

2006 National *Phytophthora ramorum* (Pr) Forest Survey (03/28/2006)

Objectives: Early detection of Pr. Survey the forested perimeter of all suitable Pr-confirmed trace forward nurseries; and survey at least one location in each high and moderate risk hex nationwide.

Methods Overview

There is a minimum target number of 30 sampling locations/state or state cluster. More locations may be justified or less required, depending on local conditions. The maximum number is determined by the number of confirmed trace forward nurseries. A sampling priority table has been prepared.

Plots are 4-100 meter long transects at each location. Transect width varies with density of vegetation (the less dense, the more you can see and the further you should meander to collect suitable symptomatic host materials). Fix GPS points at the beginning and end of each transect so we can return for additional sampling, if necessary.

Nursery survey locations are provided by the state departments of agriculture regulatory personnel (<http://nationalplantboard.org/member/index.html>). In this setting, transects are arranged to sample the most favorable habitats (cool and moist). When possible, also sample the available variation in slope, aspect, prevailing wind, etc. at the forested edge of the nursery site. Suitable host type is that which is within 0.25 miles of the production site, but directly adjacent to the production fields is best.

General forest area survey locations fall in priority below Pr-confirmed trace forward nurseries. Survey these only after higher priority nursery locations are scheduled. They are chosen at random in suitable host type in hexes not sampled with ornamental nursery perimeter locations (if possible) so as to disperse locations in all available high and moderate risk hexes in a state. In this setting, transects are ideally arranged at cardinal directions around a central starting point but may be adjusted on the ground in other configurations for safety reasons or to take advantage of optimal host type and microclimate.

Host tissues to be examined are leaves or bleeding stem cankers of symptomatic plants of 11 genera (see detailed methods) included on the regulated and associated host plant lists. The target host list, by necessity, varies between eastern and western regions.

http://www.aphis.usda.gov/ppq/ispm/pramorom/pdf_files/usdaprlist.pdf

All symptomatic host tissues are collected in 2 replicates. One goes to a local diagnostic laboratory and the other to a regional diagnostic lab for mutual quality assurance. Collect samples from bleeding stem cankers immediately. Otherwise, travel the length of the transect to determine available host plants and symptoms. Collect up to 50 symptomatic leaves/twigs from each transect for a single pooled sample for each host. After all transects are surveyed and sampled, **select 2 composite samples of the best 10 symptoms from each available symptomatic foliar/twig host genus. Seal all samples in double plastic bags keeping different host genera in separate bags.** Fill out duplicates of the pre-printed sample label. Place one label in the bag, and fix the other on the outside. PCR diagnostics on samples will be performed by designated labs for each state or state cluster. In the field, keep samples in a cooler with sealed coolant. Ship overnight mail in the provided insulated mailers to the designated PCR diagnostic

lab for your state on the same day as collected, if possible. Include a chain of custody form inside a plastic bag with each mailing. Samples that can't be shipped and received by the diagnostic labs within 72 hours of collection should be frozen and shipped at the next possible opportunity. **Send copies of field forms and report cumulative survey status to your FHM regional coordinator every other week.**

Complete the 2006 SOD Database EXCEL spreadsheet at the end of the survey and submit it to your Regional FHM Coordinator, who will then submit it to the National Pr Forest Survey Technical Coordinator for forwarding to the NAPIS database.

Chain of custody form (postcard 4 1/4" x 5 1/2" 4 per 8 1/2" x 11" sheet):

State	<u>2006 Custody Form</u>			
Shipper Name:	<u>Mail with samples to</u>			
Shipper Address:	<u>diagnostic labs</u>			
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Lab Name	SiteType #	Site Name	Host Spp	Tissue
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<i>Diagnostic Lab: Enter test date, sample information, and PCR result on Lab Report Form & mail to shipper and Regional FHM Coordinator</i>				

Sample label (1 1/8" x 3 1/2" on rolls):

2006 Lab: _____	State: _____
Site Type: _____	Site ID #: _____
Site Name: _____	
Spp/Tissue: _____ / _____	