29 June - 1 July 2004 *P. ramorum* Science Panel Questions

Revised 1 September 2005 to include information from the USFS PSW SOD Science Symposium II (Jan 05) and 2005 APS meeting (Aug 05)

DRAFT NOT FOR DISTRIBUTION

Question	Response	References	Research Underway	Experts
	>>General<<			
1. Who participated in the *P. ramorum* Science Panel held between 29 June 2004 and 1 July 2004?	A contact list of the participants is available and is listed at the end of this document (Appendix 1). This panel consisted of 62 scientists and regulators from across the United States and from Canada and The United Kingdom. The scientists are experts in either *Phytophthora* species in general, or *P. ramorum* specifically, including 29 Federal employees (with the USDA APHIS, ARS and FS), 14 scientists and regulatory officials from State governments, 13 University researchers and 2 industry representatives. This meeting was a follow up to the virtual science panel held in the Fall of 2003 and as a result of the positive finds and trace forwards associated with the large Southern California and Oregon nurseries. Several important *P. ramorum* scientists were not able to attend the meeting. These scientists will be contacted along with the participants to ensure that accurate scientific information is attained about *P. ramorum*.			
2. How will this information be used? Will the scientific community be consulted in program review?	The objective of the Science Panel is to provide relevant and timely scientific information to be synthesized and provided by CPHST to the *P. ramorum* National Program. This information will be utilized to provide needed information on the biology (including basic temperature regimes and host ranges when possible), epidemiology and diagnostics associated with *Phytophthora ramorum*. This information was acquired at the request of Jonathan Jones National Program Manager for the the *P. ramorum* National Program and may be used to			

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	assess the efficacy of the Program and any protocols utilized in operations conducted by the Program. The Program review held at the end of July 2004 will be an amalgamation of science, industry, and regulatory components. Also, input from the scientific community as well as other components of the program will be examined as a whole by the program.			
3. When reconvening the *P ramorum* Science Panel, will scientists and diagnosticians representing the USDA PPQ and ARS and all affected states and provinces be included?	The program will engage scientific experts, diagnosticians, and other subject matter experts from USDA and other organizations, states, and Countries as deemed appropriate, according to the questions to be addressed by the panel. This will capitalize on the in-field experiences of each of the state and university labs that have been engaged in *P. ramorum* testing. If important *P. ramorum* scientists are not able to attend the meeting, these scientists will be contacted to ensure that accurate scientific information is attained about *P. ramorum*.			
>> Biology and Ecology <<				
1. What is the probable spore dispersal distance from an infected plant in a nursery (and in the urban landscape)?	*P. ramorum* spore dispersal has been studied using funnel spore traps (capturing rainwater) in forest settings. Spores were recovered from traps at distances of 0.5, 1.0, and 5.0m and 10m from infected (cankered) oaks, but these traps were located under the cover of bay laurels, *Umbellularia californica*. Based on extent of sporulation in forest systems, it is presumed that the trapped spores are likely to have come mainly from infected bay laurel leaves. Tanoak (*Lithocarpus densoflorus*) branches and	Davidson et al. 2001; Davidson et al. 2002 ; Davidson et al., 2005 Rizzo, et al,	Continues spore collection in forests and open fields (Rizzo, UC Davis) Laboratory and field studies are underway in the UK to determine the potential for aerial dispersal without rain (Inman, CSL)	M. Benson, J. Davidson, M. Garbelotto, N. Grunwald, E. Hanson, S. Jeffers R. Linderman, J. MacDonald J. Ristaino,

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	redwood leaves also support spore production. Spores have been collected up to 5 m away from infected trees in adjacent grasslands and in the crown of an emergent redwood that was 32 m above ground. However, the vast amount of inoculum produced in California forests is from bay laurel trees. Rain splash has been shown to move spores of other *Phytophthora* species more than several meters. Splash dispersal distances are affected by ground cover type and prevailing weather conditions. Fungal spores may be carried by wind-driven rain or become airborne and carried over longer distances. It would be possible to obtain information on other *Phytophthora* species with similar spore characteristics in nursery stock. *P. infestans* is an example of an aerial *Phytophthora* species in which both splash and airborne dispersal of sporangia is common. Both *P. infestans* and *P. ramorum* produce sporangia abundantly on the foliage of some hosts. Airborne dispersal of *P. infestans*, while only detected when very heavily infested fields are present, can be over distances of several km. We cannot exclude the possibility that in a storm or under strong wind conditions, sporangia of *P. ramorum* might be moved long distances (i.e. several km). This will only be detectable if and when *P. ramorum* sporulates very heavily in a nursery/forest environment nearby. Observation and evaluation of the incidence and spread of *P. ramorum* in the Oregon nursery setting indicated that spread in the nursery was plant to plant	2005 Erwin and Ribeiro 1996; Ristaino and Gumpertz 2000 N. Grunwald, ARS Linderman,	An EU Project (RAPRA) will also look at issues of dispersal potential. Due to start Jan 2004 (contact: J. Webber, Forest Research, UK)	D. Rizzo,

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	within blocks of plants, presumably from point sources. Some plants were heavily infected, while others had leaf and new shoot infections, possibly from recent spore dispersal during wind/rain storms. The dispersal methods were similar to earlier observations with *P. syringae* on rhododendrons where sporangia were produced on infected stem and leaf tissue and splashed to adjacent plants, initiating new infections.	ARS		
	Infections progress when conditions are conducive but likely stop when environmental conditions are not. Fallen infected leaves are also a source of splashed inoculum. Infected tissue may remain dormant for a number of weeks (perhaps months) before becoming active again during conducive environmental conditions.	Linderman, ARS		
	Wounded tissue is more susceptible to *P. ramorum*. Freshly pruned branches are at least one order of magnitude more susceptible to infection. Wounds can facilitate infection, although it is unknown how long these wounds will represent enhanced infection courts.	Garbelotto, UC Berkeley		
	In the nursery, dissemination of many *Phytophthora* species occurs via plant material and irrigation water. Propagules are moved within a nursery from a point source to other plants through runoff and recycled irrigation water and can be moved between geographical locations on infested or infected plants.	Fitt et al. 1989 Jeffers, Clemson		
	The 2 m and 10 m zones implemented in the UK are based upon distances related to the movement of			

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	splash dispersed pathogens. It is assumed that *P. ramorum* is primarily dispersed by rain and overhead irrigation splash in nurseries. In general, spores dispersed in splash droplets are deposited within 2 m of the source in still air. With wind speeds of 2-3 m/sec, distances may be increased to 4 m, or up to a maximum of 10 m downwind. However, most spores are deposited within 2 m. *P. ramorum* has been isolated from recirculated water in nurseries which could contribute to disease spread. *P. ramorum* has been recovered from irrigation ponds and infections on landscape plantings linked to the use of contaminated irrigation water. Because *P. ramorum* is a regulated pest, studying spore dispersal in nursery settings is problematic. Currently a group of Federal and University researchers (in the USDA-CSREES W501 group) have proposed research to occur in the regulated area in California. This research proposal involves creating and maintaining a nursery infrastructure far from existing nurseries where disease epidemiology in nursery environments can be studied.	C. Sansford via Eric Allen, Central Sciences Laboratory, UK Werres et al, 1995 UK Plant Health and Seeds Inspectorate USFS PSW 2nd Science Symposium		
2. Are there experimental data which provide the mean and standard deviation for the spore dispersal distance such that a	We have not seen data reported for this on *P. ramorum*, although there are on-going experiments that may shed some light on forest epidemiology of the disease. Research on *P. ramorum* in nurseries	June 2004 Science Panel	Take samples of soil and host plant tissue of trace forward plants in the	

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confidence interval can be calculated?	under eradication is problematic because of the regulatory actions that are required for eradication to occur and there are currently no experimental data from nurseries that could be used to derive this information. A proposed experimental nursery would allow some of these data to be obtained through experimentation.		environment, with attempts to determine the time frame in which the affected plants were planted (Jeffers). This will allow a snapshot in time if infested soil or plants are located nearby.	
3. Within a *P. ramorum* -host genus, what characteristics or mechanisms have shown resistance to *P. ramorum* in cases where a particular species or variety is apparently not susceptible to *P. ramorum* infection?	Mechanisms of resistance or traits linked to resistance to *P. ramorum* have not been reported, though apparent differences in susceptibility within and among both wild and cultivated host species have been noted. Differences in the ability of *P. ramorum* isolates to cause disease (virulence) have also been documented. Published results in susceptibility tests may vary between references (see *Camellia*, *Clematis montana*, *Quercus robor* on CFIA host list). Experimental parameters involved in the methods of inoculation, such as wounding, inoculum level, incubation conditions, and genotype of *P. ramorum* isolate as well as the test plant material, have significant impact on estimating the plant susceptibility. *Lonicera periclymenum* remained unaffected after stem and leaf inoculation, while *Lonicera hispidula* is susceptible (regulated host in USA). Variation exists in susceptibility of laurel tree species. *Laurus nobilis* (Italian laurel) is less susceptible to *P. ramorum* than *Umbellularia*	C.F.I.A. Plant Health Risk Assessment Unit 2003 de Gruyter et al. 2002 Garbelotto, UC Berkeley	Evaluate cultural practices/physiological state of plants relative to susceptibility to *P. ramorum* as well as other *Phytophthora* species that infect rhododendrons. First phase will be N levels in foliage (Linderman). *Vaccinium* germplasm collection is being screened for resistance to *P. ramorum*. (Parke) A soon to be published manuscript by Tooley et al examines the effects of several isolates of	M. Garbelotto, R. Linderman, J. Parke, P. Tooley

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	californica (bay laurel). The ability of *P. ramorum* isolates to cause disease in bay laurels also varies. [Note: In July 2004 *Laurus nobilis* was reported infected with *P. ramorum* and added as an "associated plant" to the "APHIS List of Hosts and Plants Associated with *Phytopthora ramorum*". Coast live oak susceptibility to *P. ramorum* appears to vary between individual trees (resistance appears to be due to multiple genes that are inherited differentially among trees). Increased risk of *P. ramorum* infection in coast live oak has been associated with several host factors that may interact with genetic resistance. Coast live oaks with high water potentials (low water stress), larger stem diameter, greater canopy dominance, and greater bark thickness have an elevated risk of developing *P. ramorum* canker in native stands where the pathogen has become well established. Plant species retain their relative *P. ramorum* host-status throughout the year, however, there is seasonal variability within individual plants. *P. ramorum* sporulates abundantly on bay laurel in California, but not in Oregon. Furthermore there are differences in susceptibility within populations of bay laurels. The genotypes of the two bay laurel populations appear to differ. Also, there are physiological differences in the leaf surfaces of California Bay laurel and Oregon Myrtlewood. The thicker cuticles of Oregon Myrtlewood may reduce the potential for leaf infection	Garbelotto, UC Berkeley Swiecki and Bernhardt 2002abc, 2004 Garbelotto, UC Berkeley Frankel, USFS USFS PSW 2nd Science Symposium	*P. ramorum* on more than 30 Ericaceous plants	
4. Do non-deciduous, broad-	Leaves support the greatest level of sporulation of *P.*	Rizzo, UC	Madrone manuscript	J. Davidson,

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leaved hosts like rhododendron and madrone (*Arbutus menziesii* Pursh) present a significantly higher level of risk of maintaining a *P. ramorum* infection in a forest community or a nursery than deciduous, broad-leaved hosts?	*ramorum*. In California, deciduous hosts leaf out at the end of the rainy period. Therefore, evergreen, broad-leafed hosts may present a stronger means of maintaining levels of inoculum in the forest community. However, inoculum can survive in duff on the forest floor and leaves in contact or near-contact to the ground can become infected from this source. It is difficult to extrapolate from forest observations to the nursery scenario because the dynamics in nurseries are quite different to those in forests, particularly the availability of free water. *Umbellularia californica* (California bay laurel or Oregon myrtlewood) is evergreen and is recognized as a major source of inoculum in California forest systems. Small twigs of tanoak also support abundant sporulation. In laboratory studies, deciduous azaleas were generally more susceptible in detached leaf assay studies than were evergreen azaleas similarly challenged. Experience with *P. syringae* on rhododendrons indicated a high probability of new infections resulting from splash dispersal of spores from detached, infected leaves under plants. Removal of fallen leaves is important in reducing inoculum. The same may be true with *P. ramorum*, only more so, because *P. ramorum* sporulates more. Sporulation on leaves of California bay laurel trees is more abundant than that detected on pacific madrone. Chlamydospores are produced in California bay	Davis Rizzo et, 2005 Garbelotto, UC Berkeley Swiecki and Bernhardt 2002abc Tjosvold et al. 2002c Linderman, ARS	currently in review; shows this species probably not a problem, because the plant tissue dies and doesn't support sporulation for long periods of time. (Rizzo, UC Davis). UK research aims to assess the potential contribution of woodland shrub/leaf hosts to potential tree epidemics in relation to factors such as: disease type (leaf blight vs. dieback, i.e. stem and/or leaf susceptibility); host type (evergreen vs. deciduous); host habit (e.g. proximity of leaves to the ground; apical growth dominant vs. shooting from base ; host susceptibility (degree of colonization and rate of spread; proneness to insect or mechanical	M. Garbelotto D. Rizzo,

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	laurel, madrone, and huckleberry leaves, but are present only on edge of lesions in bay laurel, while they are present throughout the infected madrone leaf tissue. While California bay laurel density and cover has been associated with increased disease risk in coast live oak, density/cover of other host species including madrone and Douglas fir are not associated with increased disease risk. Certain host species (bay, tanoak, pieris, viburnum) support greater proliferation of spores in lab studies than do other hosts (madrone, camellia, evergreen huckleberry).	Garbelotto, UC Berkeley Swiecki and Bernhardt 2002ab Parke et al. 2002d and unpublished	wounding; stomatal densities and presence on upper/lower leaf surfaces, etc); plant associations with potential tree host, and density (CSL).	
5. Are there significant reasons to take different or more stringent regulatory actions on the A1 mating type?	There is concern about entry of the A1 mating type of *P. ramorum* into North America, where previously only the A2 mating type had been detected. The significance of the occurrence of both mating types is that this might lead to sexual recombination (not yet observed in nature), producing phenotypes that may have increased aggressiveness or enhanced virulence. Oospores are produced as a result of mating, and in several *Phytophthora* species. Oospores are long-lived survival structures. However, in the case of *P. infestans*, when the A2 mating type was introduced into the United States and Europe in the 1980s, the more aggressive A2 strains displaced the A1 strains, and there has been limited evidence in nature of sexual recombination in these regions although recombination is known to occur in central Mexico. It is unknown what will happen in the *P. ramorum* scenario. For *P. ramorum*, no differences have been detected	Brasier, 2003 Erwin and Ribeiro 1996	Functionality of the breeding system is being investigated under UK (C. Brasier, Forest Research) and an EU project (PRA: J. Webber, FR, UK). RA Quantify infection and sporulation rates for *P. ramorum* in Oregon (Linderman and Parke). Additional comparative studies on virulence, host range and control of	C. Brasier, H. de Gruyter, N. Grunwald, A. Inman, R. Linderman, J. Parke, J. Webber, S. Werres

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	between the mating types in terms of host responses. That is, thus far all hosts susceptible to A1 have been susceptible to A2 when challenged with the mating type, and vice versa.	USFS PSW 2nd Science Symposium	EU and NA genotypes are on-going (Parke et al.).	
	Phenotype is also significant. European genotypes differ from the North American genotypes as determined by AFLP (define AFLP), and phenotypes differ with respect to their aggressiveness in nursery situations and in laboratory culture (phenotype).	Inman et al. 2002		
	Additional information indicates that the European genotype and the A1 mating types are up to 20 times more aggressive and virulent than North American A2 genotypes/mating types. Furthermore, recent research has found much phenotypic variability in the colony morphology of the North American genotype and the European genotype. European genotype colonies are more uniform in their morphological development and typically grow faster than the North American genotypes. North American genotypes appear to be more variable in culture morphology within and between isolates.	Parke, unpublished data USFS PSW 2nd Science Symposium		
	Fortunately the EU and NA (North American) phenotypes can be distinguished via different AFLP markers = genotype. I.e., AFLP provides a valuable genetic marker set for distinguishing the two 'main' genotypes of *P. ramorum*, EU and NA. But it is the differences in phenotype we need to emphasize regarding international risk issues .	Brasier, 2003		
	In wounded leaf tests using mycelial plugs, the host range of American isolates (3) and European isolates (3) did not differ. Aggressiveness was also similar	Brasier, 2003		

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	though some American isolates produced slightly smaller lesions. However, the number of isolates was small. European isolates are more aggressive on bark than US isolates. Presently, characterization of the genotype and mating type provides important information on the potential source of the infected plant materials. However, states where *P. ramorum* distribution is limited should strongly consider eradication, regardless of mating or genotype. Inoculation studies with both the European (EU, be consistent) and NA isolates of *P. ramorum* indicate the former to be more aggressive. This suggests that the risk of spread is greater in a nursery. Growth rate of the European (EU?) A1 genotype is greater than the NA A2 genotype, and sporulation appears to be more as well. Eradication of the A1 and A2 types should remove the risk of sexual recombination in the field. However, the outcome of having both mating types of *P. ramorum* may be similar to that of *P. infestans* (potato late blight) where European strains dominate NA strains when they both become established in the same location. But genetic recombination cannot be excluded as the worst case scenario, even though to date this has not occurred with *P. infestans.* Tests for pathogenicity of EU vs. NA isolates in UK involved robust tests on inner bark of mature tree stems (i.e. not seedlings) of a susceptible host, *Quercus rubra*. Tests were of 16 and 30 isolates respectively in two experiments (8 reps per isolate).	Brasier et al. 2002; Pogoda and Werres 2002 de Gruyter et al. 2002 Grunwald, ARS Brasier, 2003		

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	On average EU isolates were about 50% more aggressive, but with considerable overlap. Another large test with a slightly different objective now nearing completion. In experiments performed in Holland no differences in aggressiveness between A1 and A2 isolates were detected. Host plants tested were *Quercus rubra*, *Quercus robur*, *Fagus sylvatica*, *Vaccinium.* However, in the experiments only two US-isolates, coded US 04 and US 13 (A-2 mating type) were compared with two European isolates. Other researchers have suggested that at least 12 isolates of each genotype should be used for such comparisons. Data shows that isolates from the wild in North America (NA) and isolates from Europe (EU) represent not only distinct populations, but distinguishable lineages. Multilocus linkage analyses based on our AFLP data confirms the two groups are not and have not recombined for a significant period of time. This isolation is the likely explanation of the significant phenotypic differences between North American and European groups. By using AFLP's, isolates from Oregon (OR) nurseries that were placed into the European lineage, although in their own subclade (fragment, please make a full sentence). It was found that these isolates would be fertile with A2 from the US. They are inter-fertile. Isolates were used from a WA nursery for this test. These isolates belonging to the two different lineages were grown next to each other. Isolates were undoubtedly inter-fertile. Non-	Brasier *et al* 2002 USFS PSW 2nd Science Symposium De Gruyter, Boogert, Van Kuik; Van Leeuwen (PPS-Holland): Garbelotto, UC Berkeley		

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	germinating oospores ranged from 50 to 95%, and number of oospores produced ranged between 4 and 990, with plenty of viable oospores produced in the matings. However, recent work in Europe suggests the mating group system with *P. ramorum* is barely functioning.			
	Within NA isolates, there is a great deal of phenotypic variability (as high as 40X) both among and within the same genotype. Geographically isolated *P. ramorum* having the same AFLP pattern differed in relative virulence. This suggests movement of isolates within an infested area may be problematic.	USFS PSW 2nd Science Symposium Garbelotto, UC Berkeley		
	Virulence of 3 Oregon nursery isolates (EU genotype, A1 mating type) was compared to that of 3 Oregon forest isolates (NA genotype, A2 mating type) on non-wounded intact plants (5 species). On some hosts, e.g. rhododendron, the nursery isolates were more virulent than the forest isolates. Nursery isolates with EU genotype have a faster growth rate and sporulate more abundantly in vitro as compared to NA genotype.	Parke, OSU		
	Still, more research is needed using many isolates of both A1 and A2 to fully understand the differences in the two mating types and genotypes.			
	Currently, *Phytophthora ramorum* is a regulated plant pest in the United States, and while there is concern that the introduction of a new mating type could cause a shift in aggressiveness or virulence of the pathogen, there is currently not enough scientific	P. ramorum		

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	evidence to warrant additional restrictions below the species level.	National Program Staff CPHST A1/A2 analysis		
6. Are there significant differences in susceptibility to infection among *Rhododendron*, *Camellia* and *Viburnum* cultivars?	Field observations and laboratory/greenhouse testing suggest that there are differences in susceptibility of cultivars of various plant species. Detached leaf assays correlate well with field observations, but should be considered as preliminary indicators of *P. ramorum* susceptibility. Presently, host range studies are being performed under greenhouse or growth chamber conditions using intact plants that are not artificially wounded. Lab studies and field observations suggest differences in susceptibility among *Acer, Rhododendron, Vaccinium, Viburnum* species and among *Acer palmatum* cultivars; however, this has not been demonstrated in controlled field, laboratory, or greenhouse experiments involving non-wounded intact plants. There is no *a priori* reason to discount inoculation studies on wounded plants. Wounding due to shearing, pruning, propagation practices, insect damage and mechanical damage happens in nurseries. Some plant species develop ramorum blight without wounding, but other plants require a wound for symptoms to develop. Results from both wounded and non-wounded plants, and from different inoculation methods, provide important information	Linderman, Tooley, Parke, unpublished data Tooley, Shishkoff, ARS Parke et al. 2002b; Parke et al. 2002a; Parke et al. 2002c Tooley, ARS Linderman, ARS	EU project (RAPRA) will investigate susceptibility of species/cultivars of some important ornamental genera, namely: Rhododendron, Viburnum, and Camellia. (CSL)	J. Parke, R. Linderman, S. Tjosvold, P. Tooley

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	on host susceptibility (complete the thought). Differences in the susceptibility of *Vaccinium* species were observed in growth chamber inoculations of non-wounded intact plants *(V. ovatum, V. macrocarpon,* and *V. corymbosum).* Detached leaf studies also indicate differential susceptibility among blueberry cultivars. Deciduous azaleas were more susceptible in detached leaf assay studies than were evergreen azaleas similarly challenged. Differences in Rhododendron cultivars and tree species susceptibility to *P. ramorum* have been demonstrated. There is great variation in susceptibility of *Viburnum* spp. in detached leaf tests in Oregon. Young tissue appears to be more susceptible than older, more mature shoots/leaves for several species. UK research also showed differences in susceptibility of *Viburnum* spp. *V. tinus* had stem and leaf susceptibility (wound tests); *V. davidii* produced slower growing leaf lesions and stem infections did not expand much beyond the wound.	Parke et al., 2003 Tooley, et al, 2004; J. Parke, OSU Tjosvold et al. 2002c Tooley, et al., 2004 Tooley and Parke, unpublished data Tooley, Parke Inman et al. 2002 Central Sciences Laboratory, UK		
7. What is the time/ temperature/ humidity relationship for predicting *P. ramorum* activity? How does this affect development, potential for	The time/temperature/humidity relationship for prediction of *P. ramorum* activity has not been defined. *P. ramorum* incidence is associated with cool temperatures with free moisture being present on leaf surfaces for 9-12 hours. (Lab studies show	Davidson et al. 2002; Maloney et al. 2002b; Tjosvold et al. 2002b; Tjosvold	Research is planned by USDA, ARS, Ft. Detrick, to evaluate conditions required for infection by *P.*	C. Brazier, J. Davidson, N. Grunwald, D. Rizzo, S. Tjosvold

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infection, ability to detect?	sporulation taking at least 24-48 hours of wet conditions; infection may require an additional few hours). Sporulation and ability to isolate *P. ramorum* from soil, leaf litter and plant material are favored by cool, moist conditions. Infection is associated with rain events. Extended periods of fog and high humidity may also be conducive to infection. Field studies indicate that it is more difficult to recover *P. ramorum* from infected plants and infested soil and litter associated with those plants under warm, dry conditions. Furthermore, interaction between requirements of free moisture and temperature need to be considered. The requirement of free moisture for sporulation and infection is a function of temperature. In the survey 2002-2003 in public greens in the Netherlands most infected Rhododendron plants were found, when bushes were situated in moist, shady areas (e.g. under trees). There is a strong relationship between California bay laurel infection, temperature, and presence of water. California bay laurel infection is strongly influenced by temperature. At 29^{o}C almost no infection occured, but at 27 & 12 ^{o}C infection occured (average of 3.5 mm in linear growth). At 18^{o}C lesions averaged 18 mm in linear length. This suggests infections are actually favored by cool to warm temperature and *P. ramorum* does not do well in too cold or too hot climates. Although California bay laurel leaves can be infected by dipping the leaf from between 1 minute and 48 hours). Size of lesion was maximum at 36 hours and	et al. 2002a; Rizzo and Garbelotto 2003 Grunwald, ARS van Leeuwen, Dutch PPS Garbelotto, UC Berkeley Garbelotto, UC	*ramorum* on some ornamental hosts. (Tooley). EU project (RAPRA) will look at temperature/moisture/ RH in relation to germination, sporulation, survival (sporangia/zoospores) for European and American isolates, plus the effect of host. Also pathogen activity will be investigated on garden and nursery sites over time. (CSL)	J. Webber S. Werres

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	significant P=0.001 from that at 6 hours. However, size of lesion at 12, 24 and 36 hours was not different. Size of lesions at 48 hours was actually less than that at 36 hours. These data suggest that when leaves remain wet for at least 12 hours, infection occurs whereas excesssive wetness may actually be detrimental). Areas where leaf wetness is shorter than 6 consecutive hours per day when temperature is between 15 and 21°C are not not likely to support significant foliar infection of California bay laurel.	Berkeley		
	Phytophthora species that attack aerial plant parts cause multi-cyclic disease, in which inoculum levels rapidly increase under suitable environmental conditions. While the availability of free moisture may drive the dynamics in forest settings, moisture is less likely to be limiting in the nursery setting due to irrigation.	Erwin and Ribeiro 1996		
	Studies have report on the growth and survival of *P. ramorum* in culture. The pathogen is reported to have an optimal growth temperature of 20°C, though there is some variation between isolates. Optimal temperature is better characterized as a range, as growth is only slightly less at 15 and 25°C. Minimum temperatures of 2-4°C are generally reported, though these temperatures are not lethal to the pathogen and trace growth at these low temperatures has been reported. Colony growth is inhibited by higher temperatures in the range of 30°C, again with some variation reported among isolates. However, periodic temperatures of 30°C may not be limiting if the pathogen can infect the host during a	Werres et al. 2001; Moralejo and Werres 2002; Rizzo et al. 2002; Browning et al. 2003; UK PRA 2003		

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	cooler period. Both soaking and chilling of material such as leaves or wood may promote recovery from these materials. Active sporulation on infected wood chips left in standing water has been reported. Detached rhododendron leaves that were dried for up to 3 months still produced sporangia upon wetting. *P. ramorum* was successfully baited from bay laurel leaves that had been dried at room temperature over a 2 week period, whereas *P. ramorum* could not be cultured or baited from coast live oak wood chips left at 20-22°C. However *P. ramorum* was successfully cultured, but not baited from wood chips maintained at 12°C, suggesting that sporulation did not occur. Additionally, P. ramorum has been recovered from forest soils after being buried for the summer, but not recovered in the leaf litter after that time. For NA isolates, optimal temperatures ranged from 19 to 24°C. One hour at 55°C, 2 hours at 45°C and 24 hours at 40°C were necessary to arrest growth of *P. ramorum* in culture. Viability of *P. ramorum* in relationship to temperature may change drastically depending on substrate.	Garbelotto, UC Berkeley, Oregon PRA 2003 Davidson and Shaw 2003 Garbelotto, UC Berkeley E. Fitchner, APS meeting Garbelotto, UC Berkeley		
8. How long are the chlamydospores viable? Do Chlamydospores lead to new infections?	This is not known for *P. ramorum*. Ranges reported for other *Phytophthora* spp. vary from 21 days to 6 years, depending on species and storage conditions. Conditions needed to induce and break dormancy are not yet defined for *P. ramorum*. Currently, practical assays are not available to detect dormant chlamydospores in woody plant tissues or to	Erwin and Ribeiro 1996	EU project will investigate chlamydospore survival potential in relation to temperature and substrate (over-wintering in northern	E. Fitchner R. Linderman J. Parke N. Shishkoff

Question	Response	References	Research Underway	Experts
	determine whether non-germinating chlamydospores are viable. Current experimentation is continuing in Beltsville and Oregon. At this time viable chlamydospores have been extracted from potting media in the absence of hosts for more than 8 months. Germination of Chlamydospores decreases with time, but seems to hold at 5-10 percent after 8 months. Experiments are continuing in both locations. New infections have not currently been attributed to chlamydospores, however, recent research in both California and Oregon has shown that chlamydospores can survive in native soils over the dry summers and chlamydospores are capable of germination to sporangia that can lead to infective zoospores. Furthermore, recent evidence suggests that P. ramorum can be detected from asymptomatic roots of Rhododendron up to 6.5 cm from thhe nearest stem lesion	 Shishkoff, unpublished data; Parke, unpublished data USFS PSW 2nd Science Symposium J. Bienapfl, APS, 2005	Europe; over-summering in southern Europe). (CSL) UK studies will also look at over-wintering of chlamydospores in and on soil under containment outside (European isolates only). Also over-wintering as infections / chlamydospores in evergreen leaves or stems (laboratory studies). (CSL)	
9. What environmental constraints would limit *P. ramorum* detection efforts in a nursery setting? Temperature ranges? Humidity?	Survey for *P. ramorum* in nursery stock is largely dependent upon symptom expression, which appears to be strongly influenced by temperature and water management (type of irrigation, drainage, etc.). *P. ramorum* is less likely to be detected in infested forest environment (water, soil, litter) during warm and dry conditions. This is likely the case for nurseries as well; however moisture is less likely to be limiting in the nursery setting.	Werres and Schroder 2003 Davidson et al. 2002; Maloney et al. 2002b; Garbelotto 2003b	We are currently investigating fungicide treatments with nursery crops in relation to infection by *P. ramorum* compared to the other *Phytophthora* spp. that can cause similar disease on rhododendrons.	M. Garbelotto, N. Grunwald, A. Inman, S. Jeffers, J. MacDonald, D. Rizzo, A. Wagner

Question	Response	References	Research Underway	Experts
	The use of systemic fungicides has been shown to suppress symptoms for other *Phytophthora* species, and therefore would likely interfere with detection of the pathogen. However, recent information has determined that if *P. ramorum* lesions are present on infected tissue that *P. ramorum* can be detected by PCR and can be cultured out of the infected tissue. Contact fungicides such as chlorothalonil can prevent infection of plant tissue by *P. ramorum* but do not prevent development of lesions on tissues already infected. Use of metalaxyl & mefenoxam in particular is very effective at preventing detection of *Phytophthora* spp., even when present. The same may be true of phosphorus acid products. Additional information presented at the 2005 APS meeting has demonstrated that plants sprayed with Mefanoxam and an unregistered product from Sipcam Agro can suppress the development of *P. ramorum*-induced symptoms on rhododendron for at least 8 weeks. Also observational data from a large Southern California Nursery that experienced a severe *P. ramorum* infestation found that *P. ramorum* could not be recovered from the soil after 3 weeks of drying.	Erwin and Ribeiro 1996; UK PRA 2003; Werres and Schroder 2003 USFS PSW 2nd Science Symposium Jeffers, Clemson R. Linderman, APS 2005 J. McDonald, UC Davis	(Linderman and Parke) UK is investigating incubation/latent period in relation to host, temperature and fungicide pre-treatment. (CSL) EU project (RAPRA) will investigate incubation/latent period and also potential for latent/cryptic infections. (CSL) A new model nursery is currently in the planning stages in the quarantine area of California. (ARS, APHIS, CSREES, UC)	
10. How long will *P. ramorum* survive in the soil and water?	Laboratory evidence has indicated that chlamydospores can survive in sterile water and on moist filter paper for 30 days (survival determined by germination). Survival of zoospores in sterile water and on moist filter paper for 30 days was also reported, though minimal after a few days. Additionally, experimental data from forest soils	Davidson et al. 2002 USFS PSW 2nd	Dutch PPS is regularly monitoring the survival of *P. ramorum* in soil/litter (on sites where infected Rhododendron	E. Fitchner S. Jeffers, J. McDonald, J. Parke, D. Rizzo N. Shishkoff

Question	Response	References	Research Underway	Experts
	suggests that up to 60% of *P. ramorum* chlamydospores can survive in forest soils through the hot and dry summers of California. Furthermore, *P. ramorum* has been baited from stream water in the eradication zone in Oregon for three years in the absence of host plants. Detection of *P. ramorum* by baiting from CA forest litter, soils, and streams is strongly correlated with the rainy season. However, *P. ramorum* survives year-round in streams. Survival of chlamydospores and conditions for breaking dormancy have not yet been determined. Reliable and rapid assays to characterize the viability of dormant chlamydospores are not available. Data regarding survival of chlamydospores of *P. ramorum* in soil is anecdotal and observational, however more data on soil survival is currently being gathered. In soils kept moist by continual or intermittent moisture (i.e. irrigation or rain on a daily basis where soil moisture is maintained) there may be a chance for *P. ramorum* to be maintained and infect new host plants or infest the potting media in which these plants are contained (data from Parke and Shishkoff both address root infection of Rhododendron). However, *P. ramorum* appears to be quite sensitive to drying. Steve Jeffers has observed that recovery of *P. ramorum* from air-dried soils (a common practice to induce germination of other Phytophthora species chlamydospores and oospores) is reduced when	Science Symposium Davidson et al. 2002; Maloney et al. 2002a; Tjosvold et al. 2002b; Tjosvold et al. 2002a Information from June 2004 Science Panel USFS PSW 2nd SOD Science Symposium Information from June 2004 Science Panel	bushes were previously removed and destroyed). EU project (RAPRA) will investigate some aspects of survival in soil/water, as will UK projects involving site studies. Hansen lab is investigating survival in soil on forest sites in Curry Co., OR. Parke is investigating survival of *P. ramorum* in forest soil and artificial potting mixes in relation to soil matric potential. Englander is studying chlamydospore biology. E. Fitchner is studying chlamydospore biology with D. Rizzo.	

Question	Response	References	Research Underway	Experts
	compared to recovery of *P. ramorum* from the same soil that is not dried. Furthermore, recovery of *P. ramorum* from the soil underneath the camellia liners in the severely infested Southern California nursery was not possible after three weeks of drying (i.e. no watering, as per CDFA referencing Jim MacDonald of UC Davis). In native soils, recovery of *P. ramorum* from known infested areas does not occur during the summer months when drying occurs due to the Mediterranean climate in California woodlands, Davidson et al., however, when *P. ramorum* inoculum was buried under forest soils in California, the organism was recovered after three months of dry weather upon re-wetting.	Davidson et al., 2004 E. Fichner, APS 2005,		
11. Should experimental and/or associated hosts be considered as "regulated hosts?" Is it necessary to complete Koch's postulates before plants species are regulated, or should we regulate any symptomatic plant species from which *P. ramorum* is identified.	A regulated host is a plant from which *P. ramorum* has been isolated from naturally infected material and subsequent to the observation, Koch's Postulates (*senso stricto*) on all regulated hosts are completed. Associated hosts are plant species from which *P. ramorum* has found in association with (usually by PCR) but for which Koch's postulates have not been completed. Experimental or associated hosts should not be considered regulated hosts. However, the use of experimentation to determine those families, genera and species at the greatest risk for developing disease symptoms from *P ramorum* infestation would provide a means to target surveys in nurseries and wildlands with the limited resources currently available. Legal issues notwithstanding, only the plant hosts that have completed Koch's postulates should be	Confirmed Nursery Protocol June 2004 Science Panel	Some testing of host range has continuing, especially in families with multiple hosts on the host and associated plant list (such as Ericaceae, Rosaceae)	R. Linderman, J. Parke, P. Tooley

Question	Response	References	Research Underway	Experts
	considered regulated hosts for this pathogen. (Note: an issue here is whether Koch's postulates are considered *sensu stricto*, i.e., isolate has to be from the host that is being tested. It has been difficult to isolate from some species; also, lack of differentiation between most US isolates makes host of origin less important. A *P. ramorum* isolate from oak that causes disease on another species and can be reisolated should potentially be considered sufficient to prove Koch's postulates.) Many Ericaceous hosts were examined through experimental inoculations and detached leaf assays and found a wide range of symptoms were found to be expressed in the Family.	Tooley, et al., unpublished data Tooley, et al., 2004		
12. What would be the ecological impact of *P. ramorum* becoming established throughout the Pacific Northwest?	Impacts include: ▪ death of select tree species, leading to increased fuel loads and greater susceptibility to/damage from forest fires ▪ increased rates of tree failure in infected oaks, leading to canopy openings and damage to targets below failed branches/trees ▪ changes in species composition (flora and fauna), due to greater impacts on particular species ▪ changes in genetic composition of some plant species/populations if variable levels of resistance are present ▪ changes in stand regeneration patterns as susceptibility differs between species and also between age classes within some species ▪ non-lethal infections likely to act as selective force and may reduce fecundity/regeneration ▪ changes in food webs (trophic cascades	Apigian and Dahlsten 2002; Apigian et al. 2002; Monahan and Koenig 2002; Tietje 2002; Rizzo and Garbelotto 2003; Swiecki and Bernhardt 2003; Zanzot et al., 2002; Zanzot et al., 2003		K. Apigian, J. Davidson, M. Garbelotto, B. Monahan, D. Rizzo, J. Zanzot

Question	Response	References	Research Underway	Experts
	possible) ▪ loss of habitat for wildlife, with potential impacts on endangered species ▪ loss of trees could have a major impact on hydrology, soil erosion, and sedimentation in streams and rivers ▪ potential extinction of endemic species with naturally limited distributions if susceptible ▪ natural selection of individuals within a species with inherent resistance to *P. ramorum*. ▪ Removal of keystone plant species ▪ Evolution of *P. ramorum* population to become specialized on different hosts (i.e. P. r. specialized on *Quercus*, Rhododendron, etc.) ▪ Possibility of adapative radiation of *P. ramorum* (by mutation and/or hybridization) to infect new host species			
	>> Epidemiology <<			
1. Should all plants retain their initial country of origin status regardless of how long they may have been grown in the US or Canada?	Capacity to track the route taken from point of origin through nursery facilities through the wholesale/retail nursery can greatly enhance the ability of regulatory programs to mitigate the risks associated with *P. ramorum* in nursery stock. Should *P. ramorum* enter the nursery stock production systems, tracking will facilitate efforts to understand how and where the organism entered the nursery industry. Source identification will provide valuable information on practices that fail to safeguard the US nursery industry and forests from import/transport of *P. ramorum*. At present, our limited understanding of the epidemiology and etiology of disease caused by *P.*		EU Plant Passport system is underway for many plants. Will allow for tracking plant from seedling/cutting to landscape planting. (S. Hunter, DEFRA, pers. communication)	

Question	Response	References	Research Underway	Experts
	ramorum in nursery crops and forest systems suggests that we should maintain tracking records from point of origin to the end-user. It is unclear how long records and tracking should be maintained. Disease outbreaks in UK nurseries were recently reported in the third growing season after Rhododendrons were planted in a nursery which had no history or known association with *P. ramorum* infestation.			
2. Considering that several nurseries infested with *P. ramorum* (estimated at 1-5.0% infection rate) supplied more than 2 million host plants to 40 states, with positive trace forwards having been detected in 176 nurseries in 21 states, and positive National Survey samples were detected in NJ, MD, CA, GA, SC, LA, and WA, what is the likelihood that the pathogen/disease is widely distributed in the United States (i.e. outside of the nursery environment)?	Much debate was offered on this point. The pathogen was likely to be widely distributed with the nursery stock and could make it into the environment. However, there was doubt that establishment of the pathogen in the environment has occurred at this point. There are many variables including weather patterns, nursery host plant infected, and aggressiveness of the isolate. More basic information on the effects of the eastern climate on this organism is needed. It seems safe to assume that 1% of million plants, namely 10,000 are infected. If a very small percentage of these end up in a landscape with good infection conditions, (assume 1% of these can survive, as a conservative estimate) then *P. ramorum* has a good chance of establishing itself (i.e. about a 100 plants). A new issue of concern is potting media, initially there seemed to be no infection underground, hence not much survival. New data show presence in root, infection via root, and an ultimate systemic infection. It has been demonstrated with Camellia leaf tissue that chlamydospores survive quite well in potting	June 2004 Science Panel Grunwald, ARS Shishkoff, Parke, unpublished data		

Question	Response	References	Research Underway	Experts
	media for at least 240 days. However, in the landscape *P. ramorum* on Camellia does not sporulate abundantly due to leaf abscission. The general impression is that the forest will require a strong source of understory inoculum. Another potential issue is latency; in tan oaks the pathogen can be present for a year (without symptom). More research (including field research in infested areas) is needed to fully understand the importance of *P. ramorum* diseases on the roots of host plants. There is not much evidence of rapid establishment with *Rhododendron* and *Viburnum* sp.; highly infected sites are usually quite restricted (ex.: largest site is a maximum of 20-30 acres with hotspots).	Parke, Linderman		
3. Should all nursery *P. ramorum* finds be tested for mating types and should A1 be handled differently? If yes, why?	The Confirmed Nursery Protocol requires any plant that tests positive for *P. ramorum* and all host plants and associated plants in a contiguous block must be destroyed until a 2 meter break of host material occurs and all host plant and associated plant material within a 10 meter buffer must be held for 90 days. Also, soil, media and water from the destruction block and buffer zone must be tested for the presence of *P. ramorum*, regardless of genotype. However, the A-1 European genotype is more aggressive than the North American A-2 type. For states where *P. ramorum* is under eradication, characterization is not relevant to regulatory action. However, characterization of mating type and genotype helps to understand the disease epidemiology. Examination of host plants that are infected in the landscape will further provide information on the epidemiology of disease and	(Linderman, ARS)		

Question	Response	References	Research Underway	Experts
	determine if spread from the urban/suburban landscape to forests is feasible. It is crucial that we monitor mating type. We need to eradicate A1 even more seriously than we are eradicating A2, because formation of oospores pose both a risk of sexual reproduction and improve survival as they also act as a survival structure (possibly better than chlamydospores). Thus far, genetic recombination of *P. ramorum* in nature has not known to have occurred.	Grunwald, ARS		
4. What single Best Management Practice would provide the most effective means of mitigating or preventing the spread of *P. ramorum* in nursery stock?	There is no single best management practice. A systems approach will be most effective in preventing the spread of *P. ramorum* in nursery stock. Increased understanding of *P. ramorum* biology and disease epidemiology/etiology will improve capacity to implement effective mitigations. At present, elements of regulatory programs might include: ▪ Establish a disease indexing program to identify infected nursery stock and establish a certification system. Restrict movement of nursery stock to plants which are shown to be free of *P. ramorum*. ▪ Cultural practices should be avoided that are conducive to *P. ramorum* infection or that may mask symptom expression of infected plant material – including clean water source, clean pots and potting material, clean parent stock (backed up by testing), clean tools, shoes, gloves, carts, tires, etc., material under pots to reduce splash, and appropriate removal of leaf and twig litter, prohibit use of prophylactic systemic fungicides that might mask infection, nurseries should not be located near natural sources of inoculum. ▪ Insect management for control of pests likely to	Erwin and Ribeiro 1996	We are exploring a "sentinel plant" program using species of *Viburnum* that may be susceptible only to *P. ramorum* and not other *Phytophthora* species. Several *Viburnum* species appear to be candidates for this purpose. We are also checking for root infections that would allow plants with no foliar symptoms to be shipped and thereby disperse the pathogen. Some fungicides may be useful to prevent infection and spread	M. Benson M. Garbelotto S. Jeffers K. Suslow

Question	Response	References	Research Underway	Experts
	cause wound sites that may enhance infection; reduced pruning activities; sanitation of pruning equipment. ▪ Keep stock separated by source and all nursery stock should be identified/labeled which would include origin and history of movement. Documentation should be maintained to allow for trace-forward and trace-back as well as a record of movement within a facility, should infection be detected. ▪ Plants that are pruned should be monitored for recurrence of symptoms that may have been removed during pruning. Leaf and branch clippings should be destroyed by burning or deep burial at a certified landfill. An integrated approach will provide the best management practice, with inspection and testing to avoid introduction of the pathogen and rapid eradication of infested or infected plant materials. Also, the feasibility of the use of fungistatic fungicides [e.g., mefenoxam, metalaxyl, fosetyl-Al, phosphorus acid, etc.] should be examined as these products do not kill the pathogen but do prevent it from being active. Symptom expression is suppressed by systemic fungicide application, although *P. ramorum* survival in the plant is not affected.	Garbelotto, UC Berkeley	within a nursery and not just mask symptoms. Another key point resulting from my work is that the symptoms caused by *P. ramorum* are virtually identical to those caused by other *Phytophthora* species (Linderman et al. 2002), making detection difficult and requiring that any suspicious symptoms should be checked out by PCR or culturing. I have confirmed this on whole plants. (Linderman)	
5. Should prohibition or a post-entry quarantine be applied to all *P. ramorum* hosts coming in from Europe? If so, for how long in each season (spring,	Prohibiting import of commercial nursery stock (hosts) and plant parts potentially infested with *P. ramorum* would reduce the risk of introducing genotypes and mating types not prevalent in the U.S.			

Question	Response	References	Research Underway	Experts
summer, fall, winter)?	Host material is imported to the US in dormant condition and as such bears no leaves from which symptoms could be observed. Offshore safeguarding efforts should require that production sites/nursery stock/floral usage be certified to be free of *P. ramorum*. The risk of introducing *P. ramorum* mating and genotypes from Europe and the UK could also be reduced if effective pre-clearance and post-entry nursery stock programs were implemented. Implementation of such programs is dependent upon validated survey, sampling, and diagnostic techniques. An Emergency Ruling is in place in Oregon requiring all shipments into the state from other states or countries be inspected within 48 hours of arrival. Receiving nurseries must notify the Oregon Dept. of Agriculture of expected shipments. Current protocols in the UK require that plants within 2m of infected plants be destroyed and that all susceptible plants within a 10m radius plus any remaining plants from the same consignment remain free of symptoms for 3 months of active growth (in periods of dormancy the clock stops and resumes when plants begin to grow). Temporal aspects of *P. ramorum* disease incidence in UK nurseries emphasize our lack of understanding of disease dynamics in nurseries.	UK PRA 2003		
6. Could delivery trucks act as a significant pathway for the dispersal of *P. ramorum* into nurseries? Are there other	Trucks are commonly used to deliver a wide variety of products (nursery stock, wood products, etc.). *Phytophthora ramorum* appears to be successfully spread by transporting infested nursery stock via			

Question	Response	References	Research Underway	Experts
environmental factors?	trucks. Since *P. ramorum* can be isolated from soil or plant debris (leaf litter, stems, etc), care should be taken to ensure that trucks are sealed during transport and that all debris is removed and properly disposed of following product delivery to reduce the potential for transport of inoculum to the nurseries or the field. Trucks are also used to transport greenwaste to composting facilities, land fills, and cogeneration plants. Historically, diseases caused by several plant pathogens have been correlated with the release of infested plant material/soil from the cargo areas of trucks. Routes taken for the transport of greenwaste to cogeneration plants in California were not associated with outbreaks of disease associated with *P. ramorum*. However, care should be exercised to ensure that infested debris is not released from trucks.	Judy Pasek, USDA APHIS, PPQ (report)		
7. How should import regulation be changed to prevent the introduction of *P. ramorum* between trading partners?	It has been confirmed that the European genotype and the A1 mating types have been detected in Oregon, Washington, and British Columbia. The origins of these detections in nurseries have not been identified. None of the detections of *P. ramorum* in nursery stock have been traced back to shipments originating in the EU or UK post-implementation of current certification requirements for *P. ramorum* hosts. Detection of *P. ramorum* in >300 UK nurseries and retail operations has been associated with the movement of nursery stock. Implementation of a significant educational program in the UK is anticipated to facilitate the UK eradication effort. (Eradication = destruction of infected plants and all susceptible plants within 2m of infected plants.	UK PRA 2003		

Question	Response	References	Research Underway	Experts
	Movement restrictions are also imposed for at least 3 months on all known susceptible plants within a 10m radius of the infected plants and any remaining plants from the affected lot). The only absolute method to prevent spread is to shut down trade involving *P. ramorum* hosts (nursery stock and plant parts). However, caution should be exercised based on current known host list, since other plant species/cultivars may be susceptible though not yet exposed to the pathogen.			
>> Control/Eradication <<				
1. Is *P. ramorum* a candidate for eradication in WA and OR and BC?	*P. ramorum* should be considered eradicable in WA, OR and BC where known infestations are considered to be of limited distribution.			N. Osterbauer E. Hanson
2. Can *P. ramorum* be eradicated, controlled or managed in nursery, urban, or forest environments? If so how?	The eradication of this pathogen in an isolated landscape planting or nursery would be feasible and possible. Control of the organism would be possible on a wider ranging basis through the judicious use of fungicides and through inoculum reduction in the urban landscape, nursery settings and homeowner environments. Eradication and control/ management of the organism and the disease it causes would be more problematic in the wild, and would be best avoided by management and control in the urban landscape and nursery settings.	June 2004 Science Panel		
3. Under what conditions and parameters can a nursery be considered "free" of *P. ramorum* and should testing include not only plants, but soil and water	Testing should include all potential inoculum sources (plants, soil, water, potting material, and pots, if reused). The source of all plants should be documented. Susceptible host material surrounding nurseries will also need to be surveyed. Bait plants	Science Panel June 2004	We are investigating "sentinel plant concept" involving *Viburnum* species that are especially	

Question	Response	References	Research Underway	Experts
sources as well?	or spore traps between susceptible surrounding vegetation and nursery stock should be considered if methods are developed. Sentinel plants (susceptible hosts) could also be placed outside the nursery operation as a means of detecting *P. ramorum* in the environment. Sources of nursery stock should be documented. Inspections need to be conducted more than once annually and during season when plants are considered to be most susceptible and prior to shipment of material (very close in time to shipment). Inspections should also be conducted at destination. Environs should be inspected for *P. ramorum* where nurseries are located in the vicinity of susceptible host material. Sentinel plants/spore traps could be placed outside the nursery to determine if conditions are conducive to disease establishment.		susceptible to *P. ramorum* for monitoring purposes (Linderman).	
4. If incineration is not an option, is deep burial, e.g. six feet, of double-bagged plant material adequate to fully minimize the risk of *P. ramorum* spread? What about deep burial of residual material that was incinerated, but not at a commercial incinerator?	Incineration is the best method of destroying *P. ramorum*-infested material. If not available, burial of double-bagged nursery stock at depths of 6 feet at certified land fills is considered adequate to minimize the risk of *P. ramorum* spread. Also, steam sterilization is an approved method of plant disposal. The term incineration means that something is burned completely to ashes. Complete destruction of residual material by incineration should be adequate to minimize the risk of *P. ramorum* spread. Provided the infested material was incinerated, it would not be necessary to couple this action with deep burial of the ashes.		We are investigating the use of air-steam to decontaminate containers that might be reused. We will be comparing *P. ramorum* with other *Phytophthora* species such as *P. cinnamomi*, P. *cactorum, P. citricola, P. citrophthora, P. parasitica*, and *P.*	

Question	Response	References	Research Underway	Experts
			syringae. Inoculum will be vermiculite cultures and infected leaves (Linderman).	
5. What is the most effective distance of host removal that would minimize necessity for on-going sampling to verify pest freedom? Would all host removal within 10m of the infection point and testing over 45 days be sufficient or 15m and 30 days? Are these distances affected by the type of cropping practices (in-ground vs. containerized), artificial environment (overhead watering vs. drip irrigation), etc. How? Can a matrix be developed?	Currently, eradication of all plants in a block removed and testing over 90 days. In facilities where plants are grown in-ground, testing of the native soils and growth medium becomes more important and the potential for soil contamination may be greater. The type of irrigation used in a facility can greatly affect the airborne and groundwater spread of the pathogen. The effects of these conditions are currently under investigation. The 90 day monitoring period called for by EU protocols was based on the observation that the latent period (period between infection and disease symptoms) in inoculation trials had not exceeded three months. This was shown to vary among plant species and is significantly influenced by conditions in each nursery. A matrix could be developed when sufficient information on the effects of various artificial environmental conditions is available.	Confirmed Nursery Protocol Netherlands PRA 2002		
6. Could artificial environmental controls be used to speed infection development and reduce quarantine times?	Hypothetically, plant material from nurseries could be placed into chambers where they were exposed to conditions that were conducive to disease development. Conditions that could be considered for such an approach might be those conditions used in pathogenicity studies performed by *P. ramorum* researchers. This approach could reduce the amount of time required for symptoms to develop and thereby reduce the amount of time required to			

Question	Response	References	Research Underway	Experts
	determine that additional crop destruction is in order. Conversely, the amount of time required under specific conditions to demonstrate that nursery stock or trees were not infested is not determined. (Proving the negative).			
7. Overhead watering systems in positive nurseries are an issue. Should they not be used once a *P. ramorum* infection has been detected? Or should they be tested and verified free-from *P. ramorum*?	Two major issues exist for irrigation systems: 1) transmission via water in general and 2) splash dispersal due to overhead watering. If the irrigation water is not free of *P. ramorum* (either contaminated surface water source or recycled), this is a pathway for infection. Testing would have to be repeated periodically. Any water contact between plants (splashing, flood irrigation systems, puddles due to insufficient drainage, etc) is a possible pathway for plant-to-plant spread. In the UK, the pathogen has been found in water samples from irrigation ponds (CSL). Once a *P. ramorum* infection is detected, overhead watering should be discontinued as *P. ramorum* has been shown to be splash dispersed. Also ground cover may be manipulated to minimize splashing (gravel, permeable ground covers not plastic, based on other *Phytophthora* spp.) Weather events such as rain/wind storms that occur during times of infection/sporulation may significantly impact disease spread on a local or regional basis.	Erwin and Ribeiro 1996; Ristaino and Gumpertz 2000	Efficacy of surface disinfestants against a variety of fungi (Copes, USDA ARS, Poplarville, MS).	
8. Is Lysol® (or Clorox®) the preferred disinfectant when conducting nursery surveys, or should we be using antibacterial	Clorox (sodium hypochlorite) is labeled for surface disinfection for plant disease-causing fungi quarantine use (0.85%-1.0% active ingredient). It is also labeled for treatment of water (~50 ppm	EPA Reg. No. 5813-50		

Question	Response	References	Research Underway	Experts
soap, and disposal gloves and shoe covers? What is labeled in each state?	available chlorine) for controlling the spread of Port Orford Cedar Root Disease (*Phytophthora lateralis*) via water used for dust abatement, fire suppression and equipment cleaning.			
	PROFESSIONAL LYSOL ® BRAND disinfectant spray is not EPA registered for surface disinfestations for *Phytophthora*. The spray contains 79% ethanol, and 0.1% phenyl phenol or 0.1% quaternary ammonium and will likely work (especially since many phenols and quaternary ammonium products are labeled for *Phytophthora* spp.). Ethyl alcohol is commonly used as a surface disinfectant for fungi, however, the efficacy of ethyl alcohol alone to disinfest equipment or hands has not been established.	Cooperative Agriculture Pest Survey program 2002		
	Lysol, Clorox and Ethanol has been used to sterilize tools artificially contaminated by dipping tools in Petri dishes rich in sporangia and chlamydospores. Extensive wiping was necessary to eliminate pathogen. Extrapolation would suggest that if soil is attached to tools, elimination of the soil is of primary concern. Using disinfectants will be much less effective than eliminating the soil with brush and/or high pressure sprayer.	Garbelotto, UC Berkeley		
	Physan 20 is registered as a surface disinfectant for *Phytophthora*	EPA Reg. No. 55364-5		
	Zerotol is registered for surface disinfestations.	EPA Reg. No. 70299-1		
	Chlorine levels of 2mg/liter or greater were correlated with control of *Phytophthora* spp. in re-			

Question	Response	References	Research Underway	Experts
	circulated irrigation systems. In the UK, Panacide-M (a.i. 30% sodium dichlorophen and alkali, 2% for at least 10 mins) is used for disinfection of surfaces (hard standing). Antec Farm Fluid S (a.i. acetic acid, dodecyl benzene sulphonic acid and hydroxy hydrindenes, 1.66% for at least 10 mins) is used for disinfection of cleaned tools, footwear.	Hong et al. 2003 Central Sciences Laboratory, UK		
9. Should a different disinfectant be used after handling plants known to be infected with *P. ramorum*, i.e. 3% sodium hypochlorite solution?	No, the strategy for use of a disinfectant is to ensure that surfaces would be rendered free of the pathogen; the same treatment should be used for all materials since you may unknowingly handle *P. ramorum* infested material. Chlorox (sodium hypochlorite) is labeled for surface disinfection for plant disease-causing fungi quarantine use (0.85%-1.0% active ingredient). Also labeled for treatment of water (~50 ppm available chlorine) for controlling the spread of Port Orford Cedar Root Disease (*Phytophthora lateralis*) for water used for dust abatement, fire suppression and equipment cleaning. Treatments reported as effective against other *Phytophthora* species include copper naphthenate for the treatment of wood surfaces, sodium hypochlorite, quaternary ammonium and hydrogen peroxide (Zerotol) for surface disinfestation, and sodium tetrathiocarbonate, methyl bromide and chloropicrin, and metam sodium (Vapam) as soil treatments.	EPA Reg. No. 5813-50 Erwin and Ribeiro 1996		
10. Would propane flaming the soil surface be an adequate treatment of a potentially infested spot	Propane flaming of soil surfaces could effectively destroy all plant debris which may harbor *P. ramorum*; however, surface flaming could not ensure			

Question	Response	References	Research Underway	Experts
where infected nursery stock was located? What other methods are available?	the sterilization of soil. Fumigation (methyl bromide, methyl bromide and chloropicrin, Vapam, and others, see Disinfectants and fumigants on the PPQ *P. ramorum* website) has been used for other *Phytophthora* spp. that cause root disease. However, they have not been evaluated for *P. ramorum*, but would likely be effective.	Erwin and Ribeiro 1996; Menge and Nemec 1997		
11. What is the rationale for assuming that limiting a "destroy-action" to *P. ramorum* symptomatic plants and those immediately adjacent prevents the spread of *P. ramorum* in a nursery situation?	The rationale for limited destroy-action is based upon our generic understanding of diseases caused by other *Phytophthora* species as well as information on *P. ramorum*. The eradication strategy for *P. ramorum* in nurseries is based upon the biology of the pathogen, the cultural practices for the nursery and the presence of hosts. A strategy is in place to remove the block containing symptomatic plants to attempt to eliminate all diseased and exposed plant material. The subsequent 90 day growing period allows detection if additional infected plants are present. This is an eradication strategy that has been used for a number of plant diseases and pests, but it requires a clear understanding of the epidemiology of the disease and nursery production practices and that fungistatic treatments are not used on the plants under observation.			
12. Based on the current understanding of *P. ramorum* biology, is the current regulatory regime sufficient to prevent the spread of *P. ramorum* to uninfected regions?	Federal regulations are under review for the purpose of modification based on the evolving understanding of the biology and epidemiology of diseases caused by *P. ramorum*.			
13. Does the current regulatory	The regulatory regime involves aspects of the nursery			

Question	Response	References	Research Underway	Experts
regime facilitate the eradication of localized *P. ramorum* outbreaks?	protocol and the Oregon program to eradicate *P. ramorum* in natural areas. These strategies appear to be effective in eradicating *P. ramorum* in these areas. APHIS is still gathering data and fine tuning these programs. Concern exists regarding the focus of surveys in diverse plant nurseries or environs, where symptoms may be observed on plant species or cultivars that were not previously known to be a host or associated with *P. ramorum*. This could jeopardize regulatory actions designed to prevent the spread of *P. ramorum* through movement of nursery stock. Furthermore, there is currently some concerns over the efficacy of the confirmed nursery protocol (CNPcurrently in place. The CNP is constantly being improved based on the available science. Several suggested additions to the current CNP have included and enhanced delimiting sampling regime, monitored exits and entrances into the destruction block to include foot baths to disinfest shoes, establishment of litter cleanup protocols, and additional delimiting surveys during the next conducive season (in addition to the yearly survey).			
14. Is regulating affected plant parts as opposed to regulating whole plants scientifically justifiable for preventing the spread of *P. ramorum*?	Regulatory programs are focused to mitigate risk associated with pathways that may be associated with the spread of *P. ramorum*. At present, plant parts have been demonstrated to be infested with *P. ramorum* and may be infectious, thereby posing risk. Those parts that have not been found associated with the disease are not regulated as they are not considered to represent a means of disease spread.			

Question	Response	References	Research Underway	Experts
	There are some host species in which a systemic response to *P. ramorum* infection has been reported, in particular Douglas-fir and redwood. It is unclear whether this is due to the production of systemically translocated compounds either by the host, the pathogen, or both, or instead by the direct action of the pathogen. Further research for conifers is needed to ensure the "plant part" concept is correct.	Garbelotto, UC Berkeley		
15. What is the best way to dispose of infected material and what site characteristics should be considered?	BMPs for disposal have not yet been determined. Also, the risks associated with shipping contaminated material is characterized as high. Some work has been done on heat treatment as well as composting for disposal of green waste, with promising results. Work has been done at multiple sites and times, both for windrow piles and static forced air ones. But as methods for testing dormancy/viability of chlamydospores have not yet been worked out, it remains to be proven that these methods kill chlamydospores. Visible bursting of chlamydospores has been demonstrated under temperatures that occur in the composting process. Tolerance to high temperature or composting is unknown for oospores of *P. ramorum*. Should both A1 and A2 mating types become established in North America and/or Europe, sexual recombination could occur resulting in the production of oospores. Further testing of composting as mitigation for *P. ramorum* would be required if oospore production is documented. Oospores of *P. infestans* have been shown to survive	CPHST Pathway analysis Garbelotto and Rizzo 2001; Swain et al. 2002; Garbelotto 2003a Garbelotto, UC Berkeley Grunwald, ARS	Air-steam treatment of used containers and lethal temperatures for killing *P. ramorum* and other Phytophthora species is being determined (Linderman). In the Netherlands eradication of *P. ramorum* by composting is being studied (Van Leeuwen, Dutch PPS). Note: Composting systems under evaluation in the Netherlands are based on closed forced-air systems. (Kaplan)	

Question	Response	References	Research Underway	Experts
	only at temperatures up to about 45 °C. Oospoes did not germinate after exposure for 2 hrs at 46 °C or 12 hrs at 40 °C. (see Fay and Fry). Thus composting might be adequate for *P. ramorum* as long as compost is mixed to ensure that all material is heated to >50 °C. This needs further study for *P. ramorum*. In the UK, composting is not considered appropriate for plant material containing quarantine organisms, particularly those like *P. ramorum* that produce hardy resting spores. On site burning has been used in Oregon. Site characteristics that would be important (incomplete list) would include surrounding vegetation (if hosts are present), water flow out of site that might carry spores, likelihood of future disturbance (if material is buried). Greenwaste can be safely transported to cogeneration plants where it should be quickly utilized in an area that is monitored for disease prevalence. Heat and vacuum were effective in reducing viability of *P. ramorum* in a relatively short time in bay leaves. Only 12 hours with a single peak at 55 °C vs. potentially a week constantly at 55 °C.	Central Sciences Laboratory, UK Garbelotto, UC Berkely	Research on re-isolation of *P. ramorum* from uncured and curing compost is currently underway.	
16. Is the treatment of soil and water at a *P. ramorum* infested nursery site required to prevent the spread of *P. ramorum*?	*P. ramorum* is transmissible through both media. Appropriate treatment protocols for *P. ramorum* have yet to be established and validated, though treatments are likely to be similar to those for other *Phytophthora* spp. (i.e. heat treatment or fumigation of soil, chlorination or filtering of water). Additional	Erwin and Ribeiro 199)		

Question	Response	References	Research Underway	Experts
	information is actively being sought to determine the effects of drying of nursery soil on the survival of *Phytophthora ramorum*.			
17. When does an infected nursery plant installed in a landscape shift the situation from a limited outbreak to a quarantine incident?	The infected nursery plant by itself constitutes a limited outbreak. Evidence that the disease has spread to other established plantings or surrounding natural vegetation shifts the situation to a quarantine incident.	Confirmed Nursery Protocol		
18. Should highly susceptible but untreated sentinel plants (i.e. *Viburnum plicatum* var. *tomentosum* "Mareisii') be used to determine if *P. ramorum* is still present?	The use of sentinel plants may be an effective means of detecting *P. ramorum* in the environment. The relationship of a positive find on a sentinel plant to indicated regulatory actions is unclear. A positive finding suggests that a nursery may be at risk, but establishment of disease by *P. ramorum* requires more than just presence of the pathogen. Furthermore, the presence of a highly susceptible host plant could lead to dissemination of the pathogen to non-sentinel nursery plants if greta care is not taken. Other strategies that might be considered would include spore traps or baiting with pear or leaf pieces for detection in air or litter/ soil/ water. These strategies are preferable since they do not lend themselves to production of air-borne inoculum. They also provide an indication of the presence of *P. ramorum* without promoting establishment of the disease. Furthermore, in the UK 10% of all susceptible hosts within a nursery are left untreated with fungicide for easier detection of *P. ramorum*.	Science Panel June 2004 Stephen Hunter, (UK DEFRA)	Numerous *Viburnum* species, especially evergreen species, are being tested to identify sentinel plants. Results have varied depending on the method of inoculation and the age and physiological state of the plants. *V. plicatum* var. *tomentosum* 'Mariesii' and *V. davidii* appear to be good in detached leaf tests, but did not perform as well using intact plants inoculated with other *Phytophthora* species. Research continues (Linderman/Parke). W501 group proposes	

Question	Response	References	Research Underway	Experts
	The usefulness of sentinel plants in a US nursery is logistically difficult. In order for sentinel plants to be part of an efficacious monitoring system, they must be treated in the manner that all other nursery stock is treated (i.e. watering regime, nursery placement, etc.) and yet not be exposed to pesticides that would reduce the usefulness as a monitoring device. The practicality of having a single plant in a block (or several plants scattered in a block) not be sprayed with pesticides is impractical and would require the plant be separated from plants being treated in the block with pesticides (and thereby being treated differently and reducing the potential usefulness). Also, nursery plants are constantly moved around and the identity of sentinel plants may be lost Finally, any infected, untreated sentinel plant may act as an inoculum source for the disease to spread in the nursery.		to find a facility/location in a regulated county in California to create a *Phytophthora ramorum* infested nursery for epidemiological research (this nursery is still in the planning stage.) (Grunwald/Parke)	
>> Survey and Monitoring <<				
1. How long should nursery plants be placed on hold/be held for observation in lieu of testing?	For regulatory purposes, there is no testing option available for use to release a nursery plant prior to the 90 day observation period. Nursery stock must be visually inspected by properly trained inspectors at least twice over the 90 day period during environmental conditions conducive to disease development. Current EU regulations call for 2 negative visual inspections during 3 months of active growth. The 90 day monitoring period called for by EU protocols was based on the observation that the latent period	UK PRA 2003 Netherlands PRA 2002	Effects of cultural practices on symptom development. Variation in plant physiological state appears to affect its susceptibility and symptom expression. Different species or cultivars express different symptoms making monitoring	

Question	Response	References	Research Underway	Experts
	(period between infection and disease symptoms) in inoculation trials had not exceeded three months. However, the latent period will vary with the host and time of year and therefore more information is required on this aspect of the pathogen x host x climate interaction to more accurately determine the minimum holding period, not to mention the potential latency associated with the use of fungicides Canadian Nursery Action Plan specifies that all host plants within the infected facility must be sampled on a monthly basis for a period of no less than 90 days following the last detection of an infected plant. There is a possibility that growing conditions could be manipulated to promote symptom development, but this hasn't been sufficiently tested or validated.	Central Sciences Laboratory, UK C.F.I.A. 2003	difficult (Linderman). Growing conditions could extend the latency of symptom expression for *P. ramorum*. In order to be comfortable with the 90 day recommendation, we should: a) monitor symptom expression in affected North American nurseries, collecting observations and data in some organized fashion, and b) set up a controlled trial evaluating symptom expression in a range of nursery host species across a range of environmental conditions (Eric Allen, CSL).	
2. How should a "lot" or a "block" of nursery stock be characterized? By physical proximity, (e.g. host plants of different species or varieties separated by a walkway)? Or by	A lot or block of nursery stock is defined as a contiguous group of host plants identified as being a unique cultivar, genus or species divided by non-host plants or a distinct physical separation of land that is no less than 2m.	Confirmed Nursery Protocol		

Question	Response	References	Research Underway	Experts
common origin (e.g. a group of rhododendrons made up of a single variety of rhododendron which came from another nursery)? What about the effect of cultural practices at the nursery (the moving of plants, overhead watering vs. drip irrigation, in-ground cultivation, etc.?	"Blocks" as defined by nurseries are often based on plant type, age, pot size, and irrigation unit. A block of plants (species, cultivar, etc.) may originate from different sources of propagation stock. Moving plants can spread the pathogen in the nursery and complicate regulatory action. Overhead irrigation and poor water management practices that favor the use of untreated water and puddling in the nursery are conducive to disease establishment and spread.			
3. As *P. ramorum* can be asymptomatic, what would be the best protocol for nursery survey?	We are currently in the process of developing science-based statistically sound survey and sampling strategies for host plant tissue with characteristic symptoms of *P. ramorum* in nurseries. The US Forest Service has developed sampling strategies for natural areas. To date, *P. ramorum* has not been detected on asymptomatic host plant tissue above-ground and tissue that was infected with *P. ramorum* prior to spraying contact fungicides still developed lesions. Surveys will involve the visual inspection of known hosts and related species and are to be conducted at the time of year when symptoms are expressed by inspectors trained specifically to recognize symptoms of *P. ramorum* on known hosts; when environmental and growing conditions favor detection of the pathogen and symptom expression. The 2005 National Nursery Survey Protocol is available for use. The sampling protocols in the in place for the national survey assume that only 75% of	Science Panel, June 2004 USFS PSW 2nd SOD Science Symposium		

Question	Response	References	Research Underway	Experts
	the plants infected with *Phytophthora ramorum* will show symptoms. The plant number that is required to be visually inspected is increased to account for the differents and ALL symptomatic plant tissue found during the inspection is required to be taken (i.e. there is not a minimum of 40 samples required, but every visually inspected plant with symptoms MUST be sampled).			
4. Are field surveys the best that they can be? Should a truly random sample of all plants in a nursery or at least a stratified random sample of host plants be conducted rather than keying-in on symptomatic host plants?	Survey strategies are always subject to improvement. Their implementation is strongly influenced by available program resources. The likelihood of survey success will also be dependent upon disease incidence, environmental conditions and the detection ability of the inspector. Current sampling strategy is to only sample symptomatic tissue, since symptomatic tissue is more than 10 times as likely to contain P ramorum than asymptomatic tissue, based on a recent study at an infected nursery prior to destruction of the infected material. Extensive experience in nursery sampling has been attained in the UK. PPQ will ask DEFRA if they have compared random vs. targeted sampling strategies for the ability to detect *P. ramorum* in nurseries. DEFRA visually inspects every plant within a nursery and sample any symptomatic tissue. Targeting known hosts and those most likely to show symptoms makes good sense. Any survey needs to favor detection. Surveys should include sites with multiple host species and be timed when symptomology is most likely. Additionally, even though plants might not be sporulating, leaf pieces from plants with lesions (either water-soaked or necrotic) could be detached and incubated in a moist	Stephen Hunter, DEFRA Garbelotto, UC Berkeley Grunwald, ARS	A comparative study looking at ease of infection of leaves (number of sporangia x environmental requirements) is needed to understand which hosts really mark the beginning of an epidemic in nature and which are just natural "baits' when inoculum is abundant. (Garbelotto, UC Berkeley)	

Question	Response	References	Research Underway	Experts
	chamber under controlled conditions to see if lesions sporulate. This could be an inexpensive method of monitoring nurseries.			
5. During *P. ramorum* survey when the weather is warm and humidity is low it has been said that plants are asymptomatic. Will they still test positive for *P. ramorum* and with what procedure?	Infected nursery plants should test positive for *P. ramorum* using DNA-based and ELISA-based diagnostics during periods when symptoms are absent. The challenge would be to identify an effective sampling strategy. This would require a focused research program and would likely vary by plant species. Initial studies indicate that in plants that have been treated with fungicides three days after infection with P. ramorum that detection of the pathogen is 100% by ELISA and by nested PCR, but less than 30% by isolation on selective media (PARP). Determining the effects of environmental conditions on detection will require experimentation in a nursery environment that is still being planned.	Science Panel June 2004 Shishkoff, APS, 2005		
6. Currently, *Rhododendron* and *Camellia* are the only hosts included at the genus level on the *P. ramorum* host list. Within other genera (e.g. *Viburnum*), which include known host species, should other species within those genera be surveyed with the same intensity as known host species?	For survey purposes it is appropriate to inspect known hosts and related species since we do not have a clear understanding of the entire host range of *P. ramorum*. Current surveys in Oregon include many plant species and genera, but focus on *Rhododendron* and *Viburnum*. Suggestions have been made for more known species/genera to be inspected than recommended; limited human and fiscal resources prevent looking at more.	Science Panel, June 2004 Linderman, ARS	Research on host range is being conducted on many families of plants that appear to be more susceptible to *P. ramorum*.	
7. Would it not be better to	Ideally it would be better to regulate nursery stock at	C.F.I.A. Plant	Testing to determine	

Question	Response	References	Research Underway	Experts
regulate genera as Canada does? What was their rationale?	the genus level as it is a more absolute method of reducing risk. However such regulations need to be practical and effective. It is apparent that there are differences in host susceptibility at the species and variety level in some genera. Thus, regulation at the genus on a unilateral basis would unnecessarily commit program resources and adversely impact diverse industries. Eleven species of *Viburnum* are hosts: either regulated, associated or experimental. This would suggest that it also would be prudent to regulate *Viburnum* at the genus level in nurseries. Canada chose to regulate *P. ramorum* hosts at the genus level for a number of quarantine considerations 1. At present we understand that *P. ramorum* is capable of infecting a large range of non-related plants (at least at the family level). We believe that it is reasonable to assume that related untested congeneric species could also be susceptible. If, in the future, individual species are shown to be resistant, they will then be removed from the list. 2. In the quarantine world, our concern has to be on pathways. If a plant is capable of transporting infection to a site where conditions favor the disease, then this becomes a quarantine concern to us. 3. We know that the disease has been transported from one nursery site to another, as well as from one natural habitat to another even though regulatory controls	Health Risk Assessment Unit 2003 (Shane Sela, CFIA, personal communication)	the susceptibility of various species of conifers to *P. ramorum* (Chastagner, WSU).	

Question	Response	References	Research Underway	Experts
	have been in place. Given this, is it sufficient to regulate only naturally occurring species? The uncertainties associated with the pathogen necessitate measures that protect un-infested areas.			
8. Is surveying or regulating plants at the variety level scientifically justifiable?	Using highly susceptible varieties as targets in surveys increases the likelihood of detection. At present there are two plant species that are regulated at the variety level based on science-based pathogenicity studies (Koch's postulates) and on their native distribution. Several genera are also regulated based on almost uniform susceptibility. A better understanding of host/pathogen interactions is needed. Currently CPHST is attempting to determine which cultivars and varieties have been responsible for the majority of P. ramorum finds throughout the country and determine if this is related to varietal popularity or varietal susceptibility.	Science Panel June 2004		
9. Has any artificial inoculation of 'azalea' shown symptoms similar to or like those in the 'rhododendron' group of *Rhododendron*?	Twenty commercially available cultivars or species were tested for susceptibility. Zoospore inoculation of detached leaves resulted in small lesions forming on all cultivars. Deciduous azaleas were generally more susceptible in detached leaf assay studies than were evergreen azaleas similarly challenged. In detached leaf and whole plant assays, under laboratory/greenhouse conditions, azaleas are as susceptible as other rhododendrons to P. ramorum. In leaf tests with species in the Ericaceae, azalea and rhododendron controls were susceptible. However, a wide range of difference in symptoms and reactions to *P. ramorum* inoculation in Ericaceous plants has	Tjosvold et al. 2002c Tooley et al., 2004 Tooley and Englander 2002		

Question	Response	References	Research Underway	Experts
	been observed.			
10. What survey elements are required for a detection program to succeed in finding *P. ramorum* in a nursery setting if some host plants do not express *P. ramorum* symptoms?	Survey protocols will have to consider proximity to symptomatic plants/inoculum source (as spore dispersal distances for *P. ramorum* have not been determined). Detection of *P. ramorum* in plant tissue that do not show symptoms has not been highly effective. Survey protocols also must consider contact through water (splash, puddling, recycling), tools, and other cultural practices known to be involved in the transmission of *Phytophthora*. To certify nurseries, testing of asymptomatic plant material may be required. In the UK, 10% of all host and associated plants are managed without fungicides to ensure that disease development will occur if the pathogen is present.	Science Panel June 2004 S. Hunter, DEFRA		
11. Can *P. ramorum* be recovered (detected at a level sufficient for regulatory action) in robust, asymptomatic plants?	A variety of *Phytophthora* species can be detected around symptomless ornamental plants, field soil, and bulk container mix in nurseries using a baiting bioassay. This assay is especially important for infested soil and potting media. To date, however, recovery of *P. ramorum* from asymptomatic host plant tissue has not occurred. Extensive sampling would be required to determine if *P. ramorum* were present in robust, asymptomatic plants. Such plants would not be suspected of being infected with *P. ramorum* unless they were associated with an outbreak of disease or infestation of a nursery by *P. ramorum*. In such instances, plant material would be held for 90 days and the plants	Ducharme and Jeffers 1998 Science Panel, June 2004	Tests are currently underway to determine efficacy of these bait assays compared to tissue sampling from an infested nursery in	C. Blomquist, N. Osterbauer

Question	Response	References	Research Underway	Experts
	observed for symptom development. However, if the potting media associated with the plants were found to be infested with *P. ramorum*, regulatory action may occur. Conversely, if *Phytophthora ramorum* was not baited out of the potting media associated with the plants did not necessarily mean plants were not infected, but that the infection was not detected.		California.	
12. In relation to monitoring a nursery following destruction of an infected block of plants, what factors would be necessary to take into consideration, i.e. irrigation system type, damp areas, soil type, proximity to infected plants, etc.	▪ history of plant: inter- and intra-nursery movement ▪ ground coverings – effects on inoculum survival and dispersal (puddling, splashing, etc.) ▪ sanitation of all equipment (tools, carts, PPE,) and pots if reused and walkways, etc. ▪ sources of water and potting material ▪ storage conditions of potting media, fertilizer, etc. ▪ disposal of culled material ▪ plant debris/soil in and on vehicles ▪ landscape setting of nursery – surrounding plants, topography, water and wind flow, etc. ▪ if burial of material is to be considered on site, double check water table, etc.			
>> Diagnostics <<				
1. What new technologies are currently being evaluated for their potential usefulness in the *P. ramorum* program?	Several new DNA-based molecular techniques have been considered or are currently under consideration for full validation by the National Plant Germplasm and Biotechnology Laboratory in Beltsville, MD (NPGBL) directed by Dr. Laurene Levy. Real-time PCR utilizing CSL's ITS region primers from the *P. ramorum* genome will be the next available validated diagnostic and is currently in the final stages of validation. Other potential methods will likely target different regions of the genomic or mitochondrial DNA to provide a different area of the *P. ramorum* genome to test. These targets include the Cox I, Cox	USFS PSW 2nd SOD Science Symposium	Research continues in the United States, Canada, Great Britain, Germany and the Netherlands and different targets that might prove useful for development of diagnostic tests for regulatory purposes. Currently a program is underway to	K.Ivors P. Bonants C. Hong F. Martin K. Hayden K. Hughes G. Bilodeau S. Doyle B. Tyler

Question	Response	References	Research Underway	Experts
	II, β-tubulin, elicitin, and CBP (Cellulose Binding Protein). Amplified fragment length polymorphism (AFLP) has been used successfully by researchers to differentiate genotypes of *P. ramorum*. This technique involves using traditional PCR to amplify segments of DNA then using restriction enzymes to ligate the DNA. Microsatellite analysis utilizes short segments of DNA that are repeated variously within the non-coding genomic DNA. The technique provides a tool to examine closely related organisms that might have slightly different segments repeats. This technique has been used in Europe to differentiate the European genotype from the North American genotype. Other technologies that might be utilized include elicitin (species-specific proteins that are secreted by *P. ramorum in planta*) that could prove useful in new species specific immunoassays.		examine several of these assays in a number of different laboratories with approximately 400 different isolates of *Phytophthora* species.	
2. Can genotypes of *Phytophthora ramorum* be distinguished using currently available techniques?	Yes. The molecular tools are currently in development by researchers in the United States and Europe that would allow fast, accurate differentiation of the two major genotypes, North American and European. These include AFLP and Microsatellite applications. The regulatory significance and potential use of these technologies still needs to be evaluated.	USFS PSW 2nd SOD Science Symposium		K.Ivors P. Bonants C. Hong F. Martin
3. Why is ELISA used first to pre-screen samples before further testing?	The ELISA used in the validated protocol doesn't detect *P. ramorum* specifically, but is relatively cheap and easy to use. Most state diagnostic labs have the facilities and expertise to perform these	Agdia web site http://www.agdia.com/cgi_bin/catalog.cgi/9260	Which plant parts (for each host/associated host) gives best ELISA	Art Wagner Chet Sutula

Question	Response	References	Research Underway	Experts
	tests. What this ELISA detects is the *Phytophthora* genus of pathogenic organisms, many of which are found throughout the U.S. Some species of *Pythium* are also detected. The reason ELISA is performed first is to quickly eliminate the relatively large number of samples that may be sent to labs for diagnosis that are NOT infected. ELISA singles out potentially infected samples for further testing, but does not determine that samples are positive for *P. ramorum*. Only further testing of ELISA positive samples by other tests can determine if they are positive for *P. ramorum*. New *P. ramorum* specific ELISA tests have been proposed based on elicitin proteins specific to *P. ramorum* that are translocated in the plant and may be detected in plants that have very small lesions. To date, however, only genus specific ELISA tests exist for *Phytophthora* species.	0 http://www.aphis.usda.gov/ppq/ispm/sod/ELISAprotocol.html USFS PSW 2nd SOD Science Symposium	results? What is the spatio-temporal effects of infected plants for ELISA detection?	
4. Why isn't culturing of P. ramorum used as the means of determining whether a plant is infected?	Culturing *P. ramorum* on PARP is not used to determine that a plant is not infected for two main reasons: 1) culturing is a relatively insensitive assay, and may not yield an isolated culture if the sample is highly contaminated, collected at the wrong season or sampled just after a pesticide treatment. 2) there are several hosts of *P. ramorum* that consistently fail to yield isolated cultures even though the host is known to be infected. In some cases, only nested PCR can quickly determine if the pathogen is present.	Hayden et al. 2004. Davidson et al., 2003 Plant Health Progress 'Pathogen Isolation'		
5. Why doesn't the ELISA detect only *P. ramorum*?	The Phytophthora diagnostic ELISA kit was originally designed to detect the late blight pathogen in potato (*Phytophthora infestans*). However, the	http://www.aphis.usda.gov/ppq/ispm/sod/ELISA	Possibility of generating *P. ramorum* species	Art Wagner

Question	Response	References	Research Underway	Experts
	antibodies used for this assay also detect many other *Phytophthora* species, including *P. ramorum* as well as a few *Pythium* species. New *P. ramorum* specific ELISA tests have been proposed based on elicitin proteins specific to *P. ramorum* that are translocated in the plant and may be detected in plants that have very small lesions. To date, however, only genus specific ELISA tests exist for *Phytophthora* species.	protocol.html USFS PSW 2nd SOD Science Symposium	specific antibodies through the use of elicitin proteins	
6. Can other *Phytophthoras* serve as an ELISA control if you don't have *P. ramorum*?	Since the diagnostic ELISA kit can detect many *Phytophthora* species, any *Phytophthora* culture or infected sample should produce a positive result. However, since the kit hasn't been tested on all *Phytophthora* species, it may be necessary to run an experiment prior to screening to determine if the control used will give suitable readings for testing purposes.	http://www.agdia.com/cgi_bin/catalog.cgi/92600		
7. Almost all of our lilac samples index as positive by ELISA, are they all infected?	The Phytophthora diagnostic ELISA kit sent to the state diagnostic centers for the *P. ramorum* trace forwards and national surveys originally used a buffer that resulted in high background readings in healthy *Syringa* sp. (lilac) leaves. A new buffer system is now available and can be obtained free of charge to these labs that had purchased kits for *P. ramorum* screening. The original ELISA positive samples of lilac should be retested with the new buffer or the DNA should be extracted and forwarded to the NPGBL in Beltsville.	Phil Berger		Agdia
8. How do genus-specific primers differ from species-specific primers?	The DNA sequence of a pair of primers determines their specificity. Genus specific primers are comprised of DNA sequences that are common to an entire genus of organisms, such as Phytophthora.	Frank Martin web site http://pwa.ars.usda.gov/salinas/	Use of cox I and II regions for nested PCR	Garbelotto group Frank Martin

Question	Response	References	Research Underway	Experts
	Species-specific primers are comprised of sequences that are highly conserved within one species. The specificity of the primers chosen (genus or species) can profoundly affect the sensitivity and overall specificity of PCR detection of *P. ramoru*m. The current validated nested PCR protocol uses primers in the first round that are specific to *P. ramorum* (based on known Phytophthora sequences). The primers in the nested round are also specific to *P. ramorum*, with a few exceptions. However, those exceptions occur in only one of the primers, so that a positive result even with these exceptions should not be observed when the nested PCR is performed.	cipru/frank/phyto.htm		
9. What does it mean if an assay gives a false positive result?	A false positive result is produced when the assay identifies a sample as being *P. ramorum*, but the sample is not infected with *P. ramorum*. In the nested PCR assay, this is frequently caused by contamination of sample DNA with target DNA (usually from positive controls or cross-contamination with an infected sample). Each experiment is run with numerous control reactions to detect this occurrence and provide information of the contamination source. In addition, there are relatively straightforward analyses that can be done to detect and diagnose a false positive. A common source of false positives is cross-contamination of samples. Diagnostic labs should be sure to take measures to maintain the integrity of all samples brought in, since it is difficult to trace and correct false positives if samples are contaminated. Although the cultural characteristics of *P. ramorum* are distinct enough for correct identification and the	Davidson et al., 2003		

Question	Response	References	Research Underway	Experts
	rate of false positives by PCR is expected to be quite low, these parameters have not been quantified. Furthermore, because culturing the organism is not very sensitive, the use of the validated PCR protocols at the PPQ Beltsville Laboratory or other APHIS-approved laboratory is required for positive identification of the organism.			
10. What about a false negative?	A false negative result is produced when the assay indicates that a sample is negative, but the sample is actually positive (infected). In the nested PCR assay, this can be caused by samples where the DNA is too dilute or contains PCR-inhibiting contaminants or is otherwise of poor quality. In the nested PCR, DNA integrity is checked by a parallel assay (the multiplex PCR assay). A sample is not analyzed unless the DNA is of sufficient quantity and quality to support amplification by PCR. Recent research indicates that false negatives can be found in samples in certain natural situations when it was previously established that the plants were infected. Although conditions used to generate these data were not readily transferable to the current diagnostic protocol, these results do serve as a warning that a certain rate of false negatives could be present and would be very hard to detect under the current program conditions. However, sampling protocols are more likely to contribute to false negative results than the PCR test itself. The rate of false negatives using only culture isolation of *P. ramorum* to identify infected plants would be expected to be high – probably higher than using PCR - because of the reasons discussed in question 2.	Winton and Hansen, 2001 Hayden et al., 2004		

Question	Response	References	Research Underway	Experts
11. What does it mean when an assay is presumptive positive?	A presumptive positive is obtained when a lab has determined that a sample could be positive for *P. ramorum*. In most situations, this would occur if a diagnostic lab isolated what appeared to be a *P. ramorum* culture based on morphology. This culture would need to be confirmed by the NIS before it is recognized as positive by PPQ. (i.e., in this example, the identity of the culture is confirmed by the PPQ National Mycologist. A positive ELISA result would NOT indicate a presumptive positive, since there are many organisms that could produce a positive result. The ELISA results are useful only in screening out negative samples and identifying samples that require further testing.)			
12. What does it mean when an assay is confirmatory?	To confirm means to validate or verify something believed to be true. e.g., a diagnostician believes that an organism isolated is *P. ramorum*. This observation is *confirmed* by NIS. In other words, a confirmatory test could be a PCR test on DNA from a culture, a second extraction of DNA from a sample followed by a PCR test, culturing of the organism from a sample that was positive by PCR, etc.			
13. Are one or two diagnostics needed? Should two protocols be necessary for every determination?	Two assays would be better for confirmatory purposes for a variety of scientific (and perhaps legal) reasons. Two positive results using completely different assays would strengthen the determination that the find is not a false positive. If one of the diagnostics is culture, then a living record of the infected tissue could be kept for subsequent examination. If both of the diagnostics are derived from PCR, then two separate genomic targets for the organism should be used to determine a positive. These two targets should ideally be species specific			

Question	Response	References	Research Underway	Experts
	and not be related to each other either in function or in terms of genetic locus. Having one target located in the nucleus and the other in the mitochondria, for example, could provide a good system.			
14. What area of a symptomatic plant part is best for sampling by 1) ELISA, 2) culture isolation, 3) nested PCR?	It was postulated by the science panel that there could be differences in the best target areas of symptomatic leaves depending on the assay used. This is possible because each of the major assays target a different portion of the organism. Culturing targets, intact hyphae or sporangia, ELISA targets proteins produced by the organism, and PCR targets DNA. Each of these can occur in various concentrations in the infected leaf. To date, tissue without lesions have not been demonstrated to harbor P. ramorum either thought PCR, ELISA or culture.		This entire question needs to be addressed in a systematic, scientifically documented way.	Kim Seong Hwan (ELISA) Nancy Osterbauer (culture) Garbelotto group, (nested PCR)
15. Is it possible to run the molecular diagnostics for *P. ramorum* detection using an automated, high throughput system? Or, is it possible to perform portions of the diagnostic tests, such as plant DNA extraction, using automated systems?	Many in the science panel felt that this approach would be most desirable, because it would speed up the reporting of samples, prevent potential backlogs, and reduce the workload of the people performing the assay. Although there are hurdles to be overcome in the deployment of high throughput systems, and extra quality control steps would need to be implemented and deployed to ensure the fidelity of the results. There are several governmental and commercial operations already in place that could conceivably be employed for this purpose.		Several organizations are being contacted to determine suitability for automated analyses of samples.	Jean Ristaino Frank Martin

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