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Understanding the Role of Avian Vectors in the Spread of *Phytophthora ramorum*

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EXECUTIVE SUMMARY

Phytophthora ramorum is a destructive plant pathogen which has been responsible for the deaths of hundreds of thousands of oak (*Quercus* spp.) and Tanoak (*Lithocarpus densiflorus*) trees in coastal woodlands of the western U.S.A. The pathogen was first detected in Great Britain in 2002 and has since spread to a large number of locations across England, Wales, Scotland, Northern Ireland and the Channel Islands, but with a concentration of outbreaks along the west coast of Great Britain. As a damaging, non-native species in the U.K., *P. ramorum* is subject to statutory control under plant health legislation, with a compulsory requirement for eradication and containment. Responsibility for this falls to the Food and Environment Research Agency (Fera) of the Department for Environment, Food and Rural Affairs (Defra). There is consequently a need for the mechanisms by which the pathogen is spread to be understood so that effective control measures can be put in place.

A considerable amount is already known about the means by which *P. ramorum* can spread to infect new hosts and new sites. Spores of the pathogen can be picked up by rainwater and then transported to new host plants in mist or rain driven by the wind and in watercourses. Viable spores can also persist in infected soil and leaf litter, which can then be transported to new areas on hikers' boots, vehicle and bicycle tyres, *etc.* Many cases of spread of *P. ramorum* can be attributed to these means of dispersal and to other human activities, particularly the movement of host plants for the nursery trade. However, it has long been suggested that animal vectors may also play a part in long distance transport of *P. ramorum*, partly due to the swift and somewhat patchy nature of the spread.

Birds are suitable candidates as potential long distance vectors of *P. ramorum* as they can move large distances and there is also a degree of correlation between the migration paths of some species and the main concentrations of *P. ramorum* occurrence in the U.K. It is possible that birds may spread spores of the pathogen through their faeces, or through carriage of spores on their feathers and/or feet. However, there have been very few studies carried out to examine these possibilities and there is currently little evidence for whether or not avian vectors may be transporting spores or contributing to the spread of the pathogen.

There exists a potential opportunity for this lack of knowledge to be addressed through taking and analysing samples from birds caught during ongoing bird ringing efforts in the U.K. We therefore considered those bird species which are likely to come into contact with *P. ramorum* spores in order to identify possible candidates for the transmission of the pathogen from which samples could be taken. Species which may associate with spore-producing plant hosts include Siskin (*Carduelis spinus*), Starling (*Sturnus vulgaris*), Blackbird (*Turdus merula*), Fieldfare (*Turdus pilaris*), Redwing (*Turdus iliacus*), Chaffinch (*Fringilla coelebs*), Brambling (*Fringilla montifringilla*) and Greenfinch (*Carduelis chloris*), and those which may transport *P. ramorum* along the west coast during migration include Garden Warbler (*Sylvia borin*), Blackcap (*Sylvia atricapilla*), Chiffchaff (*Phylloscopus collybita*) and Willow Warbler (*Phylloscopus trochilus*).

In order to understand if birds may be vectoring *P. ramorum*, it is suggested that feather, faecal and foot swab samples could be taken from these candidate species caught for ringing in the west of Great Britain (in spring, when birds are migrating and spores are being produced) by BTO volunteer ringers (supported by BTO staff) and then analysed in a laboratory for presence and viability of the pathogen. Carrying out more detailed analyses of the movements of candidate bird species using BTO datasets could also help to determine if these species are likely to be contributing to the spread of the disease.

1. BACKGROUND TO *PHYTOPHTHORA RAMORUM*

Phytophthora ramorum is a species of oomycete (often referred to as water moulds), a class of eukaryotic micro-organisms distinct from animals, plants and fungi and closely related to diatoms and brown algae (Tyler *et al.* 2006). Oomycetes include species known to be some of the most destructive plant pathogens, particularly within the *Phytophthora* genus, which translates as "plant destroyer" in Greek (Lamour *et al.* 2007). *Phytophthora infestans*, for instance, causes late blight disease in Potato (*Solanum tuberosum*), which was responsible for the Irish potato famine between 1845 and 1847 (Ristaino 2002), whereas *Phytophthora sojae* causes root and stem rot and prevents germination or survival of seedlings in Soya Bean (*Glycine max*), a major cause of crop loss (Tyler 2006).

P. ramorum itself is responsible for the disease commonly known as Sudden Oak Death (Rizzo *et al.* 2002), though in fact the pathogen affects many trees and shrubs besides oaks and does not cause death in all host species (Anacker *et al.* 2007). There are two main categories of host. Foliar hosts display symptoms such as leaf necrosis, shoot dieback and wilt (Storer *et al.* 2001). These hosts often act as sources of inoculum for the further spread of *P. ramorum*, as infected leaves are the primary site of sporulation (Davidson *et al.* 2005; Defra 2005; Sansford and Woodhall 2007). Foliar hosts are therefore considered important in the disease cycle, however they rarely die from the infection (Davidson *et al.* 2003; Anacker *et al.* 2007; DiLeo *et al.* 2009). Bark canker hosts, on the other hand, rarely show foliar symptoms but instead develop inner bark necrosis leading to bleeding cankers on mature trees (Davidson *et al.* 2003; Defra 2006). This can eventually kill all of the adjacent phloem tissue and girdle the tree, then resulting in often rapid tree death through complete wilting of the canopy (Grünwald *et al.* 2008). It was rapid death in this manner of hundreds of thousands of oak (*Quercus* spp.) and Tanoak (*Lithocarpus densiflorus*) trees in Californian coastal woodlands which gave *P. ramorum* its popular name of Sudden Oak Death (Davidson *et al.* 2003; Anacker *et al.* 2007). However, these bark lesions are not considered to produce spores, and so such hosts are usually deemed to be terminal hosts with no significant role in disease spread (Davidson *et al.* 2005; Defra 2005; Sansford and Woodhall 2007; Grünwald *et al.* 2008).

2. *PHYTOPHTHORA RAMORUM* IN THE U.K.

In the U.K., as in the rest of Europe and the U.S.A., *P. ramorum* is considered to be a non-native species thought to have possibly originated from Asia (Sansford and Woodhall 2007; Brasier *et al.* 2010). The pathogen thrives in cool, wet climates; hence in the U.S.A., coastal forests have formed the primary habitat, though spread of the disease away from this preferred environment has proved possible where the microclimate is suitable (Magarey *et al.* 2007; Sansford and Woodhall 2007). Similarly, in the U.K., when occurrences in nurseries and retail sites are excluded (see 3 below), there has been a concentration of outbreaks along the cooler, wetter, west coast of Great Britain, particularly in Devon and Cornwall, South Wales, and in counties in the northwest of England (Defra 2009; Fera 2009; Forestry Commission 2010a).

P. ramorum was first detected in Great Britain in a garden centre in 2002, and since then it has been found in a large number of geographically scattered locations, mostly infecting ornamental shrub species such as *Rhododendron* spp., *Viburnum* spp. and heathland plants such as Bilberry (*Vaccinium myrtillus*), also known as Blaeberry and Winberry (Defra 2009; Forestry Commission 2010b). More than 850 outbreaks have been confirmed to date; roughly 80% of these in England with the majority of the remainder being shared in broadly equal proportions between Scotland (approximately 64 outbreaks), Wales (46) and Northern Ireland (37), with presence also having been noted in the Channel Islands (Fera 2009; DARD 2010; Fera 2010; Forestry Commission 2010a; Scottish Government 2010). Whilst approximately two thirds of all outbreaks have been in nurseries and garden centres, there have also been large numbers of infections in gardens, woods and heaths (Defra 2009; Fera 2009; Scottish Government 2010).

The first infection of a tree in the U.K. (a mature Southern Red Oak, *Quercus falcata*) was confirmed in 2003, and the pathogen was subsequently determined as the cause of disease affecting numerous Beech (*Fagus sylvatica*) and a smaller number of individuals of other tree species at various sites, but with the largest concentration in Cornwall (Fera 2010; Forestry Commission 2010b). Unlike the species in the U.S.A., the native British oaks *Quercus robur* (Pedunculate Oak) and *Quercus petraea* (Sessile Oak) are proving to be fairly resistant to infection, both in laboratory tests and in terms of the number of incidences recorded from infected sites (Sansford and Woodhall 2007; Forestry Commission 2010b). Until late 2008, the total number of affected trees of all species, across the U.K., remained fewer than 100 and most were found to be growing in very close proximity to infected *Rhododendron* spp. (Fera 2010; Forestry Commission 2010b; Scottish Government 2010). Rhododendrons, and particularly *Rhododendron ponticum*, are amongst the most significant spore-producing hosts in England, Scotland, Wales and Northern Ireland and have therefore been identified as having a major role in the spread of inoculum, and hence infection (Forestry Commission 2010c; Scottish Government 2010).

In 2009 however, *P. ramorum* was found to be infecting large numbers of Japanese Larch (*Larix kaempferi*) and other conifers in commercial plantations in southwest England, and later (in 2010) in South Wales and eastern Northern Ireland (DARD 2010; Forestry Commission 2010a,b; Scottish Government 2010). Large numbers of larch spp. trees have now died or are dying at these outbreak sites, although the pathogen has not yet been found to be affecting any tree species in Scotland (Forestry Commission 2010b; Scottish Government 2010). This spread to Japanese Larch is a cause of serious concern, not just because *P. ramorum* is now, for the first time, affecting a commercially important conifer species (Forestry Commission 2010b), but because many of the outbreak sites are not close to rhododendrons (as the usual source of inoculum for tree infections in the U.K.), and so it is not certain how they have come to be infected (Forestry Commission 2010c). It has also recently been discovered that infected Japanese Larch in fact produce five times as many spores of *P. ramorum* as do rhododendrons, and that these spores can possibly be carried as far as 50km in wind-blown rain (see 3 below), meaning that the Japanese Larch themselves are potentially a huge threat to much further spread of the pathogen (Forestry Commission 2010b); Scottish Government 2010).

3. MEANS OF TRANSMISSION

A considerable amount is already known about the means by which *P. ramorum* can spread to infect new hosts and (though to a lesser extent) new sites. Spore producing bodies (sporangia) are produced on the leaf, and sometimes shoot lesions, of foliar hosts (Davidson *et al.* 2005; Defra 2005; Sansford and Woodhall 2007). These sporangia are deciduous, and the infective spores within them (zoospores) are released in cool, moist conditions, playing a major role in dispersal (Davidson *et al.* 2002; Sansford and Woodhall 2007). Whilst it is not thought that these spores can be carried directly on the wind (Moralejo *et al.* 2006), it has been demonstrated that they are picked up by rainwater, which can then transport the pathogen to new host plants either through rainwater splash from the foliage of nearby, infected individuals, or through mist or rain driven by the wind (Davidson *et al.* 2002; Davidson *et al.* 2005; Turner *et al.* 2006). There are also numerous examples of viable *P. ramorum* inoculum having been recovered from watercourses, particularly those draining affected sites (Davidson *et al.* 2005; Turner *et al.* 2006; California Oak Mortality Task Force 2010). Whilst detection in a watercourse does not necessarily appear to lead to contraction of the disease by host plant species nearby (California Oak Mortality Task Force 2010), it is nonetheless possible that transport of viable spores in this manner may contribute to the spread of *P. ramorum* to new sites over larger distances.

Longer-lived spores of *P. ramorum* (chlamydospores), also produced in infected foliar tissue (Defra 2005; Sansford and Woodhall 2007), have further been isolated from soil and leaf litter samples and have been shown to remain viable therein across seasons (Davidson *et al.* 2005; Defra 2005; Turner *et al.* 2006; Kliejunas 2007). Such infected soil or litter again has the potential to be carried by the wind to introduce *P. ramorum* to new plants and possibly new sites (Kliejunas 2007), and it has been demonstrated experimentally that it is possible for inoculated growing media to cause plants to become infected with the pathogen through disease transmission through the roots (Parke 2007). There is also extensive evidence, both from the U.S.A. and the U.K., that soil transported by human activities can spread *P. ramorum* to new areas, being carried on hikers' boots, vehicle tyres, bicycle tyres, dogs' paws, horses' hooves, *etc.* (*e.g.* Davidson *et al.* 2005; Webber and Rose 2007; Cushman and Meentemeyer 2008).

Many cases of spread of *P. ramorum* to new areas can, in fact, be attributed to human activities, and in particular to the widespread movement of plants (especially ornamental foliar host species, such as *Rhododendron* spp., *Viburnum* spp. and *Camellia* spp.) for the nursery trade (Sansford and Woodhall 2007; Goss *et al.* 2009). Certainly this has been the case historically, with many diseased plants (particularly in residential or managed garden settings isolated from any nearby source of natural inoculum), both in Europe (including the U.K.) and the U.S.A. having been traced back to a supply from a nursery, or infected plants having been identified by tracing forward supplies distributed from infected nurseries (Sansford and Woodhall 2007; California Oak Mortality Task Force 2010). There is also some evidence that this may have introduced the pathogen to 'wild' trees nearby (Davidson and Shaw 2003; Brasier and Jung 2006). Controls are now in place to screen known host species for infection prior to any movement of plant stock, however to what extent these controls are proving fully effective is still uncertain as *P. ramorum* has continued to be found on some controlled material within the E.U. (Slawson *et al.* 2007; Scottish Government 2010).

It has also long been suggested that animal vectors, such as insects or birds, may play a part in the long distance spread of *P. ramorum* to new areas significantly geographically separated from sites of existing infection, partly due to the swift and somewhat patchy nature of the spread (*e.g.* Blomquist *et al.* 2002; Ralph *et al.* 2003; Defra 2005; Kliejunas 2007). Certainly movement of vectors is, in general, very important as a process by which diseases expand geographical range (Williams *et al.* 2002), and there are potentially a number of means by which animals could pick up *P. ramorum* spores and transport them to new sites; for instance, through contact with infected plant tissue, leaf litter, soil or rainwater, or possibly through ingesting infected foliage, seeds or berries.

For insects, the majority of studies carried out to date have provided no evidence for dispersal of *P. ramorum* via these potential vectors (S Frankel, *pers. comm.*; Kanaskie *et al.* 2002; Defra 2005; Turner *et al.* 2006; Kliejunas 2007; Sansford and Woodhall 2007). However, at least two studies have demonstrated that, in laboratory conditions, spores of the pathogen ingested by invertebrates (fungus gnat sp. larvae, shore fly sp. larvae, snail sp., and *Derocerus reticulatum* and *Ariolimax columbianus* slugs) remain viable on excretion and that faeces can then go on to infect plant tissue of host species (Parke *et al.* 2008; Hyder *et al.* 2009).

4. A ROLE FOR BIRDS?

Birds are perhaps more suitable candidates as potential long distance vectors of *P. ramorum* than are invertebrates as they tend to move further, particularly when compared to gnat and fly larvae, snails and slugs; currently the only invertebrates known to have demonstrated some evidence of potential to act as possible transmitters of the pathogen (Parke *et al.* 2008; Hyder *et al.* 2009). There is also a degree of correlation between the migration paths of some bird species and the main concentrations of *P. ramorum* occurrence in the U.K., although it is not clear if this is causal (see 6 below).

4.1 Vectoring via feathers

The possible mechanisms of such avian vectoring are threefold. Firstly, it may be that birds could carry spores on their feathers, these having been introduced by inoculated rainwater. Zoospores would be unlikely to remain viable once feathers have dried out, however it is thought that chlamydospores could possibly remain so (Davidson *et al.* 2002; C Blomquist, *pers. comm.*). These spores could then be washed out in new rain and transferred to new hosts. Funding for a study to test this possibility in the laboratory using captured Feral Pigeons (*Columbia livia*) to be misted with artificially inoculated rainwater, was initially made available to scientists at the California Department of Food and Agriculture and the Wilderness Conservation Society (Blomquist *et al.* 2002; USDA Forest Service 2002). However the work did not go ahead due to an unexpected disease risk associated with keeping the wild pigeons in a human environment and due to the then uncertainty as to how to successfully germinate chlamydospores (C Blomquist, *pers. comm.*), and so the hypothesis currently remains untested.

4.2 Vectoring via feet

The second possible mechanism is via soil/debris containing inoculum being carried on birds' feet. The fact that *P. ramorum* is able to be transported elsewhere through soil picked up on vehicle tyres, human feet, and on the feet of some other animals (as well as the capacity for inoculum to remain viable in the process) is well established (*e.g.* Davidson *et al.* 2005; Webber and Rose 2007; Cushman and Meentemeyer 2008). Consequently there is certainly potential for any ground-foraging species of bird with some association with sites containing *P. ramorum* infected plants to pick up spores of the pathogen on their feet whilst walking over/foraging through soil containing inoculum, which they could then potentially transport to new areas (C Blomquist, H Cushman, S Frankel and M Bielka, *pers. comm.*).

However, as with the hypothesis that *P. ramorum* spores could be carried on feathers, this theory has yet to be tested conclusively for birds. The California Department of Food and Agriculture and the Wilderness Conservation Society, along with their planned study to test the ability of Feral Pigeons to transmit the pathogen via their feathers, were also to conduct experiments whereby the birds would walk across *P. ramorum*-infested mud and then have residue samples taken from their feet and cultured for *P. ramorum* growth (Blomquist *et al.* 2002). But the same problem with the unexpected disease risk again prevented this from being completed (C Blomquist, *pers. comm.*). Additionally, there was a similar pilot project carried out by scientists at both the University of North Carolina and the Sonoma State University in California, to assess the role which wild turkeys (*Meleagris gallopavo*) may play as dispersal agents of *P. ramorum* in western U.S.A. It was thought that the birds, which spend a large amount of time moving back and forth between the soil surface and the canopies of spore-producing California Bay Laurel (*Umbellularia californica*) trees, could possibly transport the pathogen between host plants and around infected sites (Cushman and Meentemeyer 2008). However, the work again proved very challenging and did not yield meaningful results (H Cushman, *pers. comm.*).

It is not thought likely that birds could pick up *P. ramorum* spores simply by perching on infected twigs/stems because a) in most cases spores are produced on the plant leaves themselves (Davidson *et al.* 2005; Defra 2005; Sansford and Woodhall 2007), which birds are unlikely to perch on, and b)

sporulating *P. ramorum* is not usually particularly sticky (C Blomquist, *pers. comm.*), and if it were it would be likely to be preened off by birds before they had travelled very far from picking it up.

4.3 Vectoring via ingestion

The third possible mechanism of *P. ramorum* being spread through avian vectors is by birds ingesting inoculum and subsequently excreting this in their faeces once having flown to new areas where further susceptible host plants are present. For this to be a plausible means of transmission there are two main requirements: 1) birds must ingest matter containing *P. ramorum* inoculum, and 2) this inoculum must remain present and viable on excretion. It has been documented that it is possible for *P. ramorum* to be present in the reproductive tissues (and hence potentially the seeds or fruits which birds may eat) of host plants. Chastagner *et al.* (2008) isolated the pathogen from the base of an old flower stalk of a species of mistletoe (*Phoradendron serotinum macrophyllum*) and from flowers growing on California Bay Laurel (*Umbellularia californica*) trees, although infection of the former may have occurred from the surrounding soil or debris while the mistletoe (which had fallen out of the host tree) was on the ground as opposed to whilst it was living on the tree. These flower parts also proved susceptible to *P. ramorum* infection in the authors' laboratory tests. Despite this however, there has been no evidence that the pathogen is carried or transmitted by seeds or fruits, or indeed that any of the *Phytophthora* species are spread in this manner (C Blomquist, *pers. comm.*).

As it has been demonstrated that spores of *P. ramorum* ingested by at least some invertebrates can survive the digestive process (Parke *et al.* 2008; Hyder *et al.* 2009), an alternative means by which some birds may perhaps ingest the pathogen is by consuming invertebrates which have fed on infected foliage. However, whilst a very small number of invertebrate species which can fall prey to birds have been shown to consume *P. ramorum* growing on foliage in the laboratory (Parke *et al.* 2008), this has yet to be demonstrated as natural behaviour in the field. Therefore, although ingestion of *P. ramorum* by birds is possible, the mechanisms are uncertain and it is perhaps most likely for species with an invertebrate component to their diet.

There is some evidence of oomycete spores of species closely related to *P. ramorum* surviving the digestive process following ingestion by birds. Chlamydospores of *Phytophora cinnamomi*, for instance (which causes root rot, dieback and cankers in many plant species), after having been fed to two species of Australian forest whistler birds (*Pachycephala pectoralis* and *Pachycephala rufiventris*) in laboratory experiments, were found to be both present and viable in the birds' faeces (Keast and Walsh 1979). The only confirmed study to have been carried out examining the same for *Phytophthora ramorum* was led by scientists at the California Department of Food and Agriculture and the Wilderness Conservation Society. This experiment involved feeding captured Feral Pigeons *P. ramorum* mixed in with their food, with all the faeces then being collected and plated onto culture medium as they were produced over a number of days. At the end of this period the birds were then killed and dissected, and the contents of their crops, stomachs and intestinal tracts likewise cultured. Faeces and crop contents were also examined microscopically. Although control cultures grew well, no evidence of any presence of *P. ramorum* was found in any of the pigeon samples, however the study did involve only ca. three birds (Blomquist *et al.* 2002; Ralph *et al.* 2003; C Blomquist, *pers. comm.*).

Although the study detailed by Parke *et al.* (2008; see 3 above) is one of very few known to have demonstrated that *P. ramorum* inoculum can remain viable following ingestion and excretion by some invertebrates (in this case slugs - *Derocerus reticulatum* and *Ariolimax columbianus*), the findings also indicated that this viability can still be dramatically reduced (by as much as 99%) when compared to the viability of spore cysts not having passed through slugs. Therefore viability of inoculum is only likely to be further decreased in any instances where it, in essence, goes through two digestion processes if ingested by birds via the consumption of invertebrates. There is currently no evidence for *P. ramorum* inoculum remaining viable following ingestion and excretion by birds, however there have as yet been very few studies carried out which examine this viability.

5. GATHERING FIELD EVIDENCE

The studies detailed above are the only ones known to have been carried out on birds regarding dispersal of *P. ramorum*, therefore it is apparent that, as with insects, there is currently little evidence for whether or not avian vectors may be contributing to the spread of the pathogen (also confirmed by S Frankel, *pers. comm.*). However, there exists a potential opportunity for this lack of knowledge to be addressed through taking and analysing samples from birds during ongoing bird ringing efforts in the U.K. By taking foot swabs (to test for possible transmission via soil or debris carried on feet), faecal swabs (for transmission via ingestion and excretion) and/or feather samples (for transmission via carriage on plumage) from birds caught for ringing and then analysing these for *P. ramorum* presence and viability, it may be possible to determine whether wild birds are, indeed, carrying the pathogen and whether such carriage may be contributing to the spread of the disease[1].

The main advantages of such a method of study are that, firstly, samples taken from birds in the field have the potential to demonstrate conclusively the actual carriage and transmission of *P. ramorum* by wild birds (should this be found to occur), whereas any similar findings in laboratory experiments would be restricted to indicating only the potential for birds to act as vectors in a natural setting. Secondly, networks of bird ringing sites run by volunteer ringers are already in place across the U.K. Therefore, by utilising these pre-existing networks in sampling birds for *P. ramorum*, there should be no need for new field stations to be established or for new staff to be recruited to carry out surveys. This would, however, be dependent on existing ringing groups being agreeable to undertaking sampling for the pathogen, and such agreement would first need to be sought in all cases, though it is not thought that this would prove problematic. Ringers willing to participate would then also require instruction prior to undertaking any new sampling method, and would need to be licensed before carrying out feather sampling, and licensed and trained before carrying out faecal swabbing (including in relation to health and safety management whilst taking samples). It is also possible that supplementary sampling by professional staff could be necessary in some instances (see 6 below).

5.1 Sampling strategy

Unfortunately, due to so few studies having currently been conducted which examine birds (or non-human animals in general) as potential vectors of *P. ramorum* (see 4 above), there is also a great deal of uncertainty regarding what the encounter rates for the pathogen are likely to be for the three sampling techniques and for various sample sizes of birds (that is if birds do, indeed, transport inoculum by any of these means). Of the possible means of carriage which sampling efforts should detect, transport of inoculum on the feet of vectors is that which has been most studied (*e.g.* Davidson *et al.* 2005; Webber and Rose 2007; Cushman and Meentemeyer 2008). However, whilst evidence for *P. ramorum* transmission in this manner is strong, there has still been a great deal of variation in encounter rates between studies. Tjosvold *et al.* (2002), for example, found *P. ramorum* in soil on the boots of up to 95% of hikers, whereas Davidson *et al.* (2005) found this figure to be 33% to 50%, and Webber and Rose give the encounter rate as just 3% to 4.5% for both *P. ramorum* and another *Phytophthora* pathogen (*P. kernoviae*) combined. There were some differences in conditions between these studies (and sample sizes ranged from as low as 30 to as high as 400), however this level of variation nonetheless demonstrates the difficulty in predicting the likely frequency of the pathogen's occurrence.

This uncertainty poses a problem in surveying for whether *P. ramorum* is carried by birds; if the number of birds sampled is too small, carriage of the pathogen by a species or population may then be

[1] A study similar to this (to use mist-netting and swabbing to establish a field method of surveying for presence of *P. ramorum* on wild birds, and also to identify the likely species involved, incidence rates and timing) was due to be carried out in the U.S.A., to be led by scientists at the Redwood Sciences Laboratory in Arcata (USDA Forest Service 2002; Ralph *et al.* 2003). However, following preparatory work and the establishment of mist-netting sites in 2002 (Ralph *et al.* 2003), the study was not subsequently completed due to personnel issues (E Jules, *pers. comm.*).

missed by chance rather than because the birds are not vectoring the disease. Taking samples from every single bird caught for ringing in the U.K. would be the best strategy for counteracting the uncertainty, as this would maximise sample size. However, such an approach would clearly be cost prohibitive and also impractical, as over 900,000 birds are ringed each year, and all ringers would then need to be agreeable to taking the samples and would all need to go through a training process for the sampling techniques. Therefore a far more suitable strategy would be to a) take samples only from birds likely to come into contact with spore-producing *P. ramorum* host plants, and b), as short distance spread of the disease is more likely to be explained by abiotic factors (see 3 and 4 above), only take samples from those birds likely to travel significant distances and which may therefore have a role to play in dispersal of the pathogen to new areas. Based on these criteria, candidate species and areas for sampling are discussed under 6 below.

5.2 Sampling techniques

Feather samples can be taken from the contour feathers on the backs of caught birds. These feathers are positioned such that they should receive sufficient exposure to any rain-borne inoculum which the birds may come into contact with, but they are also not crucial to flight, and so loss of a small number through sampling would not be detrimental to the birds. Soil/debris swabs can be taken from the feet of caught birds, and faecal samples from the cloaca.

Acquiring rain-borne *P. ramorum* spores on the feathers is a possibility for any bird which spends some degree of time close to any sporulating host plant. Therefore it would be appropriate to take feather samples from all target species (see 6 below) caught during ringing efforts. Conversely, there is much uncertainty concerning the likelihood of *P. ramorum* presence or viability in the faeces of either insectivorous birds or those which feed mainly on seeds, berries or fruits (see 4 above). Therefore, whilst so little is known about the likelihood of encountering the pathogen in the faeces of any bird species, it would be also appropriate to take faecal samples from all target species caught. Foot swabbing may likewise be appropriate for most species, since the majority of passerines likely to be caught during ringing efforts will spend at least some time on the ground, and so may come into contact with infested soil or leaf litter if they frequent areas with inoculum-producing hosts.

5.3 Analysis of samples

Laboratory analysis would then be required for all samples for the detection of any *P. ramorum* present. There are field diagnostic kits available for confirming the presence or absence of the pathogen in field samples (such as the SPOT√CHECK LF™, for example: http://www.agrifoodtest.nl/files/media/Spotcheck-LF.pdf), however these are designed for identifying the disease in plant tissue and so may not necessarily be easily adapted to 'recognise' the pathogen in the samples taken from birds. The ability to detect the presence of *P. ramorum* in these samples in the field would also be of limited value, since to act as vectors birds not only need to be carrying the pathogen but also need to be doing so in a manner which preserves its ability to go on to infect new host plants. Therefore the samples would need to be analysed for *P. ramorum* viability as well as presence, which can only be achieved in a laboratory.

As a result of this, measures would need to be taken to preserve any viability of the samples during their initial storage and transportation to the laboratory. Studies have indicated that both sporangia and chlamydospores of *P. ramorum* are able to survive well and retain a high percentage of viability at temperatures between 0°C and 20°C. Experiments funded by Defra (2005), for instance, demonstrated ca. 75% to 85% germination of chlamydospores and ca. 95% to 100% germination of sporangia at these temperatures. Consequently there should be no need for samples taken from birds to be kept refrigerated during transport and storage, unless ambient temperatures would be likely to exceed 20°C. Spores of *P. ramorum* are, however, very vulnerable to desiccation, with experiments conducted by Davidson *et al.* (2002) resulting in death of both zoospores and chlamydospores when suspensions were added to dry filter paper and crisped in a dryer at room temperature. Survival was increased by suspending the spores in water, or on moist filter paper. Any samples taken from birds

would therefore need to be kept moist to preserve spore viability, and results from the same study indicate that this could be achieved through storage with moistened filter paper in closed, screw-cap tubes. Swift transfer (*i.e.* preferably within 2-3 days) of the samples to the laboratory for analysis would, however, be desirable to avoid the linear reduction in viability observed by the authors over a 30 day period.

Analysis of the samples would need to be carried out in collaboration with a microbiological laboratory, and laboratory staff would be best informed as to what analytical methods would be most appropriate for testing for presence and viability of *P. ramorum*. However, such analysis could involve plating the samples onto *Phytophthora*-selective media (PAR: pimaricin-ampicillin-rifampicin or PARP: pimaricin-ampicillin-rifampicin-PCNB), culturing for growth, and then examining this microscopically for identification of any *P. ramorum* present. In the case of the feather samples, this would necessitate rinsing the feathers with a small volume of distilled water and then culturing the solution. This technique was used successfully by Parke *et al.* (2008), for instance, in culturing *P. ramorum* from the faeces of slugs which had fed on the pathogen. Alternatively, a Polymerase Chain Reaction (PCR) technique could be employed, as used frequently to diagnose *P. ramorum* in infected plant tissue, but as also suitable for identifying the pathogen in other material (Davidson *et al.* 2003; Ralph *et al.* 2003).

When cultured using PAR/PARP media, a measure of the viability of the *P. ramorum* sample can also be obtained through assessment of the proportion of spores which germinate (*e.g.* as monitored by Parke *et al.* (2008) in *P. ramorum* cultured from slug faeces). However, this constitutes a somewhat artificial measure of spore viability due to the fact that, in order to ensure that any *P. ramorum* present in samples becomes detectable, it is cultured on *Phytophthora*-selective media, which does not represent realistic conditions for growth which the pathogen is likely to encounter when it comes into contact with a host plant. An alternative method of establishing viability could be to introduce the material sampled from birds (*i.e.* faeces, soil or debris from foot swabs, and the solution obtained from rinsing feather samples with distilled water) to wound sites on the leaves, stems or bark of susceptible plants and to then monitor these for signs of infection. This method is far more appropriate when considering the means by which *P. ramorum* may be transmitted in the field, and it has been used by Parke *et al.* (2008) and by Hyder *et al.* (2009) to demonstrate that spores of the pathogen ingested by invertebrates can remain viable in faeces on excretion and can subsequently go on to infect host plant tissue (see 3 above). It is only by establishing both presence and such functional viability of *P. ramorum* in samples taken from wild birds that a role for avian vectors in the spread of the pathogen can be firmly demonstrated.

6. CANDIDATE SPECIES IN THE U.K.

The distribution of *P. ramorum* in the U.K. is at least partly influenced by climate (see 2 above), however this does not preclude birds from potentially being involved in the spread of the pathogen within and between suitable climatic areas. Short distance dispersal of *P. ramorum* could potentially be facilitated by resident bird species which do not move far. However, migrant species (or those that disperse) are more likely candidates for spreading the pathogen to completely new areas through movement along their migration routes.

To identify possible bird candidates for the transmission of *P. ramorum*, we considered those bird species which associate with, or which are likely to come into contact with, the main spore-producing host plant species and which are therefore most likely to pick up *P. ramorum* inoculum. In addition, as sporulation in *P. ramorum*, occurs during periods of high rainfall, and late winter/early spring is thought to be the peak time for spore production in the U.K. (Turner *et al.* 2006), we looked at those bird species that are most likely to come into contact with spores of the pathogen at that time of year. We therefore considered:

- Species of bird which associate with, or which come into contact with, Japanese Larch and/or *Rhododendron* spp. during late winter/early spring;

- Species of bird which, at that time, are migrating or dispersing along a route which completely or partly follows the west coast of Britain.

It currently seems unlikely that transfer of *P. ramorum* by birds would be by ingestion of parts of infected plants, as the tissues involved in the production of inoculum are unlikely to be eaten. Mechanical transfer on feet or plumage therefore seems more likely. However, it is possible that there is transfer via the digestive tract if bird species feed on insects which have, in turn, fed on infected plants. It may also be possible for birds to ingest *P. ramorum* from water bodies, but it is unknown if this is the case.

6.1 Bird species associated with Japanese Larch

Japanese Larch was introduced to Great Britain in 1861 and was found in the 'wild' from 1957 (Preston *et al.* 2002). It is mainly planted as a forestry crop for timber and especially fencing (Preston *et al.* 2002). Japanese Larch and Hybrid Larch (a cross between Japanese Larch and European Larch, *Larix decidua*) have largely replaced European Larch in forestry (Avery and Leslie 1990).

There are generally fewer bird species in coniferous forest compared to broad-leaved forest, although the differences are dependent on the mix of trees present (Fuller 1997). A study in Hamsterley Forest, County Durham (which, at the time, contained both Japanese and European Larch) found nine main species (in order of frequency: Coal Tit *Periparus ater* 32%, Chaffinch *Fringilla coelebs* 17%, Blue Tit *Cyanistes caeruleus* 16%, Goldcrest *Regulus regulus* 12%, Great Tit *Parus major* 6%, Siskin *Carduelis spinus* 4%, Crossbill *Loxia curvirostra* 3%, Treecreeper *Certhia familiaris* 2% and Long-tailed Tit *Aegithalos caudatus* 1%) (Peck 1989). Fuller (1997) gives a longer list of bird species associated with woodland, but few of these are migrants (see Table 1 below). Of those birds particularly associated with coniferous woodland, only Siskin is both widespread and shows widespread movement, with both residents and winter visitors tending to move south in winter (Figures 1 and 2).

Figure 1. Movements of ringed Siskins present in Great Britain and Ireland in the breeding season. Dots show winter position, indicating southward movement in winter. From Wernham *et al.* (2002).

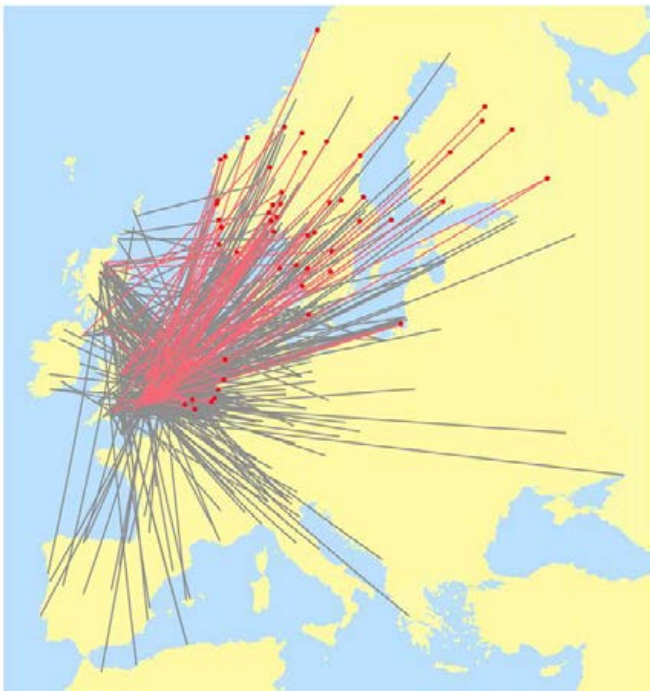

Figure 2. Movements of ringed Siskins between Great Britain and Ireland and abroad, showing the wide range of movements made. Red lines show birds that were abroad in the breeding season, grey lines those abroad in other seasons. From Wernham *et al.* (2002).

Siskins move out of forests by the end of July or early August, returning between February and mid-April (Wernham *et al.* 2002). They could therefore be mechanical vectors of *P. ramorum*, moving the organism on either their feet or plumage. A detailed study of the movements of Siskin based on recoveries of rings could help to understand if this species is likely to be a vector. Sampling of wild birds (feet, plumage and faeces) may also help us to understand if this is the case.

6.2 Bird species associated with *Rhododendron* sp.

Rhododenron ponticum was introduced to cultivation in the U.K. in 1763, with most of the plants being from Spanish stock (Preston *et al.* 2002). It was first found in the wild in 1894 then spread widely in the 20th century and is now widespread and stable (Preston *et al.* 2002). It is widely regarded as a menace (Mabey 1997) as it shades out other ground plants and can prevent tree regeneration (Rackham 2006). In addition, the understorey of rhododendron has few breeding birds, again probably because it is too dense (Fuller 1995). However, species that feed in other areas (Starling *Sturnus vulgaris*, thrushes and finches) use woodland and scrub, including the dense vegetation of rhododendron thickets, as roost sites (Fuller 1995). Birds roost communally overnight outside the breeding season, although some also use roosts in summer (Hill & Cresswell 2010). Thus species that use rhododendron as a roost site are possible vectors for mechanical transfer of *P. ramorum*, either on their feet or plumage.

6.2.1 Starling

Starlings are both resident and winter visitors to Great Britain. The resident population is largely sedentary, although juveniles disperse (Wernham *et al.* 2002). The birds returning to the Continent do so in March and April (Wernham *et al.* 2002).

6.2.2 Thrushes

Thrushes roosting communally include the resident and winter visitor Blackbird (*Turdus merula*), and the winter visitors Fieldfare (*Turdus pilaris*) and Redwing (*Turdus iliacus*) (Cramp 1988).

Blackbirds breeding in the U.K. are largely sedentary. Although migrants from northern breeding areas pass through Great Britain, most are in the east (Figure 3) and there are few reports of ringed birds between the west of Britain and other countries (Wernham *et al.* 2002). Return movements of migrant Blackbirds start in February, but mainly take place in March and early April (Cramp 1988). Blackbirds may therefore be candidates for local transfer, but seem unlikely to be involved in long-distance movement of *P. ramorum*.

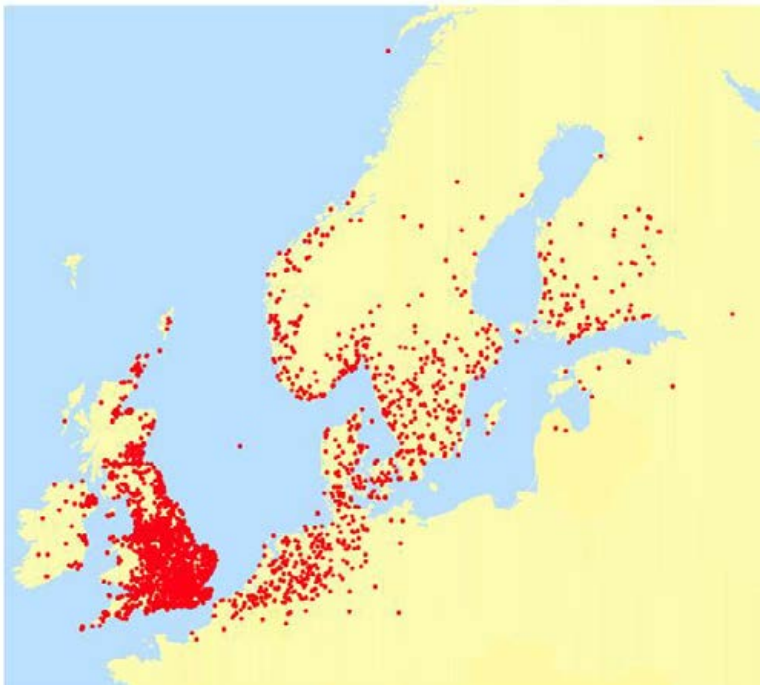

Figure 3. Locations in the breeding season of Blackbirds present in Great Britain and Ireland in winter, showing the presence of migrants that breed to the north. From Wernham *et al.* (2002).

Fieldfares breed to the north of the U.K. and are winter visitors here, arriving in late autumn and winter and departing from late March to early June, initially making local movements before moving north to breed (Cramp 1988).

Redwings are also winter visitors, with spring migration concentrated in April (Wernham *et al*. 2002).

6.2.3 Finches

Chaffinches are resident in the U.K., but the population doubles in winter as visitors move in, mainly from Fennoscandia (Wernham *et al.* 2002). The immigrants are mainly in central Britain and Ireland (Cramp and Perrins 1994) and spring migration takes place in February to May (Cramp and Perrins 1994).

Bramblings (*Fringilla montifringilla*) are winter visitors to the U.K., but winter flocks disperse or return to the Continent by mid April (Wernham *et al.* 2002).

Greenfinches (*Carduelis chloris*) in the U.K. are largely sedentary residents, although there are small numbers of winter visitors. The winter visitors are largely from Norway and tend to be found in the north and east of Britain (Wernham *et al.* 2002).

Winter visitors roosting in rhododendron could potentially be vectors of *P. ramorum*, although those moving to the northeast to breed may migrate across Britain. Detailed study of their movements and sampling of feet, plumage and faeces would potentially help to understand if these species are likely to be vectors of the pathogen.

6.3 West coast migrants

Birds moving north up the west coast of Great Britain in spring could potentially be vectors of *P. ramorum*. However, they would have to be species associated with woodland in order to pick up the pathogen. Many species of birds are associated with woodland (Fuller 1997); the following list (based on Fuller 1997, with migratory status from Wernham *et al.* 2002) details migrants from the south that come to Great Britain and Ireland to breed. These migrants move north along the west coast of Britain in spring, when they would have the greatest chance of collecting spores on their feet or plumage and potentially transporting them to the north.

Species	Migration strategy	Distribution
Garden Warbler (*Sylvia borin*)	Long distance migrant	More abundant in the south than north of Britain, rarer in Ireland
Blackcap (*Sylvia atricapilla*)	Long distance migrant	More abundant in the south than north of Britain, rarer in Ireland
Chiffchaff (*Phylloscopus collybita*)	Long distance migrant	Scarce in northern Britain
Willow Warbler (*Phylloscopus trochilus*)	Long distance migrant	Widespread

Table 1. List of migrants associated with woodland which could potentially move *P. ramorum*. Based on Fuller (1997) and Wernham *et al.* (2002).

More detailed studies of the movements of these species and sampling from them could again help us to understand if they have a role to play in the spread of *P. ramorum.*

7. CONCLUSIONS AND SUGGESTED APPROACH FOR FURTHER WORK

It is apparent that transport by avian vectors is not the sole means by which *Phytophthora ramorum* is transmitted to new host plants or spread to new areas. However, there have been very few studies carried out to examine the possible means by which birds may carry *P. ramorum* spores and there is currently little evidence for whether or not they may be acting as vectors. Therefore the evidence to date does not preclude birds from potentially having a contributing role in the spread of the pathogen.

We have identified a number of species of bird that could potentially move *P. ramorum* north along the west coast of Great Britain. In order to understand if this could be the case, and so to examine whether these species may be contributing to the spread of the disease in the U.K., we suggest two approaches for further work:

i) Sampling candidate species in the west of Great Britain in spring (when birds are migrating and spores are being produced) and then carrying out laboratory analysis of samples for presence and viability of *P. ramorum*. We would suggest that samples are taken from the feet, plumage and faeces of candidate species caught for ringing by the already existing workforce of BTO volunteer ringers. It may also be necessary to supplement this with capture of birds by professionals.

ii) Where there are sufficient data, carrying out detailed analyses of the movements of candidate bird species using already existing BTO datasets on the movements of birds to help to determine if these species are likely to be contributing to the spread of the disease.

It is only by establishing both presence and viability of the pathogen in samples taken from wild birds that a functional role for avian vectors in the spread of *P. ramorum* may be firmly demonstrated.

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