

***Phytophthora ramorum* Technical Working Group responses to second set of questions**

<p>1. Is the sampling methodology for plant tissue appropriate? --should more plant tissue be taken? --is taking only plant tissue that looks unhealthy appropriate? --should a certain amount of healthy plant tissue be sampled? if so, how much?</p>		
No.	Comments	Suggestions/Recommendations
1.	<p>Sampling of asymptomatic tissue has been shown NOT to be an effective method. Oregon clearly has proven that it is not worth the time and effort. Additionally, it flies in the face of the basic principles of plant pathology, which is based in symptomology. There would be no guidelines on what to collect if there were no symptoms. Swabbing the leaves for the presence of DNA, dead or alive, is not practical or scientifically valid.</p>	
2.	<p><i>Is the sampling methodology for plant tissue appropriate?</i> It appears that a minimum of 850 plants will be inspected in each block. This seems like a suitable number which is apparently based on statistical considerations. <i>Should more plant tissue be taken?</i> It says that a minimum of 5 leaves should be taken, which seems like enough.</p>	<p><i>Is taking only plant tissue that looks unhealthy appropriate?</i> Perhaps some samples of healthy looking tissue should also be taken, as studies in the UK have shown that <i>P. ramorum</i> can be present in apparently healthy tissue. <i>Should a certain amount of healthy plant tissue be sampled? if so, how much?</i> It would probably depend on how many symptomatic samples are being found. If few symptomatic samples are found and there is thus 'room' to process a certain number, then asymptomatic samples could be taken. Maybe 10-20 random samples from each block would be a suggestion in general so as not to end up with hundreds extra to process. Another idea would be to bulk the asymptomatic samples, process in larger groups and then if any bulked sample turned up positive you would know that at least one of the samples had contained <i>P. ramorum</i>. I see that composite samples are being used for the soil sampling.</p>
3.	<p>Sampling methodology of plant tissue seems adequate. I do not see a need for additional samples to be taken. Most inspectors will take more than 40 samples if additional symptoms are seen after they have collected the obligatory 40. I guess one could go around and sample every plant on the property, but I don't think it would necessarily provide more assurance. Of course, that cannot be done. At this time the 40 samples appear to be adequate, as California has not shipped a positive plant out in 2 years (I think... except for the mail order plant that went to Texas.) The pathogen is being detected with the current protocols; no need to increase. As a side, but not necessarily relevant to the science question, many states do not have the resources to be doing much more than they are now.</p>	

No.	Comments	Suggestions/Recommendations
4.	<p>Maybe foliar and soil sampling strategies are not at the root of the repeat nursery issue. Maybe contaminated water recirculating in systems, or illicit movement of plants are responsible for most of the problems. What portion of the repeat nurseries are retail nurseries? Might the difference in the size of the quarantine blocks between the protocols explain any difference in the rates of reinfestation?</p>	
5.	<p><i>Is taking only plant tissue that looks unhealthy appropriate?</i> I think appropriate. Sure there is a chance that you can have asymptomatic leaves, but your chances are much less than with unhealthy leaves. <i>Should a certain amount of healthy plant tissue be sampled? if so, how much?</i> I'm not sure. I would consult the state lab diagnosticians to see if the data tells us how likely this is. Consider the effort and expense involved versus the probability of detection. My gut, is that this is not worth it.</p>	<p>Recently dropped, non-senescing, lesion-free leaves should be considered in the symptomatic category.</p> <p>I suspect an inspector's time would be best spent looking for suspicious leaves.</p>
6.	<p>The sampling methodology for plant tissue is appropriate. No healthy plant tissue should be sampled. This practice has been shown to be unproductive and a waste of resources. Also, If a nursery is found to have many symptomatic plants, the sampling number typically increases based on the Ag dept determination. The regulations only establish a minimum number of samples that should be taken. They do not limit the maximum number that can be taken.</p>	

2. Is the soil sampling methodology appropriate?

--should more soil be removed? less?

--what is the best way to detect *P. ramorum* propagules in soil samples?

--what is the best way to break dormancy of *P. ramorum* propagules in soil samples?

No.	Comments	Suggestions/Recommendations
1.	<p>When I was involved in baiting greenhouse soils for Pythium, we tested each variable of our assay: how many leaf disks to add, what kind of leaf, how many hours to leave the bait in the assay, how much water to use with what surface area, etc. It didn't take that much tinkering to make sure our assay would do what we wanted it to.</p>	<p>It would seem essential to test the assay with soil baited <i>P. ramorum</i> propagules, otherwise I can't imagine how one would know if the assay worked or not. Was any such testing done for this assay? If not, my recommendation would be that someone sit down and do it.</p>
2.	<p>The sampling methodologies look thorough however they seem quite rigid. Assuming there is science to support variables like the required radius of the inspection area, would not this science have shown that <i>P. ramorum</i> may, under some circumstances, spread further than say the 10 meters currently designated? Or that under some conditions symptoms may not appear for more than 90 days?</p>	<p>Perhaps some guidance in the methodology about conditions that should be avoided or would require extension of one or more of the critical parameters may be needed.</p>
3.	<p>As always, proven over many years on many soil borne pathogens and nematodes, soil sampling is always a crap shoot. You can sample to death, and if you don't find it, it is no proof it is not there. This has been the problem with Karnal Bunt, white rot, and many others.</p>	<p>I have no recommendation on changes to the soil sampling protocol, but definitely feel new methodologies for detection of <i>P. ramorum</i> or any other pathogen are needed.</p>

No.	Comments	Suggestions/Recommendations
4.	<p><i>Is the soil sampling methodology appropriate?</i> It appears to be thorough enough and well thought out. <i>Should more soil be removed? less?</i> Seems about right to me.</p> <p><i>What is the best way to detect P. ramorum propagules in soil samples?</i> Dilution plating is most quantitative, but is also very laborious, or baiting which is less laborious. Pear baiting is a method that is not in general considered appropriate by some Phytophthora experts. Perhaps there is word of mouth as to it not detecting as well as other methods. I am not aware of any published studies comparing different baiting methods, however. In bait selection, no particular cultivar of Rhododendron is recommended, so it will result in non-standardization of the baiting protocol done by different groups.</p> <p><i>What is the best way to break dormancy of P. ramorum propagules in soil samples?</i> Some believe that dormancy in <i>P. ramorum</i> chlamyospores may not truly exist, as it has never been proven experimentally. But observation has shown that recovery from the same site can vary with time of year, etc. and nobody knows why so the theory of dormancy is invoked. I am curious how the 1 month at 4° C recommendation came about...was it experimentally determined?</p>	<p>It is hard to recommend how to break dormancy when we are not sure it truly exists.</p>
5.	<p>Soil sampling is problematic because there are not obvious symptoms to show where best to sample. I think we're doing as well as possible with soil detection. We have had many issues about treating soil, where there really isn't effective treatment for below the surface disinfecting. Steam won't reach as far as needed and removing soil entails risk of spillage or infesting new areas.</p>	<p>Paving and/or raising plants on benches seem to be the only viable treatments.</p>

No.	Comments	Suggestions/Recommendations
6.	<p>The biggest problem with both protocols is their inflexibility. No two nurseries are alike (although retail operations can be more similar than wholesale). We actually had greater success eradicating <i>P. ramorum</i> from positive nurseries when we were allowed to customize the eradication protocol to the nursery. Since the various versions of the CNPs have come out, we have seen a greater problem with recurrence of <i>P. ramorum</i> within treated nurseries.</p> <p>The soil vernalization period continues to present challenges for state regulatory officials and for the nurseries. For example, a block of plants (over 10-m from known infested plants) has been surveyed during delimitation and the plants test negative for <i>P. ramorum</i>. However, soil samples were also taken from the block and the first round of testing comes back negative. Right now, the CNPs currently allow the nursery to move product from that block (greater than 10-m from the known infected plants, tested negative so far for <i>P. ramorum</i>). But, let's say after vernalization the soil comes back positive. Now the nursery needs to be re-surveyed, potentially infested plants may have been shipped, et cetera. This creates a lot of difficulties for everyone. I'm not sure of the best way to address it, but it needs to be addressed.</p> <p>Everett Hansen and others have looked at other means of breaking dormancy in the chlamydospores.</p> <p>I think specifying how much and what plant tissue to sample hamstrings your inspectors (see comment above).</p>	<p>I would strongly suggest that APHIS reconsider the one-size-fits-all approach.</p> <p>We have greatest success using "wounded" leaf baits to pull <i>P. ramorum</i> out of the soil. Our baits of choice are <i>Viburnum davidii</i> and <i>Rhododendron</i> spp. 'Unique' leaves. <i>P. ramorum</i> is one of the few <i>Phytophthoras</i> that can really nuke <i>V. davidii</i>, so that makes this bait almost more selective for the pathogen. Rhody 'Unique' is a magnet for everything.</p> <p>We have greatest success detecting <i>P. ramorum</i> when we just tell our inspectors to go for it (100% visual inspection and grab anything even vaguely suspicious that you see) instead of specifying how of each block to look at, etc.</p>
7.	<p><i>What is the best way to detect P. ramorum propagules in soil samples?</i> Appropriate as stated.</p> <p><i>What is the best way to break dormancy of P. ramorum propagules in soil samples?</i> <i>P. ramorum</i> is a tricky beast. The chlamydospores do not have an environmental switch that turns them on or off. Moisture and cool temperatures are the most important factor to promote the breaking of dormancy it seems. Therefore, dormancy-breaking is sort of an extended-slow-release.</p> <p>I think the 2 meter regulatory rationale is okay. As long as retailers know this they could think about locating HAP plants to reduce the effect of regulation.</p>	

No.	Comments	Suggestions/Recommendations
8.	I think the critical limitation on current sampling for the detection of <i>P. ramorum</i> in our various ecosystems is the lack of a cost-effective, reliable and sensitive method for soil samples. <i>P. ramorum</i> has the capacity to survive for many years without a host. I JUST RECOVERED a 2002 submerged SAMPLE FROM A WATER TUBE STORED AT 16C. This is remarkable and clearly illustrates this species ability to persist as dormant cells. So soil needs to be effectively sampled. <i>P. ramorum</i> may exist at low levels in different nursery situations and once a suitable host and conditions arises it can multiply rapidly. This can explain the reoccurrences in nurseries, etc.	We need a cheap and effective methodology for handling large soil or potting compost samples (such as composite samples from the forest floor or nursery) to produce DNA. I envisage something that could handle a 2 liter sample reducing it to a much smaller DNA enriched fraction that can be purified (removal of PCR inhibitors) for MOLECULAR DETECTION TECHNOLOGIES.

Document Edits

Official Regulatory Protocol for Retail Nurseries Containing Plants Infected with Phytophthora ramorum Version 1.0 December 19, 2007

The 2 meter regulatory rationale is okay. As long as retailers know this they could think about locating HAP plants to reduce the effect of regulation.

Definitions: Nursery Site (p. 4)

A geographically separate location of a Nursery/Facility that has a distinct physical address and appropriate biosecurity measures to prevent the movement of P. ramorum between locations.

The definition states that each site has appropriate biosecurity measures to prevent the movement of *P. ramorum* between locations. This would be ideal but I don't believe it's true in many cases. I think it could be defined as having the capability to apply appropriate biosecurity measures to prevent the movement of *P. ramorum* between locations. One way retail nurseries become infested is by transfer of infested plants from its primary production nursery, owned and operated by the same entity but possibly physically removed. Biosecurity methods are feasible but not always in place, resulting in the positive find at a nursery site.

NINETY (90) DAY QUARANTINE ACTIVITIES (p. 16)

"If water, soil and/or media samples tested positive for P. ramorum during the delimiting survey and the water, soil, and/or media was subsequently treated, samples of the infested water, soil, and/or media material will be taken and tested during each of the two quarantine period nursery inspections per the protocols detailed in the Appendices 6 and 7."

Is this statement needed? Does this mean the nursery had a choice other than disposal?

RELEASE THE NURSERY (p. 18)**Alternative Release Strategy**

*The nursery must destroy everything (all plants, pots, media, etc.) in the **destruction radius (radii)** >and quarantine hold< by approved methods, (see Appendix 8). The nursery operator may also choose to destroy plants that have been placed under quarantine at any time within the 90 day quarantine period, however inspection and sampling must take place prior to destruction; **and** This is not clear. I believe what is intended is the nursery can avoid the SOD quarantine by voluntarily destroying the 4m hold radius.*

*Inspectors must sample and test soil of destruction and quarantine radius (radii) and drainage or recirculated irrigation water if not previously tested and determined to be negative, as per Appendices 5, 6, and 7. If soil and water samples taken are negative for *P. ramorum* the nursery can be released; **and***

Testing another 2m out might make more sense than testing what will be destroyed.

Inspectors must revisit the nursery after approximately 90 days and conduct at least a nursery level survey inspection (per the current Nursery Survey Manual) to include sampling of the soil in the destruction radius (radii). Also, the nursery is subject to “Post Eradication Monitoring”.

What is the experience using this approach? Any failures?

Soil Baiting in the Laboratory (p. 28)

*...To address this dormancy potential and to better enable the diagnostician to detect *P. ramorum* when present, mix the soil well and split the soil samples when they arrive in the laboratory**.* ...

***This applies only to initial soil samples at a location (quarantine block, destruction block, cull pile, etc.) at the infested nursery site.*

I would think this should be required for nursery release also, if soil tested positive previously.

JOB AID (p. 47)**Workflow Check List for rCNP**

What about requiring that the nursery post notice of a recall, in case infested plants have been sold?

Official Regulatory Protocol for Wholesale and Production Nurseries Containing Plants Infected with *Phytophthora ramorum*
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DISCLAIMERS (p. 3)

RETAIL SITES: We recognize that we need a protocol for >In recognition that retail nurseries face different challenges, a separate protocol has been developed (give url).< ~~Until that can issued, regulatory officials must use this protocol and apply it to each situation.~~

DEFINITIONS (p. 4)

Delimitation survey: A survey done to determine the extent of the infestation within a nursery site. The quarantine period Begins when all delimitation sampling is completed, and results have outlined the known infested area.

Nursery site quarantine: This is a period of time during which host plants and associated plants shall not be moved within or out of the quarantine block (see Appendix 2). This **quarantine period** begins when the Nursery Delimitation Survey is completed (Results in?) and lasts for 90 days during which proscribed activities must occur.

Nursery stock: Any plants for planting, including houseplants, propagative material, ^ and tree seedlings for reforestation ^ that are grown in a nursery.

TRIGGER EVENTS FOR USE OF PROTOCOL (p. 7)

This protocol shall be implemented by APHIS-PPQ and/or its State Plant Regulatory cooperators when the presence of *P. ramorum* has been confirmed in a nursery from samples collected as part of a trace forward survey*, trace back survey*, *P. ramorum* nursery survey*, or found by other means.

What does "found by other means" mean? Doesn't the detection have to be done by a regulatory official? If so, say so here.

SECURE THE NURSERY (p. 11)

- If any plants not on hold are showing symptoms consistent with diseases caused by *P. ramorum*:

Why would they not be on hold if they have symptoms?

SURVEY THE NURSERY AND PERIMETER (p. 12)

- The HAP (note: not all plants nor all HAP genera) in the destruction block shall be destroyed in an appropriate manner (see Appendix 8) Has this ever resulted in a repeat nursery?

NINETY (90) DAY QUARANTINE ACTIVITIES (p. 16)

If irrigation water is found to be positive, then any portion of the nursery that has been irrigated with the *P. ramorum* infested water shall be placed on hold and the irrigated area de-limited.

Would it make sense to require some sampling of symptomless HAP in this instance, maybe roots?

RELEASE THE NURSERY**Alternative Release Strategy:**

How often do production nurseries choose this option? If destruction block is large, and recent weather has been conducive, nursery plants outside the quarantine block might have latent infections. Should they be left available for sale for 90 days? Seems like a loophole.

Soil Baiting (p. 37)

This baiting can be done in conjunction with the final baiting required fore the quarantine release survey.

Change “fore” to for.

APPENDIX 11 (p. 52)

Mitigations for Nurseries Found with *P. ramorum* More Than Once

How well is water baiting working in nurseries? Is it really as efficient as inspection?

3. *The presence of *P. ramorum* in soil or water may contribute to the re-occurrence of disease in the nursery and puts the local area at risk. Thus it is necessary to conduct these sampling and testing procedures and if found, eradication must take place.*

7. **For one year following the second occurrence, pre-shipment notification is required to the office of the SPRO of all shipments containing any plants of the genera, *Rhododendron*, *Camellia*, *Viburnum*, *Pieris*, and *Kalmia*.**