F. sylvatica* and *A. procera* (all <5 years old) and 30 *Sorbus aucuparia* (20 years old); K2 plot contained a block of ca. 200 *Picea sitchensis* (45 years old) and 5 scattered *S. aucuparia* (15 years old).
- W1 plot contained four *F. sylvatica* (15 years old) along with several naturally regenerated *L. kaempferi* and *Pinus contorta* (all <5 years old). Prior to the study W2 plot was not known to be infected and contained ca. 40 *L. kaempferi* (50 years old), some of which were found to be symptomatic when the plot was set up. It was not subject to any eradication measures during the study.

Following eradication measures the most common ground vegetation consisted of *Rubus fructicosa*, *Pteridium aquilinum and Digitalis purpurea.* Plots K2 and W2 also contained scattered *Vaccinium myrtillus*, and plot W2 also had some *Ilex aquifolium.*

The plots were visited at monthly intervals between August 2013 and September 2015 and samples consisting of symptomatic plant material, baiting leaves, baiting plants and soil samples were collected and returned to the lab for testing. The daily rainfall, and minimum and maximum temperatures between August 2013 and September 2015 were downloaded from the Met Eireann (www.met.ie) historical weather archive. The closest Met stations to site K (Kildalton Agricultural College), site T (Cashel - Ballydoyle house) and the W site (Glenealy - Kilmacurragh park) were used. All weather stations are within 20 km of the forest sites used.

Spore trapping/sample baiting

At the start of the monitoring three permanent rainwater trapping stations similar to those used in Turner et al. (2006) and Elliot (2013) were established in the plots. Each trapping station contained a high level trap (HLT) and a low level trap (LLT). HLT consisted of a 1 lt plastic bottle with 12 cm diameter funnel (giving a sampling surface area along a plane of 452cm^2) fixed to at a height of 1 m above ground level . LLT consisted of a 20 x 30 cm plastic container (giving a sampling surface area of 600cm^2) placed at ground level with wire mesh secured in place. The surface area for trapping of the HLT was 75% that of the LLT. Trapping at two different heights was used because it was assumed that the HLT would only detect *P. ramorum* spores dripping from the canopy of the forest (i.e. from canopy sources) while the LLT would detect spores from both the canopy and also from soil splash (canopy and terrestrial sources). Early in the sampling (September 2013) five of the six spore traps in the W2 plot detected *P. ramorum*. The number of trapping stations in this plot was increased from three to six from November 2013 to the end of the monitoring in order to collect extra data from this actively infected plot.

Each rainwater trap contained a *Rhododendron caucasicum* × *ponticum* 'Cunningham's White' leaf, with the traps (i.e. bottle, funnel, and *Rhododendron* baiting leaf) changed on each plot visit and the previous months' *Rhododendron* leaf returned to the lab for testing. A *R. caucasicum* × *ponticum* baiting plant was also placed at each plot, next to one of the spore trapping stations. Symptomatic and asymptomatic leaves from these plants were removed on each visit and tested in the lab. If the baiting plant was found to be infected with *P. ramorum* it was replaced with another *R. caucasicum* × *ponticum* baiting plant. The *Rhododendron* used for baiting or as baiting plants were grown in a glasshouse for 2 years before the start of the project during which time they were monitored regularly for signs of *Phytophthora* infection. Only soft *Rhododendron* leaves from these plants were used in rainwater traps, and in soil and watercourse baiting. Leaves from these plants were also used as internal laboratory negative control leaves during the normal regulatory phytosanitary testing taking place in the Plant Health Laboratory of the Department of Agriculture, Food and the Marine, Ireland.

Soil sampling

Three ca. 200 ml soil samples were collected from random locations within the bounds of the plot on each visit. The location of these samples was marked with GPS so that sampled points were not re-sampled. Soil samples were taken to a depth of up to 10 cm, and plant litter was included in the samples. The samples were placed into 10 × 10 cm Ziploc plastic bags. Upon return to the lab, the bags including the samples were inundated with distilled water and baited using a single *R. caucasicum* × *ponticum* leaf. After 3-5 days at 17-22°C the leaves were removed and symptomatic areas of the leaves tested for *P. ramorum* (see *Phytophthora* isolation below). Random sampling of leaf sections was undertaken if leaves were asymptomatic.

Watercourse sampling

Watercourses near to plots W1, W2, T1 and K1 were baited with a *Rhododendron* leaf inside mesh sacks attached to a weight (Turner et al. 2006). Each watercourse received one baiting sack, with baiting sacks changed at monthly intervals. The watercourse baited in the T site was 1 km from both the T1 and T2 plots, and did not receive run-off water from either plot. The watercourse in the K site was situated ca. 200 m from the K2 plot, and was running parallel to the K2 plot. Two watercourses were baited in the W site, one storm drain situated 150 m from the W2 plot, the other was a stream 200 m from the W1 plot. Both these watercourses received run-off water from their nearest plot. In addition, an area of standing water in plot W2 was also baited. The standing water was ca. 2 x 2 m in area and 20-30 cm in depth and fed from water flowing through the plot.

Footwear sampling

To test if footwear became contaminated with *P. ramorum* after work within the sites, the boots of the researchers involved were washed after site visits and run-off collected in a 2 litre plastic bottle. One boot was washed with water, while the other was washed with a general purpose disinfectant (Jeyes Fluid®, Jeyes group, Active substance: Chlorocresol 6%). The run-off was baited by adding a *Rhododendron* leaf into the plastic bottle and baiting for 5 days at 17 – 22°C in the laboratory, and the leaf tested for *Phytophthora* presence using the isolation method described below.

Phytophthora *isolation*

All plant samples were washed in either dH_2O for 5 minutes or in NaOCl (1%) for 2 minutes followed by dH_2O for 5 minutes. Symptomatic pieces of plant material were aseptically removed, blotted dry with tissue and plated onto PARP (Jeffers and Martin 1986), then incubated at 17-22°C on a lab bench for up to 14 days and checked daily for the presence of *Phytophthora*-like mycelium. Inoculum plugs of *Phytophthora*-like cultures were transferred from PARP to carrot piece agar (modified CPA; Werres et al. 2001) and incubated at 17-22°C and confirmed as *P. ramorum* if the distinctive semi-papillate caduceus sporangia and abundant chlamydospores could be seen on both PARP and CPA plates. Other *Phytophthora*

and *Phytophthora*-like species isolated were identified using PCR, sequencing of the ITS region (White et al. 1990) and BLAST comparisons (see O'Hanlon et al. 2016).

Results

A total of 1282 samples were tested for *P. ramorum*, with samples collected on monthly intervals between August 2013 and September 2015. *Phytophthora ramorum* was detected on 65 occasions across the plots (5% of samples), with a marked variation in the number of detections between the plots (Table 2). In addition to *P. ramorum*, other *Phytophthora* species were detected and these comprised *Phytophthora gonapodyides*, *Phytophthora plurivora* and *Phytophthora syringae*. Several other Pythiaceae (*Elongisporangium anandrum*, *Pythium aquatile*, *Pythium* sp., *Pythium torulosum* and *Elongisporangium undulatum*) were also detected. The highest daily amounts of rainfall recorded at the weather stations near the sites were 59, 40 and 57 mm at the W, K and T sites, respectively. The number of days with temperatures below 0°C were 6, 49 and 42 at the W, K and T sites, respectively. Given the low number of detections of *P. ramorum*, no attempt was made to draw correlations between *P. ramorum* detections and weather patterns.

Plots T1 and T2

Phytophthora ramorum was never detected in plot T2. The spore trapping stations and the baiting plant in Plot T2 were all in open areas, with no overhanging trees. Plot T1 contained two positive mature *F. sylvatica* trees (bark samples found positive on August 2013, December 2013; April 2014). These trees were asymptomatic when the original eradication felling took place in 2011 (Table 1), and as *F. sylvatica* is not known to be a host supporting sporulation for *P. ramorum* it was not cleared from this plot. One of the spore trapping stations in this plot was beneath one of the infected *F. sylvatica* trees, while another trapping station was in an open area with no trees overhead. The final spore trapping station, which included a *Rhododendron* baiting plant, was placed beneath a pair of naturally regenerated *L. kaempferi* trees. These two trees had probably been missed during the original eradication treatment as they were surrounded by mature *F. sylvatica*. None of the spore traps in this plot yielded any *P. ramorum*. A soil sample from within plot T1 in March 2014 tested positive for *P. plurivora*. *Phytopythium montanum* was isolated from a *F. sylvatica* bark sample from this plot, while *Pythium aquatile* was isolated from the H_2O footwash sample from the T plots in January 2015. *Elongisporangium undulatum* was isolated from a watercourse bait in this site in July 2015. The symptomatic and asymptomatic plant material samples tested from the T site consisted of *F. sylvatica* (n=10), *Ilex aquifolium* (1), *L. kaempferi* (12), *Picea sitchensis* (1) and *Vaccinium myrtillus* (3).

Plots K1 and K2

Phytophthora ramorum was not detected in plot K1, in which all of the spore trapping stations were in open areas with no trees overhead. *Pythium torulosum* was detected in a LLT in K1 in June 2015. Two of the spore trapping stations in K2 were in open areas with no trees overhanging. The other spore trapping station and the baiting plant for the K2 plot was beneath a canopy of one *Pseudotsuga menziesii* and two *P. sitchensis* trees. *Phytophthora ramorum* was detected in a LLT in an open area in K2 (December 2013), and from a watercourse (March 2014, July 2015, September 2015) near K2. *Phytophthora syringae* was isolated from an LLT in K2 (February 2015), while *P. gonapodyides* was isolated from the watercourse near K2 (July 2014).

Plots W1 and W2

The W1 and W2 plots returned the most positive results of all the plots (Table 2, Table 3). The spore trapping stations and the baiting plant in W1 were all situated in open areas with no trees overhead. At W1 there was a positive HLT and LLT in January 2014, while the *Rhododendron* baiting plant was also positive in 2014 (June and July). The stream bait near this plot was positive for *P. ramorum* in July 2014; and also in September 2015. In March 2015 *P. ramorum* was isolated from bleeding cankers on two *F. sylvatica* trees in this plot. These trees were asymptomatic at the time of the original confirmed infection of this plot in 2010 (Table 1). Similar to plot T1, these trees were not removed in the eradication measures as *F. sylvatica* is not known to be a host supporting sporulation for *P. ramorum*. *Phytophthora gonapodyides* was detected in the stream at this plot in January and August 2015.

Within plot W2, four trapping stations and the baiting plant for this plot were situated directly underneath infected *L. kaempferi* trees (infection confirmed August 2014). These trees were not removed during the eradication measures at this site in 2011 as they were not symptomatic or within the buffer zone of an infected tree. The remaining two trapping stations at this plot were situated 5 m and 20 m distance from the nearest *L. kaempferi* tree, beneath a *P. sitchensis* over-story. The trapping station (i.e. traps 2.4L, 2.4H) furthest away from the symptomatic *L. kaempferi* canopy in this plot was never positive (Table 3). Detailed results for the traps and baits in this plot are given in Table 3. A noticeable decline in the crown foliar density of the *L. kaempferi* trees was seen between the spring 2013 and spring 2014 seasons. The stream bait near the W2 plot was positive for *P. ramorum* in June 2015. *Phytophthora gonapodyides* was also detected in this stream in July 2014, August 2015 and September 2015. There was also an infected *L. kaempferi* (infection confirmed April 2014) identified 265 m from the nearest W2 trapping station. The symptomatic and asymptomatic plant material samples tested from the W site consisted of *Blechnum spicant* (n=1), *F. sylvatica* (11), *I. aquifolium* (1), *L. kaempferi* (8), *P. sitchensis* (4) and *V. myrtillus* (4).

Soil, footwash and watercourse sampling

A total of 295 soil samples were baited for the presence of *P. ramorum* but all were negative except for those taken from under the area of standing water (Table 2). Furthermore, there was a noticeable lack of other Pythiaceae taxa baited from our soil samples (just *P. plurivora*

and *E. anandrum*). Only one footwash sample was positive for *P. ramorum* out of a total of 32 samples, and this positive came after walking through the standing water area in W2. The watercourse in the T site was baited from June 2014 till July 2015. On the sampling visits to the site in March and April 2015 the water bait could not be found, therefore giving a total of 10 baiting occasions tested for the T site. The watercourse in the K site was baited from March 2014 to September 2015, giving a total of 11 baiting occasions. The watercourse in W1 plot was baited from March 2014 to September 2015, while the watercourse in W2 plot was baited from June 2014 to September 2015. The W1 watercourse bait could not be found on the plot visit in September 2014, giving a total of 13 baiting occasions in W1, and 12 in W2. Of the 45 water baiting samples tested, only 6 were positive over the two years of sampling and originated from only three of the plots (K2, W1 and W2)(Table 2).

Discussion

This is the first published study that reports that viable sources of *P. ramorum* in previously infected *L. kaempferi* forests have been markedly reduced in sites that have been treated to eradicate *P. ramorum*. In the five plots cleared of hosts supporting sporulation (i.e. *L. kaempferi*) there were only two detections of *P. ramorum* in the spore traps, one of which was in a HLT. This could have resulted from the aerial spread of the pathogen from a nearby or distant canopy source rather than splash contamination from ground level sources in soil or litter. In contrast, for the *ad-hoc* positive control plot (W2) there were 4 detections in HLTs and 16 in LLTs. The HLT detections were most likely due to *P. ramorum* spread in rainwater from the infected *L. kaempferi* canopy overhead. The general decline in positive rainwater traps at W2 over the course of the monitoring (Table 3) is presumably due to the death of many of the trees at this plot at the end of 2013. Sporadic detections in the HLT and LLT in plot W1 could putatively be linked to nearby symptomatic *L. kaempferi* trees. The positive LLT in K2 could not be linked to any nearby canopy source, and could represent an example of long distance dispersal, or of inoculum splash from surrounding the soil and litter.

Phytophthora ramorum was not detected in any of the 295 soil samples taken across all plots. However, *P. ramorum* could almost consistently be isolated from the area under standing water in the W2 plot, indicating that persistent standing water may be important for its survival in *Larix* forests. Glasshouse trials in the USA have shown that there is a strong positive relationship between *P. ramorum* survival in soil and litter and the moisture content of the matrix (Fitchner et al. 2007). Turner et al. (2006) sampled soil for *P. ramorum* soon after eradication measures had been applied to a heritage garden containing infected *R. ponticum* in southeast England. They found very low levels of *P. ramorum* detections, with a maximum of 7% of the plots positive (10 of 147 quadrats in outbreak site 1) at any one time. Also in England, Harris (2014) used *Rhododendron* leaf baiting to detect the presence of *P. ramorum* in litter samples from a *L. kaempferi* site which also had an understorey of *Rhododendron.* She found that 6 months post eradication measures (i.e. removal of *L. kaempferi* and *Rhododendron*) *P. ramorum* was detected in 67% of her quadrats, with this dropping to 39% 18 months post clearance. Harris (2014) speculated that regrowth of infected *Rhododendron* probably contributed to the high recovery levels (ca. 40%) of *P.*

ramorum 30 months after eradication measures were applied. Isolation success of *P. ramorum* from soil and litter has also been shown to decrease over time in eradication treated tanoak forests in Oregon (Goheen et al. 2010, 2015), and in infected *Rhododendron* leaves buried less than 6 cm below the surface in Californian forests (Fitchner et al 2007). Overall, the lack of positive findings from the soil samples in our study is in agreement with the findings of McCracken et al. (2015) which found that a few months after removal of hosts supporting sporulation (i.e. *L. kaempferi*), *P. ramorum* could not be isolated from soil or litter samples from forests in Northern Ireland. There was also a notable absence of other Pythiaceae, with just *P. plurivora* and *E. anandrum* isolated from soil samples across our plots. This is in stark contrast with the study of Jung et al (2016) that baited 23 *Phytophthora* taxa from soil samples in European coniferous forests, and even from our previous work in a range of habitats across Ireland (O'Hanlon et al. 2016). The isolation success rate for *P. ramorum* from *L. kaempferi* samples is very low (Harris and Webber 2016), and it is possible that the soil and leaf litter in *L. kaempferi* associated sites has a suppressive effect on *P. ramorum*, as has been found in redwood forests in California (Fitchner et al. 2009). The lack of positive findings from soil samples was mirrored in the lack of positive findings from the footwash samples take in this study. Studies in other ecosystems have directly (Davidson et al. 2005; Webber and Rose 2008) or indirectly (Cushman and Meentemeyer 2008) linked footwear or tyres with spreading *P. ramorum* infection via attached soil/litter. This study has found that 24 months after eradication measures were applied to infected *L. kaempferi* forests in Ireland, there is only a low phytosanitary risk from residual *P. ramorum*.

Evidence which indicates how effective the eradication policy applied to *P. ramorum* has been in Ireland and the UK since the first findings on *Larix* is still accumulating. Unfortunately, we do not have any monitoring data to suggest what the levels of *P. ramorum* in soil and watercourses at our sites were before the eradication measures. Experience of the effectiveness of eradication measures against *P. ramorum* in the Pacific Northwest of the USA, and in particular Oregon, is of longer standing. Here the disease, known as Sudden Oak Death (SOD), and associated eradication efforts have been well documented since the start of the infestation in the early 2000s (COMTF 2/2014, 11/2014, 9/2015, 6/2016; Goheen et al. 2002, 2003, 2006, 2008, 2010, 2015; Hansen 2015; Hansen et al. 2006; Kamvar et al. 2015; Kanaskie 2016; Kanaskie et al. 2002, 2008, 2010, 2013, 2015; Peterson et al. 2014a, 2014b, 2015). The aim of the SOD programme in Oregon focuses on eradicating spot infections, before they can become sources of inoculum (Hansen 2015). Despite the noteworthy efforts of the scientists, inspectors and regulatory staff, the area affected by SOD has increased every year. However, Peterson et al. (2015) have concluded that the eradication efforts have probably slowed the epidemic significantly. Genotype analysis of the Oregon *P. ramorum* population supports this conclusion as the eradication measures have led to the extirpation of one of the genotypes that was widespread during the early stages of SOD in Oregon (Kamvar et al. 2015).

Several of the lessons learned in the Oregon experience with *P. ramorum* may be useful to apply to the Irish and UK policies for eradication:

1. Clearing infected hosts as soon as possible, as well as asymptomatic nearby hosts that can support sporulation, is the most effective method to contain pathogen spread. Delays in taking action have been shown to lead to a drastic increase in the number of new infected hosts (Kanaskie et al. 2010, 2013; Peterson et al. 2015).
2. Despite evidence that *P. ramorum* can remain viable in litter/soil on some sites for up to 8 years post eradication (Kanaskie et al. 2013), this is rare and inoculum levels generally reduce markedly with increasing time after eradication measures.
3. Vegetation control is important if hosts supporting sporulation can regrow post eradication (Goheen et al. 2008).
4. Stream baiting in watercourses near previously infected forests is an excellent tool for early detection of infected sites (Kanaskie et al. 2010).

This study and the work of McCracken et al (2015) and Harris (2014) all provide evidence which confirm the Oregon findings and emphasise their applicability to the Irish and UK situations. Given the infrequent nature of long-distance dissemination events, the currently practiced buffer zone of 250 m seems a reasonable balance between the phytosanitary, environmental and economic concerns of forest management. Although our results cannot be used to confirm decreasing persistence of *P. ramorum* in soil and litter over time, the work of Harris (2014) does indicate this. On point 3, although vegetation control was not identified as an issue in the *L. kaempferi* plots in this study, the study of Harris (2014) has shown that vegetation control is important in *Larix* forests with *Rhododendron* present. The infective potential of *P. ramorum* in watercourses was identified in this study, albeit at a low frequency across all watercourses sampled. A major difference between our watercourse baiting procedure and that of other researchers (e.g. Reeser et al. 2011; Sims et al. 2015) was that our baits were left *in-situ* for a longer period than is generally used (1 month vs. 1 week) and used just one baiting leaf (*Rhododendron*). This extended baiting period may account for our low diversity of watercourse *Phytophthora* species (just *E undulatum* and *P. gonapodyides*) compared to other studies in temperate forest watercourses. In California, genetic analysis has found that watercourse populations of *P. ramorum* are not regularly found causing significant over-story disease (Eyre et al. 2013; Eyre and Garbelotto 2014), while analysis has found that watercourse detections are not always linked with an increase in plant detections downstream in Oregon (Peterson et al. 2014a, b). Taken together, these studies suggest that watercourse baiting may best be used to supplement other detection methods to provide early detection of *P. ramorum* infestations (Peterson et al. 2014a).

This research has demonstrated that from two years after eradication measures in *L. kaempferi* forests, findings of *P. ramorum* in rainwater, soil and plant material are very low suggesting this management strategy is effective. Furthermore, the preliminary results of replanting trials using 9 species (*Quercus petraea*, *F. sylvatica*, *Picea abies*, *P. sitchensis*, *Pinus sylvestris*, *Pseudotsuga menziesii*, *L. kaempferi*, *L. decidua* and *Rhododendron caucasicum* × *ponticum*) in two previously infected sites in Ireland (at the T and K sites from this study) and a site in Northern Ireland indicate that residual levels of *P. ramorum* in eradication treated sites is only a concern for the latter three hosts (R. O'Hanlon unpublished

data; McCracken et al. 2015). Early detection and rapid eradication of infected sites is vital to containing the *P. ramorum* epidemic on *Larix*. The long-distance dispersal capability of *P. ramorum* (Peterson et al. 2015), its ability to asymptomatically infect *L. kaempferi* (Harris and Webber 2016), and the causes of the difficulties in isolating *P. ramorum* cultures from *L. kaempferi* material (Harris 2014) are areas where future research is needed in order to increase the effectiveness of the eradication and control efforts in Ireland and the UK.

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Table 1 Details of the plots used in the study

	T1	T2	K1	K2	W1	W2
Plot size (m)	100 × 150	55 × 150	130 × 170	45 × 240	90 × 150	65 × 95
Longtitude and latitude	N52° 23.454' W07° 57.455'	N52° 24.002' W07° 57.541'	N52° 20.747' W07° 07.587'	N52° 21.019' W07° 08.465'	N52° 50.703' W06° 07.453'	N52° 50.915' W06° 07.860'
Height above sea level (m)	175	100	208	147	120	172
Soil type	grey brown podzol	grey brown podzol	acid brown earth	acid brown earth	acid brown earth	acid brown earth
Infection history prior to 2012	*Phytophthora ramorum* detected in *Larix kaempferi* in 2011	*P. ramorum* detected in *L. kaempferi* in 2010	*P. ramorum* detected in *Abies procera*, *Fagus sylvatica* and *L. kaempferi* in 2010	*P. ramorum* detected in *Larix kaempferi* in 2010	*P. ramorum* detected in *F. sylvatica* and *L. kaempferi* in 2010	No tests for *P. ramorum* carried out on material from this plot
Age at detection; Eradication treatment year	15; 2012	13; 2011	42; 2011	42; 2011	56; 2010	>50; N/a
Eradication treatment	Removal of all tree species supporting sporulation (i.e. *L. kaempferi*) and other symptomatic hosts (i.e. *F. sylvatica*)	Removal of all tree species	Removal of all tree species supporting sporulation (i.e. *L. kaempferi*) and other symptomatic hosts (i.e. *A. procera*, *F. sylvatica*)	Removal of all tree species	Removal of all tree species supporting sporulation (i.e. *L. kaempferi*) and other symptomatic hosts (i.e. *F. sylvatica*)	No eradication treatment
Tree species in the plot at start of sampling 2012	*F. sylvatica, L. kaempferi, Sorbus acuparia*	*S. acuparia*	*L. kaempferi*, *A. procera*, *F. sylvatica*	*Picea sitchensis*, *Pseudotsuga menzeisii*	*L. kaempferi*, *F. sylvatica*, *P. contorta*	*L. kaempferi*

Table 2. *Phytophthora ramorum* detections across the sample types from the six plots. Numbers in parenthesis indicate the total number of samples of that type from that plot.

Plot → Sample ↓	T1	T2	K1	K2	W1	W2	**All plots**
High level traps	0 (53)	0 (47)	0 (44)	0 (48)	1 (57)	4 (116)	5 (365)
Low level traps	0 (46)	0 (49)	0 (43)	1 (47)	1 (57)	16 (113)	18 (355)
Soil samples	0 (48)	0 (48)	0 (40)	0 (44)	0 (51)	5 (64)	5 (295)
Plant material	4 (23)	0 (4)	0 (10)	0 (16)	3 (15)	1 (14)	8 (82)
Bait plants	0 (15)	0 (14)	0 (14)	0 (8)	2 (18)	5 (21)	7 (90)
Footwash	0 (9)		0 (9)		1 (14)		1 (32)
Running water baits	0 (10)		3 (11)		3 (25)		6 (46)
Standing water baits	-		-		15 (18)		15 (18)
All samples	4 (382)		4 (360)		57 (581)		65 (1283)

Table 3 *Phytophthora ramorum* detections in the W2 plot using different sampling methods. Cells with "Y" and shaded were positive for *P. ramorum*, "N" negative, and "-" cells indicates months when samples were not taken. Spore trap samples followed by an L indicate low traps (ground level), with H indicate high traps (1 m above ground level).

Date → Sample ↓	09-13	10-13	11-13	12-13	01-14	02-14	03-14	04-14	05-14	06-14	07-14	08-14	09-14	10-14	11-14	12-14	01-15	02-15	03-15	04-15	05-15	06-15	07-15	08-15	09-15
Spore trap 2.1L	Y	Y	Y	N	Y	N	Y	N	-	N	N	N	N	N	-	Y	N	-	N	-	N	N	-	N	N
Spore trap 2.1H	Y	Y	N	N	N	N	N	N	-	N	N	N	N	N	-	N	N	-	N	-	N	N	-	N	N
Spore trap 2.2L	Y	Y	N	N	Y	Y	N	N	-	N	N	N	N	Y	-	N	Y	-	N	-	N	N	-	N	N
Spore trap 2.2H	Y	Y	N	N	N	N	N	N	-	N	N	N	N	N	-	N	N	-	N	-	N	N	-	N	N
Spore trap 2.3L	Y	Y	N	N	N	N	N	N	-	N	N	N	N	N	-	N	N	-	N	-	N	N	-	N	N
Spore trap 2.3H	N	N	N	N	N	N	N	N	-	N	N	N	N	N	-	N	N	-	N	-	N	N	-	N	N
Spore trap 2.4L	-	-	N	N	N	N	N	N	-	N	N	N	N	N	-	N	N	-	N	-	N	N	-	N	N
Spore trap 2.4H	-	-	N	N	N	N	N	N	-	N	N	N	N	N	-	N	N	-	N	-	N	N	-	N	N
Spore trap 2.5L	-	-	N	N	N	Y	N	N	-	N	N	N	N	N	-	N	N	-	N	-	N	N	-	N	N
Spore trap 2.5H	-	-	N	N	N	N	N	N	-	N	N	N	N	N	-	N	N	-	N	-	N	N	-	N	N
Spore trap 2.6L	-	-	N	N	Y		N	N	-	N	N	N	N	N	-	N	N	-	N	-	N	N	-	N	N
Spore trap 2.6H	-	-	N	N	N	N	N	N	-	N	N	N	N	N	-	N	N	-	N	-	N	N	-	N	N
Bait plant		Y	Y	N	N	N	N	N	-	Y	Y	-	Y		-	N	N	-	N	-	N	N	-	N	N
Standing water bait	N	N	Y	N	N	Y	Y	Y	-	Y	Y	Y	Y	Y	-	Y	Y	-	Y	-	Y	N	-	Y	Y