Project Title: **Report on research undertaken between October 2009 to March 2010, on *Phytophthora ramorum* incited dieback of larch (*Larix kaempferi)***

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Summary

Phytophthora ramorum is a recently introduced pathogen that poses a threat to trees and native heathland species, but can also damage a wide range of ornamental shrubs and trees. Recent findings suggest that *P. ramorum* could have the ability to cause stem and branch cankers on some species of conifer grown in commercial forestry plantations in Britain, thereby causing tree mortality. This would mark a major shift in the disease as up until 2009, all tree infections in Britain have been close to infected rhododendron and only broadleaf tree species have been affected.

This study assessed the cause of dieback in *Larix kaempferi* (Japanese larch) growing in commercial plantations in the west country and concluded that *P. ramorum* was the cause of the symptoms. *P. ramorum* was isolated from both resinous cankers and foliage of affected trees, although isolation success was probably hampered by the high tannin content and resins in the bark. Symptoms on many mature trees were extreme. Heavy crown dieback and up to *c.* 80 separate resin bleeding cankers forming on branches and stems of some affected individuals. Dendrochronological analysis of twelve affected trees revealed that most suffered a catastrophic decline following the 2008 growing season, and at least one of the trees had died before the start of the new growing season in 2009. It remains a possibility that winter cold damage in early 2008 exacerbated bark killing caused by *P. ramorum*, but the more likely cause of the dieback was the extensive bark colonisation by *P. ramorum*, probably resulting from multiple stem and branch infections.

Monitoring spore numbers using rain traps placed in the vicinity of larch with crown dieback, revealed that *P. ramorum* was detected consistently and at high levels from October through to November-December at two forest sites (Largin and Plym). The maximum level of spores detected in both forests occurred at the beginning of November with 25,119 spores/250 mL at Largin and 1,000,000 spores/250 mL at Plym. Far fewer spores were detected in the traps located away from areas with infected larch but results show that spores were detected up to 1 km distance from the infected area. Spore levels at Plym were reliably higher than at Largin consistent with the higher levels of disease at Plym compared to Largin. There was no rhododendron present at Plym suggesting these inoculum loads were generated predominantly by the infected larch. A marked reduction in frequency of spore detection at both sites coincided with winter needle drop from the larch.

Laboratory tests indicated that the foliage of *L. kaempferi* (both wounded and unwounded) was susceptible to infection by *P. ramorum* thereby satisfying Koch's Postulates. Inoculated needles generally developed blackened lesions, and the pathogen could be readily reisolated from these. It also became apparent that infected needles supported high levels of sporulation; many individual needles had between 250-900 sporangia on their surface, with a maximum of 2685 counted on a single needle. In addition, *Larix* shoots showed some susceptibility to *P. kernoviae* but the sporulation potential was 10 fold lower than for *P. ramorum*. Field-based log tests to assess the susceptibility of *L. kaempferi* bark to *P. ramorum* compared with the known bark susceptible host, *Fagus sylvatica* (beech), were inconclusive. However, the bark on logs of both beech and *L. kaempferi* were colonised by *P. kernoviae* when exposed to natural inoculum from *P. kernoviae* infected *Rhododendron ponticum* bushes. Judged from the levels of colonisation, bark of *L. kaempferi* was less susceptible to *P. kernoviae* than the bark of beech.

A limited study did not provide any evidence that *P. ramorum* over-wintered in buds of affected *L. kaempferi* thereby resulting in infected foliage soon after bud-burst. In contrast, larch needles shed in 2009 and forming the litter layer in infected plantations were heavily contaminated with *P. ramorum*. Consequently, the pathogen is likely to persist on affected sites even after the removal of the infected trees. However, timber from such affected trees probably poses only a low biosecurity risk as *P. ramorum* was only isolated from the wood of infected trees infrequently, and only penetrated 1-2 mm into the outermost layers of sapwood.

On the basis of the findings of this study various recommendations for future work are made. These include:

- A continuation of spore monitoring work to quantify the full effects of the eradication action in relation to seasonal and weather factors.
- Testing other *Larix* species commonly grown in Britain as commercial plantation species for susceptibility to *P. ramorum* and sporulation potential.
- Genotype analysis of *P. ramorum* isolates involved in the larch affected areas in order to determine if there are any links between in the local or distant outbreaks.
- Further analysis of the over-wintering potential of *P. ramorum* in infected larch

1 Background

Phytophthora ramorum and *P. kernoviae* are both recently introduced pathogens to Britain. Both pose a threat to trees and native heathland species, but also cause damage to a wide range of ornamental shrubs and trees. *P. ramorum* (Pr) is better known as the cause of Sudden Oak Death (SOD) in California and Oregon in the USA, where over a million oaks (*Quercus*) and tanoak (*Lithocarpus densiflora*) have been killed by this disease (Frankel, 2007). Although conifer hosts such as Douglas fir (*Pseudotsugae menzeisii*) and pine *(Pinus*) are a key component of some of the forest systems affected by Pr in west coast USA, the impact of this pathogen on conifers is much less significant compared with the intensity of disease on broadleaf hosts. Virtually all conifer hosts suffer only from foliar infections (Rizzo et al., 2005), and only Pacific yew has occasionally been found with branch dieback caused by Pr (Bienapfl et al., 2006). However, recent findings suggest that *P. ramorum* could have the ability to cause stem and branch cankers on some species of conifer grown in commercial forestry plantations in Britain, thereby causing tree mortality. This marks a major shift in the disease as up until 2009, tree infections in Britain have been confined to locations close to areas of infected rhododendron and only affected broadleaf tree species.

In 2009 extensive areas of Japanese larch (*Larix kaempferi*) in the west country were found with symptoms of branch death and crown dieback. In some areas the dieback was extensive, and tree mortality not uncommon. Both mature and young (thicket to pole stage) larch were affected (see Fig. 1 below). The dieback occurred at a number of locations widely spread across the FC Estate in the west country, as well as some privately owned forests. Three of the affected locations on the Public Forestry Estate (PFE) with very high levels of damage are Largin in the Glynn Valley, Cornwall; Plym Forest, near Plymouth, Devon; Canonteign, near Exeter, Devon, all part of the Peninsula Forest District.

Fig 1: (a) Extensive area of plantation grown Japanese larch showing crown dieback (Plym, Devon); (b) stem canker and underlying lesion on young tree with associated resinosis.

During a visit to the Largin site to diagnose the cause of the larch dieback, *Rhododendron ponticum* bushes with symptoms typical of *P. ramorum* or *P. kernoviae* were seen and the symptomatic leaves gave a Phytophthora LFD positive. Young thicket-stage larch next to the symptomatic rhododendron also had foliar symptoms of withered shoots and purplish discoloured needles and also turned out to be LFD positive. Foliar samples of both larch and rhododendron from Largin were later confirmed as positive for *P. ramorum* following laboratory analysis at Forest Research. A sample of bark from a beech (*Fagus sylvatica*) with a bleeding lesion from Upper Largin (some distance away from the first samples) also proved to be positive for *P. ramorum*, but rhododendron cover on this part of the site was

sparse. This area also had mature larch with extensive dieback. Later on, samples of sweet chestnut foliage (*Castanea sativa*) from the same Upper Largin site were also found to be positive for *P. ramorum*.

During the same time period, a mature beech with bleeding lesions growing in a hardwood stand at Cann Woods, Plym, next to a plantation of young Japanese larch with extensive symptoms of dieback was also sampled and found to be *P. ramorum* positive. There was no rhododendron under or close to the beech, and therefore it was not anticipated that it would be positive. A few days later, three sweet chestnuts were sampled from the same area (although not close to the beech) and all were found to have foliar infections caused by *P. ramorum.* Once again there was no rhododendron near any of these trees although they were close to mature larch with symptoms of dieback. One of the larch was felled and tests on a bark sample with necrotic phloem, taken from the main trunk but high in the crown of the tree, yielded *P. ramorum.*

Finally, during a visit to Canonteign (west of Exeter), again to look at the dieback of mature larch, a sweet chestnut with a lesion on the stem was sampled and proved to be positive for *P. ramorum*. A more detailed visit of the site on 9th September 2009 revealed rhododendron infected with *P. ramorum*, although the extent of this was sporadic. There were also several sweet chestnut trees with Pr symptomatic leaves and another with bark lesions. Again the larch on this site showed varying degrees of dieback. Subsequently, western hemlock (*Tsuga heterophylla*) trees at Plym were also found to have resinous lesions on stems and branches, and *P. ramorum* was isolated from the lesions.

There is widespread but sporadic *P. ramorum* infection on rhododendron in Peninsula Forest District, particularly in the Glynn Valley which may have acted as the foliar host for some of the stem infections that have developed on various tree species. However, for many of the affected trees, no clear *P. ramorum* inoculum source was evident, as little or no infected rhododendron was nearby. This indicated that other significant foliar hosts could be supporting this epidemic. One of these could be sweet chestnut, as it is known to support significant levels of sporulation in laboratory tests (Denman et al., 2006). The new finding of larch as a foliar host also raised the question of how significant it was and whether it could be a sporulator.

1.1 Study objectives

Research was undertaken to investigate these findings with several specific objectives:

A. To determine whether the dieback of larch is caused by *P. ramorum* or possibly a combination of *P. ramorum* and another biotic or abiotic factors acting on both young and mature larch trees.
B. To evaluate the potential of larch foliage to support sporulation by *P. ramorum*, and therefore act as the source of inoculum for other known susceptible tree hosts in the absence of any infected rhododendron.
C. To evaluate the level of susceptibility of *Larix kaempferi* to *P. ramorum*.
D. To determine if *P. ramorum* can overwinter in the buds of deciduous larch, and initiate new foliar infection after bud break.
E. To investigate the level of *P. ramorum* infestation on in detail on larch plantation sites, particularly in relation to the implications it may have for spread to other areas as well as decisions on replanting affected sites.
F. To consider if the harvesting of larch timber from compartments with dieback poses a risk of spread by *P. ramorum* and could also jeopardise salvage and sale of potentially infected trees.

2 Results of the study

Field research was focussed on sites in Devon and Cornwall, south west England, where larch was significantly affected by dieback and mortality and where *P. ramorum* had been confirmed as present affecting a range of trees, including larch (*L. kaempferi*).

2.1 Symptom expression and the presence of *Phytophthora* (Objective A)

From September 2009 onwards, the severity of the symptoms on trees was assessed and tissue isolations made to determine the causal agent of the extensive dieback and mortality in mature (30-40m tall) and juvenile (5-12 year old) plantation grown Japanese larch (*Larix kaempferi*. Symptoms observed on the trees fell into two categories:

- Foliar symptoms consisting of needle necrosis with black or purple to brown discolouration especially to tips, aborted bud flush, wilting and senescence of dwarf shoots coupled with needle loss.
- Copious resin bleeding on the trunk, branches and side shoots of many trees, as well as dieback of branches and sometimes the entire crown. In some cases sunken cankers were often evident on branches as well. When the outer bark associated with the cankers or resinosis was removed, extensive underlying phloem lesions consisting of necrotic tissue were visible. These were usually bright pink to maroon-red at the margins and highly resinous whereas in older lesion areas the phloem was a rusty-brown to cinnamon brown, and usually much drier and less resinous than at the lesion margins.

At the same sites the other tree species were also surveyed. Cankers (usually bleeding) were found to be frequent on the stems of beech (*Fagus sylvatica*), sweet chestnut (*Castanea sativa*) and birch (*Betula pendula*), and occasionally on oak (*Quercus* spp), *Nothofagus obliqua*, western hemlock (*Tsuga heterophylla*), Douglas fir (*Pesudotsuga menziesii*) and Lawson cypress (*Chamaecyparis lawsoniana*). There were also symptoms on the foliage of understorey rhododendron (*Rhododendron ponticum*) and sweet chestnut, consistent with those of infection by *Phytophthora* spp.

Isolations were undertaken from all these symptomatic species, using *Phytophthora* selective medium and other agar media. All foliar tissue was surface sterilised (70% ethanol for 30 seconds then rinsed in sterile distilled water) to remove any surface contaminant spores before plating onto agar. The most frequently isolated agent from all the sampled trees (both foliage and bark) was *Phytophthora ramorum* although the success of the isolation from the various tree species, as well as for bark vs foliar tissue, varied markedly (see Table 1). *P. ramorum* was also isolated from the symptomatic rhododendron when it occurred as an understorey plant associated with the larch. Birch, hemlock, Douglas fir and Lawson's cypress were all new host records for *P. ramorum*. Verification of *P. ramorum* identity was based initially on morphological characters and then confirmed by PCR sequencing of the rDNA ITS region.

During isolation, *P ramorum* was never obtained from pink-maroon margins of larch phloem lesions, although it could be isolated from older lesion areas. In addition, the success rate for culturing from larch tissue was significantly less than most of the other broadleaf species (Table 1), particularly species with low levels of bark extractives such as beech and birch. The bark tissues of tree species such as oak, sweet chestnut, Japanese larch and western hemlock are particularly high in tannin (Aaron, 1982), and more recent studies have also shown that extractable tannins from oak and bay laurel can markedly reduce growth and sporulation of *P. ramorum* at (Manter et al., 2009). The red colour of bark lesions is also strongly indicative of the release of tannins, and the mechanical action of cutting bark samples which ruptures bark tissue also releases tannins and causes a considerable increase in the red-colour intensity of tannin-rich bark (Aaron, 1982). These features are

Table 1: Success of *Phytophthora* isolation from symptomatic tree species associated with *Larix kaempferi* dieback areas (August 2009-March 2010)

Tree species	**n**	**Percent successful isolations**		**Negative isolations**
		Phytophthora ramorum	**Other *Phytophthora* spp**	
Fagus sylvatica (European beech)				
Bark lesions	77	46.8%	29.9%	23.4%
Larix kaempferi (Japanese larch)				
Bark lesions	55	25.5%	0%	74.5%
Foliar necrosis	28	39.3%	0%	60.7%
Nothofagus obliqua (Roble beech)				
Bark lesions	10	10.0%	80.0%	10.0%
Castanea sativa (sweet chestnut)				
Bark lesions	8	50.0%	0%	50.0%
Foliar lesions	15	93.3%	0%	6.7%
Betula pendula (silver birch)				
Bark lesions	8	85.7%	0%	14.3%

likely to make it difficult to isolate *P. ramorum* from samples taken from tree species with high bark tannin levels, such as larch and sweet chestnut, even though tests with LFDs frequently indicate that *Phytophthora* is present in the sample. Larch bark is also very resinous compared with most other conifer species, especially after any mechanical damage, and the resin is also likely to inhibit the outgrowth of *Phytophthora.*

In the context of all the isolations it was also significant that other species of *Phytophthora* were frequently isolated from stem cankers on beech trees study (mainly *P. pseudosyringae* and *P. gonopodyides*) emphasising the susceptibility of European beech to *Phytophthora*.

2.1.1 Impact of *P. ramorum* infection on *L. kaempferi*

In an attempt to quantify the extent of *P. ramorum* infection on trees, 16 semi-mature to mature trees (24-40 years old) were felled at two sites: Canonteign and a private estate. The extent of the crown symptoms was quantified and the number of resinous lesions visible on the trunk and branches counted. Attempts were made to isolate the pathogen from some of the lesions.

These assessments confirmed that the damage to trees was very extensive and that in some cases at least 25-50% of the inner bark was necrotic or dying on individual trees, and dieback of 70-80% of the entire crown was common. Observational evidence suggested that initial infections could have occurred mainly on shoots and twigs, and that bark killing by the pathogen then extended back along the branch and eventually to the main trunk as many of the resinous lesions on the main stem of affected trees often had a dead branch at their centre. However, again it was often difficult to isolate *P. ramorum* from the trees even when a positive Pocket Diagnostic® LFD was obtained (see Table 2). It should be noted that multiple lesions were commonly observed on a single tree and as many as 83 resinous lesions were observed on a single tree, suggesting that prolific numbers of infections can also occur under high inoculum pressure.

Table 2: Characteristics of a sample of affected *Larix kaempferi* trees, assessed immediately after felling

Tree identifier	Diameter at breast height (cm)	Crown condition	No. of resinous lesions	Presence of *P. ramorum* confirmed
CTT03	35	Severe dieback	83	*P. ramorum* confirmed
CTT05	38	Severe dieback	27	*P. ramorum* confirmed
CTT06	30	Some defoliation	3	Not confirmed
CTT07§	40	Serious dieback	9	Not confirmed
CTT08	41	Some defoliation	10	Not confirmed
CTT09	31	Some defoliation	21	Not confirmed
CTT10	35	Severe dieback	7	*P. ramorum* confirmed
CTT11	35	Some defoliation	2	*P. ramorum* confirmed
CTT12	42	Severe dieback	59	*P. ramorum* confirmed
CTT13	40	Some dieback	0	Not sampled
CND02	18	Severe dieback	15	*P. ramorum* confirmed
CND03	21	Some dieback	8	Not confirmed
CND04	17	Severe dieback	4	Not confirmed
CND05	31	Severe dieback	14	*P. ramorum* confirmed
CND06	14	Dead	22	*P. ramorum* confirmed
CND07	18	Some dieback	5	Not sampled

§ Tree codes shown in bold were used in growth increment studies described below

2.1.2 Possible association of *P. ramorum* infection with cold damage

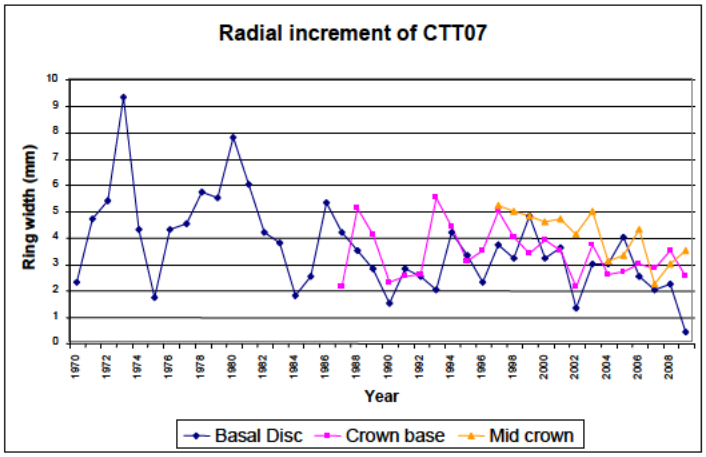
Forest managers had suggested that in some parts of the west country cold weather damage had occurred to larch during February 2009 which could also have contributed to the dieback visible in the crowns of some trees. Japanese larch has long been known to be very susceptible to frost damage (Peace, 1962) and as the new seasonal activity of the cambium can be very localised and occur early in the year, patches of bark death can occur if the cambium suffers extreme cold. There is also an interaction between frost damage to the active cambium and damage caused by the larch canker pathogen *Lachnellula willkommii*; indeed the latter is sometimes considered only as a coloniser of frost injured tissues. Therefore to investigate whether frost damage could be a factor in the larch crown dieback, six trees from Canonteign and six from the private estate were subject to a detailed dendrochronological analysis, to detect any cambial damage or the formation of trauma rings.

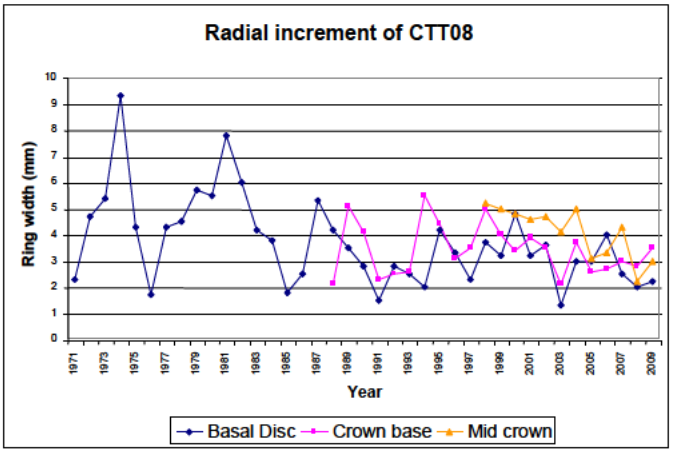
Discs were cut from three or four positions on each tree as follows: position 1 – basal disc; position 2 – base of crown; disc 3 – mid-crown; and,where possible; disc 4 taken at a point just below any dieback at the top of the tree. Graphs of the incremental growth for each disc taken from each tree over the life of the tree are shown in Figs. 2 and 3.

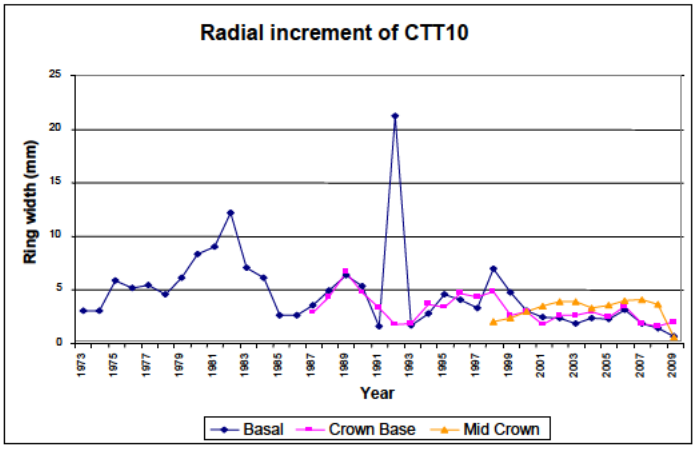
The analysis revealed that a number of the trees at both sites had suffered a catastrophic growth decline after the 2008 growing season, which was evident during the 2009 season. This was particularly apparent with trees CTT10, CTT12, CND02, CND04, CND06 and CND07, which showed no (CND06) or virtually no growth increment during the 2009 growth season. This contrasted with trees such as CTT08 and CTT11, which had less serious crown symptoms, and showed relatively good growth during 2009. Some trees (CTT10 and CND04) had trauma rings, indicating that some cold-related cambial injury had occurred. However, the poor growth evident in many of the discs taken at the base of the tree as well as cessation of growth in uppermost discs was also evidence of a widespread bark death that far exceeded what would be expected as a result of cold damage. Some trees also had very large stem lesions (eg 6m, 4m and 3m long for trees CTT09, CTT05 and CTT10 respectively) often on the lower trunk, that again was not consistent with cold damage.

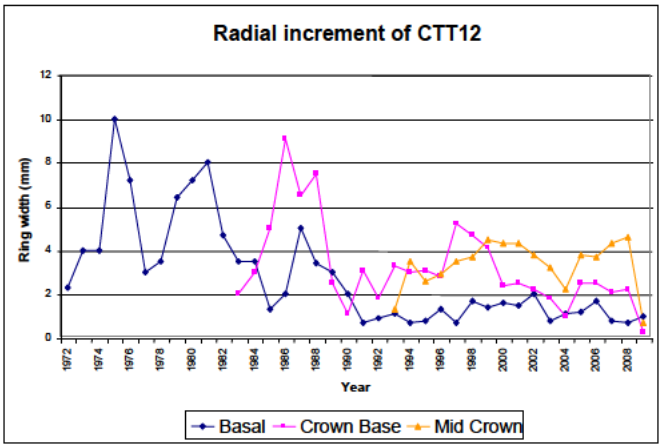
Overall, it cannot be ruled out that frost damage in early 2009 may have facilitated colonisation of affected trees by *P. ramorum*, but some trees already showed a marked decline in growth during 2008, and then died very quickly at the end of that growing season. Thus the more likely cause of the dieback is the extensive bark colonisation by *P. ramorum*, probably resulting from multiple stem and branch infections, as suggested by the many discrete areas of resinosis on some trees (Table 2).

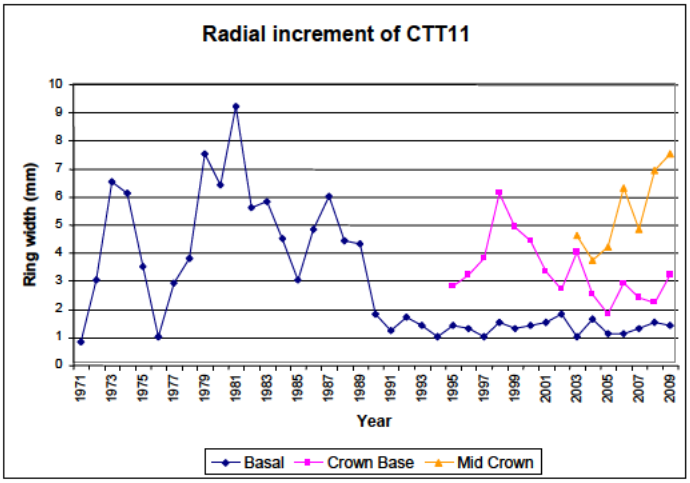
Fig. 2: Growth rates of six *Larix kaempferi* trees at Canonteign, Devon











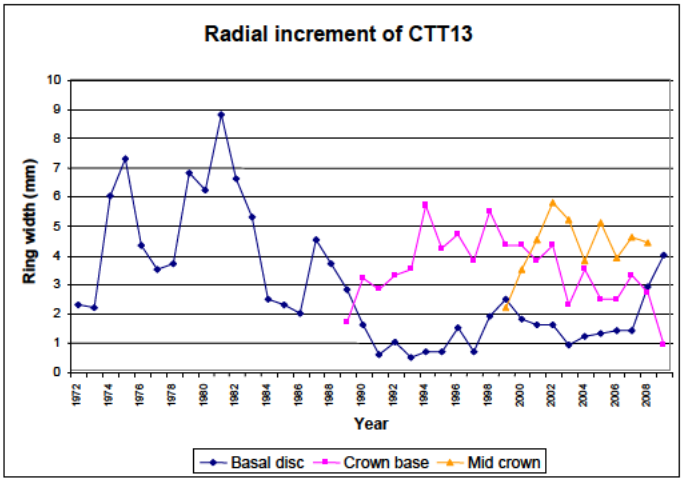
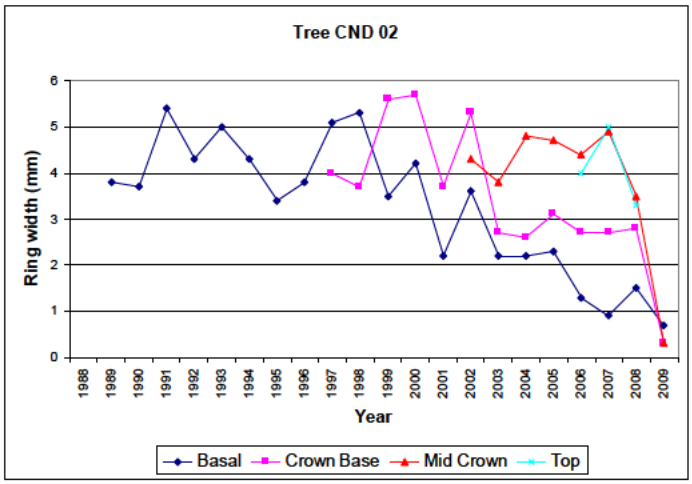
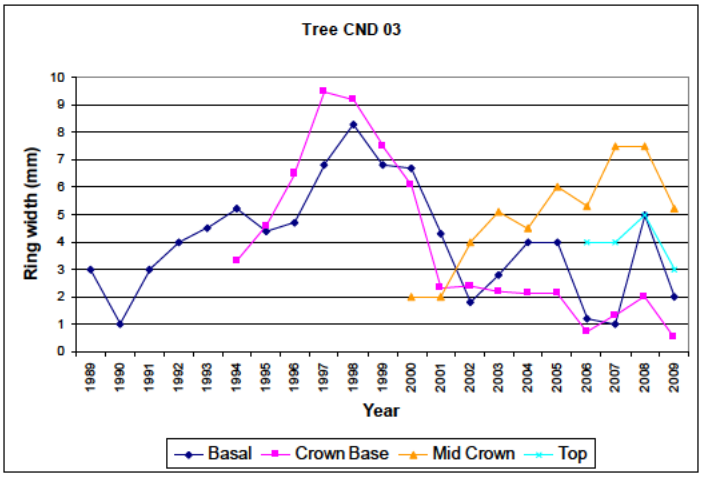
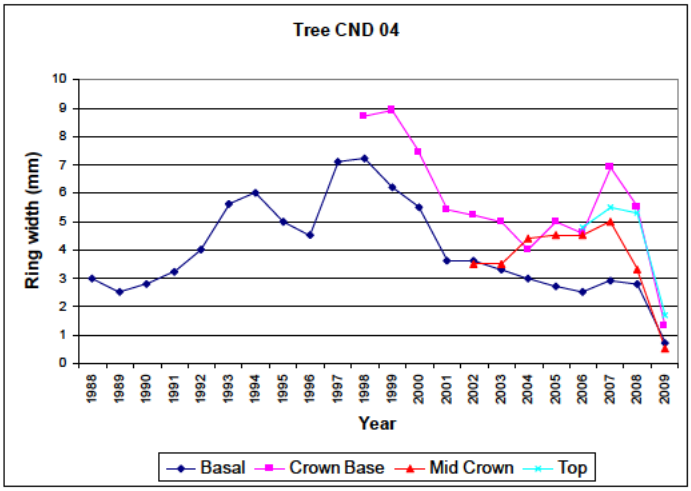
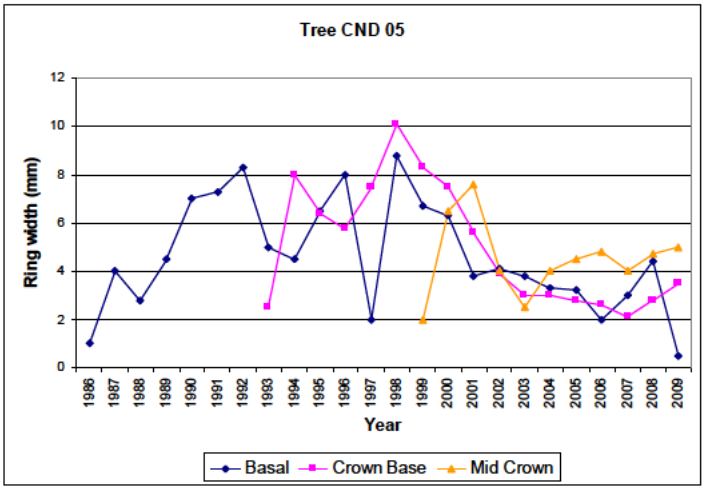


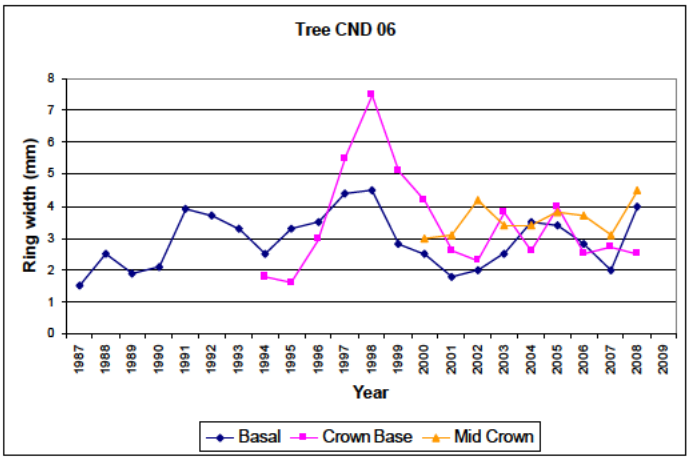
Fig. 3: Growth rates of six *Larix kaempferi* trees at Private Site 1, Devon

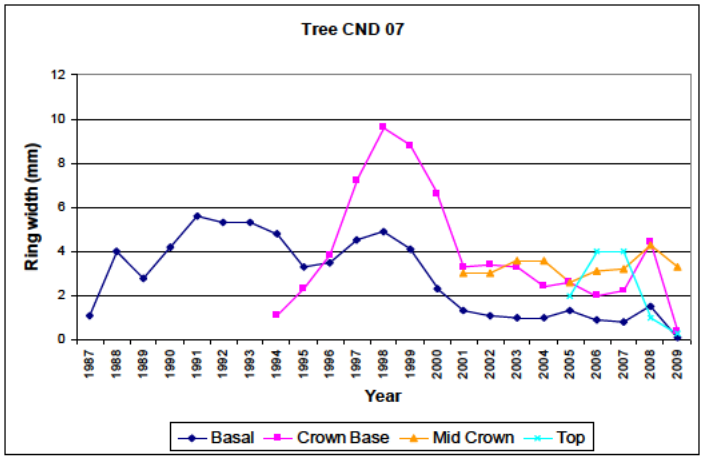












2.2 Sporulation of *P. ramorum* associated with *Larix kaempferi* (Objectives B)

2.2.1 Levels of *P. ramorum* sporulation associated with naturally infected larch

High level rain traps (~1m above ground level) were set up to monitor spore numbers of *P. ramorum* in two woodlands (Plym and Largin) in areas where the larch dieback was extensive. In addition to the rain traps, a Burkhard aerial spore trap was located in one woodland (Plym) where Japanese larch (*Larix kaempferi)* had been confirmed to be infected by *Phytophthora ramorum*. Rain traps at both sites were collected following significant levels of rainfall and sent to the laboratory at Fera for determination of *P. ramorum* spore levels (spores/250 mL rain water) using TaqMan PCR. To ensure DNA levels (and hence spore counts) were not artificially increased by the presence of *P. ramorum* infected needle/leaf material, all rain water samples were pre-filtered to remove the needles or other leaf material.

Spore numbers were monitored for a period until affected trees were felled, and then again after felling and harvesting, to determine if this had any impact on the number of spores.

Plym Wood

Initially four high level rain traps (HLRT) were set up on the 9th October 2009 (labelled HLRT1–4) (two under uninfected and two under infected larch (Fig. 4)) and a further eight on the 12th October 2009 (labelled HLRT5-12) (two under uninfected Douglas fir, two under infected beech, one under uninfected sweet chestnut, one under infected sweet chestnut and two under uninfected beech (Fig. 4). Traps were not sampled during tree felling (end of January to mid Feb 2010).

The Burkhard spore trap was placed out in Plym during November 2009. Trap tapes were changed weekly and examined microscopically in the laboratory for the presence of *P. ramorum* sporangia.

Soil and litter samples were also taken from around traps 1 to 4 on the 9th October and traps 5 to 12 on the 12th October 2009. Samples were analysed in the laboratory for the presence of *P. ramorum* using rhododendron bait tests.

Largin wood

Eight HLRT were positioned in Largin wood on the 8th October 2009 (Fig. 5). Traps were labelled HLRT1 to HLRT8, with traps sited as follows: two under infected larch with no rhododendron understory (HLRT1 and 2), two under infected larch with infected rhododendron understory (HLRT3 and 4), two in open areas not overhung by branches (HLRT5 and 6) and two at a distance from any *P. ramorum* infection (HLRT7 and 8). The commencement of felling in the wood meant that the last trap sampled was on the 26th January 2010 (traps 1-4 only). Four of the original trap locations (HLRT1, 5, 7 and 8) were reinstated in the wood and two additional traps added (HLRT9 and 10) at the beginning of May 2010 (Fig. 5).

Soil and litter samples were taken from around all eight traps on the 8th October 2009. All samples were analysed in the laboratory for the presence of *P. ramorum* using rhododendron bait tests.

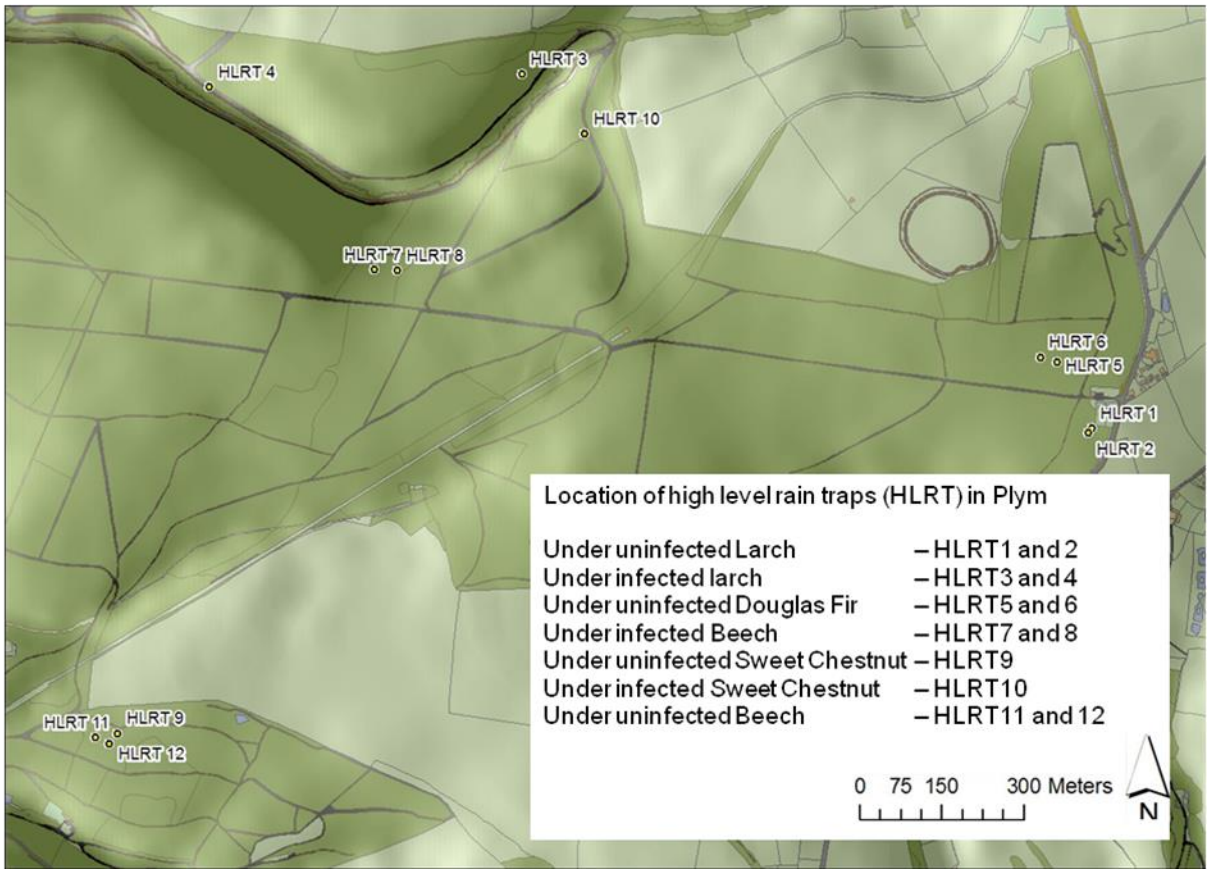


Fig. 4: Location of high level rain traps sited in Plym forest.

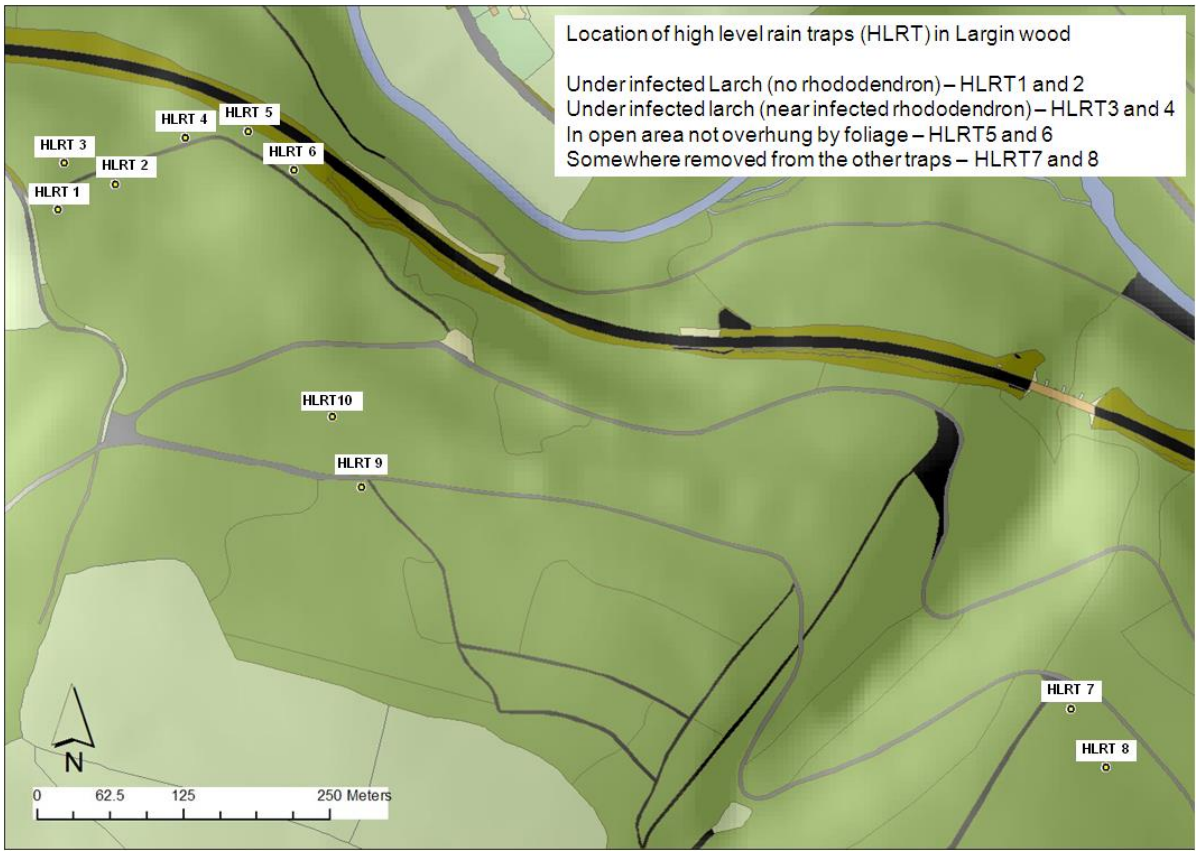


Fig. 5: Location of high level rain traps sited in Largin forest.

Spore monitoring revealed that *P. ramorum* was detected consistently and at high levels in the rain traps from the start of capture until the end of November and beginning December at Plym and Largin respectively (Figs. 6 and 7). The maximum level of spores detected in both forests occurred at the beginning of November with 25,119 spores/250 mL at Largin and 1,000,000 spores/250 mL at Plym. Spore levels at Plym were consistently higher than at Largin. The higher spore load at Plym was consistent with the higher levels of disease at Plym compared to Largin (Ben Jones, Pers. comm.). It should also be noted that there was no rhododendron present at Plym suggesting these inoculum loads were generated predominantly by the infected larch. The reduction in frequency of spore detection at both sites coincided with the end of larch needle drop (or at least with the end of needles being found in the rain traps).

At Plym (Fig. 6), *P. ramorum* was also detected in rain traps placed under infected sweet chestnut (23rd and 27 h October, and 2nd and 23rd November 2009), infected beech (2nd, 16th and 23rd November 2009) and uninfected Douglas fir (16th November and 1st December 2009). No larch needles were noted in the rainwater samples collected from under the infected sweet chestnut or beech, suggesting the spore levels detected were being generated by these tree species. In contrast, high levels of larch needles were noted in traps under the Douglas fir, as a result it is less certain whether the *P. ramorum* spore levels detected came from infected larch needles blowing into the traps or from infection on the Douglas fir. For the traps located under sweet chestnut and beech, where spores were detected, levels ranged between 5 and 200 spores/250mL for sweet chestnut and 4 and 35 spores/250mL for beech. The level of detection in the trap beneath the infected sweet chestnut and beech decreased as the number of leaves in the tree canopy reduced.

Results from Largin indicate that during the time period when these traps were deployed, there was both short distance (Fig. 7 - traps 5 and 6) and long distance (~1km) (Fig. 7 - traps 7 and 8) movement of spores.

Since the completion of larch needle drop and the felling of trees at both sites, few rain traps have been positive for *P. ramorum*. No *P. ramorum* spores have been detected on any of the Burkhard aerial spore trap tapes returned to the laboratory. Results indicate a major reduction is the level of inoculum being dispersed in the local environment since the end of November 2009. This is likely to be due to a combination of factors including the felling and removal of infected hosts, lack of foliage over the winter on the larch, the prolonged dry spell during the spring, and the infected needle litter being churned beneath the soil surface due to the action of heavy machinery. Further monitoring of the two locations will determine the relative impacts of these and other factors on ongoing inoculum levels at the sites.

Inoculum was detected in samples collected in early October in litter from around traps 1, 2 and 4 in Largin and 3, 4 and 7 in Plym. The positive litter samples from Largin were from under infected larch (traps 1 and 2) or under infected larch with infected rhododendron understory (trap 4), and those from Plym from under infected larch (traps 3 and 4) and infected beech (trap 7).

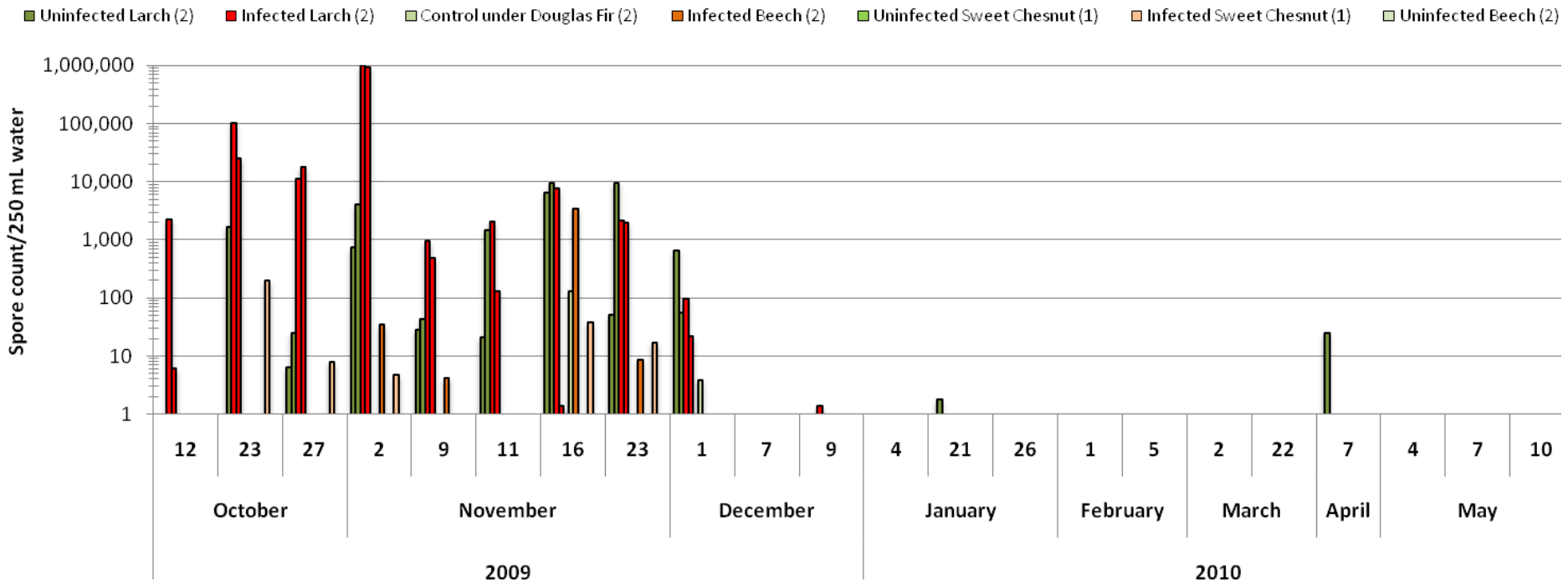


Fig. 6: Spore counts for high level rain traps sited in Plym. Dates on x axis indicate when traps were returned following significant rain, no bars indicate that P. ramorum was not detected in the trap. Large gaps between sample dates indicates a lack of rainfall during that period e.g. between 7th April and 4th May 2010.

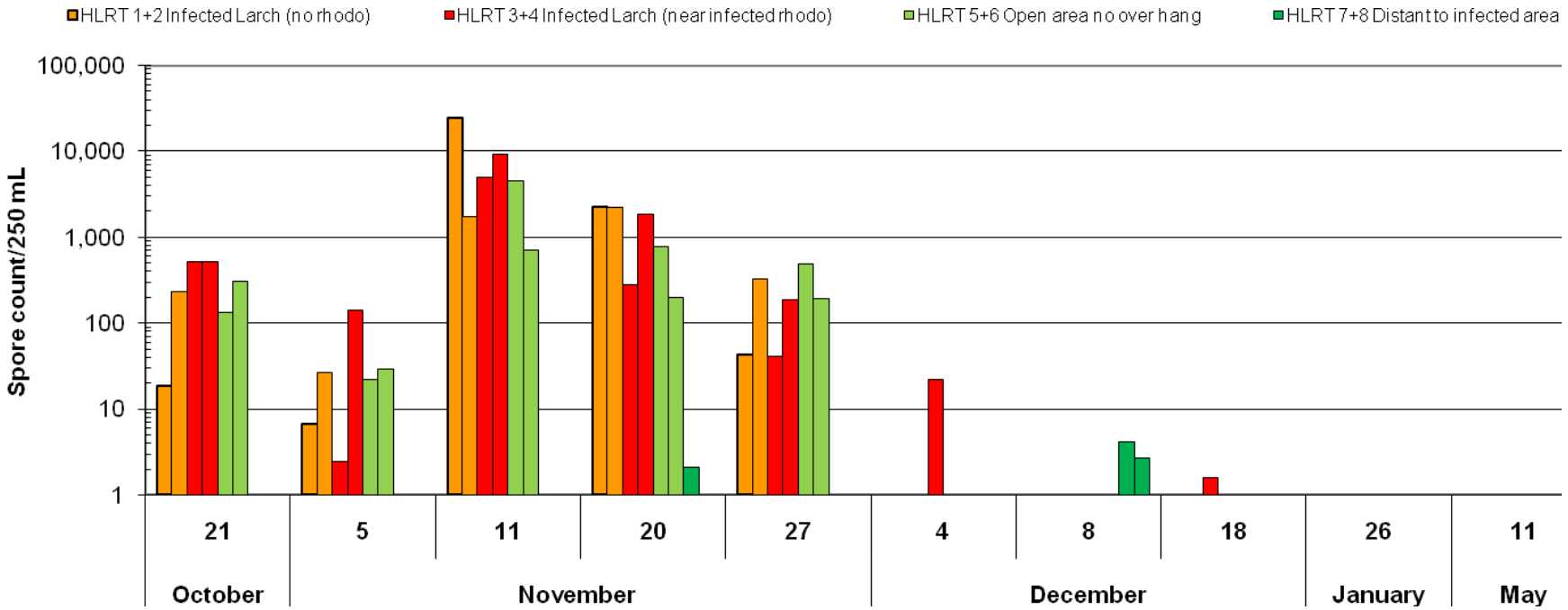


Fig. 7: Spore counts for high level rain traps sited in Largin. Dates on x axis indicate when traps were returned following significant rain, no bars indicate that *P. ramorum* was not detected in the trap. The large gap between the end of January and the beginning of May 2010 was a result of traps being removed due to felling.

2.2.2 Foliar susceptibility and sporulation potential of *L. kaempferi* Test 1

Foliage of larch was tested for susceptibility to *P. ramorum* based on the method developed by Denman *et al* (2006). In October 2009, 36 green shoots of *L. kaempferi* , each about 12-15 cm in length, were cut from 20-30 year old trees growing in a species trail area at Alice Holt research station, returned to the laboratory and placed in water immediately. Needles on half the shoots were wounded by cutting the tips. The lower half of each shoot was then dipped into a freshly prepared zoospore suspension ($1x10^4$ zoospores ml^{-1}) using an isolate of *P. ramorum* recently obtained from an infected individual of *L. kaempferi* at the Plym site. Immediately after dipping, each shoot was drained, stood in a vial of water and then all the shoots were placed in damp chambers at 18°C under alternating light and dark periods. After 7 d, each shoot was assessed for symptoms, and all needles placed in a symptom category. The categories were: partially or fully blackened along the length of the needle; browned or banded needles; chlorotic needles; green needles. At least one needle per shoot of each symptom category was retained for examination using a microscope. The remainder of all the blackened needles and between a third to a half of the needles in the other categories were then surface sterilised (70% ethanol for 40 s and washed in sterile distilled water) and plated onto Phytophthora selective medium. Any *Phytophthora* colonies growing out from the needles were then subcultured and their identity confirmed based on morphological criteria. The outcome of this test is shown in Table 3.

Table 3: Symptom development on larch (*Larix kaempferi*) following shoot dipping in a spore suspension ($1x10^4$ zoopsores ml^{-1}) of *P. ramorum*

Inoculation with *P. ramorum*	Number of needles				Needle total
	Blackened	Brown/banded	Chlorotic	Green	
Unwounded shoots, dipped in zoospores	26	68	41	154	289
% yielding *P. ramorum* after surface sterilisation	64%	6%	0%	0%	14%
Wounded shoots, dipped in zoospores	60	79	31	95	265
% yielding *P. ramorum* after surface sterilisation	68%	40%	14%	0	36%
Unwounded control shoots dipped in water	0	189	30	59	278
% yielding *P. ramorum* after surface sterilisation	0%	0%	0%	0%	0%

Even following surface sterilisation to remove any superficial spores remaining from the dip-inoculation, *P. ramorum* was isolated consistently from needles in some of the categories. Both unwounded and wounded needles that had symptoms of blackening yielded *P. ramorum* at high frequency, but a smaller proportion of the brown/banded needles were also infected with *P. ramorum*. This experiment satisfies Koch's Postulates and confirms that *P. ramorum* is capable of infecting and causing visible symptoms on foliage of *L. kaempferi* (Webber et al., 2010).

The retained needles were mounted individually on slides in cotton blue diluted in lactic acid and examined microscopically approximately 24 h later, after some clearing of the needle tissue had occurred. Many sporangia were visible on the needles (Fig. 8), especially those with symptoms of blackening, and the total number of visible sporangia was counted over the entire length of the needle on both upper and lower surfaces. The data for one needle per category for 6 unwounded shoots, 5 wounded shoots and 3 control shoots are shown in Table 4. Blackened needles frequently exhibited high levels of sporulation. Some of the

browned or banded needles also supported abundant sporulation; and a maximum of 2685 sporangia was counted on a single unwounded needle in this category. Occasionally chlamydospores were also visible on the needle surfaces along with the sporangia (see Fig. 9), but always these occurred much less frequently than sporangia. Moreover, even some of the chlorotic or green needles supported sparse levels of sporulation, indicating that for the green needles at least some sporulation occurred without any associated symptoms.

Table 4: Number of sporangia forming on individual needles of larch (*Larix kaempferi*) following shoot dipping in a spore suspension of *P. ramorum*

	No. of needles	Sporangial numbers on each individual needle			
		Blackened	Brown/banded	Chlorotic	Green
Unwounded needles					
Shoot 1	4	939	2685	63	7
Shoot 2	4	23	57	21	15
Shoot 3	4	3	12	4	12
Shoot 4	4	509	4	9	0
Shoot 5	4	301	683	20	0
Shoot 6	4	648	3	5	4
Wounded needles					
Shoot 1	4	389	250	5	0
Shoot 2	4	15	11	4	3
Shoot 3	4	612	1	1	3
Shoot 4	4	12	57	5	3
Shoot 5	4	47	13	5	0
Control needles					
Shoot 1	3	-	0	0	0
Shoot 2	3	-	0	0	0
Shoot 3	3	-	0	0	0

Fig. 8: Spores of *P. ramorum* visible on the surface of a needle of *L. kaempferi*; (a) evidence of directly germinating sporangia, (b) massed sporangia along the mid-rib of the needle.

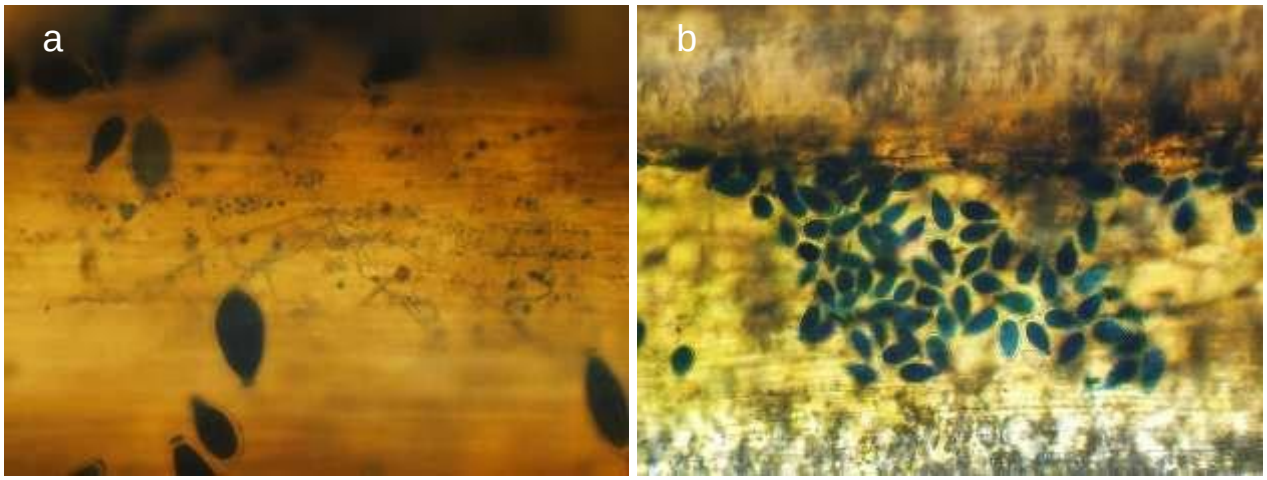
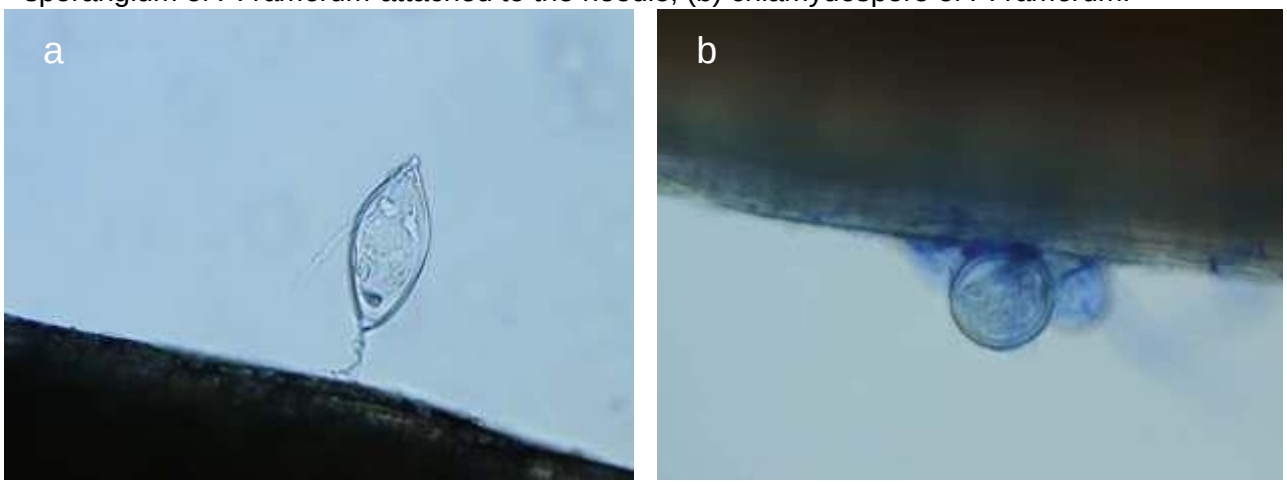


Fig. 9: Spores of *P. ramorum* visible on needle surfaces of *L. kaempferi*; (a) caducous sporangium of *P. ramorum* attached to the needle, (b) chlamydospore of *P. ramorum.*



2.2.3 Foliar susceptibility and sporulation potential of *L. kaempferi* Test 2

In a second experiment comparing *P. ramorum* and *P. kernoviae* sporulation on larch, two larch stems (20 cm in length) were sampled from healthy larch trees in East Yorkshire at the beginning of November 2009. It was unknown if the larch was Japanese, European or a hybrid. The unwounded stems were dipped into a zoospore suspension either containing $1x10^3$ zoospores ml^{-1} of *P. ramorum* (not sourced from larch) or the same concentration of *P. kernoviae*. Stems were left in the suspension for one minute and allowed to drip dry for two minutes before incubating in a humidity chamber at 18°C under alternating light and dark periods. After 10 days incubation, 10 needles were removed from each stem and the spore levels per needle counted.

These laboratory tests (Table 5) confirmed the susceptibility of larch to *P. ramorum* and the high level of sporulation from infected lesions. Measurements indicated an average of 50 spores/needle for *P. ramorum* under laboratory conditions. The results also indicated that larch is susceptible to *P. kernoviae* but the level of susceptibility and sporulation potential is 10 fold lower than for *P. ramorum*, at 5 spores/needle.

These results are put into context with previous studies on both sporulation potential in the laboratory and spore monitoring in the wider environment, and these data are shown together in Table 5.

Table 5: Comparative results - laboratory tests on sporulation potential and rain trap spore counts for *Phytophthora ramorum* and *P. kernoviae*.

	Sporangial counts/cm^2 of lesion (laboratory)		Rain trap spore counts /250 mL of rainwater (max count)	
	P. ramorum	*P. kernoviae*	*P. ramorum*	*P. kernoviae*
Larch	50/needle*	5/needle*	1,000,000	-
Umbellularia *californica* (California bay laurel)	216	-	32**	-
Rhododendron ponticum	8	4	25	661
Magnolia	-	-	39	10,500
Drimys winteri	10	166	-	38,000
Vaccinium myrtillus	45	-	#	#

* average results

** US results as colony forming units (Davidson et al., 2005)

No high level trap data available. Figures for sporangial counts from lesions in the natural environment were 9.3 sporangia /cm of lesion for *P. ramorum* and 5.8 sporangia/cm for *P. kernoviae*.

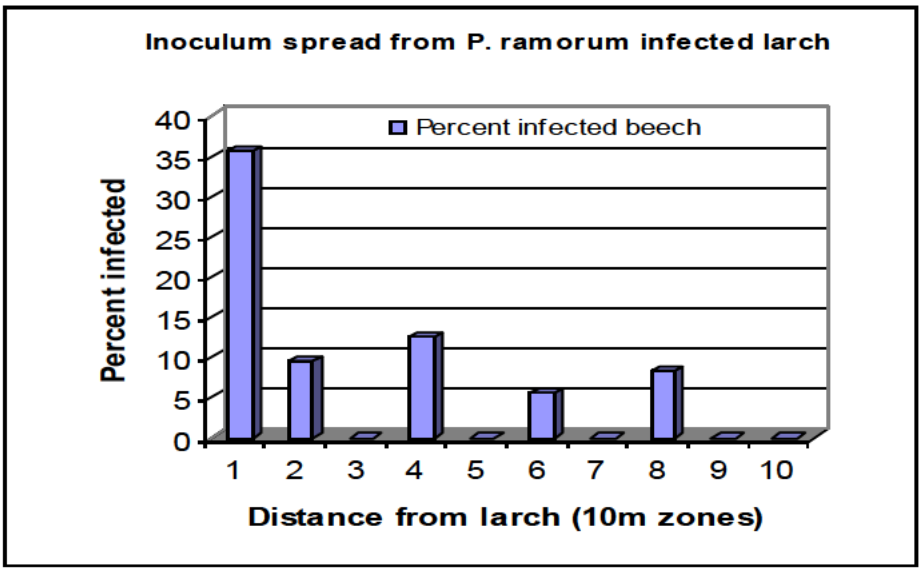
2.2.4 Impact of larch sporulation on other susceptible tree species

Further evidence of the intensity of sporulation on foliage of *L. kaempferi* and the impact on nearby susceptible tree species has come from a field assessment of mature 30 m tall beech (*F. sylvatica*) growing next to a compartment of 30m tall, *P. ramorum* infected larch. The compartment of larch was to one side of a mixed species block that included about 300 beech trees; the latter extended over a stretch of 150-200 m. Beech is known to be one of the most susceptible tree hosts to *P. ramorum* and it was notable that multiple bleeding cankers were visible on at least a third of the beech trees in a 10m wide zone immediately neighbouring the larch (Fig. 10). Most of these stem cankers occurred at between 7-11m above ground-level. However, at increasing distance from the larch, the proportion of beech with bleeding cankers decreased (Fig. 10).

Thus at a distance of ~10 m, 35% of the beech were infected but at 80-90m only 8% had stem lesions; and beyond this distance all were free of stem cankers (see Fig. 4). In addition, stem cankers on the beech further away from the larch tended to be at a much lower height – usually only 3-4 m above ground level. To confirm that *P. ramorum* was the cause of the bleeding lesions on the beech, samples were taken from almost 90% of the affected trees and, of those, more than 85% yielded the pathogen.

These data suggest that aerial dispersal of *P. ramorum* from infected larch foliage at mature canopy height can pose a risk to susceptible tree species, such as beech, up to 80m away. Infection over longer distances may well be possible, but would depend on wind direction, inoculum load, host susceptibility and suitable climatic conditions for infection.

Fig. 10: Percentage of *P. ramorum* infected beech (*Fagus sylvatica*) at increasing distances from larch (*L. kaempferi*) also infected with *P. ramorum*



Taken together, results reported in 2.2 - 2.2.4 demonstrate that *L. kaempferi* has the potential to support very high levels of sporulation in the field. Although the number of individual needles that become infected in a tree canopy may be relatively low, the number of sporangia generated from these needles is very substantial and may exceed the inoculum levels of *P. ramorum* produced from California bay laurel (*Umbellularia californica*) in California. The data from spore trapping supports this, although there are currently no data

to compare how sporulation from larch and other hosts may vary due to seasonal and weather factors. Larch is a deciduous host and the ability of the pathogen to survive over winter is a critical factor in assessing the overall risk of larch as a significant sporulator and source of inoculum for disease spread.

2.3 Bark susceptibility of *L. kaempferi* (Objective C)

Testing susceptibility of the bark of larch can be addressed in two ways; (1) by wound inoculation of freshly cut logs and incubation under controlled conditions in quarantine chambers or by exposure of similar logs to naturally produced inoculum of *P. ramorum* in the field. In this instance, the latter method was chosen as it has been effective in assessing the susceptibility of Pacific North West conifer species to *P. kernoviae* (Brasier and Brown, 2008). In September 2009, freshly cut 70cm length logs (12-15cm diameter) of *L. kaempferi* and *F. sylvatica* were exposed on two sites in Cornwall under bushes of infected *R. ponticum* infected with *P. ramorum* or *P. kernoviae.* The aim was to compare the bark susceptibility of larch to *P. ramorum* with beech (a susceptible control host) and to investigate the potential for larch to succumb to infection by *P. kernoviae.*

After 10 weeks of exposure, the logs were assessed for the number and position of infection points and extent of lesion development. Unfortunately, unusually dry conditions during September 2009 apparently reduced inoculum production from infected foliage of *R. ponticum*. Very few lesions developed on beech compared with previous trials and none on the larch logs at the *P. ramorum* site (Table 6). Better inoculum production occurred at the *P. kernoviae* site and numerous lesions developed on the beech logs, some of very substantial size, with fewer and smaller lesions developing on the larch (Table 6). These results indicate that beech bark is more susceptible to both *Phytophthora* pathogens than larch, but also suggest that *L. kaempferi* bark has some susceptibility to *P. kernoviae* and could become infected under natural conditions if exposed to high enough levels of inoculum in the field.

Table 6: Extent of colonisation of larch (*L. kaempferi*) and beech (*F. sylvatica*) logs exposed in the field to natural inoculum of *P. ramorum* and *P. kernoviae*

***Phytophthora kernoviae* site**					
	Beech log 1	**Beech log 2**	**Larch log 1**	**Larch log 2**	**Larch log 3**
Block A					
Mean lesion area (cm^2)	296.6	283.3	20.3	25.9	14.5
Log surface area (cm^2)	3150.9	3091.5	2640.0	2880.0	2940.0
Block B					
Mean lesion (cm^2)	47.6	68.6	0	37.1	0
Log surface area (cm^2)	3045	28734	-	2770	-

***Phytophthora ramorum* site**					
Block A					
Mean lesion area (cm^2)	124.9	60.6	0	0	0
Log surface area (cm^2)	3300	3060	-	-	-
Block B					
Mean lesion (cm^2)	20.9	0	0	0	0
Log surface area (cm^2)	3240	-	-	-	-

2.4 Potential for over-wintering of *P. ramorum* in buds of larch (Objective D)

Earlier work has shown that both *P. ramorum* and *P. kernoviae* are able to overwinter in the buds of some ornamental plants such as *Magnolia* species (Denman et al., 2009). If this occurred on larch the buds could act as a source of inoculum for foliage infection in the following year. Therefore, buds from dormant *L. kaempferi* were examined for the presence of *P. ramorum.* Branches and epicormic shoots taken from trees felled for the assessments described in section 2.2, plus some additional material from other sites, was the source of material for this study. Up to 50-80 buds were removed from each branch, with a total of 14 branches cut from the same number of symptomatic trees. The outer buds scales were removed from each bud and the remaining tissue assessed for the presence of *P. ramorum* using direct isolation, and water baiting of batch samples of ten buds.

In all, although over 1000 buds were assessed, *P. ramorum* was not found associated with any of them. Some fungal growth was observed on few buds but was identified as a species of *Fusarium*. Therefore, this limited study did not provide any evidence of *P. ramorum* overwintering in buds. It may be that infection of larch foliage has to occur *de novo* each year via aerial sources of inoculum. It is also possible that *P. ramorum* from needle infections in one season perennates on the dwarf shoots beneath the needle rosettes, and reinfects needles in the following season. However, since it is difficult to isolate *P. ramorum* even from visibly infected larch foliage (Table 1), levels of infection may be underestimated, especially when trying to detect the pathogen at low levels and in dormant tissue.

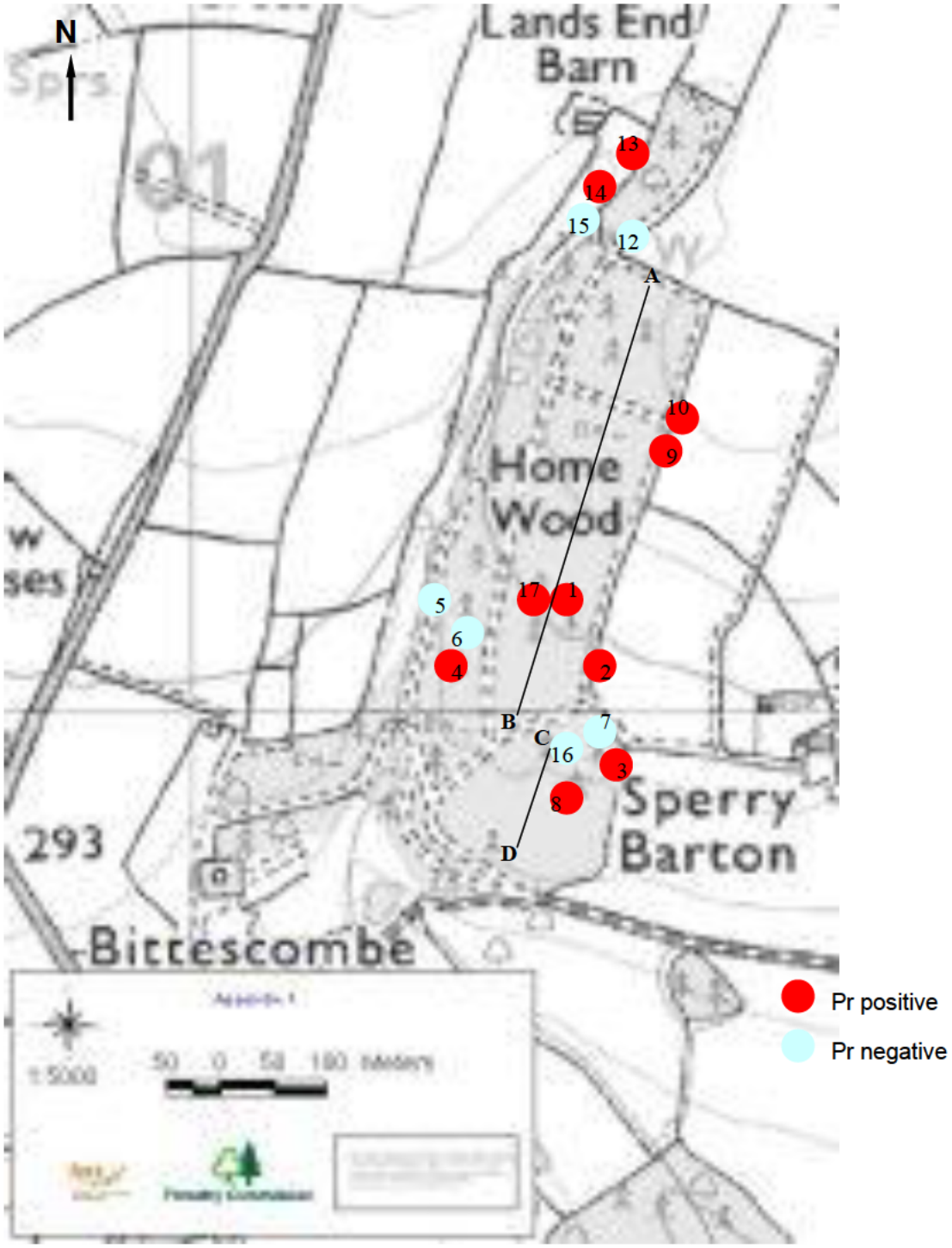
2.5 Assessment of *P. ramorum* infestation on infected larch sites (Objective E)

One privately owned larch plantation site in the West Country was studied in detail during November 2009 to determine the extent of the infestation, the impact on neighbouring trees and the extent of contamination across the site. The site was relatively small, approx 3.6 ha, with a central block of about 700 larch, the majority of which showed symptoms of crown dieback and stem resinosis.

Table 7: Outcome of sampling at privately owned larch plantation in the west country

Tree species	Tree code	Symptom/material sampled	Sampling outcome
Larix kaempferi	LK01	Symptomatic foliage	*P. ramorum*
Larix kaempferi	LK01	Bark – resinous canker	Negative
Fagus sylvatica	FS02	Bark – bleeeding canker	*P. ramorum*
Chamaecyparis lawsoniana	CL03	Bark – resinous canker	*P. ramorum*
Fagus sylvatica	FS04	Bark – bleeeding canker	*P. ramorum*
Fagus sylvatica	FS05	Bark – bleeeding canker	Negative
Fagus sylvatica	FS06	Bark – bleeeding canker	Negative
Larix kaempferi	LK07	Bark – resinous canker	Negative
Larix kaempferi	LK07	Symptomatic foliage	Negative
Castanea sativa	CS08	Symptomatic foliage	*P. ramorum*
Castanea sativa	CS08	Bark – stem canker	Negative
Fagus sylvatica	FS09	Bark – bleeeding canker	*P. ramorum*
Fagus sylvatica	FS10	Bark – bleeeding canker	*P. ramorum*
Fagus sylvatica	FS12	Bark – bleeeding canker	*P. ramorum*
Fagus sylvatica	FS13	Bark – bleeeding canker	*P. ramorum*
Fagus sylvatica	FS14	Bark – bleeeding canker	*P. ramorum*
Fagus sylvatica	FS15	Bark – bleeeding canker	*P. ramorum*
Fagus sylvatica	FS16	Bark – bleeeding canker	*P. ramorum*
Fagus sylvatica	FS17	Bark – bleeeding canker	*P. ramorum*

Fig11: Location of sampled trees and litter transects within a private *L. kaempferi* plantation



Some symptomatic *Rhododendron ponticum* bushes were present in the southern part of the plantation but absent from the main part of the wood; foliage from two of these bushes was sampled and yielded *P. ramorum*. The larch plantation was contained within a remnant wooded area, various broadleaf species occurred across the site as well as along the plantation boundary including some unusual trees such as *Chamaecyparis lawsoniana* along with beech, sweet chestnut and oak (see Fig. 11). On the west side of the site other conifers such as *Picea abies* had been planted. Seventeen trees were examined in some detail; all had either foliar symptoms or stem lesions. Results of the sampling are shown in Table 7. In addition, the larch litter layer in the wood was sampled along two transects (A-B, C-D), to determine levels of *P. ramorum* infestation on the site.

The majority of the trees sampled yielded *P. ramorum*, either from the bark or foliage. The positive finding of *P. ramorum* infecting a 13m tall Lawson's cypress (*C. lawsoniana*) was a new record, and the tree had lesions extending over 2m in the bark of the crown. The source of the infection was most probably from the foliage of a symptomatic, mature larch standing <3m away, which was in direct contact with the stem of Lawson's cypress. However, samples taken from the larch (LK07) did not yield *P. ramorum*, consistent with the difficulty described in above (section 2.1) of isolating *P. ramorum* from symptomatic larch tissue. Only two of the trees sampled were in close proximity to the infected *R. ponticum* (ie within 5m) were trees CS8 and FS16; all others were all close to symptomatic larch. Of the two larch trees sampled, only one was confirmed as *P. ramorum* infected.

The fallen larch foliage in the litter layer consistently yielded *P. ramorum*. In transect A-B, 22 litter samples were taken along the length of the transect, with samples taken at approximately 20m intervals. Of those samples, 19 out of the 22 contained *P. ramorum*, and there was no rhododendron in this area. For transect C-D where some *R. ponticum* was present, a further 8 litter samples were taken and all except one contained *P. ramorum.* Based on this information, and the large number of infected broadleaf trees, levels of *P. ramorum* foliar infection in the larch canopy on this site were extremely high. It is also likely that inoculum of *P. ramorum* will be present and continue to persist in the litter layer and soil on the site even after the removal of the infected *L. kaempferi* trees.

2.6 Impact of harvesting of larch timber from infected sites (Objective F)

There is now strong evidence of extensive infection of *L. kaempferi*, based both on the number of symptomatic trees, the number confirmed *P. ramorum* infections from infected foliage or bark, and the *P. ramorum* spore levels detected in spore traps placed close to larch with dieback. This raises biosecurity issues surrounding the harvesting of the timber from affected stands, and the movement of the timber in the wood chain. Two key questions arise from this: (1) is *P. ramorum* associated only with bark and foliage of infected *L. kaempferi* or can the pathogen be found in the sapwood of infected trees as well? (2) how effective are Pocket Diagnostic® LFDs at detecting *P. ramorum* infection in the bark of symptomatic *L. kaempferi* standing trees and harvested timber, and thus their value as a field diagnostic for conifers?

Out of the 55 bark samples taken from *L. kaempferi* between August 2009 to March 2010, 13 yielded *P. ramorum* (~25%), and *P. ramorum* could be isolated from the sapwood in only three of those 13 trees. In all instances, where *P. ramorum* was isolated from wood it was very superficial and at most penetrated only 1-2mm into the sapwood below confirmed *P. ramorum* bark lesions. Based on this small dataset, the conclusion is that there is potential for *P. ramorum* to be present in conifer sapwood, but its occurrence is likely to be infrequent and only in the outermost layers of wood.

In a comparison of (1) the usefulness of LFDs in flagging up infected material, (2) the potential to convert LFDs positives to a *P. ramorum* diagnosis using PDPlus and (3) how this

compared with isolation success, samples were taken from *L. kaempferi* trees at three private estates in February 2010. A similar process was undertaken with harvested logs of *L. kaempferi*, cut from infected larch plantations in Plym forest. An LFD diagnostic was then run on every sample. Based on prior experience only LFDs with a strong or moderate band result were sent off for PDLus analysis by Foresite Diagnostics, Sand Hutton, York, and isolation was attempted from each larch sample as well. The results of the comparisons are shown below in Table 8.

Table 8: Comparison of LFD tests, PDPlus analysis and direct isolation of samples taken either from standing trees of *L. kaempferi* or logs stacks of harvested timber of *L. kaempferi*

Location	Sample type	No. of trees/ logs sampled	LFDs with moderate/ strong result	PDPlus result	Isolation success
Estate 1	Standing trees	8 trees	7	4 Pr +ve	1 Pr +ve (1)*
Estate 2	Standing trees	12 trees	8	6 Pr +ve	1 Pr +ve (1)*
Estate 3	Standing trees	16 trees	12	7 Pr +ve	5 Pr +ve (3)*
Plym	Logs	40 logs	26	6 Pr +ve	No data

* In brackets number of positive isolations that corresponded with PDPlus confirmation of *P. ramorum*

Not all the suspect samples gave a strong or even moderate LFD reaction, and in most cases only strongly reacting LFDs yielded a *P. ramorum* positive with PDPlus. Also, a moderate to high proportion of LFDs with strong reactions failed to confirm a *P. ramorum* diagnosis. Most often this was when the membrane in the LFD was discoloured with the bark extractives from the larch bark sample. Only a small amount of discoloration was enough to result in a failed DNA analysis of the LFD membrane. It was also apparent that the proportion of failed PDPlus LFDs increased markedly for the tests run on logs in comparison with the material tested from standing trees, even when the LFD had a strong test line. As bark on the logs tended to be highly resinous, this probably permeated the LFD membrane and affected the PDPlus diagnostic success rate. Overall, providing that LFDs have strong reactions and no discoloration of the membrane, the level of positive diagnosis using PDPlus exceeded the number of positives that can be generated by direct isolation, at least during the winter months. However, the combination of PDPlus and direct isolation can give a higher overall number of positives, as sometimes *P. ramorum* could be isolated from samples that failed to convert from a strong LFD result to a positive *P. ramorum* PDPlus diagnosis.

3 Conclusions

- *P. ramorum* is the cause of both dieback and foliar symptoms visible on *Larix kaempferi* (Japanese larch) growing in commercial plantations in the west country. For most of the plantations examined there was little or no evidence that the larch has been infected for a very prolonged period of time or that the rhododendron in the same area had shown any symptoms prior to the autumn of 2009. However, some of the larch were very severely affected with one tree having over 80 lesions evident. Symptoms on the rhododendron understory indicated that the source of infection was most likely to have come from the larch.

- Both laboratory tests and the evidence of spore trapping have shown that *L. kaempferi* can act as a primary host sporulator for *P. ramorum*. The extent of sporulation from

naturally infected *L. kaempferi* also placed other species within 100m distance at high risk of infection including known susceptible species such as beech, sweet chestnut, *Nothofagus* and rhododendron, as well as less susceptible species such as silver birch and Lawson cypress. However, the deciduous nature of the larch is likely to have reduced the risk of disease transmission over the winter.

- The extent of symptoms on the larch and rhododendron and the evidence from the spore monitoring indicated that this outbreak was likely to be a major inoculum source for further spread within the environment, placing not only other larch plantations at risk but also other habitats. Factors such as the extent of the symptoms on trees across both sides of the valley and the height of the trees increased the risks of further spread. Spore monitoring showed that inoculum could be detected at low levels at a distance of up to 1 km from the infected area.

- Visual evidence suggests that the original infection of the larch occurred through aerial transmission, from a remote source via wind and rain.

- Litter and soil layers under larch with dieback are heavily contaminated with *P. ramorum*; this is likely to have an ongoing impact, probably for several years, on what tree species can be used for replanting and future land use. Current evidence suggests it would be unwise to replant these areas with any *Larix* species, and in particular *L. kaempferi*.

- Preliminary evidence suggests that although there is a small likelihood of *P. ramorum* penetrating the sapwood of larch, this is likely to be infrequent and only in the outermost layers of wood. Therefore, logs harvested from infected larch can be used in the wood chain if appropriate biosecurity protocols are applied to infected bark and the 1-2mm zone of underlying sapwood.

- There is evidence to suggest that *Larix* bark and foliage could also be susceptible to *P. kernoviae*, although at a lower level compared with *P. ramorum*, so there is a risk that larch may also become a sporulating host for *P. kernoviae.*

- The use of spore monitoring equipment (rain traps) has been shown to be a very effective approach in rapidly quantifying inoculum generation within outbreaks. This, coupled with the evidence of the high sporulation potential of *L. kaempferi* needles, provided robust evidence on which to base policy decisions to fell the larch.

- It is recommended that the spore monitoring work is continued at the two locations to quantify the full effects of the eradication action in relation to seasonal and weather factors. In addition, monitoring within ongoing larch outbreaks would provide detailed data on seasonal, weather and host factors affecting the frequency, duration and timing of peaks in spore release to further assist contingency planning and inform disease management actions. It should be noted that the very high levels of spore production in infected larch forest were measured in late autumn during needle drop and further work will need to be undertaken to investigate whether these levels of sporulation occur all year round or only under certain conditions.

- Further research needs to address the persistence of inoculum in needle litter compared to litter in rhododendron and vaccinium dominated environments. In addition, further work is recommended on the over-wintering and re-infection potential of Pr in larch plant tissues including work on production of chlamydospores in plant tissues as this is critical to the assessment of risks posed by larch for further disease spread.

- It is recommended that all isolates involved in causing larch outbreaks in the south west (and elsewhere if this arises) are tested to identify their genotype in order to determine any links between outbreaks in the local or distant areas and to investigate the sources of these outbreaks in larch.

- It is recommended that other *Larix* species commonly grown in Britain as commercial plantation species are tested for susceptibility to *P. ramorum* and their sporulation potential also determined (are there any other genera we need to test?).

- During the study, in addition to *L. kaempferi*, a number of new hosts were found to be naturally infected with *P. ramorum* (birch, hemlock, Douglas fir and Lawson cypress). It is recommended that Koch's postulates are satisfied for these new hosts to underpin any listing of these species as regulated hosts.

- Further research needs to solve the issues of low success rates of isolation from larch tissues which are suspected to be infected with *P. ramorum*. Use of a wider range of approaches including molecular methods could assist in resolving some of the problems and increase the speed of diagnosis to support management action.

4 Acknowledgments

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Forestry Commission Scotland
Coimisean na Coilltearachd Alba

ACTION PLAN FOR RAMORUM ON LARCH IN SCOTLAND (2013/14)

Context

Actions for the health and biosecurity of Scotland's trees, woods and forests are set within the context of the overarching, sustainable forest management principles set out in the UK Forestry Standard[1] and the Scottish Forestry Strategy[2].

Sound evidence is a prerequisite for tree health actions. Scotland is well placed to provide this through its existing research providers and through the Scottish Government's support for joint strategic research initiatives such as the LWEC Tree Health and Plant Biosecurity Initiative[3].

This Action Plan supports delivery of the Disease Management Response Plan for *Phytophthora ramorum* in GB[4] which is set within the context of the Forestry Commission's interim Tree Health Biosecurity Strategy[5] and the Defra/Forestry Commission Action Plan for Tree Health and Plant Biosecurity[6]. It relates primarily to Japanese, European and Hybrid larch but also includes linkages with other key host species such as *Rhododendron ponticum* and blaeberry (*Vaccinium myrtillis*). Its delivery will be dependent on a wide range of partners in the state and private sectors, NGOs and the third sector.

The Plan will be reviewed annually or earlier if disease progression escalates significantly in-year.

Current situation

The fungus-like pathogen *Phytophthora ramorum* (*Pr*) was first detected in GB in 2002 but not seen in Scotland outside the nursery trade until 2007. Its global host range is wide (numerous species in over 70 host genera, representing at least 33 different plant families). Until 2009 *Pr* was mostly found to be infecting shrub species such as Rhododendrons (particularly *R. ponticum*), Viburnum, Pieris etc. It is referred to as

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[1] www.forestry.gov.uk/ukfs

[2] www.forestry.gov.uk/sfs

[3] www.lwec.org.uk/node/512

[4] Currently under revision by the GB *Phytophthora ramorum* Outbreak Management Team. General guidance is also available at: www.forestry.gov.uk/forestry/INFD-8XLE56 and www.forestry.gov.uk/website/forestry.nsf/byunique/infd-66ths4

[5] www.forestry.gov.uk/pdf/TreehealthStrategyMinisters.pdf/$FILE/TreehealthStrategyMinisters.pdf

[6] www.defra.gov.uk/food-farm/crops/plant-health/action-plan/

ACTION PLAN FOR RAMORUM ON LARCH IN SCOTLAND

Sudden Oak Death in the USA, but this is a misnomer in the UK as it rarely affects our native oaks. However, several other tree species, including some conifers, are more susceptible. In the UK, the only trees on which this pathogen is currently known to sporulate (reproduce) are sweet chestnut, ash, Holm oak and the larches. Japanese larch is particularly vulnerable and can die within one to two seasons, with consequential economic, environmental and amenity impacts. High *Pr* inoculum levels from infected Japanese larch also represent a wider risk to biodiversity and the historic environment: for example, blaeberry has been shown to be susceptible and *Pr* can have serious consequences for important gardens and designed landscapes. *Pr* was first detected on Japanese larch in south west England in 2009, followed by Wales, Scotland and Northern Ireland in 2010/11. There are currently 137 sites in Scotland with confirmed larch infection (primarily Japanese larch), amounting to 420 ha (out of 65,000 ha of larch woodland) but further infections are inevitable.

Strategic objective

Manage and control the rate of spread of *Pr* on larch to significantly reduce economic impacts to the forestry, nursery and ornamental garden sectors and to protect the health of trees and heathland.

Action Plan

Research

Support work to:

- develop a better understanding of the etiology, pathology and epidemiology of *Pr* in Scotland (with a particular focus on the EU2 lineage).
- support GB *Pr* modelling to support spatial disease management recommendations in Scotland.
- identify resistant larch trees in Scotland that could aid molecular work on resistance and which might provide to future breeding potential for 'resistance durability'.
- investigate the potential for cost-effective treatments, including resistance enhancements, for *Pr* on larch.
- investigate alternative, cost-effective surveillance techniques (e.g. use of remote sensing, spore trapping, water baiting etc).
- further develop rapid field diagnostic techniques for *Pr* in soil, water and plants.
- understand the wider biodiversity implications of *Pr* on larch and consider ways to mitigate negative impacts.

Detection

- Expand aerial surveys (by helicopter) to cover CEH-modelled high risk sites in the north east of Scotland.

ACTION PLAN FOR RAMORUM ON LARCH IN SCOTLAND

- Enhance FCS capacity to accelerate aerial survey photograph analysis, ground-truthing surveys and disease confirmation.
- Continue to explore the potential for ROAV[7] and remote sensing technology to assist with disease detection.
- Through the Phytophthora Scotland Steering Group, continue to enhance *Pr* survey capacity for heathland.
- Maintain current nursery and garden inspection regimes for *Pr* (Scottish Government Horticulture & Marketing Unit).

Precautionary measures

- Destruction of infected plants at nurseries.
- Continue to develop proportionate biosecurity measures for *Pr*.
- With partners, explore funding opportunities for the prophylactic removal of *Rhododendron ponticum* in high risk[8] areas.
- Revise the Scotland larch *Pr* risk zones[9] and supporting guidance on felling licensing (in the winter period) and replanting.
- Consider financial support measures for disease management (including opportunities in the next Scotland Rural Development Plan).

Dealing with infected stands

- Maintain current default policy[10] of felling/killing all larch within a 100m radius of confirmed larch infections and revise guidance on where felling/killing out to 250m would be desirable.
- Continue to offer targeted support measures to facilitate rapid removal/killing of infected larch stands.
- Facilitate prompt revision of Felling licences/Forest Plans to enable cost-effective and rapid harvesting of infected larch stands.
- Through the FC GB Phytophthora Outbreak Management Team, review and implement appropriate biosecurity measures in the timber supply chain[11], including a risk-based approach to the handling of non-larch timber from infected sites.

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[7] Remotely operated aerial vehicles

[8] Based on modelling work for the Scottish Government by the Centre for Ecology & Hydrology (CEH)

[9] These are based on the risk of pathogen spread: from 'high' in Risk Zone 1 to 'low' in Risk Zone. See: www.forestry.gov.uk/pdf/Pramorum_risk_zones_Oct11.pdf/$FILE/Pramorum_risk_zones_Oct11.pdf

[10] In exceptional circumstances, such as intensively managed sites where individual trees can be under constant observation by expert staff and where wholesale felling would be of major detriment to e.g. internationally recognised plant collections or recreation facilities, alternative disease management regimes can be considered.

[11] E.g. www.forestry.gov.uk/website/forestry.nsf/byunique/infd-849e4r

ACTION PLAN FOR RAMORUM ON LARCH IN SCOTLAND

- Actively encourage more processors to become authorised for handling timber from infected larch stands.
- Through the Scottish Timber Market Impacts Group, explore the implications of *Pr* for the production and marketing of timber from Scottish forests in the short term and over the next 25 years.
- Continue to develop and provide advice to the sector (including nurseries) on the use of alternative species in areas where larch is at high risk of infection by *Pr.*

Awareness-raising

- Align *Pr* within a refreshed FCS communications strategy for tree health issues in Scotland, particularly focusing on stakeholders associated with potential disease pathways (such as the 'active recreation' sectors).
- Support regular tree health seminars to raise awareness of this and other tree pests and diseases (stakeholders in the east of Scotland to be reminded of the need for continuing vigilance).
- Continue to work with partners to embed biosecurity awareness in the forestry, environmental and recreation sectors.
- Publish a broad assessment of the potential environmental, economic, landscape and social impacts of *Pr* on larch in Scotland.

Forestry Commission Scotland
27 March 2013

Summary of current knowledge: *Phytophthora ramorum* disease on larch in Scotland

A number of factors influence disease levels each year in the epidemic of *P. ramorum* on larch. These include (1) climate and-year-on-year weather conditions, (2) levels of spore production from infected hosts and the capacity to remove these 'sporulators' (primarily larch and rhododendron) from the landscape and (3) the lineage (either EU1 or EU2) or even specific genotypes of *P. ramorum*.

Climate and weather: For more than a decade it has been known that the mild wet conditions typical of western Britain favour the disease. Particularly in autumn, mild and wet conditions provide the ideal environment for *P. ramorum* to produce huge numbers of spores on the needles of infected larch trees, and the same conditions also favour infection of intact bark tissue. In addition, winters that are warmer than average probably also favour disease development. Evidence for this comes from laboratory experiments which have shown that *P. ramorum* is almost as capable of attacking and killing larch bark tissue when growing at a constant 10^{o} C, than it is at 20^{o} C which is usually considered its optimum temperature for growth. On that basis, it can be concluded that during mild/warmer than average winters, *P. ramorum* is likely to be more damaging to larch following on from infection initiated the previous year or years, compared with disease progression under consistently colder and drier conditions.

Sporulating hosts: Phytophthora ramorum sporulates prolifically on the leaves and shoots of certain hosts (i.e. green tissue) but not on lignified bark tissue although it will colonise and cause extensive bark cankers on some trees. The sporulating hosts drive epidemics caused by *P. ramorum.* There is no doubt that larch (mainly Japanese larch), has the ability to produce very large numbers of spores when infected by *P. ramorum*. These can amount to 100s or 1000s from a single infected larch needle, compared with 10-100s produced from an infected rhododendron leaf. Removal of symptomatic infected trees and those without symptoms (which may not yet have been exposed for long enough or under the right conditions to develop symptoms) in a buffer zone around infected trees is essential to limit disease spread and reduce wider impacts. Apart from larch and rhododendron, there has been much debate about what other broadleaf and conifer tree species might be significant sporulators and as a consequence could drive a *P. ramorum* epidemic in the absence of larch. Sweet chestnut is now also starting to play that role in some parts of England but as it is not a common broadleaf species in Scotland, is unlikely to be a significant host here.

The likelihood of other significant sporulating hosts arising is a constant concern and stems from the unexpected host jump to larch. A recent study funded by FC Scotland compared the potential of various conifer hosts to sustain sporulation by the pathogen on infected needles, given that they are known to have some level of bark susceptibility to *P. ramorum* and can be occasional hosts in the wider environment. The studies, limited to laboratory tests, compared sporulation on infected foliage of Sitka spruce, western hemlock, grand fir, Douglas fir and coastal redwood against a baseline of sporulation on rhododendron and Japanese larch. The latter was always the outstanding sporulator compared with all the other species, with a *ca.* 17-fold difference in the levels of sporulation between larch and rhododendron. Some sporulation, however, did occur on the artificially infected needles of the other conifers although it was always less than rhododendron when the mean values were compared. Overall, rhododendron was the highest, then hemlock and coastal redwood, followed by Sitka spruce, Douglas fir and grand fir. Based on this, there is little evidence to suggest that any of these conifer species could take the role that larch or rhododendron currently play in driving the *P. ramorum* epidemic, especially as the

significance of a sporulating host not only depends on its ability to produce spores, but how few spores are needed to cause infection. In this respect rhododendron is outstanding, only a few spores can initiate infection and thereby cause further disease cycles.

Spore dispersal: In general dispersal of *P. ramorum* spores leading to infection occurs over relatively short distances of just a few metres, especially when spore release is from foliage in the understorey or from isolated plants. Spore dispersal over longer distances is facilitated if sporulation occurs high in the canopy and is generated from a super-sporulator such as larch. Work by Fera has shown dispersal distances of up to 1 km in south west England, whilst *P. ramorum* dispersal in forest ecosystems in Oregon, suggests dispersal over a few kilometres is not unusual, but tens of kilometres is unlikely. The significance of any long distance spore dispersal will be influenced by dilution effects and the number of spores required for infection once spore deposition has occurred. Rhododendron and *Vaccinium* leaves can be easily infected with just a few zoospores, whereas on oak thousands of spores are required to infect through intact bark. In instances where trees develop stem or branch cankers (ie bark infection) after exposure to spore plumes from heavily sporulating larch, in general only trees within a 100 m radius of the sporulating larch are likely to develop stem cankers, as has been observed with beech, fir, Douglas fir and western hemlock. The 'infection radius' is probably wider in relation to foliar infections because fewer spores are required to infect susceptible foliage but it will depend on the host species and has not been quantified.

Outcome of infection:
Mostly Japanese larch (*Larix kaempferi*) is affected by *P. ramorum* although European (*L. decidua*) and hybrid larch (*L. x eurolepis*) are also susceptible. Trees of all ages can be quickly killed by girdling cankers on branches and stems; eve with mature trees death can occur rapidly within 2-3 years if trees suffer multiple infections throughout the crown and branches. This is not unusual within compartments of larch, where build-up of spore inoculum can be intense and the distance for spore dispersal is small. As *P. ramorum* invades larch bark, it not only incites copious resin flow from infected tissues, but the freshly invaded phloem tissue takes on a striking pink-red colour at the margins, only changing to a typical brown-coloured necrosis in older lesion regions. Chemical changes in the phloem tend to be concentrated in these red-coloured areas, with resin acids and other compounds such as α-pinene and 3-carene produced at high levels apparently as an induced resistance response of the larch to invasion by *P. ramorum*. However, the response may be overwhelmed when multiple infections occur on individual trees, especially during the epidemic peak, hence the rapid tree death. In contrast, when the spore source is removed and the number of infection points on a tree is limited, colonisation progress by *P. ramorum* can arrest and trees may even recover albeit with some damage.

In some trees, however, the infection can become chronic with symptoms of crown dieback developing over several years. In most instances, trees which suffer this type of infection have been exposed to massive spore inoculum from nearby larch – they are known as terminal hosts because although they can become infected they do spread the pathogen to other hosts. Nevertheless, even with the removal of the larch and infectious spore source, *P. ramorum* sometimes remains viable in the bark when such terminal host, causing expanding stem lesions on the main stem for several years. This is can be evident as gradual crown dieback on affected trees, often from the top down because infection frequently appears to be initiated where the bark is thinner in the upper part of the crown. Beech, fir, Douglas fir and western hemlock are most prone to this form of chronic *P. ramorum* infection after removal of nearby sporulating hosts.

Lineages of the pathogen: Currently, *P. ramorum* is known to exist in the form of four near-clonal lineages, indicative that it has arrived via a series of separate introductions: NA1 and NA2 – only known in North America and EU1 and EU2 which are present in Europe, but with the EU2 so far only found in Scotland and Northern Ireland and not in England or Wales or elsewhere in mainland Europe. Evidence suggests that the EU2 was introduced into Northern Ireland prior to its arrival in Scotland. If so, spread from Northern Ireland to Scotland is more likely to have occurred through movement of ornamental plants rather than aerial spread, due to distances involved.

Behaviourally the lineages differ with slightly different optimum and lethal temperatures which define growth, illustrating their individual gene x environment interactions. Genetic analysis has also shown that the divergence of the four lineages is relatively ancient (hundreds of thousands of years), and that the EU2 may be an ancestral form. Biologically, the EU2 is also more aggressive at attacking larch bark than the EU1 but slightly poorer at sporulating on larch needles, and the different behaviour between lineages may be a 'trade-off' for the pathogen. Although the EU2 is a more effective bark killer of larch, on average it produces fewer spores on larch needles than the EU1, which as a consequence is likely to reduce its dispersal potential or even limit the likelihood for infection compared with the EU1. Moreover, although the EU2 is almost exclusively found in the Management Zone, where it is undoubtedly very damaging, the EU1 (with other significant behavioural and genetic differences) is also capable of being highly damaging. Therefore, any upsurge in disease is most likely to be weather related, and not due to either the EU2 or additional host species acting as significant sporulators.

Since the first finding of the EU2 lineage of *P. ramorum* in Scotland, the spread of both lineages has been monitored and the EU2 still has a relatively limited distribution and is mainly but not exclusively found in south west Scotland (see maps below). This relatively slow spread and the genetic uniformity of the EU2 lineage, suggests it is a much more recent introduction than the EU1, that it may be spreading from a few foci or just one, and the current management efforts are containing its spread. Its limited distribution also suggests it is not moving in the plant trade as the EU1 has done (and continues to do although regulation limits this), although currently routine lineage testing of *P. ramorum* infected nursery plants is not undertaken.

Finally, there is evidence for at least the EU1 that through mutation some genotypes (ie, particular individuals of *P. ramorum*) are emerging that are better adapted to larch and becoming dominant. This adaptation takes the form of being able to sporulate even more heavily on larch, and therefore increases the potential for spread and infection by the EU1 in larch plantations. However, this process of adaptation to larch may be countered to some extent as *P. ramorum* cycles between larch and other hosts.

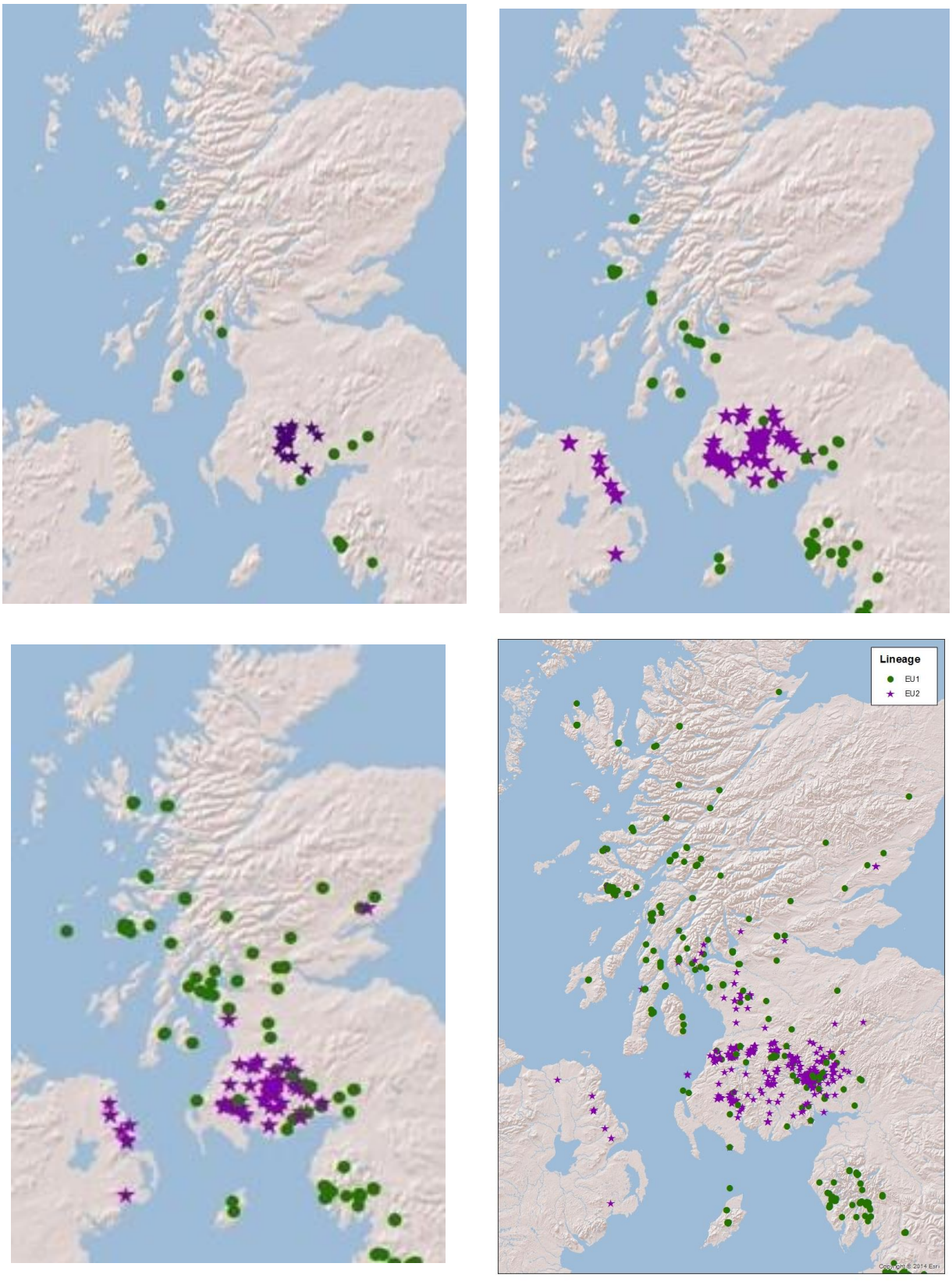
Origins of P. ramorum: The origins of *P. ramorum* are now known to be south east Asia. This is evidenced by findings in 2017 of the pathogen in Vietnam close to the boarder with China. Some of its closest *Phytophthora* relatives also appear to be native to Taiwan and Japan, strengthening the likelihood that *P. ramorum* is native to this region. It also raises a question about the pathways that might have allowed *P. ramorum* to spread from this region into both Europe and North America, and what other lineages might also be present. A population of *P. ramorum* dispersed across this region with many geographically separated habitats, may also explain why at least four genetic lineages of the pathogen exist and have driven the genetic divergence that is evident from genetic analysis. It also raises the possibility of the

arrival of more forms of *P. ramorum* into Europe and North America and, as a consequence, opportunities for genetic recombination and greater variation.

Surveys of P. ramorum in Scotland: During survey years 2014 up to 2018, the number of samples taken primarily for the detection of *P. ramorum* in Scotland has increased markedly. In 2014, 140 tree and rhododendron samples were tested (40% positive for *P. ramorum*); in 2015, 114 samples were tested (48% positive); in 2016, 162 samples (31% positive); in 2017, 538 samples (51% positives); 237 samples (62% positive). The variation in the number of positive samples can reflect the experience of those undertaking the sampling, but often is influenced by wider surveys of hosts more likely to return negative results, or checks of previously ramorum-free areas where the sample material is likely to be negative. Apart from *P. ramorum* various other *Phytophthora* species are occasionally identified but most commonly include: *P. pseudosyringae* and *P. plurivora*.

Research underway or planned by Forest Research

- Tracking the expanding range of the EU1 and EU2 lineages across Scotland.
- Evaluating the susceptibility of Sitka spruce to both the EU1 and EU2 lineages of *P. ramorum* and the spore numbers likely to lead to infection.
- Likely interaction with *Ips cembrae* due to the growing resource of larch that could be used as breeding material.
- Possible resistance in Japanese larch to *P. ramorum.*
- Dispersal distances of *P. ramorum.*
- Relationship between tissue moisture content of larch bark and aggressive colonisation by *P. ramorum.*



Changing EU1 (green dots) and EU2 (purple stars) over time. Top left, known distribution in 2013; top right, 2015; bottom left, 2016; bottom right, 2018

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Spore dispersal