Phytophthora ramorum and *Phytophthora kernoviae*: Key findings from UK research

This is a summary of key research findings up to June 2008. For on-going projects, data should be considered as provisional and may be amended as more information is obtained.

Additional information can be found in:

- Defra final research reports on the Defra website: http://randd.defra.gov.uk/

- Forestry Commission research reports: http://www.forestry.gov.uk/forestry/KIRN-5LDLRQ

- The updated *Phytophthora ramorum* UK datasheet (dated November 2007) and *P. kernoviae* Pest Risk Analysis (dated February 2008) prepared for the Defra and Forestry Commission policy consultation:

  http://www.defra.gov.uk/planth/pra/pram.pdf

  http://www.defra.gov.uk/planth/pra/pker.pdf

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SASA	Over-wintering potential of *P. ramorum* in soil/litter in quarantine contained experiments	**Biology**

1. DEFRA PLANT HEALTH DIVISION FUNDED WORK

Project Short Title	Summary Objectives	Key findings
PH0192: Comparison of *Phytophthora ramorum* populations from Europe & USA **STATUS:** Completed	- Comparison of isolates from US & EU. - Growth rates under different environmental parameters. - Mating systems of the two populations, if they interbreed and, if so, under what conditions.	- EU isolates grew faster than US isolates in most environments tested. No isolates grew at 31°C. EU isolates were significantly more aggressive than US isolates on bark of *Quercus rubra.* - EU isolates were consistently uniform and characteristic of 'wild' type colony morphology, exhibiting only limited variation in growth rate. US isolates were either of a similar wild type or fell into a range of morphologically variable, often slow growing, colonies. They were found to be developmentally unstable and could change from wild type to an unstable non wild type form in culture. These differences may reflect differing origins and/or evolutionary history. - EU isolates drawn from several countries (UK, NL, DE, BE, FR) were all of A1 mating type with the exception of a single isolate from Belgium which was A2. - A method for enhancing mating between A1 and A2 individuals of *P. ramorum* in culture has been developed. This has revealed that successful mating tends to be sporadic, with fewer sexually produced spores (oospores) than is usually seen in A1 x A2 pairings in other *Phytophthora* species. It is still unclear whether the sexual breeding system of *P. ramorum* is functional. However, there remains the possibility that non-sexual recombination could occur between *P. ramorum* isolates from different lineages or populations and this could lead to further adaptive variation. - It is not clear how these two groupings (European and American) came about but probably as a result of two separate introductions from an, as yet, unknown origin/s. Researchers suggest that they should be considered as distinct American and European population types. Furthermore, at some point they may need to be considered as separate subspecies of *P. ramorum.* - Final report available from: http://randd.defra.gov.uk/ - Further up-to-date information on populations and mating type issues can be found in the revised UK datasheet (November 2007): http://www.defra.gov.uk/planth/pra/pram.pdf
PH0193S: Non-tree host range & diagnosis of *P. ramorum* **STATUS:** Completed	- Molecular methods for detection and diagnosis. - Host range studies: potential of US & EU isolates to infect a variety of under-storey shrubs, ornamental shrubs & heathland/moorland plants. - Sporulation potential on heathland hosts.	- A DNA-based detection assay (TaqMan® PCR) was developed and is now used routinely as part of laboratory-based diagnoses. In comparative tests between laboratory isolation methods and direct PCR on plant material, the two methods gave virtually identical results on over 300 samples. - Molecular fingerprinting techniques were developed and used to confirm the origin of a variety of important UK/EU findings, e.g. confirming that the Belgian A2 mating type isolate belonged to the European population (all other European isolates have been A1). Isolates from various new UK hosts, including tree species, were also confirmed as isolates belonging to the European population. - Non-tree host range tests (using wounded leaves and mycelial plugs) predicted various potential ornamental hosts, several of which have subsequently been found as natural hosts, e.g. *Camellia*, *Syringa*, *Leucothoe*, *Pieris, Fraxinus.* - For ornamentals, cherry laurel and Portuguese laurel (often used as replacement planting for removed rhododendrons) were tested using zoospore inoculations, which represent a more natural infection mechanism. Leaves developed lesions, but these expanded relatively slowly and the leaves responded by attempted or successful abscission the lesions. Wounded cherry laurel fruits and

		racemes were also infected. - Important woodland under-storey/hedgerow species which have natural susceptibility potential (zoospore-dipping tests) in laboratory tests included *Rhododendron ponticum,* elder, bilberry, dog rose, ash, honeysuckle and wych elm. The majority of these were predicted as only potential leaf blight hosts as stems were rarely significantly colonised; exceptions included *Rhododendron* and *Vaccinium* that are known or potential dieback hosts, respectively. - For heathland plants, *Vaccinium* species varied in their susceptibility and sporulation potential: bilberry (*V. myrtillus*) was highly susceptible and had a high sporulation potential; cowberry (*V. vitis-idaea*) was much less susceptible and produced very few spores. *Arctostaphylos* (bearberry: now a recorded natural nursery host in the USA) was susceptible when wounded but had a low sporulation potential; *Empetrum* (crowberry) was not infected, even when wounded. *Calluna vulgaris* (wild and ornamental plants) had a significant natural susceptibility potential (and is now a recorded natural nursery host in Europe), though infections rarely progressed from leaf to leaf or through the stem; however, it had a significant sporulation potential. Some *Erica* species had limited susceptibility, primarily when wounded. - Of the heathland hosts, bilberry in particular could also be a significant source of inoculum for susceptible trees where it occurs in woodland. - Final report available from: http://randd.defra.gov.uk/
PH0194: *P. ramorum* epidemiology: sporulation potential, dispersal, infection and survival. **STATUS:** Completed	- Inoculum sources: sporulation potential of susceptible non-tree and tree leaf hosts; survival potential under UK conditions. - Natural infection potential of tree species: infection through unwounded bark, susceptibility of young trees in woodlands/nurseries. - Dispersal potential: airborne & rain splash; longevity of sporangia; pathogen distribution in infected sites; potential dispersal by insect & vertebrate species - Latent periods of symptom development.	- Light, temperature, humidity and nutrient status all appear to be important for sporulation. Decreased nutrient availability and increased light and air flow (reduced humidity) result in increased sporangial production. - There is no relationship between lesion size on different hosts and the number of sporangia produced. - Sporangial production was most abundant on lilac, (exceeding production on all other hosts tested by a factor of at least of 28) followed by Californian bay laurel (*Umbellularia californica*), *Pieris japonica*, *Camellia japonica* and *Rosa canina.* Sporangial production on rhododendron was moderate and was particularly low on wild crab apple, ivy and yew in laboratory tests. - Chlamydospore production was generally less abundant than sporangial production. Chlamydospores (considered to play a survival role) were produced on less than half of the host species with highest levels recorded on lilac, only slightly higher than the number produced on Californian bay laurel. *Viburnum opulus* and ash supported production of relatively moderate numbers of chlamydospores whereas production on *R. ponticum* was low in laboratory tests. - Sporangia and chlamydospores are robust and are able to survive extremes of temperature and pH. No chlamydospores germinated following exposure to 40°C for 24 hours or -25°C for just 4 hours. No sporangia survived a 2-hour exposure to these temperatures. - Sporangia were found to survive up to 6 hours in moisture-free conditions. - Chlamydospores survived the winter season (under quarantine containment) on both lilac and rhododendron leaf material as either surface leaf litter or buried in soil at sites in northern England (Defra CSL studies) and Scotland (SASA studies). In Scotland, the SASA study continued over a second winter and the pathogen successfully survived the two consecutive winters. - Experimentally, no evidence was found for latency in infection: leaf symptoms on most hosts

		appeared after three days under optimum conditions. Symptom expression can be delayed by up to 7 days under cooler conditions; it may be longer under field conditions or on other plant parts. - Susceptibility of three-year-old saplings of tree species varied according to season (summer/winter) and whether the plant was wounded or not. No stem/shoot symptoms developed on any unwounded saplings. - Under summer conditions using wounded saplings, Douglas fir, sweet chestnut, red oak, English oak, Sitka spruce, beech and wild cherry were most susceptible. - Under winter conditions, Douglas fir, sweet chestnut and beech were most susceptible whilst Sitka spruce, horse chestnut and hornbeam did not develop symptoms. - Saplings of eight conifer species were susceptible to *P. ramorum* isolates from both Europe and the USA, with Douglas fir, Sitka spruce and western hemlock the most susceptible species. - Sporangial production on leaves was most abundant on ash and sweet chestnut and least abundant on three oak species. However, the leaves were tested at the time of year when susceptibility to *P. ramorum* was at its lowest. Also, young foliage was likely to be more susceptible to infection. Several other tree species have foliage that was susceptible to colonisation by *P. ramorum* in laboratory tests (e.g. horse chestnut, elm and holly), but these hosts were much less effective at supporting sporulation by *P. ramorum*. - Wounding was not required for infection to take place in tests using mature logs of beech, red oak, sweet chestnut, English oak, Sitka spruce and Douglas Fir (see TL8850a). - Bark thickness and bark resin appeared to play a part in limiting infection or reducing persistence of the pathogen. Necrosis occurred most frequently in the thin-barked species (red oak, beech and sweet chestnut). - Final report available from: http://randd.defra.gov.uk/ - Other tree-related data available at: http://www.forestry.gov.uk/forestry/KIRN-5LDLRQ
PH0195: Epidemiology of natural outbreaks of *Phytophthora ramorum* **STATUS:** Completed (continued under PH0414)	- Monitor disease symptoms & outbreak development at infected sites in England. - Determine natural mechanisms of spread. - Investigate latency or symptomless infections. - Inoculum production: variation with plant species/variety and season. - Longevity of spore survival.	- Three main outbreak sites were studied: 1 south-east site (SE) and 2 south-west (SW) sites; the degree of contamination and inoculum pressure varied between them. - Although the outbreak at the SE site had a significant number of infected plants, the outbreak was well controlled by the eradication action taken. - Complete removal of the infected plants was the most effective eradication strategy. At the SE site, there have been no significant new plant infections since October 2003, though inoculum persists in soil and water. At the SW sites, there have been no new plant infections in the direct vicinity of the eradication action. - Levels of soil contamination were generally low after removal of infected plants and leaf litter. - Pruning (with fungicides applied during the pruning action) did limit disease development but did not impact on contamination levels in leaf litter and soil. - Re-growth of rhododendron is particularly susceptible to infection. Symptoms indicate that infection can occur through: contact within infected soil or stumps; infection of the shoot as it grows through the bark; or contact between plant stems or leaves. - None of the host plants examined in this study showed any evidence of truly latent infection. - *P. ramorum* can survive in leaf debris and soil for at least 3 years in southern England. - Increased persistence of inoculum in soil appeared associated with the presence of roots and

		stumps. Removal of stumps/roots reduced levels of inoculum below detectable levels. - *P. ramorum* was more persistent in soil than *P. kernoviae*. - Watercourses were widely contaminated with *P. ramorum*. Inoculum levels fluctuated with season and environmental conditions, with lowest levels detected in summer. The significance and risk from contaminated watercourses is unknown. - There was no evidence for dispersal of inoculum of *P. ramorum* by either insect or vertebrate vectors (excluding humans). - Inoculum dispersal occurred during rain. Traps showed greatest dispersal occurring as splash-borne inoculum near the ground; much less inoculum was detected 1m above the ground. All spore sampling via volumetric air samplers was negative (see also PH0414 for additional spore trapping results). - Monitoring of inoculum in soil, watercourses and dispersal during rainfall indicated that the peak for inoculum dispersal was during winter/spring and is linked to periods of high rainfall. - Final report available from: http://randd.defra.gov.uk/
PH0308: Management & containment of *P. ramorum* (see also the EU RAPRA Project below). **STATUS:** Completed	- Susceptibility (to both EU and US isolates) of tree & non-tree species. - Research on *P. ramorum* epidemiology to elucidate the risks of spread & establishment in the EU. - Evaluate active ingredients for *P. ramorum* control in ornamentals. - Collate information on epidemiology & control chemicals to develop coordinated management practices. - Contribute to a European Pest Risk Analysis (PRA) for *P. ramorum.* - Provide information to underpin and advise both UK and EU plant health policy. [Match funding for CSL component the EU project RAPRA]	- The RAPRA project website publishes key outputs, including databases for natural and experimental hosts: http://rapra.csl.gov.uk - Only work done by CSL under Defra matched funding is reported here; the EU Pest Risk Analysis is due to be completed by autumn 2008, pending production of Deliverable Reports form Partners. Epidemiology of *Phytophthora ramorum* in relation to risk and policy - Differences in humidity had most effect on sporangial production and zoospore germination whereas sporangial germination was less sensitive to changes in water availability. - Maximum levels of sporulation and zoospore germination occurred at 100% humidity or water potentials of 1. - Temperature optima for sporulation and germination ranged from 20 to 30°C depending on the conditions of the experiment. Management of outbreaks of *P. ramorum* through experimentation: Fungicide tolerance and development of tolerance. - Robust baseline data on the *in vitro* sensitivity of a range of *P. ramorum* isolates to selected fungicide active ingredients was established. - The range of *in vitro* tests illustrated that a range of fungicide modes of action are active against the pathogen. These could be utilised within control programmes to minimise the risk of further insensitivities developing should the restriction on the use of fungicides be lifted. - Of the products tested, metalaxyl-M (as SL 567A) was consistently the most effective active against both mycelial growth and zoospore germination of *P. ramorum*. - Products containing metalaxyl-M showed both protectant and eradicant activity. - None of the fungicides used caused symptoms of phytotoxicity. - A number of isolates have already developed reduced sensitivity to metalaxyl-M. Should fungicides become part of the management strategy they would need to be used within robust treatment programmes, which minimise the risk of developing resistance. Evidence in this project confirms that plant infections caused by these resistant isolates are difficult to control using metalaxyl-M. - Consento could be a key product within spray programmes for both management of the disease

		and minimisation of resistance development. - The most effective fungicides were lethal to the fungus and not fungistatic i.e. they killed the pathogen rather than merely halting it's development. - There was no evidence from this work of fungicides causing latency in plant infections. <u>Pathogenicity studies:</u> - Resistance has already developed to metalaxyl-M in a number of isolates, particularly those originally collected from nursery situations. However, fungicide resistance was not evident in all tests and the effectiveness *in planta* cannot necessarily be predicted from these laboratory tests. - All rare *Rhododendron* species/hybrids tested, were susceptible to *P. ramorum*. It is therefore unadvisable to use these varieties in future planting schemes where *P. ramorum* is known to be present. It would also be prudent to protect the established bushes of these rare plants by removal of inoculum sources of *P. ramorum* from infected sites in the near vicinity. - Root infection studies were done on rhododendron & viburnum using a variety of inoculation approaches. Results suggested that roots are not a primary pathway for infection of above ground parts of these ornamental species, though asymptomatic colonisation of roots was demonstrated. - Report on defra website: http://randd.defra.gov.uk/
PH0310: Development of a PCR assay for *P. kernoviae* and profiling of isolates **STATUS:** Completed	- Develop and validate molecular techniques to identify *P. kernoviae*. - Molecular profiling of isolates collected from the infected areas.	- A PCR assay was developed and implemented for routine laboratory use on cultures and plant material; comparative tests between isolation methods and direct-PCR on plant samples have been completed and showed that direct PCR was comparable to culturing. - The assay has been adapted for use in an on-site PCR system (SmartCycler) and has been deployed when needed. - DNA fingerprinting techniques (AFLP) have shown very little or no genetic variability between isolates from sites in Cornwall, south Wales and from the single nursery finding in NW England. This potentially suggests a single, relatively recent introduction. - *P. kernoviae* is now also known to occur in New Zealand: DNA-based studies indicate that UK isolates appear to have a single base-pair difference in their ITS sequence compared to a single NZ isolate; in DNA-fingerprinting (AFLP) studies using AFLP techniques, the single NZ isolate studied conformed to one of the two UK AFLP types. - Final report available from: http://randd.defra.gov.uk/
PH0312: Plant Health Fellowship: *Phytophthora* species boundaries (TL8851) **STATUS:** Completed (report in preparation)	- Assess the phenotypic, chromosomal & molecular variation in the target *Phytophthora* species of trees. - Assess the extent of genetic barriers to gene flow between the target *Phytophthora* species affecting trees. - Assess the phenotypic and molecular diversity of progeny resulting from experimental inter-specific hybridisation. - Determine the extent to which inter-specific fusions can occur, and their traits, to help assess risks posed by	- The species included in hybridisation studies were those commonly found on trees in Cornwall, e.g. *Phytophthora ramorum*, *P. kernoviae*, *P. ilicis*, *P. cambivora*, *P. cinnamomi*, *P. citricola* and *P. gonapodyides*. - *P. kernoviae* has a haploid chromosome number of 10 and completes a normal meiotic cycle. - The historically invasive species *P. ilicis* has a haploid chromosome number of 14 to 18; this, together with high abortion rates among the sexual spores and other instabilities in culture, suggests a possible historic hybrid origin for the species. - Drug-resistant forms of all the phytophthoras except *P. ilicis* were developed. Protoplasts were generated for each of these drug-resistant forms, and successful intra-specific protoplast fusions were achieved for several of the species combinations. - The inter-specific protoplasts germinated to produce 11 putative hybrid isolates, representing 6 different species combinations. These were analysed to determine if they were 'true hybrids'.

	potential hybrid *Phytophthora* species to native forest systems.	- Phenotypic evidence (gametangial structure, growth rates, colony morphology, temperature tolerance, spore production, oospore viability, etc.) suggests that two are probably true hybrids, differing noticeably from both parental isolates. - Examination with molecular markers (RAPDS, ITS sequences), however, indicates the `putative hybrids are identical to one or other of the parental isolates. Additional marker analysis would be needed to confirm their status.
PH0315: Pre-symptomatic detection of *P. ramorum* **STATUS:** Completed.	- To develop laboratory and on-site capabilities to detect pre-symptomatic infection in plant material. - Detection of inoculum and infection prior to symptom development. - Technology transfer to PHSI.	- Time course experiments have shown that symptoms are observed on leaves of rhododendron, viburnum and camellia within 3 days of inoculation with 500 *Phytophthora ramorum* zoospores. - By comparison, *P. ramorum* is detectable by real-time PCR within 5 hours of inoculation. - A DNA extraction method was developed which allows the detection of one pre-symptomatic inoculated leaf tip in a bulk sample of 300 leaf tips of mixed genera (including *Rhododendron*, *Viburnum*, *Camellia*, *Pieris*, *Skimmia*). - Pre-symptomatic testing has been performed on-site with the Plant Health and Seeds Inspectorate (PHSI), but large bulk samples are more easily tested in the laboratory. - Final report available from: http://randd.defra.gov.uk/ - See also projects below (e.g. EU PORTCHECK project and HSFP-funded Projects) for other SmartCyler® and on-site PCR diagnostic research outputs.
PH0316/408 Conservation of rare plant species threatened by *Phytophthora ramorum* or *P. kernoviae* in SW England. STATUS: PH0408 On-going	- To develop micropropagation techniques for plant taxa threatened by *P. ramorum* and *P. kernoviae.* - To generate pathogen-free material that can be safely released back into gardens.	- Juvenile vegetative growth from ancient rhododendron trees (over 150 years old) was difficult to propagate from due to contamination with micro-organisms. - Rhododendron floral buds could withstand harsher decontamination protocols compared with shoot material; shoot regeneration from rhododendron florets was more prolific and gave a faster rate of development compared to vegetative shoots. - A total of 356 rhododendrons have been received from 17 Cornish gardens and one Devon garden: >70% have been successfully propagated; 50 accessions have been acclimatised to greenhouse conditions in growing media and hardened off; 19 micropropagated, pathogen-free accessions have been returned to their original owners. - 27 magnolia species and cultivars have been processed from young vegetative shoots; 6 have been successfully decontaminated and are proliferating. - 20 other potentially threatened plant species have been received for potential micropropagation. - Report available for PH0316: http://randd.defra.gov.uk/
PH0317: Detection of *P. ramorum* in watercourses **STATUS:** Completed	-To evaluate detection methods for *P. ramorum* in watercourses. -To carry out a pilot study to determine the robustness of methods. Aim: to assist in the detection of sources of inoculum and new outbreaks in Defra and FC surveys. - To contribute to the development of modelling approaches for *P. ramorum.*	- Preliminary studies comparing detection methods have shown that the three methods tested gave comparable results: rhododendron leaf baits; autoclaved rhododendron leaf baits; and synthetic media baits. - Rhododendron leaf baits have the advantage that they can be pre-prepared. This is currently the preferred method. - The methodology is sensitive to at least 1 sporangium per litre in laboratory experiments. - Bait bags can be stored in the freezer for at least one month prior to use. - Baits can be used up to one week after receipt (stored at room temperature). - Baits are effective in water temperatures between 4^{o}C and 25^{o}C; *P. ramorum* was not detected in water containing sporangia tested at 0^{o}C. - The bait method was evaluated in a pilot study of 10 selected watercourses, at sites with a

		ramorum history, in spring 2005: 41% of baits were positive by direct PCR; flow rate of the water appeared to have a significant effect on detecting *P. ramorum* such that fast or slow flowing waters gave a significantly greater number of positive findings than still water; in any stretch of water of approximately 1000m in length 1 in 3 baits were positive for *P. ramorum*. - A more detailed national study of various catchments and watercourses started in autumn 2005. - Sites and specific bait locations were chosen for the national study and delivered to the appropriate inspector for testing through November 2005. 707 out of 716 baits were returned to CSL. All baits were tested by direct Taqman PCR. - *P. ramorum* was detected from 32 (4.6%) baits. The majority of these findings (66%) were from the site with a previous history of *P. ramorum* and the river catchment area from this site (19%). *P. ramorum* was not detected at the majority of sites tested (5/9 sites) and at a low level at the remaining three sites (19%, 9% and 6% respectively). - *P. rmaorum* was not therefore widely distributed throughout England and Wales based on the sites studied. - Final report available from: http://randd.defra.gov.uk/
PH0318 Eradication strategies for *P. kernoviae* in the natural environments **STATUS:** Completed (continued under PH0414)	-To establish the effectiveness of clearance of infected under-storey plants and associated leaf litter to protect at-risk trees in two small woods in the SW of England. - Complementary laboratory and field investigations into key aspects of *P. kernoviae* epidemiology. - To investigate potential cultural and chemical control strategies to protect at-risk trees.	Pre-eradication and on-going tree studies: - Patterns of *P. kernoviae* infection within the two woods indicated possible spread via air movement or wind-driven rain along natural pathways through the under-storey. - Inoculum was detected in rain traps 1m from the ground but not at 3m (nor in volumetric samplers); inoculum dispersal was relatively localised and associated with rain. - Levels of contamination of leaf litter and mineral soil were highest in areas of highest disease. Highest levels of mineral soil contamination were found around the roots/flares of infected trees. - All infected trees were closely associated with infected rhododendron (typically direct contact) and areas of highest disease pressure within the rhododendron under-storey. - Despite the high disease pressure, no other under-storey species were affected and no foliar symptoms were found on holm oaks in the study woods. Post-eradication studies: - Post-eradication monitoring of inoculum dispersal during rainfall showed no detectable inoculum 1m above ground. - With two exceptions, monitoring of residual contamination in soil in May, September and November 2005 showed no detectable inoculum. - Re-growth from a proportion of rhododendron stumps occurred during spring/summer 2005 and infections by *P. kernoviae* were confirmed on these in September 2005. Further soil contamination occurred as a result of the infections on the re-growth and *P. kernoviae* was detected on the bark and roots of one stump. - Inoculum was consistently detected in rain-traps on the ground, deployed following the detection of infected re-growth, indicating that localised splash dispersal was common between November 2005 and March 2006 (greatest levels recorded in November/December). - Bait plants deployed following eradication did not become infected, indicating that inoculum dispersal was not occurring at levels that could establish new infections on susceptible hosts. - Cankers on 2/3rd of the infected trees increased in size during a 2-year period; lesions increased by an average of 2-3 times. Bark lesions were not associated with wounds. At least beech tree has died at the study site.

		- Two beech trees developed *P. kernoviae* bleeding cankers post-eradication, but were probably already infected prior to the removal of infected rhododendrons although not then showing external symptoms. - There is evidence of lesions caused by *P. kernoviae* on roots of some of the infected trees. - No sporulation was detected on beech bark. *P. kernoviae* was isolated from the xylem of two of the infected beech trees and persisted in tissues even after the bark had been removed for over a year. - There was no evidence of vector transfer of inoculum by rabbits or badgers; however, inoculum could be detected on the boots (prior to disinfection) of workers on the site. - Baiting of nearby streams showed no detectable contamination by *P. kernoviae.* <u>Additional experimental work:</u> - Sporulation was greatest on rhododendron, magnolia and camellia, lower on holm oak and lowest on *Michelia* spp. - Quantitative, highly sensitive DNA-based techniques showed that inoculum of *P. kernoviae* was still present in the soil but at very low levels, despite being undetectable by traditional baiting methods. These levels of inoculum are likely to be epidemiologically insignificant with respect to tree bole infections. - Lateral Flow Devices (LFDs) were proven to be robust and effective for pathogen detection on symptomatic field samples. - During laboratory investigations, inoculation of rhododendron leaves with *P. kernoviae* led to the production of oospores; oospores have not been observed in naturally infected tissues to date. - Final report available from: http://randd.defra.gov.uk/
PH0412 Detection and identification of *P. ramorum* and *P. kernoviae* using LFDs **STATUS:** Ongoing until July 2008	- Attempt to develop species-specific antibodies for *Phytophthora ramorum* and *P. kernoviae.* - Develop laboratory-based molecular (DNA-based) methods for identifying *Phytophthora* species from genus-specific LFDs (lateral flow devices).	- This project has resulted in the production of a *Phytophthora* genus-specific MAb (monoclonal antibody) with specificity similar to the current Neogen antibody. Introduction of this antibody into the LFD system gave results similar to the Neogen antibody. Further optimisation and validation of the new genus-specific antibody in the LFD format is underway. A new genus-specific LFD will ensure future supply and reduced costs. - *P. ramorum* specific MAbs could not be produced. The production of a potential *P. kernoviae* specific MAb is still under investigation - DNA from the pathogens within LFD devices could be used as a template in a real-time PCR reaction to give specific detection of *P. ramorum* or *P. kernoviae* from a positive genus-specific LFD. Further work will be performed on the stability of the DNA present within the LFD membrane to investigate if the positive devices could be returned to the laboratory for species discrimination using either real-time PCR (TaqMan) or DNA sequencing. This could potentially be used as an alternative to returning leaf samples for DNA extraction, potentially speeding up testing and reducing sampling errors. In addition it could form the basis of an extraction system for more simplified/specific DNA-based tests in the field.
PH0414 Post-eradication strategies for managing contaminated	- Investigate sources and levels of residual inoculum at outbreak sites (one in the SE; several in the SW). - Examine selected control strategies for persistent inoculum at nursery and	- A method has been developed to extract and quantify DNA of *P. ramorum* and *P. kernoviae* from soil and water samples. This method is more sensitive than normal bait testing but does not indicate if the pathogens are viable. <u>Monitoring at the SE outbreak site (started in PH0195):</u> - *P. ramorum* was isolated from 15% of the baits deployed in autumn 2006 every 10m along a stream

substrates **STATUS:** Completed (Final report in preparation)	environmental outbreaks. - Monitor the sporulation potential and spatial and temporal aspects of infection development on rhododendrons infected with *P. ramorum* and *P. kernoviae* and compare and contrast seasonal effects on disease development and spread.	(c.f. 64% in summer 2006); most positive baits were in one area of the stream. Inoculum levels were unusually high during the summer (sampling was done following thunderstorms after prolonged dry weather) whereas levels in the autumn were relatively low compared to previous seasons (sampling done after prolonged heavy rainfall). Quantitative PCR tests directly on water samples from 3 areas with positive baits indicated that levels of inoculum were very low. - A consistently contaminated gravel pathway near a previous infection (see PH0195) was used to test a 1:20 dilution of Panacide M) for control of residual contamination. Samples were taken prior to treatment and 24 hours and two months post-treatment. All pre-treatment samples were positive for *P. ramorum* by baiting and PCR. PCR tests on post-treatment samples showed that pathogen DNA was still detectable (but possibly non-viable) and bait tests indicated no viable inoculum. Samples taken after two months showed that inoculum levels had returned to those detected prior to disinfection. - Locations monitored at this site (see PH0195) were revisited and soil samples tested by baiting and quantitative PCR. Of the 19 samples, none were positive using the bait test and only one was positive by PCR. The previous eradication measures have therefore been very successful. <u>Monitoring at SW sites - Site 1:</u> - Monthly monitoring at one Cornish garden of rain traps placed on the ground (low level) and 1m above ground (high level) was done at 5 locations close to infected plants. - Up to December 2006, only *P. kernoviae* (Pk) was detected from high-level rain traps; all traps were negative for both pathogens between July and September. - In December 2006, *P. ramorum* (Pr) was detected for the first time in high level traps at two sites and Pk was detected more frequently than during the summer. - Samples from a high-level rain trap on open ground tested negative for Pr and Pk between June and November 2006 but positive both Pr and Pk in December and Pk only in January 2007; this indicated possible dispersal of Pr/Pk spores at this site over a distance of at least 50m. - Low-level rain traps have detected both Pr and Pk more consistently in the autumn and winter than in summer. <u>Monitoring at SW sites - Sites 2 & 3:</u> - Monthly monitoring of two woodlands (see PH0318), where Pk-infected rhododendron has been cleared, was continued. - No inoculum of Pk was detected in low-level rain traps between May and August, whereas 10% and 60% of traps were positive in late September and October respectively. - Soil samples were tested by quantitative PCR analysis. Larger levels of DNA (and therefore potentially inoculum) were detected where disease levels were highest before eradication measures were taken and where re-growth had become infected. - Measurement of the spatial distribution of infections by Pk on a diseased rhododendron in a nearby wood in October showed increased disease development since June. <u>Monitoring of residual inoculum sources at selected nurseries:</u> - Three nurseries were visited in Yorkshire, Lancashire and Cornwall respectively in 2007. In Lancashire, there had been no outbreaks since August 2004 but *P. ramorum* was detected in soil at three locations (1 by baiting; 2 by PCR). In Yorkshire, there had been no outbreaks since March

		2006 but *Pr* was detected in soil at two locations where susceptible plants were no longer located. In Cornwall, *Pr* was detected in gravel at one location. Latest annual report on defra website: http://randd.defra.gov.uk/

2. SMALL PROJECTS FUNDED UNDER THE DEFRA HORIZON SCANNING AND FUTURE PROOFING PROGRAMME AT CSL (2003–7)

Project Title	Summary Objectives	Key findings
HSFP1 Measuring the value of trees at risk from *P. ramorum* **STATUS:** Completed	- To develop experience in using Contingent Valuation (CV) methodology. Preparation for larger scale CV studies. - Estimate the public's economic value of tree species susceptible to *P. ramorum*. Publish the results in a peer-reviewed journal. - Establish CSL capability for valuing environmental properties using CV.	- The average person in the study was willing to pay £55 over a five year period to protect trees at risk.
HSFP2 Validation (in the USA) of the Smartcycler for in-field diagnosis **STATUS:** Completed	- Trial on-site DNA extraction method on host species not readily available in the UK. - Test TaqMan assay against naturally infected host material infected with American isolates of *P. ramorum,* not present in Europe. - Enhance relations with USDA personnel helping to integrate & promote UK test methods with methods already established in the US.	- This built on the previous Forestry Commission funded project which developed a *P. ramorum* Smartcycler assay. - Robust DNA extraction methods and field-stable reagents have been developed and evaluated on-site in the UK and USA for non-woody host tissue. - On-site detection has proven as effective as laboratory-based PCR diagnoses. - PHSI have since been trained in the use of the Smartcycler and have deployed it when required at outbreak sites for both *P. ramorum* and *P. kernoviae* - see also PH0315.
HSFP3 Development of a Lateral Flow device for *P. ramorum* **STATUS:** Completed	- Develop a LFD for the rapid field detection of *Phytophthora* spp., using antibodies in the Neogen ALERT and AGRI-TEST ELISA kits. - With the assistance of PHSI evaluate the suitability of a *Phytophthora* LFD for on-site use.	- A lateral flow device for *Phytophthora* spp. has been developed which enables field staff to conduct an initial screen on material showing suspect symptoms. - Both *P. ramorum* and *P. kernoviae* are detected; the LFDs are not species specific but detect all phytophthoras. - There was 84.5% agreement with samples evaluated in 2003-5 (634 samples checked by LFD and by isolation). Sensitivity was 87.6%; specificity was 82.9%. - The LFD's are being used as an aid to in-field detection and sampling by Defra and FC inspectors. - See also PH0412 for subsequent work.
HSFP4 DNA extraction methods for	- Extend capability to extract nucleic acid from various tree tissues (e.g. conifer needles, bark, woody twigs and	- A comparison of a large range of real-time PCR reagents from different manufacturers identified suitable alternatives to the reagents already in use for routine *P. ramorum* testing, which gave equivalent performance together with a reduction in cost of up to 50%.

molecular diagnosis of *P. ramorum* & other quarantine fungal pathogens of woody hosts **STATUS:** Completed	outer sapwood) to aid diagnosis of quarantine fungal pathogens for either laboratory or on-site use. - Methods to improve the cost effectiveness of using molecular diagnostics (extraction and assay) as a primary screen.	- A semi-automated DNA extraction method was developed which allowed more efficient processing of samples than the existing method in use for routine testing, with a reduction in cost of around 50-60% per sample. -The semi-automated extraction method was found to be effective for the extraction of DNA from various tissue types including leaves, conifer needles & bark. However, successful extraction from bark material was dependant on the sample's condition. - *P. kernoviae* also was identified by real-time PCR testing of extracts of *Liriodendron tulipifera* and *Fagus sylvatica* prepared using the extraction method developed.
HSFP5 Microsatellite analysis of *P. ramorum* genetic diversity **STATUS:** Completed	- Analyse genetic diversity of UK isolates to investigate patterns of spread and identify if the pathogen is evolving.	- 118 *P. ramorum i*solates, from a large range of outbreaks in both nurseries and gardens/woodlands, were analysed. - Microsatellite analysis detected genetic variation in the UK *P. ramorum* population. However, the vast majority of UK isolates (92%) were of a single genotype. A low level of six other genotypes was also identified. The identification of three new genotypes in 2007 suggests that the UK *P. ramorum* population may be evolving. - The existence of rare genotypes offers the opportunity to study pathogen dispersal far more closely and to follow pathways of spread.

3. SMALL PROJECTS FUNDED UNDER THE DEFRA PHYTOPHTHORA RAMORUM/KERNOVIAE PROVISION (2005–8)

Project Title	Summary Objectives	Key findings
PROV1 Investigation of Alternative Eradication Control Methods for *P. ramorum* and *P. kernoviae* on/in plants. **STATUS:** Completed	- Determine cardinal and lethal threshold temperatures for growth of *P. ramorum* and *P. kernoviae* propagules. - Determine the effect of pathogen lethal threshold temperatures on the growth of selected host plants. - Examine hot water and dry heat treatments for sanitation and prevention of infections.	- Results indicate that mycelium of *P. ramorum* does not survive at 50°C for more than 15 minutes. - Mycelia of both *P. ramorum and P. kernoviae* were more resistant to dry heat treatment than sporangia; this was reversed when using wet heat treatments. Dry heat: A 30 min. dry heat treatment at 60°C or 60 min. at 52.5° was required for the eradication of *P. ramorum*. A 30 min. dry heat treatment at 60°C or a 120 min. treatment at 50° was required for *P. kernoviae*. Wet heat: This was more effective than dry heat treatment, with successful eradication of both *Phytophthora* species after a 10 min. treatment at 42.5°C. The effective kill temperature could be reduced by using longer exposure times: 60 min at 40°C for *P. ramorum* and 90 min. at 37.5°C for *P. kernoviae*. Whole plants (uninfected): *Camellia*, *Rhododendron* and *Viburnum* plants were subjected to wet heat ranging from 45 - 60 °C. *Viburnum* was the least heat tolerant, a 45°C treatment caused severe damage. *Camellia* and *Rhododendron* showed little or no damage after a 20 min. treatment at 45°C. Plants were killed completely after a 20 minute dry heat treatment at 55°C. Detached leaf assays - wet heat: Wet heat treatment (20 minute at 45°C) was 100% effective at sanitising leaves inoculated with either *P. kernoviae* or *P. ramorum* in detached leaf assays of *Camellia*, *Rhododendron* and *Viburnum*. Report on defra website: http://www.defra.gov.uk/planth/ramorum/phe2122reports.pdf

PROV2 Investigation of dry-heat treatment methods for sanitisation of *P. ramorum* and *P. kernoviae* on/in plants. **STATUS:** Completed.	- Determine lethal threshold dry heat temperatures for growth of *P. ramorum* and *P. kernoviae* in detached leaf assay. - Determine the effect of pathogen lethal threshold temperatures on the growth of selected host plants. - Examine dry heat treatments for sanitation and prevention of infections.	Detached leaf assays – dry heat: - Initial tests were carried out on detached rhododendron, camellia and viburnum leaves to determine kill times for dry heat treatments at temperatures of 37.5, 40, 42.5 or 45°C. Leaves were inoculated with *P. ramorum* and then heat treatments applied according to the following regimes: (a) two hours after inoculation; (b) approx. 12 hours after inoculation; (c) 24 hours after inoculation; and (d) four days after inoculation. - No growth of *P. ramorum* was observed at temperatures of 45, 42.5, 40 and 37.5°C at duration times of 60, 80,100 and 240 min respectively. - No growth of *P. kernoviae* was observed at temperatures of 45, 42.5, 40 and 37.5°C following exposure times of 40, 60 and 100 and 240 min respectively. Whole plants (uninfected) - dry heat: - Whole *Rhododendron*, *Camellia* and *Viburnum* plants have been exposed to the dry heat treatments regimes determined to be most effective for the pre-symptomatic sanitisation of both *P. ramorum* and *P. kernoviae* (240 min at 37.5°C, 120 min at 40 and 42.5°C, and 100 min at 45°C). Plants were not adversely affected by any of the treatments used either immediately post treatment or during subsequent monitoring of the plants – all plants subsequently produced fresh growth and some flowered. Whole plants (infected) – dry heat: - Rhododendron, camellia and viburnum plants infected with *P. ramorum* were treated for 240 min at 37.5°C, 120 min at 40°C and 42.5°C, and 100 min at 45°C. All the heat treatments were ineffective at the temperatures and timings tested. - The test was repeated at 45°C with the treatment time increased to 130 min. The control achieved was much improved compared to the 100 min treatment. However, the treatment still was not 100% effective. - It was concluded that heat treatment was unlikely to find practical application for control of *P. ramorum/P. kernoviae* in plants. Report on defra website: http://www.defra.gov.uk/planth/ramorum/phe2122reports.pdf
PROV3 Susceptibility of heathland species to *P. kernoviae* **STATUS:** Completed	- Determine susceptibility of selected heathland species to *P. kernoviae.*	- Detached leaves of several heathland species, both wounded and unwounded, were inoculated with isolates of *P. kernoviae* and their relative susceptibilities determined. - The two wild *Vaccinium* species tested (*V. myrtillus* and *V. vitis-ideae*) were found to be very susceptible; cranberry was not. - The *Erica* species tested were tolerant with only a slight necrosis on wounded leaves. - *Calluna vulgaris* was only slightly susceptible. - Isolates did not differ in virulence. - In sporulation potential tests, smaller leaved species, e.g. *Empetrum nigrum*, *Erica tetralix* and *Erica cinerea* produced more sporangia per cm^2 on wounded test plants than *Rhododendron catawabiense* or *Umbellularia californica*; *Vaccinium vitis-idaea* and *V. myrtillus* produced less sporangia per cm^2 than any of the other plant species tested. - Report on defra website: http://www.defra.gov.uk/planth/pkernovii5.htm

PROV4 Datamining **STATUS:** On-going	- Formalise and standardise approaches to the analyses of Defra's PHSI DOMERO dataset. - Carry out a scoping study to determine possible analyses. To be considered are: risk factors and drivers; efficiency of phytosanitary measures; spread between nurseries and adjacent environment (and *vice versa*) - Carry out analyses that are possible from the data.	- Comprehensive datasets of all *P. ramorum* and *P. kernoviae* visits (up to end March 2008) were collated and standardised. - Approximately 47% of supplier records can be, and have been, matched to existing clients. Work is ongoing to determine whether the quality of information is sufficient to identify potential trade routes. - The initial scoping study concluded that analyses exploring the effectiveness of phytosanitary action (as 2m destruction & 10m holds) were not possible from the Defra PHSI Domero datasets. - Annual summaries (by financial year) of epidemic history for commercial vs non-commercial premises have been generated. - Effects of environmental variables (rainfall, altitude, distance to nearest watercourse) were examined for outbreaks in the semi-natural environment (SNE) for both *P. ramorum* and *P. kernoviae.* - For *P. ramorum*, there were significant associations with altitude (more findings at 50m and 100m classes than expected from random outbreaks) and for annual rainfall; there was no significant association with nearest distance to watercourses. - For *P. kernoviae*, there was no significant association with altitude or rainfall (though this may reflect the small sample size and the fact that findings are mostly limited to two relatively small geographical areas within the SW). There was no positive association with proximity to watercourses. - Local-area public footpath information was sourced to investigate whether *P. kernoviae* in Cornwall is being inadvertently spread via public access routes. Preliminary analyses found no significant association with *P. kernoviae* sites and public footpaths, as compared to random locations. - Analyses were done to investigate potential associations between outbreaks of *P. ramorum* in the SNE and nursery outbreaks. The data did not indicate that there was currently a high risk of nurseries becoming infected from outbreaks in the SNE. - The currently available data is being used to help identify sites of *V.myrtillus* (bilberry) that are most at risk from infection by *P. kernoviae*.

4. DEFRA AND FORESTRY COMMISSION CO-FUNDED WORK

Project Title	Summary Objectives	Key findings
AGRIFOS Use of Agrifos (experimental permit) to control *P. kernoviae* leaf & bud infections on magnolia. **STATUS:** Completed	- Assess the effect of phosphonate (Agrifos) on Pk over-wintering on infected magnolia buds and shoots. - Monitor the effect of phosphonate on infections of new foliage of mature trees that have been previously naturally infected.	<u>Sapling trials:</u> - Injection of Agrifios (phosphonate) into magnolia stems (under experimental permit) gave a good degree of control of *P. kernoviae* but also resulted in serious phytotoxicity with the rates used; further work is needed to establish optimal doses and timings. - Application of Agrifos via a bark paint gave no control due to lower uptake of the phosphonate. <u>Mature, established plant trial:</u> - At one site, four *P. ramorum*-infected magnolia trees were injected (under experimental permit) with Agrifos in April 2007. All agrifos-treated trees subsequently tested negative post-treatment up to August 2007; one untreated control remained infected, whilst the second did not test positive up to August 2007. All injected trees exhibited initial bleeding from the injection site, but subsequently healed well. - At a second site, two infected trees (1 *Pr* and 1 *Pk*) were injected with Agrifos: control was not

		completely effective, possibly due to the Agrifos not being injected deep enough into the stems; levels of phosphonate detected in leaves were lower than those that at the previous site - Report on Forest Research website: http://www.forestresearch.gov.uk/pdf/P_kernoviae_phosphonate_fungicide_report_2007.pdf/$FILE/P_kernoviae_phosphonate_fungicide_report_2007.pdf
SD0413 Epidemiological modelling for *P. ramorum* **STATUS:** Completed	- Construct a dynamic network model of the HONS trade flows in the UK . - Determine any association between *P. ramorum* spread in the nursery trade and its occurrence in wild areas. - Assess the likely long-term effectiveness of eradication as a control strategy for *P. ramorum* in the UK. - Develop the model framework so that alternative/complementary management measures can be compared for nurseries and wild outbreak areas. - Devise future strategies for management (including eradication) of *P. kernoviae* in the UK.	- There is evidence for a scale-free network which has a lower epidemic threshold than other kinds of networks; but this can result in better opportunities for epidemic control. - The network constructed by connecting affected plant genera, where these were found to be infected by *P. ramorum* at the same site, suggests that there are genera (*Rhododendron, Viburnum*) which could act as super-spreaders and could be targeted for an efficient containment strategy. - There is some limited evidence for spread from nurseries to the wild. - A policy support model has been implemented to simulate disease epidemics when the trade network is superimposed over the semi-natural environment. This model allows the user to control the key epidemiological parameters and manipulate a virtual inspectorate to investigate some simple inspection and control strategies. - Report on defra website: http://randd.defra.gov.uk/

5. FORESTRY COMMISSION FUNDED WORK

Project Title	Summary Objectives	Key findings
FC-CSL1 On-Site PCR Detection of *Phytophthora ramorum* **STATUS:** Completed	- Development of a protocol for use by field mobile CSL or FR staff to detect *P. ramorum* in plant tissues "on-site". (See also HSFP2, PH0315 and EU PORTCHECK projects)	- The PCR diagnostic protocol for *P. ramorum* has been adapted for use in a portable PCR machine (SmartCycler®). - Sensitivity of the portable assay is similar to that of the laboratory based techniques. - It has been successfully used on naturally infected leaf material and on-site in the UK. - Evaluations have also been completed in the field in the USA under a small horizon-scanning project; on-site PCR technology performs as well as laboratory-based PCR.
TL8850A Tree host range and diagnosis of *Phytophthora kernoviae* **STATUS:** Completed	- Host range studies: potential of *P. kernoviae* to infect a variety of broadleaf hosts in comparison to *P. ramorum*. - Susceptibility of mature tree stems, with particular emphasis on beech and oak, to establish the host range and compare this with the host range of *P. ramorum*.	<u>Tests on logs from mature trees:</u> Based on log tests involving wounded bark, tree species could be grouped into the following susceptibility categories: 'more' susceptible; 'less' susceptible; resistant. - For *P. ramorum*, 'more' susceptible species included: *Fagus sylvatica* (beech), *Quercus cerris* (Turkey oak), several American red oak species (e.g. *Q. rubra*), *Acer Pseudoplatanus* (sycamore), several *Nothofagus* species (southern beeches), *Castanea sativa* (sweet chestnut), *P. menzeisii* (Douglas fir) and *Tsuga heterophylla* (western hemlock) and some firs (*Abies* spp.); common oak (*Q .robur*) and sessile oak (*Q. petraea*) were less susceptible.

	- Foliar susceptibility of a range of broadleaf tree species; ability of *P. kernoviae* to persist on colonised foliage and produce inoculum.	- For *P. kernoviae*, the host range is more limited than with *P. ramorum*: *Fagus sylvatica* (beech) and *Nothofagus* species were 'more' susceptible; *P. kernoviae* is slightly more aggressive to beech than *P. ramorum*, but has very little predicted impact on other Fagaceae (e.g. native oaks, red oaks, sweet chestnut). Even species which have succumbed to natural infection by *P. kernoviae*, such as *Q. robur* and *Liriodendron*, apparently have low susceptibility, suggesting that infection occurred under field conditions because of overwhelming inoculum levels. - With *Q. robur* and *Q. petraea*, susceptibility could vary significantly with provenance. - Results on *P. ramorum* log tests with unwounded bark are reported under PH0194: infection could occur without wounding on *F. sylvatica, Q. robur, Q. rubra, C. sativa, P. sitchensis* (sitka spruce), *Ps. mensiesii*, especially on thinner-barked species. <u>Tree sapling tests:</u> see PH0194 - In general, results supported the host susceptibilities found in log tests, with only a few exceptions. - Saplings were only infected by *P. ramorum* when wounded; susceptibility varied with season. - Beech and sweet chestnut were consistently highly susceptible to both *Pr* and *Pk* in wound-inoculation tests; similarly, stems of magnolia had low susceptibility. <u>Tree foliage tests:</u> - For known foliar hosts of *P. ramorum*, foliage of holm oak had comparable susceptibility and sporulation potential to rhododendron; ash was also highly susceptible and a good sporulator; sweet chestnut and Turkey oak were moderately susceptible and moderate sporulators. - For *P. kernoviae*, the foliar host range on trees is much narrower than with *P. ramorum* and largely limited to rhododendron, holm oak and various genera/species within the Magnoliaceae (e.g. *Liriodendron*, *Michelia* and *Magnolia*); *P. kernoviae* sporulated most readily on holm oak and magnolia species. - Reports available from FC website: http://www.forestry.gov.uk/forestry/KIRN-5LDLRQ
TL8850B Studies of *P. ramorum* and *P. kernoviae* at outbreak sites **STATUS:** On-going	- Monitor disease symptom and outbreak development at infected sites. - Confirm infected trees and compile information for management plan. - Mechanisms of spread including human vectoring - Development of stem infections and process of infection.	- By June 2008, 26 trees (all in Cornwall with several exceptions: one beech tree in west Yorkshire; one beech in Staffordshire; plus one red oak in Northern Ireland and another in sussesx) had been found with *P. ramorum* stem infections. - Around 61 trees (mainly beech) have been found with *P. kernoviae* stem infections, plus further trees (holm oak, *Magnolia* and *Michelia*) with foliar infections. In addition to beech with stem infections, other infected tree species have been found: a *Liriodendron* with extensive stem bleeding and infected foliage; two English oaks (*Q. robur)*. *Pieris*, *Gevuina avellana* and *Castenopsis* have also been found with infected foliage, in addition to rhododendron. - All infected trees with either *P. ramorum* or *P. kernoviae* have been in close association with infected rhododendron (never more than 50m away from a source of infection, and most within 0-2m). - Branch points often appear to be natural infection points, apparently because rainwater contaminated with spores gathers in these locations, leading to bark penetration and infection. - There is evidence that *P. ramorum* and *P. kernoviae* infections are not limited to the phloem and cambium, but can be isolated from the xylem and appear to move within the vascular tissue, leading to multiple lesions in the overlying bark. - There is no evidence for sporulation on bark lesions.

		- *P. kernoviae* survived better in naturally infected leaves slightly buried in the litter than those exposed to the air. After 6 months the pathogen was recovered from 8.5% of leaves exposed to the air, and from 55.5% of leaves buried in the litter, but after a year the percent survival was recorded at 10% for air exposed leaves and 18% for litter embedded. Three years after rhododendron removal from Pk infested woodlands, the pathogen can still be detected in the soil and litter layer, albeit at much lower levels than immediately before eradication efforts. After the same time some of the re-growth from cut and herbicide treated rhododendron stumps has also been found to be infected by *P. kernoviae*, indicating the ability for extended persistence on sites after clearance by this pathogen. - Rhododendron seedlings regenerating on Pk infested sites have sometimes been found to yield *P. kernoviae* from their root systems although they remain symptom free. - To examine the potential for infection through intact bark in the field, freshly cut logs of *F. sylvatica, Q. robur,* and *A. pseudoplatanus* were placed under rhododendrons heavy infected with either *P. ramorum* or *P. kernoviae* at two woodland sites. Potential inoculum was assumed to be of zoospore origin. Lesions caused by both *Phytophthora* species were found only on *F. sylvatica* logs only after 6 weeks. By 11 weeks the *P. kernoviae* exposed *F. sylvatica* logs averaged 2.7 lesions per log, mean lesion area about. 23 cm_2. For the *P. ramorum* logs 6.2 lesions per log but mean lesion areas of only about 8 cm_2. Lesions were therefore more numerous with *P. ramorum* but larger with *P. kernoviae*. - Reports available from FC website: http://www.forestry.gov.uk/forestry/KIRN-5LDLRQ
TL8850C Studies on dissemination of *P. ramorum* and *P. kernoviae* at outbreak sites **STATUS:** Completed	- Assess the extent to which these phytophthoras can be moved on footwear by people moving out of heavily infected sites.	- Samples of soil and leaf litter on people's boots (prior to disinfection) have been tested from July 2004 to October 2005 for the presence of phytopthoras. The majority of samples have come from the *P. kernoviae* Management Zone. - *P. kernoviae* was found in 10-15% of samples, but was most abundant in samples taken during June-July and October-November. - Apart from *P. kernoviae* the main *Phytophthora* species detected on footwear is *P. citricola*, and overall about a third of the samples contained at least one species of *Phytophthora.* The study is on going. - Reports available from FC website: http://www.forestry.gov.uk/forestry/KIRN-5LDLRQ
TL8850D Studies on infection of tree saplings exposed to natural sources of inoculum in the field **STATUS:** Completed	- Assess susceptibility of foliar hosts exposed to naturally available inoculum. - Determine periods when tree infection is most likely to occur. - Assess likelihood of tree foliar hosts providing enough inoculum to lead to infection of other plants.	- The foliar hosts *Rhododendron ponticum* and holm oak are infected in significant numbers by *P. ramorum* and may provide inoculum throughout the year, leading to infection of previously healthy tree hosts. - Tree saplings (holm oak, sweet chestnut, ash, Douglas fir, Turkey oak with a rhododendron control) have been exposed to inoculum from naturally infected holm oak and rhododendron. Some trees succumbed to foliar infections within 1-2 weeks of exposure. - The incidence of infection on bait plants was less with *P. ramorum* than with *P. kernoviae*; this may reflect differences in inoculum levels or a greater field fitness for *P. kernoviae*. - Incidence of infection was greatest from the end of June to end of July and in mid-October 2005 for both pathogens, though infection occurred in most months. - Infection of exposed saplings was most frequent near rhododendron and with inoculum production from infected hosts occurring from June through to October.

		- Reports available from FC website: http://www.forestry.gov.uk/forestry/KIRN-5LDLRQ
TL8855 Survey for *P. ramorum* carried out by Technical Support Units (FR) **STATUS:** On-going	- Occurrence of *P. ramorum* and *P. kernoviae* in woodland areas, or areas of high risk (e.g. gardens or semi-natural environments) where one or both pathogens have been found infecting rhododendron and other ornamental plants.	- The survey concentrated on woodlands in areas of high risk, where sources of infection were nearby. The survey included an assessment of trees and the placement of water baits in rivers/streams in the two areas. The baits gave several positives in one area in the SE of England where infected rhododendron had previously been found, but all were negative in the New Forest area. - Bark samples were taken from more than 40 trees with bleeding lesions during the survey. A further 18 trees were sampled at the *P. kernoviae* / *P. ramorum* outbreak in S. Wales but all were negative. However, a pattern is emerging of other *Phytophthora* species associated with certain tree species.

6. EU FUNDED PROJECTS CO-FUNDED BY DEFRA PHD AND FORESTRY COMMISSION

Project Title	Summary Objectives	Key findings
RAPRA: Pest Risk analysis (PRA) for *P. ramorum* Matched funding from Defra (See PH0308) and FC **STATUS:** On-going until Autumn 2008	- Distribution, host range, mating types, population structure in Europe. - Susceptibility of important host species. - Disease epidemiology, potential for mating between *P. ramorum* isolates of the European and American populations. - Socio-economic & environmental impact. - Risk management through evaluation of control options. - Development of harmonised risk management strategies and contingency plans for Europe. - Development of a European PRA.	- The RAPRA project website has been launched. Development of the natural hosts database is progressing well and can be accessed from http://rapra.csl.gov.uk - See RAPRA website for project progress and key findings under PH0308 and FR projects (above) for specific UK outputs. - Work on the new EU PRA has commenced as well as work on contingency planning and socio-economic impact. These are scheduled for delivery in autumn 2008, pending production of the Project's Deliverable Reports by the various European partners. Report of Defra (CSL) experimental work within the RAPRA project available from website under project PH0308: http://randd.defra.gov.uk/
PORTCHECK: Development of generic 'on site' molecular diagnostics for EU quarantine pests and pathogens **STATUS:** Completed	- To develop generic molecular diagnostic methods (including sampling, extraction and detection) for pests/pathogens of quarantine importance (including *P. ramorum*) that could be used by authorities implementing Council Directive 2000/29/EC within the EU for detection on site and at ports of entry.	- Methods have been developed to allow trained laboratory staff to test for *P. ramorum* at outbreak sites using real-time PCR. - The reagents are stable at room temperature and are not hazardous. Results obtained in the field, whilst working alongside the PHSI, have been comparable with the best laboratory methods. - The project has worked on converting this method into a kit form that could be performed by inspection services. - A kit was ring-tested across European diagnostic laboratories in Autumn 2006.

7. HORTICULTURAL DEVELOPMENT COUNCIL (HDC) FUNDED WORK

Project Title	Summary Objectives	Key findings
HNS 123 Control of *P. ramorum* in nursery stock (COPRINS) **STATUS:** Completed	- Chemical efficacy: fungitoxicity or suppressant activity against *P. ramorum*. - Decontamination of standing areas using chemical drenches. - Produce Code of Practice for the control and spread of the disease	- SL 567A (Metalaxyl-M) was the most effective fungicide, showing both protectant and eradicant activity against *P. ramorum*. However, use of this fungicide as a single active ingredient could not be recommended due to the significant risk of the rapid development of resistance. - Co-formulations and mixtures of metalaxyl-M with other effective active ingredients need to be investigated to develop a protocol for durable fungicidal control of *P. ramorum*. Possible mixtures could include other products found to be effective in this study, including Amistar (Azoxystrobin) and Sonata (Fenamidone + Mancozeb). - There were significant differences in efficacy of fungicides on different host plant species. - Panacide M at a dilution of 1L/60L or Jeyes Fluid at 30 mL/L were the most effective disinfectants against *P. ramorum*. Antec Farm Fluid S 2% could also be used. However, Jeyes Fluid has recently changed its formulation so research results for this product are no longer valid. Antec Farm Fluid S was withdrawn in September 2006 and any remaining stocks should not be used. Panacide will be withdrawn at the end of 2007. - Code of practice produced (now incorporated into the guidance booklet entitled '*Phytophthora ramorum: A practical guide for the nursery stock & garden centre industry*' http://www.defra.gov.uk/planth/pestnote/2005/pramnurs.pdf
HNS134/PH0320: Decontamination/ Disinfection of HONS nurseries (Joint HDC/Defra PHD project) **STATUS:** Completed	- Validate baiting technique for detection of *Phytophthora* spp. on HONS nurseries. - Evaluate slow sand filtration for the removal of different *Phytophthora* species from nursery water sources. - Test effectiveness of disinfectant treatments for decontamination. - Compare the effectiveness and applicability of refined technologies under commercial conditions.	- Autoclaved rhododendron leaves were confirmed as an effective bait for detecting *Phytophthora* species (*P. ramorum* detected at a level of 0.1 spores/ml). (See also PH0317). This leaf bait method also detected *P. kernoviae* from water, however it was less sensitive than with *P. ramorum.* - Two slow sand filters have been established in quarantine glasshouses. Testing indicated that they were highly effective in removal of both *P. ramorum* and *P. kernoviae* from contaminated water. The efficacy of the filters was reduced when the schmutzdecke, the biologically active layer, was in some way disrupted e.g. pitting of the layer, cleaning of the filter or addition of disinfectant. The activity of the filter was regained once they had re-primed. - Six disinfectants (Jet 5, Menno-Florades, Unifect G, Sodium hypochlorite, Hortisept and 70% IMS) have been evaluated for decontamination of surfaces and media. Initial tests using inoculated cellophane sheets showed IMS was the most effective treatment with no growth of either *P. ramorum* or *P. kernoviae* after a 5 min treatment. Most other disinfectants were effective following a 30 min exposure. Only IMS and Unifect G were effective when infected cellophanes were buried in soil; treatments were effective after 10 and 15 min respectively. No treatment was effective (following a 6h exposure) in soils directly infected with either *P. ramorum* or *P. kernoviae*. IMS and Unifect G both effectively decontaminated Mypex inoculated with either *P. ramorum* or *P. kernoviae* (applied as a soil-based inoculum) after a 5-minute exposure time. Jet 5, sodium hypochlorite and Menno Florades were also effective. However longer exposure times were required (30 min, 60 min and after 20 h respectively). - A seventh product 'Cleankill' (produced by Camlab Ltd) has been tested against *P. ramorum* and *P.*

		kernoviae grown on cellophane sheets. This product was effective against both pathogens after a 5-minute exposure time. Further testing of the product will be carried out to determine whether the presence of organic matter affects the product's efficacy. - Water was also successfully decontaminated of *P. ramorum* using chemical treatments. A 5 min exposure to either Jet 5 or a 10% bleach solution was sufficient to decontaminate water of *P. ramorum*. A range of chlorine dioxide concentrations were used; the 500 and 50 ppm solutions of chlorine dioxide required less than 5 and 60 minutes respectively to decontaminate the water, whereas the 5 ppm solution had not decontaminated the water of *P. ramorum* after the 2h test period. - Report on defra website (*P. ramorum*/*kernoviae* outputs): http://randd.defra.gov.uk/
HNS123a: Chemical control of *P. ramorum in* HONS **STATUS:** Completed	- Test new actives. - Investigate: formulations; wetters, adjuvants & stickers; and application techniques. - Develop robust & durable control strategy.	- A range of additional active ingredients and formulated mixtures have been tested for activity against *P. ramorum*. The fungicides tested included: - cyazofamid (Ranman), - boscalid/pyraclostrobin (Signum), - tolylfluanid (Elvaron Multi), - mancozeb/zoxamide (Electis 75 WG), - fenamidone/propamocarb (Concento), - fluazinam/metalaxyl M (Epok), - mancozeb/metalaxyl M (Fubol Gold), - chlorothalonil/metalaxyl M (Folio Gold), - mancozeb/KIF230 (Cerf303). - A number of fungicides have been shown to be effective when used as a protectant treatment for the control of *P. ramorum* and *P. kernoviae.* These include SL 567A (metalaxyl-M), Epok (fluazinam/metalaxyl-M), Fubol Gold (mancozeb/metalaxyl-M), Folio Gold (chlorothalonil/ metalaxyl M) and Consento (fenamidone/propamocarb hydrochloride). - None of the additional products tested were more effective than metalaxyl-M, whereas the formulated mixtures containing metalaxyl-M were equivalent in effect compared to metalaxyl-M alone. Concento was effective when used as a protectant. - Products containing metalaxyl-M also showed eradicant activity. - None of the fungicides used caused phytotoxicity symptoms in the plant species tested (Rhododendron, Camellia and Viburnum). - As resistance to metalaxyl-M has already been detected in UK isolates of *P. ramorum*, fungicides need to be used within robust treatment programmes, which minimise the risk of resistance development. Evidence in this project confirms that plant infections caused by these resistant isolates are less easily controlled by metalaxyl-M. - Consento could be a key product within spray programmes for both management of the disease and minimisation of resistance development. - Effective fungicides were lethal to the fungus and not fungistatic i.e. they killed the fungus rather than merely halting its development. - There was no evidence from this work of fungicides causing latency in plant infections - The addition of adjuvants, especially 'stickers', to fungicide treatments can enhance levels of control

		of *P. ramorum* on certain leaf types, such as camellia. - An effective soil drench treatment for *P. ramorum* still needs to be identified. SL 567A (metalaxyl-M) was shown to be very effective but cannot be recommended due to the risks of further development of resistance within the pathogen population.

8. SASA (SCIENCE AND ADVICE FOR SCOTTISH AGRICULTURE) WORK

Project Title	Summary Objectives	Key findings
Contained over-wintering experiments to replicate the work conducted at CSL (see PH0194).	To determine over-wintering potential of P. ramorum in soil/litter in quarantine contained experiments.	The pathogen was detected at similar frequencies after over-wintering in 2003/4 at an experimental laboratory site in Edinburgh to frequencies from an Experimental laboratory site in York. It was still detected after over-wintering in the consecutive winter of 2004/5 at the Scottish site.