

**Scope of Work for *Phytophthora ramorum*
(Sudden Oak Death)
In Regulated Counties
July 1, 2010 - June 30, 2011
FY 2010/2011**

**Appendix G
Sample Invoice**

**Scope of Work for *Phytophthora ramorum*
(Sudden Oak Death)
In Regulated Counties
July 1, 2010 - June 30, 2011
FY 2010/2011**

**Appendix H
USDA/APHIS PPQ Tracking Log**

**EXHIBIT B
(County Agreement)**

BUDGET DETAIL AND PAYMENT PROVISIONS

1. Invoicing and Payment

- A. For services satisfactorily rendered, and upon receipt and approval of the invoices, the State agrees to compensate the Contractor for actual expenditures incurred in accordance with the rates specified herein, which is attached hereto and made a part of this Agreement.
- B. Unless mutually agreed, monthly invoices must be submitted within 60 days from the end of each month in which services were rendered. Invoices must include the Agreement number and submitted in triplicate to the Program Contract Manager listed in this contract.
- C. Any travel and subsistence payments authorized under this agreement shall be paid as needed to execute the work. The maximum travel rates allowable are those established in Title 2, Division 1, Chapter 3, Subchapter 1, Article 2 of the California Code of Regulations 599.619.

2. Budget Contingency Clause

- A. It is mutually agreed that if the Budget Act of the current year and/or any subsequent years covered under this Agreement does not appropriate sufficient funds for the program, this Agreement shall be of no further force and effect. In this event, the State shall have no liability to pay any funds whatsoever to Contractor or to furnish any other considerations under this Agreement and Contractor shall not be obligated to perform any provisions of this Agreement.
- B. If funding for any fiscal year is reduced or deleted by the Budget Act for purposes of this program, the State shall have the option to either cancel this Agreement with no liability occurring to the State, or offer an agreement amendment to Contractor to reflect the reduced amount.

3. Funding Sources for County Contracts (If no Federal Funds, this Section is not applicable)

An annual report of expenditures, where county payments are supported by Federal funds, will be issued by CDFA Administrative Services, Financial Services Branch. This report will be issued by September 30th for invoices submitted prior to July 31st for services rendered in the prior State Fiscal Year.

Federal and State Regulations - The County will comply with all Federal and State regulations and requirements. The County must ensure they have an adequate accounting system in place and appropriate internal controls to ensure expenditures are tracked and maintained.

All sub-recipients of Federal awards shall comply with the Code of Federal Regulations (CFR) Title 2, Part 225 - Cost Principles for State and Local Governments and Title 7, Part 3016 - Uniform Administrative Requirements for Grants and Cooperative Agreements to state and local governments.

Federal 2 CFR 225 (OMB Circular A-87) can be found at the following website:
<http://training.fws.gov/fedaid/toolkit/2cfr225.pdf>

Federal 7 CFR 3016 can be found at the following website:
http://www.access.gpo.gov/nara/cfr/waisidx_01/7cfr3016_01.html

The State's accounting standards and procedures for counties provided by the State Controller's Office are located at the following website: <http://www.sco.ca.gov/ard/manual/cntyman.pdf>

Workplan for Sudden Oak Death Statewide Emergency Response
 Regulated Counties
 FY 2010/2011
 July 1, 2010 through June 30, 2011



County: Riverside
 Date: July 21, 2010
 *Cost Per Hour: \$38

| Activity | Number of Facilities Requiring Activity | Estimated Visits/Year/Facility | Estimated Hours/Visit | Total Est. Hours | Estimated Annual Cost |
|--|---|--------------------------------|-----------------------|------------------|-----------------------|
| Eradication: | | | | | |
| Implementation of Confirmed Nursery Protocol | | | | | \$0 |
| Implementation of Retail Confirmed Nursery Protocol | | | | | \$0 |
| Trace Forward/Trace Back/Survey: | | | | | |
| Regulatory Activity for Trace Forward/Back Investigations | 50 | 1 | 3 | 150 | \$5,700 |
| Quarantine/Enforcement: | | | | | |
| Regulatory Activities at Host Nurseries | 77 | 3 | 5 | 1155 | \$43,890 |
| Regulatory Activities at Non-Host Nurseries | 50 | 1 | 2.5 | 125 | \$4,750 |
| Other: | | | | | |
| Administrative Support | N/A | N/A | N/A | 220 | \$8,360 |
| TOTAL HOURS | | | | 1650 | |
| TOTAL PERSONNEL COSTS | | | | | \$62,700 |
| Overhead (Indirect Costs) -- Not to exceed 25% of Total Personnel Costs | | | | | |
| Enter Overhead Percentage: 25% | | | | | \$15,675 |
| Miscellaneous | | | | | |
| Supplies | | | | | \$3,000 |
| Vehicle Usage | | | | | |
| Enter Estimated Miles: 7,500 | | | | | |
| Rate Per Mile: \$0.50 | | | | | \$3,750 |

*Figure must match the rounded figure on your "Cost Per Hour Worksheet". You must submit your "Cost Per Hour Worksheet" with your workplan.
 All dollars and figures entered on this page must be whole numbers with the exception of the "Rate Per Mile" for vehicle usage and "Overhead Percentage". You must round your "Overhead (Indirect Costs)" and "Estimated Annual Cost" for vehicle usage to whole dollars.

Cost Per Hour Worksheet
Sudden Oak Death Satewide Emergency Response
 FY 2010/2011
 July 1, 2010 through June 30, 2011

County: Riverside
 Date: July 21, 2010

| Title | Hourly Wage | Hourly Benefit Amount | Total Hourly Rate | Estimated Hours to be Worked | Total Cost |
|--|-------------|-----------------------|-------------------|------------------------------|-------------|
| Office Assistant III | \$18.09 | \$8.20 | \$26.29 | 95 | \$2,497.55 |
| Agricultural & Standards Investigator I | \$20.64 | \$8.77 | \$29.41 | 100 | \$2,941.00 |
| Agricultural & Standards Investigator II | \$22.94 | \$8.71 | \$31.65 | 150 | \$4,747.50 |
| Agricultural & Standards Investigator III | \$25.51 | \$9.83 | \$35.34 | 380 | \$13,429.20 |
| Agricultural & Standards Investigator IV | \$28.36 | \$12.49 | \$40.85 | 800 | \$32,680.00 |
| Supervising Agricultural & Standards Investigator II | \$32.37 | \$14.99 | \$47.36 | 25 | \$1,184.00 |
| Deputy Agricultural Commissioner | \$40.17 | \$16.24 | \$56.41 | 100 | \$5,641.00 |
| | | | *Total: | 1650 | \$63,120.25 |

**Weighted Average Cost Per Hour: \$38

*Total "Estimated Hours to Be Worked" MUST match the "Total Hours" on the Work Plan.

**"Weighted Average Cost Per Hour" MUST be entered rounded to whole dollars and entered into the "Cost Per Hour" box of your workplan. You must submit this worksheet with your workplan.

**EXHIBIT C
(County Agreement)**

GENERAL TERMS AND CONDITIONS GTC 610

1. **APPROVAL:** This Agreement is of no force or effect until signed by both parties and approved by the Department of General Services, if required. Contractor may not commence performance until such approval has been obtained.
2. **AMENDMENT:** No amendment or variation of the terms of this Agreement shall be valid unless made in writing, signed by the parties and approved as required. No oral understanding or Agreement not incorporated in the Agreement is binding on any of the parties.
3. **ASSIGNMENT:** This Agreement is not assignable by the Contractor, either in whole or in part, without the consent of the State in the form of a formal written amendment.
4. **AUDIT:** Contractor agrees that the awarding department, the Department of General Services, the Bureau of State Audits, or their designated representative shall have the right to review and to copy any records and supporting documentation pertaining to the performance of this Agreement. Contractor agrees to maintain such records for possible audit for a minimum of three (3) years after final payment, unless a longer period of records retention is stipulated. Contractor agrees to allow the auditor(s) access to such records during normal business hours and to allow interviews of any employees who might reasonably have information related to such records. Further, Contractor agrees to include a similar right of the State to audit records and interview staff in any subcontract related to performance of this Agreement. (Gov. Code §8546.7, Pub. Contract Code §10115 et seq., CCR Title 2, Section 1896).
5. **INDEMNIFICATION:** Contractor agrees to indemnify, defend and save harmless the State, its officers, agents and employees from any and all claims and losses accruing or resulting to any and all contractors, subcontractors, suppliers, laborers, and any other person, firm or corporation furnishing or supplying work services, materials, or supplies in connection with the performance of this Agreement, and from any and all claims and losses accruing or resulting to any person, firm or corporation who may be injured or damaged by Contractor in the performance of this Agreement.
6. **DISPUTES:** Contractor shall continue with the responsibilities under this Agreement during any dispute.
7. **TERMINATION FOR CAUSE:** The State may terminate this Agreement and be relieved of any payments should the Contractor fail to perform the requirements of this Agreement at the time and in the manner herein provided. In the event of such termination the State may proceed with the work in any manner deemed proper by the State. All costs to the State shall be deducted from any sum due the Contractor under this Agreement and the balance, if any, shall be paid to the Contractor upon demand.
8. **INDEPENDENT CONTRACTOR:** Contractor, and the agents and employees of Contractor, in the performance of this Agreement, shall act in an independent capacity and not as officers or employees or agents of the State.
9. **RECYCLING CERTIFICATION:** The Contractor shall certify in writing under penalty of perjury, the minimum, if not exact, percentage of post consumer material as defined in the Public Contract Code Section 12200, in products, materials, goods, or supplies offered or sold to the State regardless of whether the product meets the requirements of Public Contract Code Section 12209. With respect to printer or duplication cartridges that comply with the requirements of Section 12156(e), the certification required by this subdivision shall specify that the cartridges so comply (Pub. Contract Code §12205).
10. **NON-DISCRIMINATION CLAUSE:** During the performance of this Agreement, Contractor and its subcontractors shall not unlawfully discriminate, harass, or allow harassment against any employee or applicant for employment because of sex, race, color, ancestry, religious creed, national origin, physical disability (including HIV and AIDS), mental disability, medical condition (e.g., cancer), age (over 40), marital

status, and denial of family care leave. Contractor and subcontractors shall insure that the evaluation and treatment of their employees and applicants for employment are free from such discrimination and harassment. Contractor and subcontractors shall comply with the provisions of the Fair Employment and Housing Act (Gov. Code §12990 (a-f) et seq.) and the applicable regulations promulgated thereunder (California Code of Regulations, Title 2, Section 7285 et seq.). The applicable regulations of the Fair Employment and Housing Commission implementing Government Code Section 12990 (a-f), set forth in Chapter 5 of Division 4 of Title 2 of the California Code of Regulations, are incorporated into this Agreement by reference and made a part hereof as if set forth in full. Contractor and its subcontractors shall give written notice of their obligations under this clause to labor organizations with which they have a collective bargaining or other Agreement.

Contractor shall include the nondiscrimination and compliance provisions of this clause in all subcontracts to perform work under the Agreement.

11. **CERTIFICATION CLAUSES:** The CONTRACTOR CERTIFICATION CLAUSES contained in the document CCC 307 are hereby incorporated by reference and made a part of this Agreement by this reference as if attached hereto.

12. **TIMELINESS:** Time is of the essence in this Agreement.

13. **COMPENSATION:** The consideration to be paid Contractor, as provided herein, shall be in compensation for all of Contractor's expenses incurred in the performance hereof, including travel, per diem, and taxes, unless otherwise expressly so provided.

14. **GOVERNING LAW:** This contract is governed by and shall be interpreted in accordance with the laws of the State of California.

15. **ANTITRUST CLAIMS:** The Contractor by signing this agreement hereby certifies that if these services or goods are obtained by means of a competitive bid, the Contractor shall comply with the requirements of the Government Codes Sections set out below.

a. The Government Code Chapter on Antitrust claims contains the following definitions:

1). "Public purchase" means a purchase by means of competitive bids of goods, services, or materials by the State or any of its political subdivisions or public agencies on whose behalf the Attorney General may bring an action pursuant to subdivision (c) of Section 16750 of the Business and Professions Code.

2). "Public purchasing body" means the State or the subdivision or agency making a public purchase. Government Code Section 4550.

b. In submitting a bid to a public purchasing body, the bidder offers and agrees that if the bid is accepted, it will assign to the purchasing body all rights, title, and interest in and to all causes of action it may have under Section 4 of the Clayton Act (15 U.S.C. Sec. 15) or under the Cartwright Act (Chapter 2 (commencing with Section 16700) of Part 2 of Division 7 of the Business and Professions Code), arising from purchases of goods, materials, or services by the bidder for sale to the purchasing body pursuant to the bid. Such assignment shall be made and become effective at the time the purchasing body tenders final payment to the bidder. Government Code Section 4552.

c. If an awarding body or public purchasing body receives, either through judgment or settlement, a monetary recovery for a cause of action assigned under this chapter, the assignor shall be entitled to receive reimbursement for actual legal costs incurred and may, upon demand, recover from the public body any portion of the recovery, including treble damages, attributable to overcharges that were paid by the assignor but were not paid by the public body as part of the bid price, less the expenses incurred in obtaining that portion of the recovery. Government Code Section 4553.

d. Upon demand in writing by the assignor, the assignee shall, within one year from such demand, reassign the cause of action assigned under this part if the assignor has been or may have been injured by the

violation of law for which the cause of action arose and (a) the assignee has not been injured thereby, or (b) the assignee declines to file a court action for the cause of action. See Government Code Section 4554.

16. CHILD SUPPORT COMPLIANCE ACT: "For any Agreement in excess of \$100,000, the contractor acknowledges in accordance with Public Contract Code 7110, that:

a. The contractor recognizes the importance of child and family support obligations and shall fully comply with all applicable state and federal laws relating to child and family support enforcement, including, but not limited to, disclosure of information and compliance with earnings assignment orders, as provided in Chapter 8 (commencing with section 5200) of Part 5 of Division 9 of the Family Code; and

b. The contractor, to the best of its knowledge is fully complying with the earnings assignment orders of all employees and is providing the names of all new employees to the New Hire Registry maintained by the California Employment Development Department."

17. UNENFORCEABLE PROVISION: In the event that any provision of this Agreement is unenforceable or held to be unenforceable, then the parties agree that all other provisions of this Agreement have force and effect and shall not be affected thereby.

18. PRIORITY HIRING CONSIDERATIONS: If this Contract includes services in excess of \$200,000, the Contractor shall give priority consideration in filling vacancies in positions funded by the Contract to qualified recipients of aid under Welfare and Institutions Code Section 11200 in accordance with Pub. Contract Code §10353.

19. SMALL BUSINESS PARTICIPATION AND DVBE PARTICIPATION REPORTING REQUIREMENTS:

a. If for this Contract Contractor made a commitment to achieve small business participation, then Contractor must within 60 days of receiving final payment under this Contract (or within such other time period as may be specified elsewhere in this Contract) report to the awarding department the actual percentage of small business participation that was achieved. (Govt. Code § 14841.)

b. If for this Contract Contractor made a commitment to achieve disabled veteran business enterprise (DVBE) participation, then Contractor must within 60 days of receiving final payment under this Contract (or within such other time period as may be specified elsewhere in this Contract) certify in a report to the awarding department: (1) the total amount the prime Contractor received under the Contract; (2) the name and address of the DVBE(s) that participated in the performance of the Contract; (3) the amount each DVBE received from the prime Contractor; (4) that all payments under the Contract have been made to the DVBE; and (5) the actual percentage of DVBE participation that was achieved. A person or entity that knowingly provides false information shall be subject to a civil penalty for each violation. (Mil. & Vets. Code § 999.5(d); Govt. Code § 14841.)

20. LOSS LEADER: If this contract involves the furnishing of equipment, materials, or supplies then the following statement is incorporated: It is unlawful for any person engaged in business within this state to sell or use any article or product as a "loss leader" as defined in Section 17030 of the Business and Professions Code. (PCC 10344(e).)

**EXHIBIT D
(County Agreement)**

SPECIAL TERMS AND CONDITIONS

1. **Excise Tax**

The State of California is exempt from federal excise taxes and no payment will be made for any taxes levied on employees' wages. The State will pay for any applicable State of California or local sales or use taxes on the services rendered or equipment or parts supplied pursuant to this Agreement. California may pay any applicable sales and use tax imposed by another state.

2. **Settlement of Disputes**

In the event of a dispute, Contractor shall file a "Notice of Dispute" with the CDFA within ten (10) days of discovery of the problem. Such Notice of Dispute shall contain the Agreement number. Within ten (10) days of receipt of such Notice of Dispute, the Agency Secretary, or Designee, shall meet with the Contractor and the CDFA project manager for the purpose of resolving the dispute. The decision of the Agency Secretary or Designee shall be final. In the event of a dispute, the language contained within this Agreement shall prevail over any other language including that of the bid proposal.

3. **Agency Liability**

The Contractor warrants by execution of this Agreement, that no person or selling agency has been employed or retained to solicit or secure this Agreement upon agreement or understanding for a commission, percentage, brokerage, or contingent fee, excepting bona fide employees or bona fide established commercial or selling agencies maintained by the Contractor for the purpose of securing business. For breach or violation of this warranty, the State shall, in addition to other remedies provided by law, have the right to annul this Agreement without liability, paying only for the value of the work actually performed, or otherwise recover the full amount of such commission, percentage, brokerage, or contingent fee.

4. **Potential Subcontractors**

If Contractor subcontracts out a portion of the work required by this Agreement, nothing contained in this Agreement or otherwise, shall create any contractual relation between the State and any subcontractors, and no subcontract shall relieve the Contractor of his responsibilities and obligations hereunder. The Contractor agrees to be as fully responsible to the State for the acts and omissions of its subcontractors and of persons either directly or indirectly employed by any of them as it is for the acts and omissions of persons directly employed by the Contractor. The Contractor's obligation to pay its subcontractors is an independent obligation from the State's obligation to make payments to the Contractor. As a result, the State shall have no obligation to pay or to enforce the payment of any moneys to any subcontractor.

5. **Right To Terminate**

The State reserves the right to terminate this agreement subject to 30 days written notice to the Contractor. Contractor may submit a written request to terminate this agreement only if the State should substantially fail to perform its responsibilities as provided herein.

However, the agreement can be immediately terminated for cause. The term "for cause" shall mean that the Contractor fails to meet the terms, conditions, and/or responsibilities of the contract. In this instance, the contract termination shall be effective as of the date indicated on the State's notification to the Contractor.

This agreement may be suspended or cancelled without notice, at the option of the Contractor, if the Contractor or State's premises or equipment are destroyed by fire or other catastrophe, or so substantial damaged that it is impractical to continue service, or in the event the Contractor is unable to render service as a result of any action by a state agency directly impacting the Contractors ability to perform.

EXHIBIT E

ADDITIONAL PROVISIONS

CONTRACTS FEDERALLY FUNDED

It is mutually understood between the parties that this contract may have been written before ascertaining the availability of congressional appropriation of funds, for the mutual benefit of both parties, in order to avoid program and fiscal delays which would occur if the contract were executed after that determination was made.

This contract is valid and enforceable only if sufficient funds are made available to the State by the United States Government for the Fiscal Year 10/11- covered by this agreement for the purposes of this program. In addition, this contract is subject to any additional restrictions, limitations, or conditions enacted by the Congress or any statute enacted by the Congress, which may affect the provisions, terms, or funding of this contract in any manner.

It is mutually agreed that if the Congress does not appropriate sufficient funds for the program, this contract shall be amended to reflect any reduction in funds.

The department has the option to void the contract under the 30-day cancellation clause or to amend the contract to reflect any reduction of funds.

SUBCONTRACTORS

Contractor shall obtain prior approval from CDFA before hiring subcontractors, consultants or both. The total amount of all subcontracts shall not exceed \$50,000 or 25% of the total amount of the contract, whichever is less, unless the Contractor can provide certified documents that award was made through a competitive bidding process requiring at least three bids from responsible bidders.

All subcontractors identified shall be experts in their respective disciplines and capable of performing the tasks for which they were hired. Subcontractors shall have extensive experience in their area of expertise, with particular emphasis on prior experience on similar programs or projects that clearly illustrate their expertise in areas essential to the Contractor and to CDFA.

INSURANCE REQUIREMENTS – Contractor shall comply with all requirements outlined in the (1) General Provisions section and (2) Contract Insurance Requirements outlined in this section. No payments will be made under this contract until contractor fully complies with all requirements.

1. General Provisions Applying to All Policies

- a. Coverage Term – Coverage needs to be in force for the complete term of the contract. If insurance expires during the term of the contract, a new certificate must be received by the State at least ten (10) days prior to the expiration of this insurance. Any new insurance must comply with the original contract terms.
- b. Policy Cancellation or Termination & Notice of Non-Renewal – Insurance policies shall contain a provision stating coverage will not be cancelled without 30 days prior written notice to the State. New certificates of insurance are subject to the approval of the Department of General Services and the Contractor agrees no work or services will be performed prior to obtaining such approval. In the event Contractor fails to keep in effect at all times the specified insurance coverage, the State may, in addition to any other remedies it may have, terminate this Contract upon the occurrence of such event, subject to the provisions of this Contract.
- c. Premiums, Assessments and Deductibles – Contractor is responsible for any premiums, policy assessments, deductibles or self-insured retentions contained within their insurance program.
- d. Primary Clause – Any required insurance contained in this contract shall be primary, and not excess or contributory, to any other insurance carried by the State.

- e. Insurance Carrier Required Rating – All insurance companies must carry an AM Best rating of at least "A–" with a financial category rating of no lower than VI. If the Contractor is self insured for a portion or all of its insurance, documentation of self-insurance must be submitted and approved by the Department of General Services, Office of Risk and Insurance Management.
- f. Endorsements – Any required endorsements requested by the State must be physically attached to all requested certificates of insurance and not substituted by referring to such coverage on the certificate of insurance.
- g. Inadequate Insurance – Inadequate or lack of insurance does not negate the contractor's obligations under the contract.
- h. Use of Subcontractors - In the case of Contractor's utilization of subcontractors to complete the contracted scope of work, contractor shall include all subcontractors as insured's under Contractor's insurance or supply evidence of subcontractor's insurance to The State equal to policies, coverages, and limits required of Contractor.
 - The policy must name **The County and State of California, its officers, agents, employees and servants as additional Insureds, but only with respect to work performed under the contract.**

2. Contract Insurance Requirements

Contractor shall display evidence of the following on an Acord certificate of insurance or documentation of self insurance on county letterhead evidencing the following coverages:

- a. Commercial General Liability – Contractor shall maintain general liability on an occurrence form with limits not less than \$1,000,000 per occurrence for bodily injury and property damage liability combined with a \$2,000,000 annual policy aggregate. A "per project aggregate" endorsement is required. The policy shall include coverage for liabilities arising out of premises, operations, independent contractors, products, completed operations, personal & advertising injury, and liability assumed under an insured contract. This insurance shall apply separately to each insured against whom claim is made or suit is brought subject to the Contractor's limit of liability.
 - The policy must name **The State of California, its officers, agents, employees and servants as additional insureds, but only with respect to work performed under the contract.**
- b. Automobile Liability – Contractor shall maintain business automobile liability insurance for limits not less than \$1,000,000 combined single limit. Such insurance shall cover liability arising out of a motor vehicle including owned, hired and non-owned motor vehicles. Should the scope of the Contract involve transportation of hazardous materials, evidence of an MCS-90 endorsement is required.
- c. Workers Compensation and Employers Liability – Contractor shall maintain statutory worker's compensation and employer's liability coverage for all its employees who will be engaged in the performance of the Contract. In addition, employer's liability limits of \$1,000,000 are required.
- d. Environmental/Pollution Liability - If hazardous materials work is required, then the contractor shall maintain Environmental/Pollution Liability for limits not less than \$1,000,000 per claim covering the contractor's liability for bodily injury, property damage and environmental damage resulting from pollution and related cleanup costs incurred arising out of the work or services to be performed under this contract. The policy must include:

- The State of California, its officers, agents, employees and servants as additional insured, but only with respect to work performed under this contract. This **endorsement** must be supplied under form acceptable to the Office of Risk and Insurance Management of the California Department of General Services.

Coverage shall be provided for both work performed on site and during transportation of as well as proper disposal of hazardous materials. Proof of pollution during transportation shall be provided on an MCS-90 form or equivalent.



**United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine**



**Official Regulatory Protocol for Wholesale and Production Nurseries
Containing Plants Infected with *Phytophthora ramorum***

**Confirmed Nursery Protocol: Version 8.0
Revised March 31, 2010**

**(Appendix 1 updated March 2010, Appendices 3, 6 & 7 updated June 26, 2008;
Appendix 11 modified June 23, 2010)**

**United States Department of Agriculture (USDA)
Animal Plant Health Inspection Service (APHIS)
Plant Protection and Quarantine (PPQ)**

**Center for Plant Health Science and Technology (CPHST)
Emergency and Domestic Programs (EDP)
Eastern Region (ER)
Western Region (WR)**

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INTENDED USE

In February 2005, USDA Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) published an interim rule revising federal domestic regulations for *Phytophthora ramorum* (7 CFR 301.92). The complete text and other information may be found at the USDA APHIS PPQ web site:

http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/

Since the regulations were first published in 2002, *P. ramorum* has been detected in a significant number of nurseries. These detections prompted the need for a standard protocol for use by state and federal regulators to respond to finds of *P. ramorum* in nurseries. To ensure that there is consistency in responding to infestations of *P. ramorum*, this protocol describes the official activities performed within and around nurseries by USDA APHIS staff in cooperation with state agriculture regulatory officials.

The goal of this protocol is to ensure that any infestations of this serious pathogen are consistently and effectively addressed, mitigated, and eradicated. Cooperation by nursery management personnel is essential. Early detection and reporting of *P. ramorum* finds are critical to ensure that the infestation is contained and spread is minimized. The strategies employed in this protocol are consistent with those of the European Union, Canada, and other areas where eradications are being carried out with measures that ensure rapid suppression of infection, and which prevent the spread of the pathogen.

P. ramorum infestations in nurseries may be introduced via three critical pathways.

- The movement of infected plant material from one nursery to another;
- The natural environmental movement of spores from a nursery or infected wild plants to infect plants in a nursery;
- The transmission of the pathogen from non-plant pathways to plant material (e.g. the introduction of infested soil, water, growing media, equipment, etc.)

Other pathways are possible, but are not yet known.

Nurseries found with *P. ramorum* infestations more than once

P. ramorum infestations in nurseries may also be re-introduced by the above means, or the effort to eradicate the disease may fail. In the event that a nursery has *P. ramorum* detected on site after the initial release from the Emergency Action Notification (EAN) or state equivalent, it is necessary to implement additional measures to ensure that the risks associated with *P. ramorum* are properly mitigated. See **Appendix 11** for details of these additional measures.

GOAL

The goal of this protocol is to find and eradicate the pathogen in nurseries. Any interpretation of this protocol or its procedures that are not consistent with this goal is a misinterpretation of this protocol

DISCLAIMERS

FIELD GROWN STOCK: We have received comments that this protocol fails to adequately address situations found in nurseries with field grown stock. We recognize this limitation and leave it to field personnel to properly adapt this protocol to those situations when they occur until appropriate modifications can be incorporated.

RETAIL SITES: We recognize that we need a protocol for retail nurseries. Until that can be issued, regulatory officials must use this protocol and apply it to each situation.

CHALLENGES: *P. ramorum* is a microorganism. Thus it can be elusive and difficult to detect and difficult to eradicate. It can infect plants, infest media, soil and water and persist despite best intentions and best efforts. It can wash into nearby waterways and can be expected to do so and be present during eradication and monitoring procedures. Scientists continue to learn and report on basic biology and enhanced detection and eradication techniques. We continue to learn from science and our successes and failures and those will be reflected in updated protocols and regulations.

DEFINITIONS

| | |
|---|--|
| Associated plants: | Associated plants are those reported found naturally infected and from which <i>P. ramorum</i> has been cultured and/or detected using PCR (Polymerase Chain Reaction). For each of these, traditional Koch's postulates have not yet been completed or documented and reviewed. See Appendix 1. |
| Biosecurity measures: | Actions taken to reduce or mitigate the potential introduction or spread of <i>Phytophthora ramorum</i> from one area or site to another area or site of a nursery. See Appendix 9. |
| Compost pile: | A heap of mixture of decaying organic matter, as from leaves and manure, used to improve soil structure and provide nutrients. |
| Cull pile: | An area where discarded plant material is deposited. Also known as a waste or trash pile. |
| 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. |
| Destruction block: | Block of plants to be destroyed. Within a nursery, this is a contiguous block of HAP containing one or more plants known to be infected with <i>P. ramorum</i> . The block will be considered contiguous until there is a 2 meter break of either no plants or no HAP. |
| Emergency Action Notification (EAN): | PPQ Form 523 or equivalent State document, is used to specify the regulatory actions to be taken within a nursery. |
| Free from: | Without pests (or a specific pest) in numbers or quantities that can be detected by the application of phytosanitary procedures. (ISPM Pub. No. 10, 1999) |
| HAP: | Host and associated host plants listed on the official APHIS List of Regulated Hosts and Plants Associated with <i>Phytophthora ramorum</i> . |
| Hold block: | This term no longer in use; See Quarantine Block. |
| Host plants: | Naturally infected plants verified with completion, documentation, review and acceptance of traditional Koch's postulates and listed in the "APHIS List of Regulated Hosts and Plants Associated with <i>Phytophthora ramorum</i> ". |

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| Infected plants: | Plants officially confirmed as being infected with <i>P. ramorum</i> , based on the use of APHIS approved diagnostics, and following the PASS system. |
| Nursery/Facility: | Any location where nursery stock is grown, propagated, stored, or sold; or any location from which nursery stock is distributed, including locations that grow trees to be sold without roots, such as Christmas trees. |
| Nursery block: | A contiguous grouping of plants separated by at least two meters from other contiguous groupings of plants. |
| Nursery site: | A geographically separate location of a Nursery/Facility that has a distinct physical address and appropriate biosecurity measures (See Appendix 9) to prevent the movement of <i>P. ramorum</i> between locations. |
| 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 <u>Nursery Delimitation Survey is completed</u> and lasts for 90 days during which proscribed activities must occur. During the quarantine period, inspection, sampling, and testing must reveal no further detection of <i>P. ramorum</i> . Conducive conditions exist when climatic conditions match <u>optimum disease etiology and are likely to express disease symptoms 50% or more of the time</u> . |
| Nursery stock: | Any plants for planting, including houseplants, propagative material that are grown in a nursery and tree seedlings for reforestation. |
| Parallel quarantine: | A quarantine or regulation imposed by a State or local plant regulatory authority that is essentially the same as a federally promulgated quarantine. These regulations can be more restrictive for intrastate movement and internal controls. |
| PASS (Potentially Actionable Suspect Sample): | A presumptive positive <i>P. ramorum</i> sample diagnosed or identified by a provisionally approved laboratory or diagnostician with identification authority that would require confirmatory testing by an official APHIS Laboratory due to the nature of the plant sampled and the necessity for Federal confirmation. (For more information see: "PASS System Policy" at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/protocols.shtml) |

- Presumptive positive:** A preliminary diagnostic test result from a laboratory indicating *P. ramorum* is present.
- Quarantine block:** Area identified as a 10 meter radius around the destruction block (see Appendix 2) designed to determine if *P. ramorum* has spread beyond the destruction block. (Use of Quarantine block is an adaptation from the definition: "An area in which a specific pest does not occur, or occurs at a low level and is officially controlled, that either encloses or is adjacent to an infested area, an infested place of production, a pest-free area, a pest-free place of production or a pest-free production site, and in which phytosanitary measures are taken to prevent spread of the pest." [ISPM Pub. No. 10, 1999]).
- Quarantine period:** A minimum of 90 days that begins when the Nursery Delimitation Survey is completed and lasts until such time as both plant parts and climatic conditions conducive to disease expression have occurred. During the **quarantine period**, inspection, sampling, and testing must reveal no further detection of *P. ramorum*. Conducive conditions exist when climatic conditions match optimum disease etiology and are likely to express disease symptoms 50% or more of the time.
- Quarantine release survey:** This is the second quarantine period inspection that occurs near the end of the quarantine period. This survey includes visually inspecting all HAP genera within the nursery and sampling any unhealthy plant tissue, soil of destruction and quarantine block(s) and drainage or recirculated irrigation water, as per Appendices 4, 6 and 7, respectively. When the quarantine period is completed and all plant, soil and water samples taken are negative for *P. ramorum* the nursery can be released.
- Regulated area:** Any state, or portion of a state, in which only nurseries that ship HAP interstate are regulated to prevent the spread of *P. ramorum* and the only regulated article is nursery stock. These areas are detailed in the regulations posted at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/
- SPHD:** The State Plant Health Director of a particular state. Lead APHIS contact in each state responsible for overseeing all Plant Protection and Quarantine activities in that state.
- SPRO:** The State Plant Regulatory Official in any given state's department of agriculture. This is the person primarily responsible for plant health programs in that state. SPROs can be found listed at: www.nationalplantboard.org/member/index.html

TRIGGER EVENTS FOR USE OF PROTOCOL

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. Confirmed samples must have been diagnosed using a methodology approved by USDA, APHIS, PPQ and consistent with the Potentially Actionable Suspect Sample (PASS) protocol*.

*See http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/ for links with details on trace forward survey, trace back survey, *P. ramorum* nursery survey, and the PASS protocol.

AUTHORITIES

- For states with quarantines equivalent to the Federal regulation, State personnel will conduct specific actions required by the protocol, within and around the nursery, under State authority with Federal support.
- For States without quarantines for *P. ramorum* equivalent to the Federal regulations, specific actions required by this protocol within and around the nursery will be conducted under Federal authority, in cooperation with State personnel.

COMMUNICATE AND NOTIFY

Communicate suspect finds using the bullets below as soon as one of the following has occurred:

1. A positive PCR determination
 2. A culture that matches the morphology for *P. ramorum* (i.e. isolation of *P. ramorum*)
- Immediately notify the State Plant Health Director (SPHD) and the State Plant Regulatory Official (SPRO) of the State in which the nursery is located. The SPHD will notify the Regional Office and National Headquarters Office. See Appendix 3, Resource and Contact List.
 - SPHD's and SPRO's, shall notify facilities within their states that are impacted by the trace backs and trace forwards and provide a list of these facilities to their PPQ Regional offices. See "Conduct Investigations" Section.
 - Laboratories need to notify, the SPHD, and the SPRO, the Regional Office, National Program Manager, and the submitter. Ideally the SPRO should notify the owner of the nursery, but either the SPRO (if State authority is used) or the SPHD (if Federal authority is used) may notify the owner of the nursery.
 - The SPRO and SPHD will use state channels, including public affairs offices to make any public announcements, as necessary. The SPHD will ensure that the USDA APHIS Office of Legislative and Public Affairs is aware of any pending release, via the Regional Office and National Headquarters Office.

CONDUCT INVESTIGATIONS

Trace Forward Investigation:

Initiate trace forward investigations. Identify all domestic and international shipments of the High Risk HAP Genera: *Camellia*, *Rhododendron*, *Pieris*, *Viburnum* and *Kalmia* and HAP shipments of the infected plant species within the 6 months prior to the first positive detection of *P. ramorum* at the nursery as per the protocol. This information on shipments needs to be gathered, processed, and forwarded to Regional Office **within 10 working days**. Reporting trace forwards within 10 days is essential. If requested or necessary, Smuggling Interdiction and Trade Compliance (SITC) or Investigative and Enforcement Services (IES) may be asked to assist in the information gathering, as appropriate. The Regional Offices will forward these domestic lists to the States that have received plants. Headquarters will inform international trading partners of shipments to their countries. The plants sent to the receiving States must be inspected at the receiving nurseries.

Use the Trace Forward Protocol posted at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/

Trace Back Investigation:

Implement the current Trace Back Protocol present on the *Phytophthora ramorum* website located at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/

Nursery Sites:

Determine whether additional locations (nursery sites) are maintained by the same nursery personnel, or if HAP move to other sites or between sites.

- **Equipment:** Determine if equipment used at the site is shared with other nursery sites or field areas. Document any shared equipment utilization in different nursery sites or field areas. Equipment movement without appropriate biosecurity measures (See Appendix 9) between nursery sites requires that all nursery sites utilizing the equipment be included under this protocol.
- **Plants:** Determine if HAP move between sites. If so, than all sites receiving HAPs must be included under this protocol.

SECURE THE NURSERY

When the presence of *Phytophthora ramorum* has been confirmed in a nursery:

- All plants (including non-host plants) in the destruction block shall remain under regulatory control as per the Emergency Action Notification (EAN) or State equivalent document. All plants within the destruction block shall be cordoned off with no unauthorized access until delimitation survey is complete and all destruction block(s) is(are) defined.
- All HAP genera in the nursery are to be placed under regulatory control as per EAN. This action may also include any item that an inspector determines to present a risk of spreading *P. ramorum* within or from the nursery; and,
- A delimitation survey will take place on the nursery site as per this protocol; and,
- All HAP genera must be held until delimitation within the nursery is complete, that is, until the samples taken have diagnoses reported that allow release of blocks of HAP. This hold may also include “any other product or article that an inspector determines to present a risk of spreading *Phytophthora ramorum*, if an inspector notifies the person in possession of the product or article that it is subject to the restrictions in the regulations” (7CFR part 301.92-2) within the infested nursery site; and,
- Secure the cull pile until all testing is complete.
- Ensure that equipment used on nursery site is not moved from the site without proper disinfection.
- Any additional treatments and/or basic sanitary and precautionary measures shall be detailed on the EAN.
 - PPQ form 523, Emergency Action Notification will be used as the official Federal authorization of hold. The required treatments and/or basic sanitary and precautionary measures (e.g. bio-containment of suspected infected material, etc.) should be included in the PPQ form 523. If the State initiated action, then the appropriate State notification would be used. Stop Sales notices should be placed on the nursery by the appropriate State Regulatory Official.
- If any plants not on hold are showing symptoms consistent with diseases caused by *P. ramorum*:
 - These plants must be sampled and tested for the presence of *P. ramorum*.

SURVEY THE NURSERY AND PERIMETER

The goal of the survey is to locate *P. ramorum* in the nursery and perimeter. A detailed and thorough inspection should be conducted at the field level to determine the presence of *P. ramorum*. Samples should be collected from unhealthy looking plants, including any plants with any minute symptoms such as tiny leaf spots or brown leaf tips.

Delimiting Survey and Establishing Destruction and Quarantine Block(s):

- Inspect all plants held, for sale or propagation, of HAP genera in the nursery and decorative plants (permanent landscape plants within the nursery that are not for sale).
- Examine all HAP genera within 10 meters of the positive block(s) in the nursery as per Appendix 4. Sample any unhealthy tissue.
- All HAP genera within 10 meters of the positive block(s) shall be considered exposed to *Phytophthora ramorum* and shall be held for the quarantine period.
- Examine all plants within the nursery and sample any unhealthy plant tissue found. A minimum of 40 samples shall be taken in a small* nursery, 80 samples in a medium* nursery and 120 samples in a large* nursery. These are absolute minimums. To assure proper delimiting it is expected that the actual numbers will commonly be in the hundreds.
- Samples must be analyzed using a methodology approved by APHIS (see Appendix 5).
- The destruction and quarantine block(s) is (are) established when diagnostic results from all delimiting samples have been reported. The 90 day quarantine period begins when the delimiting survey is complete.
- Establish destruction block(s) by flagging the perimeter of the block(s) of HAP containing one or more plants known to be infected with *P. ramorum*. The block is considered contiguous until there is a 2 meter break of either no plants or no HAP.
- Limit access to destruction block. Ensure that proper sanitation measures are applied (See Appendix 8).
- The HAP (note: not all plants nor all HAP genera) in the destruction block shall be destroyed in an appropriate manner (see Appendix 8)

*A small nursery can be considered one containing less than 200,000 HAP plants. A medium sized nursery can be considered as one containing more than 200,000 and less than 999,999 HAP plants. A large sized nursery can be considered as one containing more than 1,000,000 HAP plants.

Soil and Growing Media Sampling:

- Soil from within the destruction and quarantine block(s) must be sampled, and

- Growing media from non-HAP within the destruction block(s) and from all types of plants in the quarantine block(s) must be sampled, and
- Soil and growing medium from nursery blocks down slope from destruction and quarantine block(s) must also be sampled.
- Growing media from the plant potting area shall be sampled.
- Soil is the substrate underneath pots and growing medium is located within pots with the plants in the blocks.
- If reported positive, determine the content, origin, storage and handling of growing media used at the nursery site. See Appendix 6 for detailed soil and media sampling protocol. Keep soil samples separate from growing media samples.

Water Sampling:

Determine the source of water used at the nursery site and where drainage water flows. Note the type of irrigation system(s) in use, areas of standing water and any safeguards against water back flow in the irrigation system, as well as any water treatment practices if recirculated water is used. Water is to be sampled; See Appendix 7 for detailed water sampling protocol. Water sampling is not required for irrigation water from municipal water facilities that treat their water prior to release, but any retention pond or area where water collects at the nursery site must be sampled.

Cull Pile Sampling:

Record the location of any cull piles as these may be contaminated with infected plant material or associated soil and/or growing media. Check any cull piles for *P. ramorum* symptomatic plants and plant material and sample if observed. Determine how the nursery disposes of culled plant material. Sample and test soil at the down slope edge of the cull pile for the presence of *P. ramorum*.

Compost Pile Sampling:

Record the location of any compost piles as these may be contaminated with infected plant material or associated soil and/or growing media. Check any compost piles for *P. ramorum* symptomatic plants and plant material and sample if observed. Determine how the nursery disposes of composted plant material. Sample and test soil at the down slope edge of the compost pile for the presence of *P. ramorum*.

Perimeter Survey:

The purpose of the perimeter survey is twofold: (1) to ensure that *P. ramorum* has not spread from the infested nursery to the surrounding environment and (2) to verify that the infection in the nursery did not originate in the surrounding environment. Conduct a survey concentrating on plants of all HAP genera located within 10-meters of the infested nursery for symptoms of disease caused by *P. ramorum*. Sample all plants with suspicious symptoms. Samples must be labeled and sent to a laboratory for testing using a method approved by APHIS (see Appendix 5). Detection of *P. ramorum* in the perimeter may be indicative of a more widespread infestation. In this case, notify your PPQ Regional Office immediately as further regulatory actions may be required depending on the quarantine status of the area.

DISINFEST THE NURSERY

Plant Destruction:

Where a *P. ramorum* infected plant(s) is found, all HAP and plant parts within a destruction block will be removed and destroyed using one or more of the techniques detailed in Appendix 8.

Debris Removal:

All plant debris including growth medium, leaves, stems, flowers, roots, and any other plant parts found within the destruction block will be removed and destroyed using one or more of the techniques detailed in Appendix 8.

Cull Pile Treatment:

If any plants, plant material, growing media or soil from a cull pile is positive for *P. ramorum*, all material in the cull pile shall be properly disposed. See Appendix 8 for recommended destruction/disinfestation options.

Compost Pile Treatment:

If any plants, plant material, growing media or soil from a compost pile is positive for *P. ramorum*, all material in the compost pile shall be properly disposed. See Appendix 8 for recommended destruction/disinfestation options.

Non-porous Surfaces:

Non-porous surfaces will be disinfested. See Appendix 8 for recommended disinfestation options.

Water Treatment:

If water tests positive for *P. ramorum*, treatment is required (see Appendix 8 for recommended disinfestation options) and an additional delimitation of the nursery must be completed. For nurseries with established quarantine block(s) undergoing a 90 day quarantine period, the 90 day quarantine period re-starts after the second delimiting survey is completed. Also, plants and growing media that may have been irrigated with infested water must also be resampled and retested within the new 90 day quarantine period.

Soil and Growing Media Treatment:

If soil, growing media or plant debris in a destruction or quarantine block test positive, soil treatment is required. The destruction block is the most likely area of soil or growing media infestation (underneath and around the diseased plants, and in containerized stock) and the most likely area where reinfestation of new host material would occur. See Appendix 8 for recommended destruction/ disinfestation options.

Equipment and Personnel:

See Appendix 8 for recommended disinfestation options.

Biosecurity Measures:

Biosecurity measures are designed to minimize the risk of introduction or, spread and survival of the pathogen in a nursery. See Appendix 9 for recommended biosecurity measures.

NINETY (90) DAY QUARANTINE ACTIVITIES

These concurrent activities follow completion of the delimiting survey:

- Any non-HAP that were present in a destruction block will be held in place, or moved under official supervision to a safeguarded area with a non-porous surface, during the quarantine period and be subject to the same conditions as the HAP in the quarantine block(s).
- For nurseries with HAP genera in the quarantine block(s) (see Appendix 2), these HAP genera shall not be moved within or out of the quarantine block(s) during the quarantine period. This quarantine period begins when the delimiting survey is completed (i.e. the last sample is taken and an EAN is issued) and lasts until such time as both plant parts and climatic conditions conducive to disease expression have occurred for at least 90 days. If the quarantine period (90 days) does not include climatic conditions conducive for disease development then the quarantine period shall be extended to an appropriate length to include conducive climatic conditions for a total of 90 days. During the quarantine period, inspection, sampling, and testing must reveal no further detection of *P. ramorum*.
- During the 90 day quarantine period within the 10 meter quarantine block(s):
 - No fungicides registered for *Phytophthora* control shall be applied.
 - Regulatory officials will visually inspect plants a minimum of two times, once about half-way through the anticipated quarantine period and once near enough to the end to have test results coincide with the end of the quarantine period, according to the protocol detailed in Appendix 4. This second visual inspection in the quarantine block(s) can be done at the same time as the quarantine release survey as described below. A minimum of 40 samples shall be taken in a small* nursery, 80 samples in a medium nursery and 120 samples in a large nursery. These are absolute minimums.
 - Regulatory officials will collect water, soil, and media samples and test during the quarantine period according to the protocols detailed in Appendices 6 and 7.

If found positive:

- If a plant sample tests positive for *P. ramorum*, the destruction block(s) and 10 meter quarantine block(s) shall be redefined via sampling and the quarantine period reset.
- If water, soil, and/or media samples tested positive for *P. ramorum* during the delimiting survey, it must be treated per Appendix 8. Once successfully 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 Appendices 6 and 7.

- 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 re-delimited.
- If a soil sample is found to be positive, the soil shall be treated, then any plants in the block with the infested soil are placed on hold and the area re-delimited.
- The growing media in the potting shed must be tested. Any positives for *P. ramorum* from the media in the shed confer with the Regional Program Manager.
- **A quarantine release survey of the entire nursery must be completed near the end of the 90 day quarantine period.** This survey includes visually inspecting all HAP genera within the nursery and sampling any unhealthy plant tissue, soil of destruction and quarantine block(s) and drainage or recirculated irrigation water. When the quarantine period is completed and all plant, soil and water samples taken are negative for *P. ramorum* the nursery can be released.

RELEASE THE NURSERY

Nurseries and their plants that have been placed under regulatory control may be released from regulatory control by USDA-APHIS or its designated authority after the quarantine period if the following three conditions are met:

- There are no additional detections of *P. ramorum* in nursery stock based on USDA APHIS approved plant inspection, sampling and testing protocols for the preceding quarantine period; and
- Water, soil and growing media have also tested negative for *P. ramorum* based on USDA APHIS approved sampling and testing protocols for the preceding quarantine period; and
- The quarantine release survey is negative for *P. ramorum*.

Alternative Release Strategy:

A nursery may avoid a quarantine period, through a voluntary management decision, by:

- Destroying everything (all plants, pots, media, etc.) in the destruction block(s); and
- Destroying the HAP genera and plant parts in the quarantine block(s); and
- Visually inspecting all HAP genera within the nursery and sampling and testing any unhealthy plant tissue, soil of destruction and quarantine block(s) and drainage or recirculated irrigation water, as per Appendices 4, 6 and 7, respectively. If plant, soil and water samples taken are negative for *P. ramorum* the nursery can be released., and
- Revisit the nursery after approximately 90 days of conducive conditions and conduct at least a nation-wide survey level inspection to include sampling of the soil in the destruction block.

POST ERADICATION MONITORING

Nurseries that have been infested will continue to be monitored when disease expression is anticipated for the following two years at the nursery survey protocol levels. These nurseries are not under any quarantine or regulatory action, unless there are additional detections.

CONFIRMED NURSERY PROTOCOL FLOWCHART

A flow chart of these protocols is shown in Appendix 10.

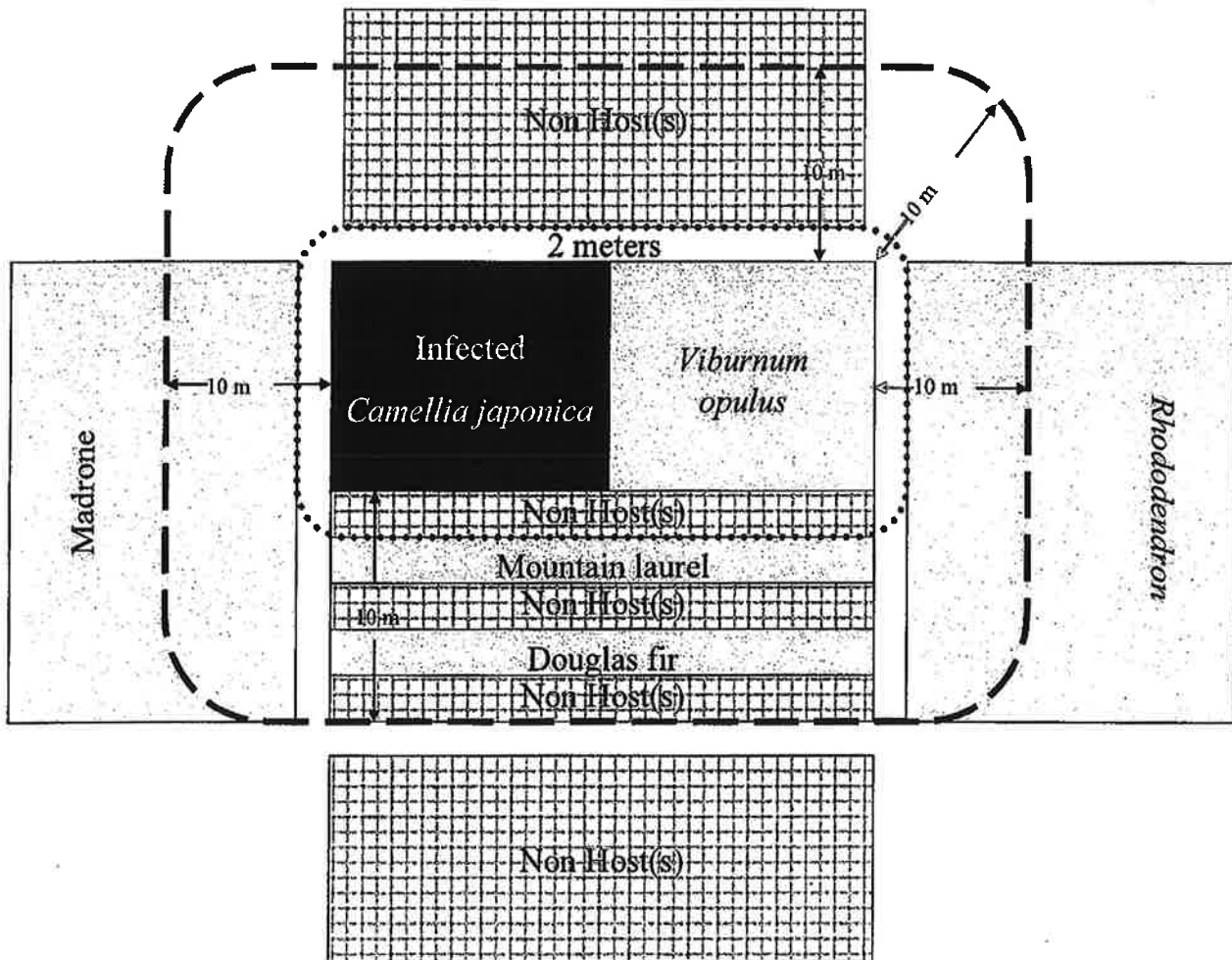
APPENDIX 1


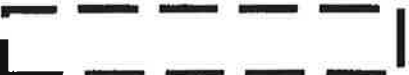
APHIS List of Regulated Hosts and Plants Associated with *Phytophthora ramorum*

A current list may be found at the USDA APHIS PPQ website at
http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/

APPENDIX 2

Schematic of Wholesale/Production Nursery with Infected Host Plant(s)
 Revised: August 31, 2006



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|  | Destruction Block Action: Destroy <i>Camellia japonica</i> and <i>Viburnum opulus</i> . Hold and monitor all non-hosts. |
|  | Quarantine Block Action: Hold and monitor all Mountain laurel and Douglas fir, as well as some Madrone and Rhododendron. |

APPENDIX 3

Resource and Contact List

Revised: May 2007

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APPENDIX 4

Delimiting Survey Protocol

Delimiting Survey Protocol to Detect *Phytophthora ramorum*
In Plants at Confirmed Nurseries
Revised: July 19, 2007

Objective:

The objective of this document is to provide guidelines for the delimiting survey in nurseries where the regulated pathogen, *Phytophthora ramorum* has been confirmed. This survey method is designed using the best available scientific principles to determine apparent freedom from *P. ramorum* in nursery plants. In order to achieve this freedom from *P. ramorum*, accurate and successful inspection of HAP (genera for wholesale/production) must be accomplished at an appropriate confidence level to ensure detection of disease.

Sampling method:

The goal is targeted sampling of plant tissue to determine the presence of *P. ramorum* with a 95% confidence of finding the disease at a very low level (0.5% of plants are infected with *P. ramorum*) by inspecting a minimum of 850 HAP plants in each block (or all the plants if there are less than 850). A physical sample of the inspected plant is only to be taken if unhealthy plant tissue is present. * ~~Do not sample asymptomatic plants.~~

*It is expected that if there is sufficient unhealthy plant tissue, the minimum sample rate, as specified in the Official Regulatory Protocol for Wholesale and Production Nurseries Containing Plants Infected with *Phytophthora ramorum*, shall be adhered to. A minimum of 40 samples shall be taken in a small* nursery, 80 samples in a medium* nursery and 120 samples in a large* nursery. These are absolute minimums. To assure proper delimiting it is expected that the actual numbers will commonly be in the hundreds.

- Inspector should contact the nursery manager to set up the inspection and find out approximately how many HAP are present in each nursery block (i.e. a nursery map).
- These visually inspected plants should be chosen at random, but if certain areas of the block contain plants exhibiting unhealthy tissue or are more prone to disease development (such as low areas where water might puddle or places where mist or fog persists) these areas should be included in the sampling process.
- Disposable rubber gloves and tyvek booties should be worn and should be changed or disinfested using 10% bleach solution or a quaternary ammonium solution (at the labeled rate) between each block. Additionally, waterproof raingear and rubber boots may be used and disinfested between each block. Washtubs with ~ 1/2 inch of disinfectant to step in for booties and 3 inches in buckets to dip gloved hands should be sufficient.

- To visually inspect a plant, carefully lift the plant from surrounding plants, if possible, and carefully examine all plant leaves and stems for unhealthy tissue particularly for the presence of water-soaked or necrotic lesions consistent with *P. ramorum* infection, however all unhealthy tissue should be considered suspect. Take care to examine the leaves on the interior as they may exist in a microclimate more conducive to disease development and may be more likely to have disease symptoms. Be sure to properly disinfest booties and gloves between all nursery blocks. Because this is a confirmed nursery, proper use of sanitation is imperative to reduce the potential for pathogen transport from an infested part of the nursery to an un-infested nursery block.
- Sample plant tissue from any and all visually inspected plants that appear unhealthy. Each sample should consist of a minimum of five leaves; for *Vaccinium* and other small leaf hosts collect the terminal last 3 inches of branch tips, if present, from each unhealthy plant. If, however, only one leaf is unhealthy include only the one leaf with lesions. Examine any other leaves on the plant for the presence of lesions, because chances are much smaller lesions may be present on other leaves of the same plant.
- Samples should be placed in a re-sealable leak proof plastic bag labeled with the appropriate nursery designation and sample number. Samples should be double-bagged in an additional re-sealable leak proof plastic bag with a completed PPQ391 form for each sample submitted.
- Keep the samples cool by placing them in a cooler (around 3° – 6° C or 37 – 43 F).
- Overnight mail or deliver the sample to the laboratory as soon as possible to preserve freshness.
- All samples must be analyzed following the APHIS diagnostic protocols.
- Continue inspecting 850 plants in each block that contains HAP (genera for wholesale/production).
- Examine all HAP (genera for wholesale/production) in cull piles for the presence of tissue symptomatic for *P. ramorum* and take symptomatic tissue from any and all plants with symptoms.

APPENDIX 5

Diagnostics

Revised: April 2007

Samples must be analyzed using a methodology approved by APHIS. See techniques posted at:
http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/

APPENDIX 6

Soil and Growing Medium Sampling Protocol Revised April 22, 2008

See http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/ for latest approved protocol.

Soil and Growing Media Sampling:

- Infested soil or growing media will look exactly the same as un-infested soil or growing media. Therefore all soil and media must be handled carefully. All tools used to collect soil or media samples must be disinfected with 10% bleach solution, quaternary ammonium solution or flame-sterilized with a propane torch between blocks. All soil and organic material should be removed from the tools prior to disinfection. Care should also be taken not to transfer soil or growing media from one block to the next on shoes or clothing. All sampling equipment should be cleaned and disinfected prior to entering a new nursery block. Care must be taken to ensure that un-infested soil or growing media is not contaminated by infested soil or growing media. If the areas of soil/media infestation are known or suspected sample these quarantine block and work toward the destruction block(s).

Preparing for sampling:

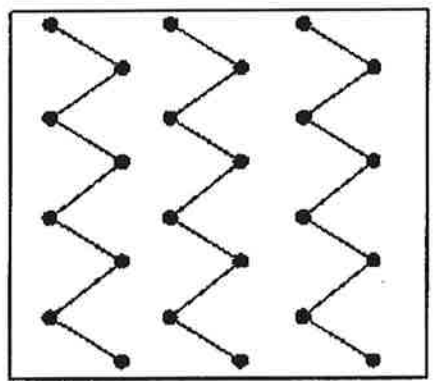
- Soil and growing media samples should be collected as composite samples. Composite samples of growing media should be kept separate from soil samples. A composite sample consists of a mixture of sub-samples. Sub-samples (See Figure 1) are small amounts of soil (or media) removed from the ground (or pot) and added together to form a composite sample. The use of sub-sampling increases the chances of finding *P. ramorum* if it is present. Samples should contain a maximum of 500-ml (volume) of soil and/or growing media (1/2 of a quart-size Ziploc bag). The number of composite samples collected will depend upon the size of the nursery block being sampled (see Table 1). There should be at least two samples, one for growing media and one for soil, unless all plants and associated growing media were destroyed or the plants are not on soil (e.g. on concrete or asphalt). If the surface of soil is covered with gravel take sub-samples from the soil beneath the gravel. If water permeable weed block is present, either covered with gravel or under gravel, the weed block should be removed prior to soil sampling.

Table 1: Number of composite samples collected based on nursery block size.

| Size of Treated Site (acres) | Sq Ft | No. of Soil and Growing Media Samples Collected (total) |
|------------------------------|-----------------------|---|
| $0.00 < n < 0.25$ | $n < 10,890$ | 5 (10) |
| $0.25 < n < 0.5$ | $10,890 < n < 21,780$ | 10 (20) |
| $0.50 < n < 1.0$ | $21,780 < n < 43,560$ | 20 (40) |
| $n > 1.0$ | $n > 43,560$ | 30 (60) |

- Each composite sample will consist of at least five sub-samples collected from soil or growing media within the targeted area. While five is a minimum, it is preferable to take 24 sub-samples of soil or growing media for each sample, provided the area is large enough (for soil samples) and enough plants are present (for growing media samples). Sub-samples should be collected according the pattern in the diagram below (Figure 1). Alternatively, if fallen leaves or other debris from the infected plants are present; sub-sampling may be targeted towards those areas. The location of each composite sample should be maintained (preferably by GPS but at least by flagging) in case follow-up treatment of the soil or growing media for *P. ramorum* is required. Composite samples may also be collected from neighboring blocks of un-infested plants using the same steps. If you are collecting from blocks of un-infested plants, collect the composite soil/growing media samples from these blocks first to minimize the risk of contaminating un-infested soil/growing media. If all potentially-infested growing media has been destroyed with the infected plants, collect composite samples from the remaining host plants within 2- to 10-m of the originally infected plants that have been placed on hold. Preferentially target the growing media of those plants that are down slope (e.g., based on watering patterns) of the originally infected plants.

Figure 1: Recommended pattern for collection of sub-samples for composite soil and/or growing media samples.



Soil Baiting

It is possible to follow the below procedure and not successfully bait and culture *P. ramorum*. This may be due to *P. ramorum* not being present, but may be due to dormancy of *P. ramorum*. 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. Once the samples are well mixed and split, place one of the split sample halves into cold storage at approximately 4 degrees C for one month. Bring samples out from cold room after one month has passed, leave samples at room temperature for two days and repeat soil baiting process. This baiting can be done in conjunction with the final baiting required for the quarantine release survey. The samples should be processed as shown below.

To prepare soil bait, briefly soak the pears (select unripe green pears) or Rhododendron leaves in a mild detergent solution to remove any pesticide residues. Rinse the baits well and drain.

Leaving the soil in the Ziploc bag, add enough sterile deionized water to saturate and cover soil with about 2.5 cm (1") of water. Do not mix the soil and water.

Use two pears or leaves per soil sample. With a black sharpie pen, label one side of the pears or leaves with the soil sample number and date processed. The USDA Forest Service recommends the following bait selection criteria in *Stream Baiting Protocol: 2007 National Phytophthora ramorum Early Detection Survey of Forests*, issued March 20, 2007. See <http://fhn.fs.fed.us/sp/sod/sod.shtm> for latest approved protocol.

Bait Selection

- Use leaves from a population of native or naturalized rhododendrons, if possible. The population should be sufficiently large to supply needed leaves for the survey duration.
- Variation in Pr susceptibility among rhododendron species/cultivars in laboratory inoculation has been published, but field and lab studies have shown that leaves of common native and naturalized species perform acceptably as Pr bait.
- Leaf size can vary considerably among species and cultivars. If bait leaves are quite small (8 cm x 3 cm at the widest point or smaller), use 2 leaves in each pocket of the bait bag.
- If the source of leaves is nursery-grown or naturalized landscape plants, ensure that they have been free of fungicides and other pesticides for a minimum of 6 weeks before using as bait.

- Source plants should be mostly free of dieback and leaf symptoms. Use 1 year-old leaves as free as possible from leaf symptoms (spots, blight, chlorosis), insect damage, and mechanical damage. Do not use newly formed, succulent leaves. Leaves formed in the present year may be used after full leaf expansion and a period of hardening in summer.
- Bait leaves wrapped in paper towels moistened with chlorinated tap or sterile water and sealed in a plastic bag may be stored refrigerated for up to 1 week before use. Do not use well water or stream water for stored leaves.

Carefully push each pear or leaf into the wet soil and water until the bait is immersed halfway. Leave the labeled side of the bait out of the water. Seal the Ziploc bag and leave bait in the soil/water mixture for at least 48- hr at room temperature.

After 48-hr, remove the baits and wash off any clinging soil into Ziploc bag. Set the bait on a moistened paper towel in a sealed container at room temperature for 7-d to let any potential disease symptoms develop. The soil/water mixture must be autoclaved before disposal.

Examine the bait daily for developing symptoms. Pears infected with *P. ramorum* will display lesions that are round, brown, somewhat leathery in texture, with undefined edges. Colorless, watery, and/or soft lesions are generally caused by other pathogens (especially *Pythium* spp.).

Rhododendron leaves that have become infected with *P. ramorum* will exhibit 'diffuse' leaf spots usually with the midvein most affected.

Under the laminar flow hood, cut eight to 10 pieces of pear or leaf from the edge of the developing lesion or leaf spot and insert into the P&R&P medium. Write the sample number and date processed on the underside of the Petri dish. Seal the dish with parafilm and incubate and treat as described in the USDA approved *Guidelines for Isolation by Culture and Morphological Identification of Phytophthora ramorum* at:
http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/protocols.shtml

APPENDIX 7

Water Sampling Protocol Revised March 2010

See http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/ for latest approved protocol.

Phytophthora ramorum is a soil-borne plant pathogen well adapted to water-logged soil environments. Described as a “water mold”, *P. ramorum* is more closely related to algae than fungi. For this reason, water samples collected from potentially infested nursery blocks can be tested for the presence of *P. ramorum* spores.

The following methods should be utilized to detect *P. ramorum* on a landscape or watershed level. A detection of *P. ramorum* does not mean that nursery stock is infected with the pathogen, but that an infestation within the watershed exists. Detection of *P. ramorum* by this method indicates a problem exists, thereby triggering a more intensive survey.

There are two methods summarized here for detecting *Phytophthora* species spores from water. Additional updates to this protocol are expected soon. The first and preferred sample collection method, for use in waterways or ponds located within and around a nursery, relies on host material (*Rhododendron*, *Camellia*, etc.) leaves contained in mesh bags or containers that function to “bait” or attract *Phytophthora* spores. This method is most effective when water temperatures are warmer than 39°F but cooler than summer-heated levels. After exposure, leaf baits are held briefly in a moist chamber to promote disease development and symptom expression.

The second method, water filtration, may be used where drainage outflows, diversion boxes, standing water and streams are found within the nursery perimeter. More time-sensitive than the first method, water is collected in one-liter plastic bottles and filtered through sterile particulate filters. Following exposure, filters are placed on specialized growth media to support pathogen establishment.

***In situ* Water Sampling with Host Material Leaf Baits**

Bait Selection

- Collect leaves that are known to be free of disease from a population of native or naturalized *Rhododendron*, *Camellia*, *Syringa* (lilac), or *Viburnum* spp. that have susceptible responses to *P. ramorum*. Source host material must not have been sprayed with fungicide within the last three months. Avoid using newly acquired host plants for this reason. Bait-source plants should be sufficiently large, robust and numerous enough to supply leaves during the entire duration of the survey.
- Use healthy leaves that have been on the plant for at least one year and are as free as possible from insect and mechanical damage. Do not use newly formed, succulent leaves.

Present year leaf growth may be used after full leaf expansion and a period of hardening in summer.

- If bait leaves are smaller in size than 8 cm x 3 cm at the widest point, use 8 leaves at each sampling location (one in each mesh bait bag). If leaves are larger than this dimension, four leaves per site can be used.
- Bait leaves may be wrapped in moistened paper towels and sealed in self-sealing plastic bags for refrigerated storage for no more than 14 days before use. Paper towels should be moistened with chlorinated tap or sterile water. **Do not use underground well water or water from a stream to moisten towels.**
- Place 4-8 leaves with the petioles (the stalk-like tissue that attaches the leaf to a stem) attached into each container depending on leaf size (discussed above) at each sampling site. Insert a uniquely numbered plastic tag into each bait bag. The dates (date when bait was established and when bait was collected), water source (location), nursery information (i.e., nursery license number), tag number, water temperatures (initial temperature when baiting was established and final temperature when bait was collected) and GPS coordinate should be recorded on a data sheet.

Baiting Techniques

Bait Bags

- Multiple-use bait bags (approximately 12 inches by 12 inches) should be constructed of a durable, coarse nylon mesh material (e.g., window screening) and fastened together on three sides to allow sufficient overlapping material to seal bag edges (Figure I-1). Single-use bait bags can be fashioned from muslin. Bait bags must have a separate pocket for each leaf to maximize surface area in the water. Exact configuration is not crucial and any bag type that can be closed and securely fastened (drawstring, flapped, rolled, etc.) is sufficient. Once leaves are placed into bag, it should be secured so leaves cannot float out and away.

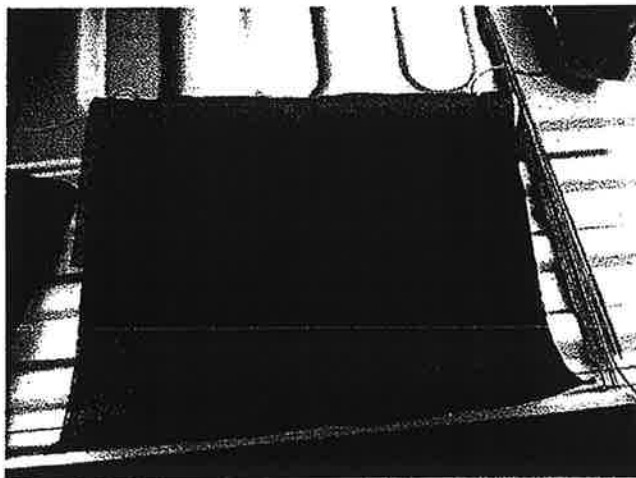


Figure I-1. Example of a bait bag construction.

- Firmly secure each bag by tethering to a stake driven into the ground or by suspending from a rope that spans the width of a stream. Bags should float near or just below the water's surface for 7 days. Bait bags should be placed in an area of the stream where water flows more slowly and pools. Do not place the bait in the fast moving current of the stream. Locate the bags such that the leaves remain submerged even if water levels fluctuate. Do not leave the bait in the water for more than seven days as the leaf tissue will degrade and baiting efficacy will be sharply reduced.
- If possible, place the bait near the site where water flows into the body of water from the nursery. When locating bait bags in waterways, priority should be given to sampling areas downstream from host material, and at the upstream and downstream edges of the nursery perimeter. When possible, shaded locations should be chosen.
- When placing bait bags in retention ponds, priority should be given to inflow and outflow points, preferably in shaded areas. A minimum of two bait bags per pond should be deployed.

Bait Stations

- An alternative to bait bags is a bait station, constructed of a PVC frame to which is attached ½ inch plastic fencing material (see below for photo and construction details). Leaves are attached with binder clips secured to the bottom of the enclosure.
- Attach the station to a stake driven into the ground or by suspending from a rope that spans the width of the stream or pond. The station should be deployed for 7 days. This period of time allows sufficient exposure time for *Phytophthora* spores to locate leaf tissues while limiting the effects of degradation and interference from the colonization of other water-mold organisms.
- If possible, place the bait near the water inflow point from the nursery. When locating bait stations in waterways, priority should be given to sampling areas downstream from host material, and at the upstream and downstream edges of the nursery perimeter. When possible, shaded locations should be chosen.
- When placing bait stations in retention ponds, priority should be given to inflow and outflow points, located in shaded areas. A minimum of two bait stations per pond should be deployed.

Bait Retrieval

- After 7 days, remove bait leaves and the tag from each bag or station and rinse using water from the stream or pond thereby reducing the foreign matter (organic and soil particles) on the bait leaves.
- Wrap leaves in a moistened paper towel and place in a 1 gallon self-sealing plastic bag. Be certain to place the numbered tag from each bait bag into the plastic bag of the

corresponding leaf bait tissues. Double bag the samples to prevent contamination or desiccation in the event a bag ruptures.

- Place all sample bags in an insulated cooler with blue ice or another sealed cooling media for transport to the laboratory. Do not place bait samples directly on the ice; cardboard can be used to separate the ice from the bait samples.
- Record the date of bait retrieval as well as the water temperature at time of retrieval. This information should be entered into an appropriate database (see Appendix G of Nursery Survey Manual).
- Multi-use bait bags should be cleaned and sterilized with either 95% ethanol or a 10% household bleach solution for at least 4 hours prior to reuse. Rinse bags thoroughly with chlorinated tap or sterile water after sterilization. Check for signs of deterioration and bag failure, replacing bags accordingly.

Sample Transport, Storage, Shipping and Processing

- Samples should be kept in a cooler on a sealed coolant bag or in a refrigerator until shipped. ***Do not permit the samples to freeze or dry out at any time.*** Contact laboratory personnel before shipping to advise them that a sample will be arriving. Ship samples via overnight courier; do not store samples prior to shipping.
- Laboratory personnel should process bait samples using the same methods as for foliage samples, according to the diagnostic protocol found here http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/culture_protocol6-07.pdf

Water Sampling for Filtration

Sample Collection

- The number of water samples collected is based upon the number of water bodies present; availability of run-off water; and the overall size of the nursery. If this sampling method is selected, a minimum of two 1-liter samples should be collected per site. More samples will be needed for larger nurseries with more water and irrigation drainage sources.
- Collect one liter of water from each sample point. Collect the cleanest sample possible by minimizing disturbance to the sediment, while avoiding plant and other floating debris. Use a 100-ml measuring cup or beaker to fill a 1-liter screw cap plastic bottle in increments rather than filling the container all at once. If possible, collect the 1-liter sample from several collection points at each sample site.
- Measure and record water temperature at each sample location. When possible, GPS coordinates should be recorded for each sample.
- Identification labels (time tape or masking tape) should be affixed both to the screw cap and the outside of each water collection bottle using a waterproof pen. Labels should be

sufficiently coded to correspond with datasheet entries for each nursery and water body and should include date collected, water source (location), and nursery (i.e., nursery license number).

- Before water is collected, rinse bottles downstream with the water about to be sampled. Maintain collected water samples in a cooler (without ice if external temperatures are cool or with enough ice to gradually cool water samples). Samples should be processed within 8 hours of collection to maximize detection of *Phytophthora* spores. Samples cannot be stored for more than 12 hours without compromising the quality of survey results.
- Log the water samples into the appropriate database. Assign a unique sample number to each bottle.
- Wash each 100-ml measuring cup and 1-liter bottles with warm, soapy water between sample collections. Thoroughly and completely rinse each item. For best results, use an automatic dishwasher with a heated drying cycle. Have several cup/bottle sets on hand to support water collection at a number of bodies of water each day. Use only clean, sanitized collection materials at each site and water source.

Sample Processing

- Most samples can be filtered through polycarbonate membrane filters with 3- μ m pores however, turbid or dirty water samples (from ponds or standing water) will need to be filtered through Durapore membrane filters with 5- μ m pores
- Place a filter funnel into a filter flask with a capacity of at least one liter and connect the flask to a vacuum source using plastic tubing; put a second filter flask as a trap between the flask with the funnel and the vacuum source (i.e., electric vacuum pump or a hand vacuum pump).
- Wet filter holder with distilled water and place a polycarbonate membrane with the shiny side up or a polyvinylidene fluoride membrane with smoothest side up. Be sure the paper between the filters has been removed, the filter is aligned over the perforated area of the funnel, and that the filter is not wrinkled. Assemble the filter funnel and clamp it in place.
- Thoroughly mix the water sample by inverting the plastic bottle and/or swirling and pour 100 ml of sample into the funnel. If the water is highly turbid, 100 ml may not be completely filtered by a single filter; conversely, if the water is extremely clear, additional water (up to 200 ml) may be processed by a single filter.
- Turn on the vacuum source to filter water subsamples. The air should be turned off immediately after each subsample is completed (approx. 10 seconds) to avoid building up excessive vacuum pressure, which could damage *Phytophthora* spores. Rinse the inner surface of the funnel with distilled water to dislodge spores that may be adhered to funnel wall.

- Gently remove sizable organic debris or soil particles trapped on the surface of the filter if it prevents complete contact of the filter with the surface of PARPH-clarified V8 agar contained in Petri plates. Using forceps, gently lift the filter and invert it so the collection side contacts the media surface. Smooth the filter with the forceps to remove air bubbles that may have formed between the filter and the agar media surface.
- Repeat steps 1 to 6 until the entire 1 liter water sample has been filtered. Unless water is very clear, this should result in a minimum of 10 agar plates per collection bottle and 20 agar plates per collection site.
- Rinse the filter funnel assembly and forceps under hot, running tap water after each sample to avoid cross-contamination between samples. Do not disinfect the funnel with a bleach solution or alcohol as any residues may affect spore viability.
- Transfer the plates immediately to a state or federally-approved processing laboratory via overnight courier, or
- Maintain the agar plates with filters at 20°C in the dark for at least three days. Because *P. ramorum* grows slower than most other species of *Phytophthora* commonly found in water, leaving the filters on the agar plates for a three day incubation period is critical for recovery of this species.
- After the incubation period, remove the filters with sterile forceps and gently rinse the surface of the agar medium with running tap water to wash off small particles and bacteria that may interfere with microscopic observation. Filters and rinse water should be treated and properly discarded.
- Check the plates at regular intervals under low magnification-using an inverted or dissecting microscope-for colonies with typical morphological characters of *P. ramorum* (e.g., coralloid hyphae, semi-papillate sporangia, and large chlamydospores).
- Contact laboratory personnel if *P. ramorum* is suspected from any samples. See Appendix 3 for shipping details in the culture and morphology protocol at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/cultur_eproto6-07.pdf

Bait Station Construction Details

Frame

- Frame is made using ½-inch-diameter PVC pipe and four elbow joints.
- Cut four lengths of PVC—two 11 ½ inch and two 10 ½ inch lengths.
- Glue pipes and joints into a rectangular shape using PVC primer and cement.

Mesh

- The mesh used is a plastic, ½-inch fence material (with 3/8-inch openings) available at any national home improvement chain store
- Dimensions are given both in inches and also based on number of squares (Figure I-2)
- Mesh is secured to frame using plastic zip-ties.
- Leave one side of mesh half secured until leaves are inserted.
- Tie four small binder clips into mesh using plastic covered twist-ties; these clips hold leaves in place by petioles. This maintains separation between bait leaves, allowing for maximum water flow exposure for each bait tissue (Figure I-3).

Water Filtration Methods Materials List

- 2, 1-liter bottles per collection site (Nalgene preferred)
- 100-ml plastic measuring cup or beaker per collection site
- thermometer (water-resistant type preferred)
- ice chest cooler (with a small amount of ice or other refrigerant if temperatures are warm outside)
- electric vacuum pump or hand-operated vacuum pump
- Sterile PARPH-clarified V8 selective medium (see protocol recipe) in disposable Petri plates; 10 plates per collection bottle or 20 plates per collection site; media plates can be stored in sealed plastic sleeves or bags in a refrigerator for two months before use
- 47-mm-diameter polycarbonate membrane filters with 3- μ m pores (e.g., Sterlitech SKU No. PCT3047100 at <http://www.sterlitech.com>)
- 47-mm-diameter polyvinylidene fluoride (Durapore) membrane filters with 5- μ m pores (e.g., Fisher Scientific #SVLP04700)
- clamp-type filter funnel (Nalgene preferred)
- two 1- or 2-liter filtering flasks (plastic or glass)
- plastic tubing
- bent-tip forceps
- Squeeze bottle containing distilled water
- inverted or dissecting microscope

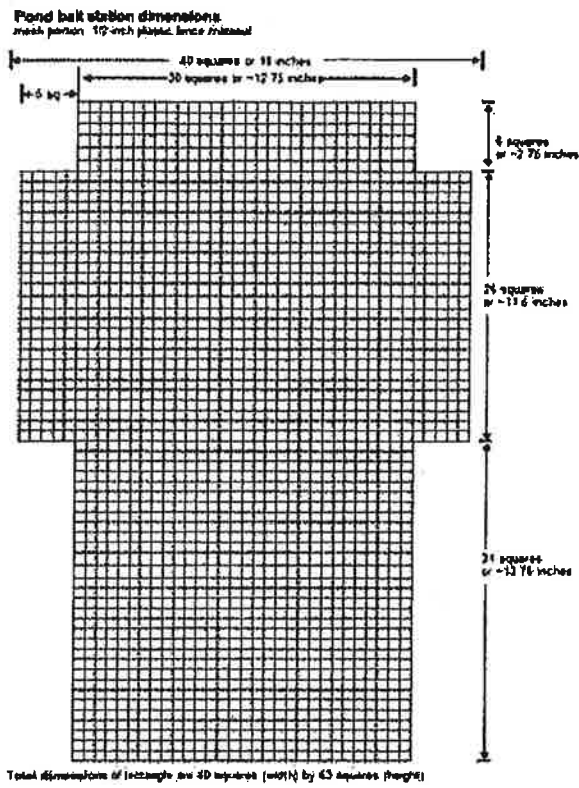


Figure I-2. Pattern of mesh screen to use for constructing a bait station.

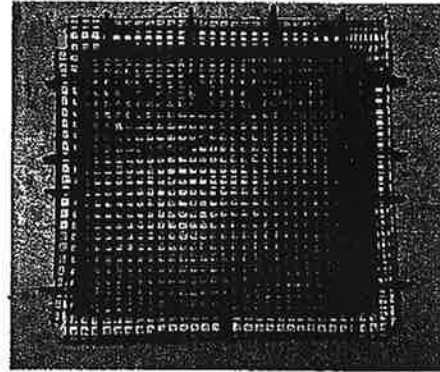


Figure I-3. Completed bait station construction. Photo courtesy of Dr. Steve Jeffers, Clemson University

PAR(PH)-V8 Selective Medium: For *Phytophthora* species. Adapted from *Jeffers & Martin, 1986; Ferguson & Jeffers, 1999.*

| Ingredient | Amount Per: | | Concentration (PPM) |
|----------------------------|------------------|-----------|---------------------|
| | 1.0 Liter | 0.5 Liter | |
| Basal Medium | | | |
| Clarified V8 Concentrate | 50 ml | 25 ml | |
| Distilled Water | 950 ml | 475 ml | |
| Difco Bacto Agar | 15 g | 7.5g | |
| Amendments | | | |
| Delvocid (50% pimaricin) | 10 mg = 0.01 g | 5 mg | 5 |
| Sodium Ampicillin | 250 mg = 0.25 g | 125 mg | ~250 |
| Rifamycin-SV (sodium salt) | 10 mg = 0.01 g | 5 mg | ~10 |
| Terraclor [75% PCNB]* | 66.7 mg = 0.0667 | 33.4 mg | 50 |
| Hymexazol* | 50 mg = 0.05 | 25 mg | 50 |

Clarified V8 concentrate is made from V8 Juice, buffered with the addition of CaCO₃ (1.0 g CaCO₃/100 ml V8 Juice) and clarified in one of three ways:

- centrifugation @4000 RPM for 20 min followed by filtration using 2 layers of Whatman No. 1 under vacuum, or;
- centrifugation @ 7000 rpm for 10 min then filtration is not necessary, or;
- vacuum filtration alone with Celite.

Following clarification, V8 should be frozen at -20°C in 50-ml aliquots (e.g., in disposable 50-ml centrifuge tubes).

* PCNB and hymexazol are optional and can be omitted (e.g., to make PAR, PARP, & PARH). PCNB is useful to inhibit soilborne fungi on soil dilution plates and hymexazol inhibits **most** *Pythium* spp. while allowing **most** *Phytophthora* spp. to grow

Directions

1. Add ingredients for **basal medium only** to a 2-L flask; thoroughly mix on a magnetic stirrer with a large stir bar in the flask
2. Autoclave for 20 min at 121 C and 15 psi; turn waterbath on to ~50°C
3. Add each amendment to separate sterile water blanks [5 ml distilled water in a 16-mm test tube]; vortex each to mix or suspend
4. Cool medium in waterbath
5. Slowly stir medium with a magnetic stirrer in laminar flow hood
6. Vortex each amendment thoroughly and add to mixing basal medium
7. Use one additional sterile water blank to sequentially rinse all amendment tubes and then add rinse water to the medium; continue mixing medium
8. Pour plates relatively thin (i.e., about 15 ml/plate = 60 plates/liter); pour molten medium so it does not quite cover the entire plate; therefore, plates will need to be swirled gently to evenly distribute medium before it hardens
9. Cool plates at room temperature
10. Store plates inverted in plastic bags in the dark in a refrigerator
11. Best if plates are used within several weeks—but they will keep for months

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APPENDIX 8

Treatment and Disinfection

Revised April 2007

The following techniques are approved by USDA APHIS PPQ for control of *P. ramorum* in nurseries found to contain plants infected with *P. ramorum*.

Infected Plants:

Note: HAP material, including leaf litter, must not be placed in compost piles or be removed from the nursery site as trash or in debris removal. HAP material should be collected and incinerated or double bagged and deep buried in a site approved by USDA, APHIS or delegated regulatory authority.

- **Incineration (burning to ash):** Infected plants, associated growth media, associated containers (i.e. pots and trays), all leaf debris in and around the area where plants were stored may be disposed of by incineration at a facility or other location (e.g. on site) approved by USDA and permitted within state and municipal statutes or regulations. Off nursery movement must be properly safeguarded and every effort to prevent plant debris or soil from being dislodged from the plants prior to incineration should be taken. Burning may be through open burning or in an incinerator.
- **Deep burial:** Infected plants, associated growth media, associated containers (i.e. pots and trays), all leaf debris in and around the area where plants were stored must be double bagged using plastic bags of 2 mil thickness or greater and buried to a depth of no less than two meters. The material must be buried at a USDA approved site, onsite, or municipal landfill, which is expected to remain undisturbed. Every effort to prevent plant debris or soil from being dislodged from the plants should be taken.
- **Steam sterilization:** Dry heat or steam commonly heated to internal temperatures of 212° F (100° C) for 30 minutes followed by burial in a landfill, or as otherwise detailed in the USDA Treatment Manual for "insect pests and pathogens in garbage", Schedule T415b.
http://www.aphis.usda.gov/ppq/manuals/port/Treatment_Chapters.htm

Non-Porous Surfaces:

Most disinfectants are not labeled for use in soil and are only useful for nonporous materials such as concrete floors, nursery pots, and plastic sheeting. A number of disinfectants are registered for use on nonporous surfaces that may effectively reduce populations of *Phytophthora* species. If it is practical, tools such as knives, pruners, water breakers, water wands and other implements used in the quarantine area should only be used in the quarantine area. If tools and other implements must be moved from the quarantine area, then regular disinfection using an appropriate disinfectant for the control of *P. ramorum* is recommended prior to removal from the quarantine block. The following table modified from <http://cpmcnet.columbia.edu/dept/ehs/decon.html> examines the effects of different classes of

disinfectants on microbial populations. This list is for explanation and information only. Few disinfectants are specifically labeled for *Phytophthora* species and are shown in **Bold**.

All labels for the disinfectants listed below must be strictly adhered to for maximum efficacy and environmental and worker safety.

Summary of Disinfectant Activities

| Disinfectant | Trade names | Comments | Contact time |
|---|---|--|---------------------|
| Alcohols (ethyl and isopropyl) 60-85% | Lysol Spray | Evaporates quickly so that adequate contact time may not be achieved, high concentrations of organic matter diminish effectiveness; flammable. | 10-15 minutes |
| Phenolics (0.4%-5%) | Pheno-cen | Phenol penetrates latex gloves; eye/skin irritant; remains active upon contact with organic soil; may leave residue. | 10-15 minutes |
| Quaternary Ammonium (0.5-1.5%) | Consan Triple Action 20 Physan 20 Green-Shield 20 | Effective for non-porous surface sanitation (floors, walls, benches, pots). Low odor, irritation. Use according to labels. | 10-15 minutes |
| Chlorine (100-1,000 ppm) | 10% Clorox 10% Bleach | Inactivated by organic matter; fresh solutions of hypochlorite (Clorox) should be prepared every 8 hours or more frequently if exposed to sunlight; corrosive; irritating to eyes and skin. Exposure to sunlight further reduces hypochlorite efficacy. Keep solution in opaque container. | 10-15 minutes |

Water:

- **For dust abatement, fire suppression, and equipment cleaning:** Clorox (sodium hypochlorite) is labeled (EPA Reg. No 5813-50) for treatment of water (~50 ppm available chlorine) for controlling the spread of *Phytophthora lateralis* via water used for dust abatement, fire suppression and equipment cleaning. The active ingredient level must be measured from water collected at the sprinkler head.

- **For irrigation:** Chlorine levels of 2ppm or 2mg/liter or greater has been correlated with the control of *Phytophthora* spp. in re-circulated irrigation systems. For irrigation purposes, recirculated, non-municipal water, must be chlorinated at an active chlorine concentration equal to or greater than 2 mg/liter of water; for facilities that recycle water, this chlorine level must be monitored.

Soil and Potting Media:

- **Potting media:** Potting media must be heated such that the temperature in the center of the load reaches at least 180 degrees F for 30 minutes. Treatment must be conducted in the presence of an inspector or treated with an approved fumigant as detailed below.
- **Soil:** Soil must be heated such that the temperature in the center of the load reaches at least 180 degrees F for 30 minutes. Treatment must be conducted in the presence of an inspector or treated with an approved fumigant as detailed below. Methyl bromide has been used for fumigating wood products, but the data on fungi and related organisms in wood are limited. However, methyl bromide has a long history of fumigation of soil in the field and greenhouse. It has commonly been used in combination with chloropicrin for control of *Phytophthora* spp. and other pests in strawberry beds. Methyl bromide has been used for soil treatment for the mitigation of *P. cinnamoni* in citrus groves. However, many of the compounds currently in use have been implicated in human and environmental risks. Solarization is not a consideration as a viable option for soil treatment.

All fumigants are restricted use and must be applied according to labels by a licensed applicator. Any use of pesticides in any manner not listed on the label is unlawful.

Summary of Labeled Soil Fumigants

| Fumigant | Trade names | Comments |
|--------------|--|---|
| Chloropicrin | Chlor-O-Pic Metapicrin Timberfume Tri-Clor | Often used in combination with methyl bromide due to its ability to be detected in small quantities. |
| Dazomet | Basamid | Methyl isothiocyanate (MITC) breaks down into cyanide gas. Granular formulation that is water activated. Requires careful soil preparation and incorporation into soil. All application must be made in accordance with labeling. |
| Metam-sodium | Busan 1020 Busan 1180 Busan 1236 Metam Vapam | Metam can be applied through irrigation. Tarping can increase efficacy. All application must be made in accordance with labeling. |

| Fumigant | Trade names | Comments |
|----------------|--|--|
| Methyl Bromide | Tri-Con Terr-O-Gas Preplant Soil Fumigant Pic-Brom | Colorless and odorless. Usually combined in various concentrations with Chloropicrin (tear gas). Use is restricted due to ozone depletion potential. |

Physical Treatment of Soil:

- Mitigation of infested soil can also be achieved by installing permanent impermeable, non-porous barriers that consist of cement, concrete or asphalt. These barriers must be constructed so that no native soil within the destruction block is visible. The barriers should be graded such that no standing water can be observed.

Equipment and Personnel (Inspectors and employees):

- Access to infested areas and hold areas should be limited, as much as possible, to officials and necessary employees. Everyone entering and leaving the nursery site must scrape off loose pieces of soil into the destruction block. Those working with, or in contact with suspected infested material (including plants), must wash hands using soap or approved disinfectant immediately after completion of task. There are no products currently labeled for use on porous materials for *Phytophthora* control.
- Personnel should not have access to other production areas of the nursery after entering the destruction block on the same day.
- A disinfectant foot bath should be placed near the exit to the destruction blocks and quarantine blocks and used by all personnel entering and exiting the quarantine block and entering and exiting the destruction block at the infested nursery site, where the contact with potentially infested soil or plant debris by footwear is likely. The foot bath must be filled with fresh disinfectant at least on a daily basis or more frequently if contaminated with soil or organic debris, in accordance with label directions. Use of disposable shoe covers may be used in lieu of a footbath, if disposed of immediately upon exiting from the quarantine block or destruction block. The disposable shoe covers must be placed in bags and incinerated, deep-buried or properly disposed in a sanitary landfill.
- The tires (or other parts in contact with the soil or plants, such as the bed of trucks) of vehicles must be cleaned of loose soil and plant debris and disinfested with the appropriate labeled products before leaving the infested site. If at all possible, vehicles should not be allowed in the destruction blocks at all. Any efficacious product labeled for use on non-porous surfaces may be used on tires or vehicle undercarriages.

- Do not visit other nursery sites in potentially contaminated work clothing and footwear. Where it is necessary that visitors enter the nursery, the nursery should ensure that every precaution is taken to prevent the movement of infected plants, contaminated soil or debris by the visitor.
- Wood surfaces suspected of contamination with *P. ramorum* should be disposed of as stated above under "Infected Plants."

APPENDIX 9

Biosecurity Measures for Nurseries April 2007

In the course of daily work, nursery personnel are frequently required to visit a number of different nurseries sites, greenhouses, fields, and facilities. These actions could potentially provide a pathway for transferring quarantine organisms from one work site to another during the work day. It should also be recognized that even if a single work site is visited per day, precautions must be taken to avoid contaminated clothing and equipment from being used at a new site the following day. Further, visitors to these same facilities present the same risks and additionally could vector disease-causing-organisms from other sites.

Biosecurity measures must be taken by nurseries and be required of nursery personnel and visitors to avoid and mitigate the spread of *P. ramorum*. The biosecurity measures described here are the minimum measures to be taken by the nursery.

Communications

All nursery personnel should be trained and visitors informed of these biosecurity requirements that have been put in place by the facility. As new scientific data and technology is learned, the facility needs to update their biosecurity requirements and retrain their personnel.

Vehicles

Vehicles can become contaminated with soil; a primary vector for quarantine pests. The following guidelines seek to reduce the likelihood of this pathway.

Avoidance:

Once at the inspection site, if possible, the vehicle should only be driven and parked on paved, concrete or gravel areas to avoid contact with soil and organic matter. Visitors should consider requesting a facility employee to drive them to their designated location with one of the nursery's vehicles. Loading of nursery stock onto other than the nursery's vehicles should be done in an area with concrete or asphalt pad located near the gate and not in the interior of the nursery.

Cleaning:

Interior of nursery vehicles should be cleaned to ensure no build-up of soil, debris or other items.

Where it is not possible to avoid the vehicle going onto the fields, the vehicle must be driven to the edge of the facility where the tires, wheel wells and accessible areas of the undercarriage of the vehicle must be cleaned of soil and organic matter with a brush or a water hose followed by a spray down with a suitable disinfectant. In situations where the undercarriage has been coated with soil it is recommended that after cleaning and disinfecting at the work site an effort be made

to go through a car wash that has the ability to clean the undercarriage before proceeding to another work site. If a car wash is not available, avoid driving onto the next work site. To ensure the entire surface of the tires are cleaned it will also be necessary to move the vehicle forward a foot or so to permit cleaning of the portion of the tire in contact with the ground.

The tires (or other parts in contact with the soil or plants, such as the bed of trucks) of vehicles must be cleaned of loose soil and plant debris and disinfested with the appropriate labeled products before leaving the infested site. Any efficacious product labeled for use on non-porous surfaces may be used on tires or vehicle undercarriages.

A portion of the vehicle must be designated as a "clean area" where clean work supplies and equipment can be kept. A designated "dirty area" of the vehicle such as the trunk of the car or a specified enclosed area of a truck bed must also be identified for use to hold double bagged clothes or dirty equipment that require cleaning. In situations where pool vehicles are used, the work site should adopt a set procedure for all personnel.

Nursery Personnel

Nursery personnel routinely come in contact with potentially contaminated soil, plants and organic matter and this requires the personnel to address a number of biosecurity measures. If the inspection site has distinct levels of biosecurity for different areas in the nursery, it is necessary to be aware of this situation. Work should always be completed working from the areas of lowest to highest risk.

Access:

Access to infested areas and hold areas should be limited, as much as possible, to personnel and employees. Everyone entering and leaving the nursery site must scrape off loose pieces of soil into the destruction block. Those working with, or in contact with suspected infested material (including plants), must wash hands using soap or approved disinfectant immediately after completion of task. There are no products currently labeled for use on porous materials for *Phytophthora* control.

- Personnel should not have access to production areas of the nursery after entering the destruction block on the same day.
- A disinfectant foot bath should be placed and used by personnel entering and exiting the quarantine area and entering and exiting the destruction block at the infested nursery site, where the movement of soil or plant debris on footwear is likely. The foot bath must be filled with fresh disinfectant at least on a daily basis or more frequently if contaminated with dirt or debris, in accordance with label directions. Use of disposable shoe covers may be used in lieu of a footbath, if disposed of immediately upon exiting from the quarantine area or destruction block. The disposable shoe covers must be placed in bags and incinerated or deep-buried.
- Do not visit other nursery sites in potentially contaminated work clothing and footwear.

Boots:

Rubber boots which can be disinfected should be worn and if they are not available disposable boot covers must be worn over work boots in any infested or possibly infested area. The rubber boots must be disinfected on arrival, even if this has been done at the time of departure from the last work site. At the conclusion of the inspection, the boots must be cleaned of soil and disinfected prior to placing into the vehicle area designated as a "clean area". Dispose of used boot covers by double bagging and place into the designated "dirty area" of the vehicle for proper disposal. After removing boot covers, the soles of the work boots must be inspected for soil and if present, must be cleaned of soil and treated with disinfectant.

Hands:

Thoroughly wash hands with soap and water before entering and after leaving the work site. Follow these four simple steps to keeping hands clean.

- Wet hands with warm running water.
- Add soap, and then rub hands together, making a soapy lather. Do this away from the running water for at least 20 seconds, being careful not to wash the lather away. Wash the front and back of hands, as well as between fingers and under nails.
- Rinse hands under warm running water. Let the water run back into the sink, not down the elbows. Turn off the water with a paper towel and dispose in a proper receptacle.
- Dry hands thoroughly with a clean towel

If a hand washing station is not available, antiseptic rubs/gels/rinses (containing a minimum of 70% ethyl alcohol and left on for 10 - 15 minutes) must be used. Follow these basic steps for using antiseptic rubs/gels/rinses.

- Remove soil from hands.
- If hands are wet, dry as much as possible.
- Apply enough disinfectant (amount about the size of a quarter) onto hands to cover all areas, including under the nails. Use a rubbing motion to evenly distribute the disinfectant product for about 15 seconds.

If antiseptic rubs/gels/rinses are used, avoid formulations with moisturizers as they leave a gummy residue. Disposable gloves may be used, however they have the tendency to rip and become uncomfortably wet after a short period. Rubber gloves which withstand more abuse than disposable gloves have the same drawbacks as disposable gloves, however will be more practical when handling materials that are sharp or jagged. If rubber gloves are used in cold weather it is recommended to purchase rubber gloves with cotton or acrylic liners. Both disposable and/or rubber gloves must be double bagged after use if working in an infested area and placed into the

“dirty area” of the vehicle for disposal or cleaning. If on-site disposal of the gloves are available this option should be chosen. After disposal of gloves, hands must be washed or sanitized. To avoid cross contamination, disinfection of hands must take place after handling any plants or other contaminated matter in the infested area.

Equipment

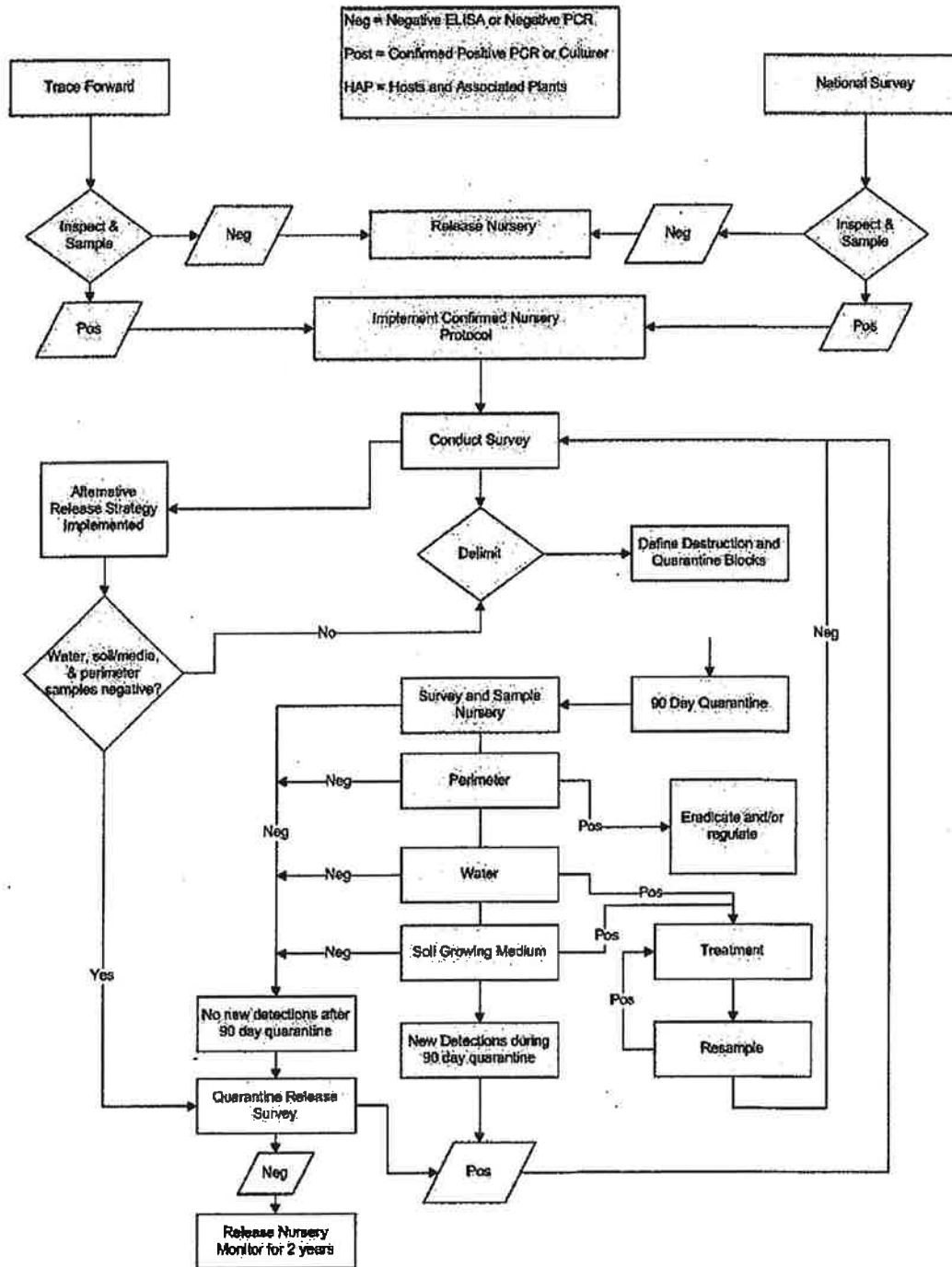
Any equipment used (pruners, measuring tapes, clipboards, pens, etc.) at a work site must be disinfected prior to leaving the work site. Where practical, equipment should be disinfected as frequently as possible at each work site. Where equipment must leave the work site for disinfection it must be double bagged and place in the designated “dirty area” of the vehicle.

Visitors:

- Access to infested areas and hold areas should be limited, as much as possible, to personnel and employees. Everyone entering and leaving the nursery site must scrape off loose pieces of soil into the destruction block. Those working with, or in contact with suspected infested material (including plants), must wash hands using soap or approved disinfectant immediately after completion of task. There are no products currently labeled for use on porous materials for *Phytophthora* control.
- A disinfectant foot bath should be placed and used by all entering and exiting the nursery site. These should be placed at all entrances and exits. The foot bath must be filled with fresh disinfectant at least on a daily basis or more frequently if contaminated with dirt or debris, in accordance with label directions.

APPENDIX 10

Confirmed Nursery Protocol Flowchart for First Time Positive Nurseries
 Revised: April 2007



APPENDIX 11

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

May 2007

(Modified June 23, 2010)

These mitigations apply for nurseries detected as positive for *P. ramorum* within one year of release from an EAN (Emergency Action Notification) or state equivalent. *P. ramorum* infestations in nurseries may be re-introduced or the effort to eradicate the disease may fail. In the event that a production or wholesale nursery has *P. ramorum* detected on site after the initial release from the EAN or state equivalent, it is necessary to implement additional measures to ensure that the risks associated with *P. ramorum* are properly mitigated. These seven additional measures are to be implemented:

1. Additional monitoring of the nursery is required. There are two methods for monitoring: water-baiting and regulatory inspection.
 - a) Water baiting
 - If there is significant water run-off and the water has not yet tested positive, conduct seasonal baiting of that water.
 - If any water sample, either draining from or within the nursery premises is determined to be positive, **then the option defaults to option b**, and a nursery inspection is required of all plants within the nursery that are listed on the APHIS list of Regulated Proven Hosts and Plants Associated with *P. ramorum*.
 - b) Regulatory Inspections of host and associated plants:
 - Conduct two additional inspections, during the two out of the three best remaining seasons that are conducive to the development of symptoms for *P. ramorum*.
 - Inspect all plants within the nursery that are listed on the APHIS list of Regulated Proven Hosts and Plants Associated with *P. ramorum*.
 - Any plants observed with the symptoms will be sampled sufficiently to represent the plants with the symptoms being expressed and those samples are to be analyzed for *P. ramorum*.
2. Appropriate biosecurity measures are to be incorporated into the EAN or Compliance Agreement and remain in place until two years of negative survey are completed.

See Appendix 9 for biosecurity measures. These contain practices which, if properly applied, can be expected to effectively mitigate risks associated with *P. ramorum* in a nursery. In areas of the country not regulated these need to remain in place for two years via the EAN. In regulated and quarantine areas these practices are to be included as part of a Compliance Agreement. In all cases, appropriate and specific timelines for implementation will be established. Additionally, these will be periodically verified, perhaps best done at the seasonal re-inspections.

3. 45 days after implementation of the CNP, a series of soil samples will be taken in the destruction and quarantine blocks as well as any water drainage areas will be baited or sampled and analyzed for the presence of *P. ramorum*.

The presence of *P. ramorum* in soil or water may contribute to the 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 is to take place. See Appendices 6 and 7 for how to conduct sampling and Appendix 8 for details on treatment and eradication procedures.

4. Fallen leaves and plant debris will be removed from pots, soil and within the immediate area of *Rhododendron* and *Camellia* on a quarterly basis to the best ability of the nursery to prevent possibly infested dropped leaves from infesting the soil or other plants. Verify this at the seasonal inspections.

Camellia and other hosts are known to shed infected and infested leaves. This may result in further infection and soil infestation with a potential for resultant spread of infection. To address this potential, it is important for these leaves and related debris be removed and destroyed or buried. The use of a blower to move these leaves away to a different location is not an appropriate mitigation.

5. Nurseries that ship interstate must undergo approved training in the risks, recognition and mitigation of *P. ramorum*. The nursery shall develop and maintain a database/list showing names of staff and date of training and make it available to regulatory officials upon request.

Appropriate nursery personnel must complete training approved by APHIS (contact the Regional Program Manager for currently approved training) and provide appropriate guidance to other nursery personnel as demonstrated by the training.

6. Nurseries are to inspect all *Rhododendron* and *Camellia* brought into the nursery. *P. ramorum* has been re-introduced to nurseries through buy-ins and customer returns. Therefore, neither of these two genera, nor any other taxa of plants found positive in the nursery, is to be returned to stock upon a customer's return or when purchased as seconds. If the nursery has a policy to accept nursery stock returns, then destroy those using appropriate methods. If seconds of these two taxa are purchased, these plants must be safeguarded, segregated, and withheld from interstate movement until the plants are officially inspected, sampled, tested and found free of evidence of *Phytophthora ramorum*.

P. ramorum is occurring in these two genera at greater levels, as compared to other genera. It is essential that *Rhododendron* and *Camellia* be carefully examined for any signs of this disease and samples provided for analysis should any be detected. If customer returns, do not return members of these genera to stock but rather destroy them appropriately. Other taxa found positive in a nursery present the same risk. Seconds of these two genera present a similar risk.

7. A one year pre-shipment notification to the office of the SPRO of all shipments containing any plants of the genera, *Rhododendron*, *Camellia*, *Viburnum*, *Pieris*, and *Kalmia*.

Upon being confirmed positive for *P. ramorum*, the nursery is required to notify the SPRO of any interstate shipment made containing these five hosts. This notification is expected to be a fax (or agreed upon equivalent) containing all the information needed to identify the shipper, receiver, contents of the shipment, expected arrival date and appropriate contact information. It is to be sent to the office of the SPRO and identified as "Pre-shipment notification of *P. ramorum* hosts as required by USDA-APHIS". SPRO contact information can be found at: www.nationalplantboard.org/member/index.html

**Scope of Work for *Phytophthora ramorum*
(Sudden Oak Death)
In Regulated Counties
July 1, 2010-June 30, 2011
FY 2010/2011**

Appendix B

Pest Exclusion Advisory 7-2008

PEST EXCLUSION ADVISORY

NO. 07-2008



STATE OF CALIFORNIA
DEPARTMENT OF FOOD AND
AGRICULTURE
1220 N Street, Room A-372
Sacramento, CA 95814

DATE: March 3, 2008

TO: All County Agricultural Commissioners

FROM: Plant Health and Pest Prevention Services

SUBJECT: Actions at *Phytophthora ramorum* Positive Locations

Effective immediately, the United States Department of Agriculture has issued a Retail Confirmed Nursery Protocol and an amended version of the Trace Back Protocol for *Phytophthora ramorum*. Please visit the CDFA *P. ramorum* site (<http://www.cdfa.ca.gov/phpps/pe/InteriorExclusion/SuddenOakDeath/>) to view the documents in their entirety.

This advisory is being issued to clarify the actions that must take place at different types of nurseries confirmed positive for *Phytophthora ramorum* and replaces instructions outlined in Pest Exclusion Advisory 02-2006.

Production Nurseries/Wholesale Brokers

Interstate Shippers in All Counties (Quarantined and Regulated)

Nurseries that are under compliance, ship, or intend to ship plants interstate:

1. Compliance agreement (if issued) must be suspended.
2. The federal Confirmed Nursery Protocol (version 8.0) must be implemented.
3. Compliance agreements for interstate shipping will be re-issued after delimitation according to the Confirmed Nursery Protocol (version 8.0) including sampling and testing with negative results (see Phytosanitary Advisory 12-2006).

Intrastate Shippers in Regulated Counties

Nurseries that do not intend to ship plants interstate:

1. The Confirmed Nursery Protocol (version 8.0) must be implemented.

Intrastate Shippers in Quarantined Counties

Nurseries located within the 14 quarantined counties that **DO NOT SHIP OUT** of the quarantined area should be dealt with using the following nursery stock cleanliness standards:

1. The stock shall be kept "free of" *P. ramorum*, California Code of Regulations (CCR) Section 3060.2 (b)(2).
2. Such stock (the lot) is subject to disposal in a manner satisfactory to the county agricultural commissioner.
3. Stock may be sold under a written agreement between the buyer and seller in accordance with CCR Section 3060.4 (a)(1)(D).

Retail Nurseries/Garden Centers

Interstate Shippers in All Counties (Quarantined and Regulated)

Nurseries that are under compliance, ship, or intend to ship plants interstate:

1. Compliance agreement (if issued) must be suspended.
2. The federal RETAIL Confirmed Nursery Protocol (version 1.0) must be implemented.
3. Compliance agreements for interstate shipping will be re-issued after delimitation according to the RETAIL Confirmed Nursery Protocol (version 1.0) including sampling and testing with negative results (see Phytosanitary Advisory 12-2006).

Retail Nurseries/Garden Centers in Regulated Counties

Nurseries that do not intend to ship plants interstate:

1. The RETAIL Confirmed Nursery Protocol (version 1.0) must be implemented.

Retail Nurseries/Garden Centers in Quarantined Counties

Nurseries located within the 14 quarantined counties that **DO NOT SHIP OUT** of the quarantined area should be dealt with using the following nursery stock cleanliness standards:

1. The stock shall be kept "free of" *P. ramorum*, California Code of Regulations (CCR) Section 3060.2 (b)(2).
2. Such stock (the lot) is subject to disposal in a manner satisfactory to the county agricultural commissioner.
3. Stock may be sold under a written agreement between the buyer and seller in accordance with CCR Section 3060.4 (a)(1)(D).

Mixed Retail/Production Nurseries in All Counties

Nurseries that have a retail and production/wholesale component:

1. The appropriate actions must be applied to any portion of the nursery where *P. ramorum*-positive plants are grown or stored.
2. Inspectors should use their judgment in delineating the retail sales area from the production/wholesale area when implementing the appropriate actions.

For questions regarding this advisory, please contact Amber Morris at (916) 654-0312 or by e-mail at amorris@cdfa.ca.gov.

**Scope of Work for *Phytophthora ramorum*
(Sudden Oak Death)
In Regulated Counties
July 1, 2010–June 30, 2011
FY 2010/2011**

**Appendix C
Trace Forward Protocol**



United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine



Trace Forward Protocol
For
Nurseries that Received Plant Material Shipped from a Confirmed
***Phytophthora ramorum* infested nursery**

6 June 2008
Version 2.1

Purpose

The purpose of this protocol is to establish a set of procedures that are to be used to determine if a nursery that received plants from a *Phytophthora ramorum* confirmed positive nursery has infected plants in their inventory, and thus has become infested themselves. *P. ramorum* is the plant pathogen that causes sudden oak death, ramorum blight, and ramorum die-back. By following the procedures in this protocol, we can ensure a consistent, science and risk based response to detections of *P. ramorum* in commercial nursery stock. For more information on this pathogen please visit the USDA, APHIS, PPQ web site at: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram

Definitions

- Associated plants:** Associated plants are those reported found naturally infected and from which *P. ramorum* has been cultured and/or detected using PCR (Polymerase Chain Reaction). For each of these, traditional Koch's postulates have not yet been completed or documented and reviewed. See Appendix A
- Block:** Within a nursery, this is a contiguous block of HAP. The block will be considered contiguous until there is a 2 meter break of either no plants or no HAP.
- Confirmed Positive:** The test result on a presumptive positive that *P. ramorum* is present based on DNA testing or culture morphology. This confirmation would be conducted by APHIS in the case of PASS samples or by the provisionally approved lab or diagnosticians with identification authority in the case of non-PASS samples.
- Cull Pile:** An area where discarded plant material is deposited. This area may also be known as a waste pile.
- Destruction block:** Block of plants to be destroyed. For precise definitions of destruction block see the Confirmed Nursery Protocol for

Wholesale and Production nurseries, and the Confirmed Nursery Protocol for Retail Nurseries.

- Destruction radius:** Block of plants to be destroyed. Within a nursery, for purposes of the **retail protocol**, the destruction radius is defined as all *P. ramorum* infected HAP and all other HAP within 2 meters of any infected HAP.
- HAP:** Host and associated host plants listed on the official APHIS List of Regulated Hosts and Plants Associated with *Phytophthora ramorum*.
- High Priority Target Plants:** These are any HAP which originated in the destruction block at the infested (source) nursery. These plants are to be identified using the best available information and to the lowest available taxonomy, (for example, if high priority target plants can be identified to cultivar, then trace forward activities may be conducted at the cultivar level). Identify all domestic and international HAP shipments within the 12 months prior to the first positive detection of *P. ramorum* for only the infected plant species and for the five high risk host genera (*Rhododendron*, *Camellia*, *Viburnum*, *Pieris* and *Kalmia*)
- Host plants:** Naturally infected plants verified with completion, documentation, review and acceptance of traditional Koch's postulates and listed in the "APHIS List of Regulated Hosts and Plants Associated with *Phytophthora ramorum*". (Also see: "Associated Plants")
- Infected plants:** Plants officially confirmed as being infected with *P. ramorum*, based on the use of APHIS approved diagnostics.
- Medium Priority Target Plants:** Any HAP located in an infested nursery which did not originate in the destruction block.
- Nursery/Facility:** Any location where nursery stock is grown, propagated, stored, or sold; or any location from which nursery stock is distributed. Locations that grow trees to be sold without roots (i.e. Christmas trees) and locations where such trees are stored or distributed are considered to be nurseries.
- Quarantine Block:** Area identified as a 10 meter radius from the destruction block (see Appendix B) designed to determine if *P. ramorum* has spread beyond the destruction block.

Quarantine radius: Block of plants to be quarantined. Within a nursery for purposes of the **retail protocol** this is an area identified as a 2 meter radius beyond the destruction radius (see Appendix C) designed to determine if *P. ramorum* has spread beyond the destruction radius.

PASS (Potentially Actionable Suspect Sample): A presumptive positive *P. ramorum* sample diagnosed or identified by a provisionally approved laboratory or diagnostician with identification authority that would require confirmatory testing by an official APHIS Laboratory due to the nature of the plant sampled and the necessity for Federal confirmation. (For more information see: "PASS System Policy" at: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/)

Retail nursery: A nursery whose business is the sale of plants to the end user, typically a home owner.

Suspected infected plants: These are plants with visible symptoms of *P. ramorum* infection; and/or HAP that are a part of an infested block or derived from an infested block or quarantine block; and/or plants that have tested positive using PCR or culturing, but have not been confirmed positive for *P. ramorum* by APHIS.

Trace Back (TB) Plants: All plants of the same taxon (such as genus, species, hybrid, variety, or cultivar) regardless of size, location or lot, back to the original propagation source if still existing.

Trace Back (TB) Site: Any location that shipped potentially infected plants to a confirmed infested nursery, residence or commercial landscape.

Trace Forward (TF) Plants: Plants identified on a trace forward list as being potentially infected with *P. ramorum*.

Trace Forward (TF) Site: Any location that received potentially infected plants from a confirmed infested source nursery; including residential or commercial landscapes.

Before inspection

1. Plan an inspection without delay. However, an inspection must be conducted when favorable climatic conditions, pathogen infectivity, and host susceptibility share an optimum time for disease development and symptoms are likely to be expressed. If conditions are not suitable for disease development when the initial inspection is

conducted, an additional inspection must be conducted when favorable climatic conditions are present.

2. For Federal inspectors, notify state officials of your plans to inspect.
3. Coordinate visit with State inspector.
4. Federal and State or County inspectors should contact the nursery owners/managers prior to the visit to determine how many plants are still in stock and to arrange for the inspection. This nursery contact would normally occur within 24 hours of the expected arrival time for the TB inspection.
5. If you are unable to visit the nursery within one day of your contact with the nursery owner/manager, send a PPQ form 523, an Emergency Action Notification (EAN) by fax to them and request that they sign and return it to you by fax.

Survey/Inspection Procedure

1. Identify yourself and agency to the nursery/facility owner/manager.
2. Explain to the nursery/facility owner/manager the purpose of your visit.
3. Obtain copies of the shipping documents that relate to the trace forward plants received from a confirmed *P. ramorum* infested nursery. Also obtain copies of those documents associated with trace forward plants that came from the confirmed positive nursery that have been shipped by the trace forward nursery to other nurseries or retail facilities.
4. Determine the presence or absence of any of the trace forward High Priority Target Plants and Medium Priority Target Plants at the trace forward site.
 - If the trace forward nursery received high priority target plants from the infested source nursery during the past 12 months, a trace forward investigation must be conducted, even if there is no longer any high priority target plants present on the trace forward nursery. If records are available, use them to determine if or what other hosts may have come in contact with the trace forward plants, and where in the nursery that contact occurred.
 - If the trace forward nursery received in the previous 12 months only medium priority target plants from the infested source nursery a trace forward investigation must be conducted if the plants are still present on the nursery.
 - If the trace forward nursery received in the previous 12 months only medium priority target plants from the infested source nursery a trace forward investigation may be conducted or the investigation may be deferred and the nursery placed on the target list for the next cycle of nursery surveys for *P. ramorum*, if the plants are no longer present on the nursery.
5. Ask owner/manager to fill out questionnaire (attached, ~~Exhibit D~~ ^{Appendix}), or complete with their input.
6. Complete an Emergency Action Notification (EAN, PPQ form 523) to place a hold on all the high and medium priority target trace forward plants from the infested (source)

nursery and other HAP, products or articles that present a risk of spreading *P. ramorum*. The *P. ramorum* host list is available at the APHIS *P. ramorum* Web site: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram

Use this language in Section 16 of the PPQ 523 – Action Required:

- All plants of the following listed species received from [INSERT Name of Nursery] during the period from [INSERT dates, one year prior to current date. Example: March 1, 2007 to February 28, 2008] are prohibited from movement pending further notification by USDA, APHIS, PPQ. Access to these plants is limited to appropriate Regulatory Officials. These plants are not to be sampled, sold, or moved within the nursery, unless under the supervision of a Regulatory Official. In addition: [Select option a. or b. below]
 - a. If this nursery is a production/wholesale nursery then add the following language to Section 16 of the EAN. The following shall also be prohibited from movement pending further notification by USDA, APHIS, PPQ:
 1. all high priority target host plants
 2. all other HAP located in the high priority target block
 3. all medium priority target plants

OR

- b. If this nursery is a retail nursery then add the following language to Section 16 of the EAN. The following shall also be prohibited from movement pending further notification by USDA, APHIS, PPQ
 1. all high priority target and medium priority target plants in the nursery.
 2. all other HAP within 2 meters of the high priority target plants.

7. Plants to be held

- For production/wholesale nurseries:
 - a. Place a hold on all high priority target plants and all other HAP in the block where the high priority target plants have been located at the trace forward nursery.
 - b. All medium priority target plants are to be held.
 - c. All other HAP in the trace forward nursery is not required to be held under this protocol because risk of spread in non-TF blocks is low. Inspectors may place other plants and other HAP, products or articles that present a risk of spreading *P. ramorum* on hold at any time per Federal and State authorities.
- For retail nurseries (because plant propagation does not occur on site risk is lower):
 - a. Place a hold on all high priority target plants and all other HAP within a 2 meter radius of the high priority target plants located at the trace forward nursery.
 - b. All Medium priority target plants are to be held.

- c. All other HAP in the trace forward nursery that are not within a 2 meter radius of high priority target plants are **not** required to be held under this protocol, however inspectors may place HAP, plant products or articles that present a risk of spreading *P. ramorum* on hold at any time per Federal and State authorities (e.g. If plants from the TF nursery have been moved within the retail nursery or commingled with other HAP in that nursery, the additional HAP must also be held.)
- d. Once inspection and sampling are complete, the held plants may be consolidated and segregated. If the plants are not consolidated and segregated, then the affected portion of the nursery must be closed to the public. With the approval of the regulatory officer, segregated plants may be moved to a site within the nursery or to a location away from the nursery. Any movement of the segregated plants must be done in a manner that will safeguard and prevent the spread of the disease at the nursery, and be conducted under the direction and oversight of a regulatory official. Segregation must include storage on a hard impermeable surface (e.g. a 45 mil thick pond liner or concrete or asphalt) and may not be within 2 meters of any other plant. The 2 meter requirement addresses the spread potential of *Phytophthora ramorum* should any TF plants be positive. The impermeable surface should ideally be situated to drain away from HAP.

8. Determining number of plants to be inspected

- Determine, if possible, all TF HAP genera at the receiving nursery and visually inspect all TF HAP genera at the receiving nursery. If TF HAP plants can not be determined, visually inspect at least 850 HAP plants of the same genera shipped from the TF nursery.
9. Visually inspect the appropriate number of HAP for unhealthy tissue. To visually inspect a plant, carefully lift the plant from surrounding plants and examine all plant leaves and stems for unhealthy tissue particularly for but not limited to the presence of water-soaked or necrotic lesions consistent with *P. ramorum* infection. Take care to examine the leaves on the interior as they may exist in a microclimate more conducive to disease development and may be more likely to have disease symptoms. Be sure to mark plant as visually inspected either with flagging with the appropriate sample number or a stake with the appropriate sample number. Also examine the leaves that have fallen off the plant for disease symptoms. A physical sample of the inspected plant is only to be taken if unhealthy plant tissue is present. **Do not sample asymptomatic plants**, but feel free to sample any and all plants exhibiting unusual or atypical plant tissue. Images of *P. ramorum* symptoms are available at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram. Keep in mind that these images should only be used as a reference, and should not be considered all inclusive for each species. Links to other sites such as <http://www.cnr.berkeley.edu/comtf/>, which provide nursery guides that describe and illustrate *P. ramorum* infections are also available at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram. These

nursery guides may be viewed and printed from these sites. Symptoms of *P. ramorum* may include:

- Leaf spots
- Twig dieback
- Stem cankers

Keep in mind that many other pathogens cause similar symptoms. Remember that other symptoms caused by *Phytophthora ramorum* as yet unseen may be detected, so sample any unusual or atypical plant symptoms.

10. ***SAMPLE PLANT TISSUE from any and all visually inspected plants that appear unhealthy.*** Each sample should consist of a minimum of five leaves; for vaccinium and other small leaf hosts collect the terminal last 3 inches of branch tips, if present, from each unhealthy plant. If, however, only one leaf is symptomatic include only the one leaf with lesions. Please examine any other leaves on the plant for the presence of lesions, because chances are much smaller lesions may be present on other leaves of the same plant. Take care to examine the leaves on the interior as they may exist in a microclimate more conducive to disease development and may be more likely to have disease symptoms, not forgetting the leaves that have fallen off. If certain areas of the nursery are more prone to disease development (such as low areas where water might puddle or places where mist or fog persists) these areas should be included in the sampling process.

For all samples:

- Fill out PPQ Form 391 (Name of host, variety, state code, facility code, etc.).
- Assign a unique sample number using the following conventions:
XX-ABC-0001
where XX is your two-letter state code, ABC is a three-letter, state-assigned facility code, and 0001 is the sample number for that facility.
- Log each sample according to the unique sample number.
- Double bag samples (e.g., symptomatic leaf tissue with associated twig intact) in plastic bags.
- Label with collection date, time, location, responsible party. Be sure to write sample number on the bag containing the sample.
- Be sure to mark sampled plants either with flagging with the appropriate sample number or a stake with the appropriate sample number.
- Refrigerate, but do not freeze specimen.
- Submit with minimal delay to your designated laboratory for analysis. All tissue samples must be analyzed by APHIS-approved or APHIS provisionally-approved Laboratory using the appropriate diagnostic protocols.
- Overnight the sample if necessary – do not send samples on Friday or the day before lab holidays – check with the lab – as they may not be received until the following Monday. Samples held in a shipper's warehouse without refrigeration may deteriorate and not be testable. Identify the sample(s) as Trace Forward (TF) Sample to distinguish the sample(s) from National Survey samples.

11. Ask owner/manager to identify cull or waste/refuse piles and compost piles. Check any piles for *P. ramorum* symptomatic plants and plant material and sample as above, if observed.
 - Include appropriate sampling and testing of soil and water. Soil should be tested underneath piles. Water should be baited and sampled from downward slopes, as well as any collected or drained reservoirs from the site.

12. If the survey requires the inspector to move among multiple greenhouses, shade houses or discrete blocks, disinfect tools, hands and shoes (or wear disposable gloves and tyvek booties) to prevent pathogen spread between areas. If using disposable gloves and booties, be sure to dispose of these after each individual greenhouse/shade house/block inspection. Disposable rubber gloves and tyvek booties can also be disinfected using 10% bleach solution or a quaternary ammonium solution (at the labeled rate) between inspecting each area. See Exhibit E for details on disinfectants and fumigants for use in nurseries. (Washtubs with ~ 1/2 inch of disinfectant to step in for booties and 3 inches in buckets to dip gloved hands should be sufficient.) Be sure to properly disinfect booties and gloves between all nursery blocks. Disposable gloves and booties should be bagged and disposed by burial or incineration, or in a landfill upon completion of inspections.

13. Sanitize/disinfest tools and shoes before leaving premises, using an appropriate disinfectant for the control of *Phytophthora spp.* (such as a 10% solution of bleach or quaternary ammonium solution at labeled rates). See Exhibit E for details on disinfectants and fumigants for use in nurseries. *Appendix*

14. Advise the owner/manager –
 - The plants will remain on hold until further notification from USDA, APHIS.
OR
 - For Production/Wholesale Nurseries:
 - a. Once plant samples are taken, the owner/manager may choose to voluntarily destroy all HAP in the nursery once sampling is completed. After the plants have been destroyed, the establishment can continue to do business without any regulatory restrictions. Destruction and disinfestation will be performed as per the APHIS, PPQ Confirmed Nursery Protocol. Destruction and disinfestation to be conducted under the supervision of a Federal or other appropriate regulatory official. If plant samples are found positive for *P. ramorum* whether the plants are remaining on the site or have been destroyed, the APHIS, PPQ Confirmed Nursery Protocol for Wholesale and Production Nurseries will be applied.
 - b. Where many samples have been taken, plants may be released on a block by block basis if *P. ramorum* has not been detected in the block after all test results for that block are available.
 - For retail nurseries:
 - a. Once sampling is completed the owner/manager may choose to voluntary destroy the HAP received from the TF nursery and all other plants within 2 meters of the trace forward plants. After the

plants have been destroyed, the establishment can continue to do business without any regulatory restrictions. Destruction and disinfestation will be performed as per the PPQ Retail Confirmed Nursery Protocol. Destruction and disinfestation to be conducted under the supervision of a Federal or other appropriate regulatory official. If plant samples are found positive for *P. ramorum* whether the plants are remaining on the site or have been destroyed, the APHIS, PPQ Confirmed Nursery Protocol for retail nurseries will be applied

- b. The high priority target plants and plants in the 2 meter quarantine radius may be treated as a quarantine block and plants may be released on a block by block basis if *P. ramorum* has not been detected in the block after all test results for that block are available.
- **If any plant samples are found positive for *P. ramorum* whether the plants are remaining on the site or have been destroyed, the APHIS, PPQ Confirmed Nursery Protocol for production/wholesale nurseries or the Confirmed Nursery Protocol for retail nurseries will be applied, whichever is applicable.**
 - **If any samples associated with the cull piles are found positive for *P. ramorum*, the APHIS, PPQ Confirmed Nursery Protocol for production/wholesale nurseries or the Confirmed Nursery Protocol for retail nurseries will be applied whichever is applicable.**

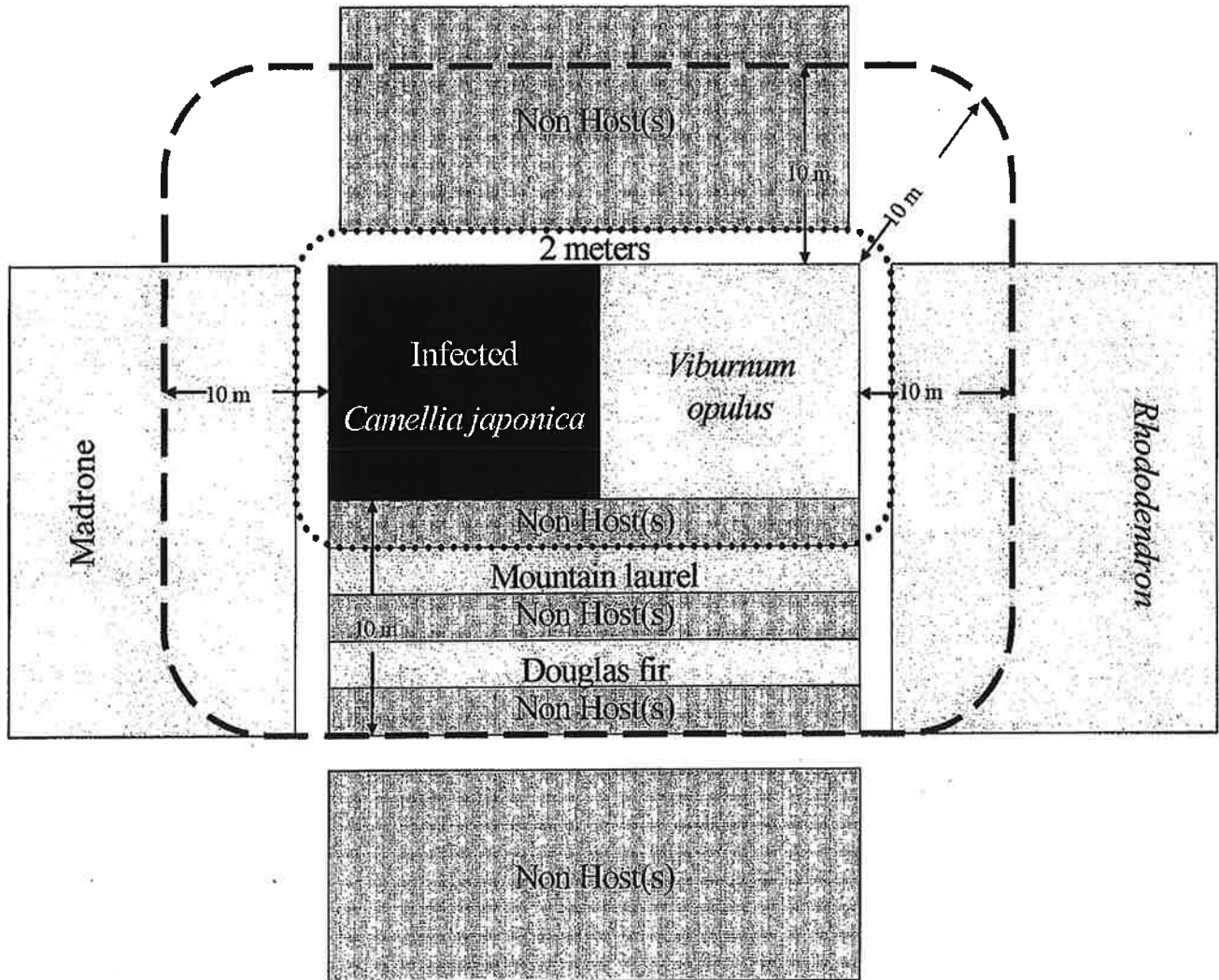
Appendix A

APHIS List of Regulated Hosts and Plants Associated with *Phytophthora ramorum*

A current list may be found at the USDA APHIS PPQ website at
http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/

Appendix B

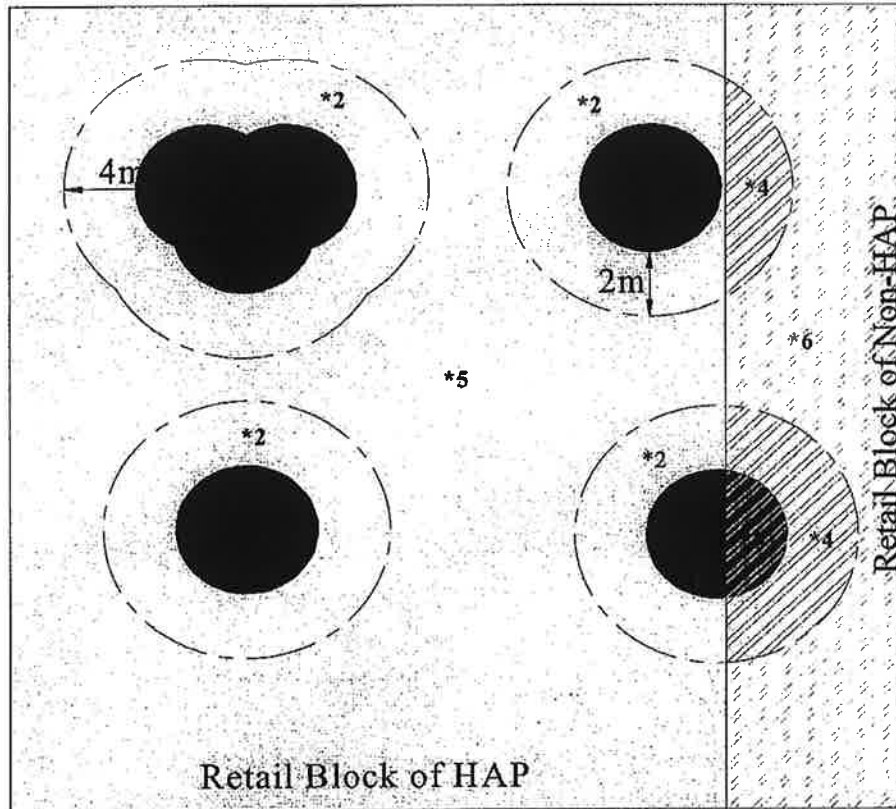
Schematic of Nursery with Infected Host Plant(s)



| | |
|--|---|
| | <p>Destruction Block Action: Destroy <i>Camellia japonica</i> and <i>Viburnum opulus</i>. Hold and monitor all non-hosts.</p> |
| | <p>Quarantine Block Action: Hold and monitor all Mountain laurel and Douglas fir, as well as some Madrone and Rhododendron.</p> |

Appendix C

Schematic of Retail Nursery with Infected Host Plant(s)
 July 9, 2007



| | | |
|------------------------|-------------------|------------------------------------|
| Red (* 1) | Destruction block | Destroy infected plant and all HAP |
| Yellow (*2) | Quarantine block | Hold HAP from sale for 90 days |
| Hatch Over Red (*3) | | Release non-HAP from sale |
| Hatch Over Yellow (*4) | | Release non-HAP for sale |
| Green (*5) | | Release HAP for sale |
| Blue Hatch (*6) | | Release non-HAP for sale |

Appendix D

***Phytophthora ramorum* Questionnaire (Property Owner or Manager): Part 1**

Name of Nursery or Garden Store: _____

Name of Owner or Manager: _____

Address of Site: _____

City: _____, State: _____, Zip Code: _____

Contact Name: _____, Title: _____

Phone Number: _____, Fax Number: _____

GIS Coordinates (if available): _____

Type of Facility (circle): Nursery Greenhouse Wholesale Retailer
 Other _____

1. Are you the owner or manager of the property or facility? If not please provide owner contact information. _____

2. Did you purchase the plant(s) in question? (If "no", seek information on individual who planted material in question) _____

3. How long ago did you purchase the plant(s)? _____

4. Did you purchase any other plants from this same nursery? _____

5. Have you noticed any other problems with plants on your property? _____

6. Have you moved any plants, received from the infested nursery, from your primary retail location to a different location? _____
 - a) What types and varieties were they? _____

 - b) How long ago was that? _____
 - c) What is the address of that location? _____

7. Did you move any plants here from a different location? _____
- a) What types and varieties were they?

- b) How long ago was that? _____
- c) What is the address of that location?

8. Do you have a landscape company that purchases plants from you? _____
9. What is the contact information for the landscape company? _____

10. What is your source of water? _____

***Phytophthora ramorum* Questionnaire (Property Owner or Manager): Part 2**

Information on suspect plant material for inspector visiting property:

1. What is the variety and number of plants? _____
2. What is the condition of the plant material? _____
3. Have the plants been trimmed or pruned? _____
4. How are the trimmings disposed of? _____
5. Did the plant material come in pots? _____
- a) Did you dispose of the pots or re-use them? _____
6. If the pots were reused or stored, describe how the pots were handled?

Appendix E

Treatment and Disinfection

The following techniques are approved by USDA APHIS PPQ for control of *P. ramorum* in nurseries found to contain plants infected with *P. ramorum*.

Infected Plants:

Note: HAP material, including leaf litter, must not be placed in compost piles or be removed from the nursery site as trash or in debris removal. HAP material should be collected and incinerated or double bagged and deep buried in a site approved by USDA, APHIS or delegated regulatory authority.

- **Incineration (burning to ash):** Infected plants, associated growth media, associated containers (i.e. pots and trays), all leaf debris in and around the area where plants were stored may be disposed of by incineration at a facility or other location (e.g. on site) approved by USDA and permitted within state and municipal statutes or regulations. Off nursery movement must be properly safeguarded and every effort to prevent plant debris or soil from being dislodged from the plants prior to incineration should be taken. Burning may be through open burning or in an incinerator.
- **Deep burial:** Infected plants, associated growth media, associated containers (i.e. pots and trays), all leaf debris in and around the area where plants were stored must be double bagged using plastic bags of 2 mil thickness or greater and buried to a depth of no less than two meters. The material must be buried at a USDA approved site, onsite, or municipal landfill, which is expected to remain undisturbed. Every effort to prevent plant debris or soil from being dislodged from the plants should be taken.
- **Steam sterilization:** Dry heat or steam commonly heated to internal temperatures of 212° F (100° C) for 30 minutes followed by burial in a landfill, or as otherwise detailed in the USDA Treatment Manual for "insect pests and pathogens in garbage", Schedule T415b (http://www.aphis.usda.gov/ppq/manuals/pdf_files/Treatment%20Chapters/05-05-T400-5.pdf).

Non-Porous Surfaces:

Most disinfectants are not labeled for use in soil and are only useful for nonporous materials such as concrete floors, nursery pots, and plastic sheeting. A number of disinfectants are registered for use on nonporous surfaces that may effectively reduce populations of *Phytophthora* species. If it is practical, tools such as knives, pruners, water breakers, water wands and other implements used in the quarantine area should only be used in the quarantine area. If tools and other implements must be moved from the quarantine area, then regular disinfection using an appropriate disinfectant for the control of *P. ramorum* is recommended prior to removal from the quarantine block. The following table modified from <http://cpmcnet.columbia.edu/dept/ehs/decon.html> examines the effects of different classes of disinfectants on microbial populations. This list is for explanation and information

only. Few disinfectants are specifically labeled for *Phytophthora* species and are shown in **Bold**.

All labels for the disinfectants listed below must be strictly adhered to for maximum efficacy and environmental and worker safety.

Summary of Disinfectant Activities

| Disinfectant | Trade names | Comments | Contact time |
|---|---|---|---------------|
| Alcohols (ethyl and isopropyl) 60-85% | Lysol Spray | Evaporates quickly so that adequate contact time may not be achieved, high concentrations of organic matter diminish effectiveness; flammable. | 10-15 minutes |
| Phenolics (0.4%-5%) | Pheno-cen | Phenol penetrates latex gloves; eye/skin irritant; remains active upon contact with organic soil; may leave residue. | 10-15 minutes |
| Quaternary Ammonium (0.5-1.5%) | Consan Triple Action 20 Physan 20 Green-Shield 20 | Effective for non-porous surface sanitation (floors, walls, benches, pots). Low odor, irritation. Use according to labels. | 10-15 minutes |
| Chlorine (100-1,000 ppm) | 10% Clorox 10% Bleach | Inactivated by organic matter; fresh solutions of hypochlorite (Clorox) should be prepared every 8 hours or more frequently if exposed to sunlight; corrosive; irritating to eyes and skin. Exposure to sunlight further reduces hypochlorite efficacy. Keep solution in opaque container. | 10-15 minutes |

Water:

- **For dust abatement, fire suppression, and equipment cleaning:** Clorox (sodium hypochlorite) is labeled (EPA Reg. No 5813-50) for treatment of water (~50 ppm available chlorine) for controlling the spread of *Phytophthora lateralis* via water used for dust abatement, fire suppression and equipment cleaning. The active ingredient level must be measured from water collected at the sprinkler head.
- **For irrigation:** Chlorine levels of 2ppm or 2mg/liter or greater has been correlated with the control of *Phytophthora* spp. in re-circulated irrigation systems. For irrigation purposes, re-circulated, non-municipal water, must be chlorinated at an active chlorine concentration

equal to or greater than 2 mg/liter of water; for facilities that recycle water, this chlorine level must be monitored.

Soil and Potting Media:

- **Potting media:** Potting media must be heated such that the temperature in the center of the load reaches at least 180 degrees F for 30 minutes. Treatment must be conducted in the presence of an inspector or treated with an approved fumigant as detailed below.
- **Soil:** Soil must be heated such that the temperature in the center of the load reaches at least 180 degrees F for 30 minutes. Treatment must be conducted in the presence of an inspector or treated with an approved fumigant as detailed below. Methyl bromide has been used for fumigating wood products, but the data on fungi and related organisms in wood are limited. However, methyl bromide has a long history of fumigation of soil in the field and greenhouse. It has commonly been used in combination with chloropicrin for control of *Phytophthora* spp. and other pests in strawberry beds. Methyl bromide has been used for soil treatment for the mitigation of *P. cinnamoni* in citrus groves. However, many of the compounds currently in use have been implicated in human and environmental risks. Solarization is not a consideration as a viable option for soil treatment for *P. ramorum* due to insufficient data on treatment efficacy.

All fumigants are restricted use and must be applied according to labels by a licensed applicator. Any use of pesticides in any manner not listed on the label is unlawful.

Summary of Labeled Soil Fumigants

| Fumigant | Trade names | Comments |
|----------------|--|--|
| Chloropicrin | Chlor-O-Pic Metapicrin Timberfume Tri-Clor | Often used in combination with methyl bromide due to its ability to be detected in small quantities. |
| Dazomet | Basamid | Methyl isothiocyanate (MITC) breaks down into cyanide gas. Granular formulation that is water activated. |
| Metam-sodium | Busan 1020 Busan 1180 Busan 1236 Metam Vapam | Metam can be applied through irrigation. Tarping can increase efficacy. All application must be made in accordance with labeling. |
| Methyl Bromide | Tri-Con Terr-O-Gas Preplant Soil Fumigant Pic-Brom | Colorless and odorless. Usually combined in various concentrations with Chloropicrin (tear gas). Use is restricted due to ozone depletion potential. |

Physical Treatment of Soil:

- Mitigation of infested soil can also be achieved by installing permanent impermeable, non-porous barriers that consist of cement, concrete or asphalt. These barriers must be constructed so that no native soil within the destruction block is visible. The barriers should be graded such that no standing water can be observed.

Equipment and Personnel (Inspectors and employees):

- **Access to infested areas and hold areas should be limited, as much as possible, to officials and employees. Everyone entering and leaving the nursery site must scrape off loose pieces of soil into the destruction block. Those working with, or in contact with suspected infested material (including plants), must wash hands using soap or approved disinfectant immediately after completion of task. There are no products currently labeled for use on porous materials for *Phytophthora* control.**
- Personnel should not have access to other parts of the nursery after entering the destruction block on the same day. If entry is unavoidable, follow disinfection procedures in this section.
- A disinfectant foot bath should be placed near the exit to the destruction blocks and quarantine blocks and used by personnel entering and exiting the buffer zone and entering and exiting the quarantine block and entering and exiting the destruction block at the infested nursery site, where the contact with potentially infested soil or plant debris on footwear is likely. The foot bath must be filled with fresh disinfectant at least on a daily basis or more frequently if contaminated with filth, in accordance with label directions. Use of disposable shoe covers may be used in lieu of a footbath, if disposed of immediately upon exiting from the quarantine or destruction block. The disposable shoe covers must be placed in bags and incinerated, deep-buried or properly disposed in a sanitary landfill.
- The tires (or other parts in contact with the soil or plants, such as the bed of trucks) of vehicles must be cleaned of loose soil and plant debris and disinfested with the appropriate labeled products before leaving the infested site. If at all possible, vehicles should not be allowed in the destruction blocks at all. Any efficacious product labeled for use on non-porous surfaces may be used on tires or vehicle undercarriages.
- Do not visit other nursery sites in potentially contaminated work clothing and footwear. Where it is necessary that visitors enter the nursery, the nursery should ensure that every precaution is taken to prevent the movement of infected plants, contaminated soil or debris by the visitor.

Wood surfaces suspected of contamination with *P. ramorum* should be disposed of as stated above under "Infected Plants". There is no effective way to test or treat wood surfaces for contamination.

**Scope of Work for *Phytophthora ramorum*
(Sudden Oak Death)
In Regulated Counties
July 1, 2010 - June 30, 2011
FY 2010/2011**

Appendix D

Federal Domestic Quarantine 7 CFR 301.92

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301.92 *Phytophthora ramorum*

Federal Domestic Quarantine

Sec. 301.92 Restrictions on interstate movement

(a) No person may move interstate from any quarantined area any regulated, restricted, or associated article or any other nursery stock except in accordance with this subpart.

(b) No person may move interstate from any nursery in any regulated area any nursery stock except in accordance with this subpart.

(c) No person may move interstate from any quarantined or regulated area any regulated restricted, or associated article or nursery stock that has been tested with a test approved by APHIS and found infected with *Phytophthora ramorum*, or that is part of a plant that was found infected with *Phytophthora ramorum*, unless such movement is in accordance with part 330 of this chapter.

Sec. 301.92-1 Definitions

Administrator. The Administrator, Animal and Plant Health Inspection Service, or any person authorized to act for the Administrator.

Animal and Plant Health Inspection Service. The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture.

Associated article. Any article listed in 301.92-2(C) of this subpart.

Bark chips. Bark fragments broken or shredded from a log or tree.

Certificate. A document, stamp, or imprint by which an inspector or person operating under a compliance agreement affirms that a specified regulated or associated article meets applicable requirements of this subpart and may be moved interstate to any destination.

Compliance agreement. A written agreement between APHIS and a person engaged in growing, processing, handling, or moving regulated or associated articles, wherein the person agrees to comply with this subpart.

Duff. Decaying plant matter that includes leaf litter, green waste, stem material, bark, and any other plant material that, upon visual inspection, does not appear to have completely decomposed.

Firewood. Wood that has been cut, sawn, or chopped into a shape and size commonly used for fuel, or other wood intended for fuel.

Forest stock. All flowers, trees, shrubs, vines, scions, buds, or other plants that are wild-grown, backyard-grown, or naturally occurring.

From. An article is considered to be "from" a specific site or location for the purposes of this subpart if it was grown or propagated in, stored or sold, or distributed from the site or location.

Growing media. Any material in which plant roots are growing or intended for that purpose.

Inspector. Any employee of APHIS, the U.S. Department of Agriculture, or other person authorized by the Administrator to perform the duties required under this subpart.

Interstate. From any State into or through any other State.

Log. The bole of a tree; trimmed timber that has not been sawn further than to form cants.

Lot. A contiguous block of plants of the same species or cultivar, of the same container size and from the same source, if known.

Lumber. Logs that have been sawn into boards, planks, or structural members such as beams.

Moved (move, movement). Shipped, offered for shipment, received for transportation, transported, carried, or allowed to be moved, shipped, transported, or carried.

Mulch. Bark chips, wood chips, wood shavings, or sawdust, or a mixture thereof, that could be used as a protective or decorative ground cover or as part of a growing media mixture.

Non-host nursery stock. Any taxa of nursery stock not listed in 301.92-2 as a regulated or associated article.

Nursery. Any location where nursery stock is grown, propagated, stored, or sold, or any location from which nursery stock is distributed. Locations that grow trees for sale without roots (e.g., as Christmas trees) are considered to be nurseries for the purposes of this subpart.

Nursery stock. All plants for planting, including houseplants, propagative material that is grown in a nursery, and tree seedlings for reforestation, except the following: Seeds; turf or sod; bulbs, tubers, corms, or rhizomes; greenhouse grown cactus, succulents, and orchids; aquarium grown aquatic plants; greenhouse, container, or field grown palms; greenhouse, container, or field grown cycads, and tissue culture plants grown in vitro; and plants meeting the definition of forest stock.

Permit. A written authorization issued by APHIS to allow the interstate movement of restricted articles in accordance with part 330 of this chapter.

Person. Any association, company, corporation, firm, individual, joint stock company, partnership, society, or other entity.

Plant Protection and Quarantine. The Plant Protection and Quarantine program of the Animal and Plant Health Inspection Service, United States Department of Agriculture.

Quarantined area. Any State, or any portion of a State, listed in 301.92-3(A)(3) of this subpart or otherwise designated as a quarantined area in accordance with 301.92-3(A)(2) of this subpart.

Regulated area. Any area listed in 301.92-3(B) of this subpart.

Regulated article. Any article listed in 301.92-2(B) of this subpart.

Restricted article. Any article listed in 301.92-2(A) of this subpart.

Soil. The loose surface material of the earth in which plants grow, in most cases consisting of disintegrated rock with an admixture of organic material.

State. The District of Columbia, Puerto Rico, the Northern Mariana Islands, or any State, territory, or possession of the United States.

Sec. 301.92-2 Restricted, regulated, and associated articles; lists of proven hosts and associated plant taxa.

(A) Restricted articles. The following are restricted articles:

1. Bark chips or mulch located in a quarantined area that are proven host plant taxa listed without asterisks in 301.92-2 (D)
2. Forest stock located or grown in a quarantined area and that are proven host plant taxa or associated plant taxa listed in paragraph (D) or (E) of this section.
3. Any other product or article that an inspector determines to present a risk of spreading *Phytophthora ramorum*, if an inspector notifies the person in possession of the product or article that it is a restricted article.

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05-22-08**(B) Regulated articles.** The following are regulated articles:

1. Nursery stock, decorative trees without roots, unprocessed wood and wood products, and plant products, including firewood, logs, lumber (**firewood, logs and lumber of species listed in paragraph (d) of this section and that are marked with an asterisk* are not regulated articles**), wreaths, garlands, and greenery of proven host plant taxa listed in paragraph (D) of this section.
2. Soil and growing media.
3. Any other product or article that an inspector determines to present a risk of spreading *Phytophthora ramorum* if an inspector notifies the person in possession of the product or article that it is subject to the restrictions in the regulations.

(C) Associated articles. The following are associated articles:

1. Nursery stock of associated plant taxa listed in paragraph (E) of this section.

(D) Proven host plant taxa. The following are proven hosts of *Phytophthora ramorum*:

- * *Acer macrophyllum*- Bigleaf maple
- Acer pseudoplatanus*- Planetree maple
- * *Adiantum aleuticum*- Western maidenhair fern
- * *Adiantum jordanii*- California maidenhair fern
- * *Aesculus californica*- California buckeye
- Aesculus hippocastanum*- Horse chestnut
- * *Arbutus menziesii*- Madrone
- * *Arctostaphylos manzanita*- Manzanita
- * *Calluna vulgaris*- Scotch heather
- * *Camellia* spp.- Camellia (all species, hybrids, cultivars)
- * *Castanea sativa*- Sweet chestnut
- Fagus sylvatica*- European beech
- * *Frangula californica* (= *Rhamnus californica*)- California coffeeberry
- * *Frangula purshiana* (= *Rhamnus purshiana*)- Cascara
- Fraxinus excelsior*- European ash
- * *Griselinia littoralis*- Griselinia
- * *Hamamelis virginiana*- Witch hazel
- * *Heteromeles arbutifolia*- Toyon
- * *Kalmia* spp.- Kalmia (all species, hybrids, cultivars)
- * *Laurus nobilis*- Bay laurel
- Lithocarpus densiflorus*- Tanoak
- * *Lonicera hispidula*- California honeysuckle
- * *Magnolia doltsopa* (= *Michelia doltsopa*) (*Michelia*)
- * *Maianthemum racemosum* (= *Smilacina racemosa*)- False Solomon's seal
- * *Parrotia persica*- Persian ironwood
- * *Photinia fraseri*- Red tip photinia
- * *Pieris* spp.- Pieris (all species, hybrids, and cultivars)
- * *Pseudotsuga menziesii* var. *menziesii* and all nursery-grown *P. menziesii*- Douglas fir
- Quercus agrifolia*- Coast live oak
- Quercus cerris*- European turkey oak

- Quercus chrysolepis*- Canyon live oak
 - Quercus falcata*- Southern red oak
 - * *Quercus ilex*- Holm oak
 - Quercus kelloggii*- California black oak
 - Quercus parvula* var. *shrevei* and all nursery grown *Q. parvula*- Shreve's oak
 - * *Rhododendron* spp.- Rhododendron (including azalea) - includes all species, hybrids and cultivars
 - * *Rosa gymnocarpa*- Wood rose
 - * *Salix caprea*- Goat willow
 - * *Sequoia sempervirens*- Coast redwood
 - * *Syringa vulgaris*- Lilac
 - * *Taxus baccata*- European yew
 - * *Trientalis latifolia*- Western starflower
 - * *Umbellularia californica*- California bay laurel, pepperwood, Oregon myrtle
 - * *Vaccinium ovatum*- Evergreen huckleberry
 - * *Viburnum* spp.- Viburnum (all species, hybrids, and cultivars)
- (E) Associated plant taxa.** The following plant taxa are considered to be associated with *Phytophthora ramorum*
- Abies concolor*- White fir
 - Abies grandis*- Grand fir
 - Abies magnifica*- Red fir
 - Acer circinatum*- Vine maple
 - Acer davidii*- Striped bark maple
 - Acer laevigatum*- Evergreen maple
 - Arbutus unedo*- Strawberry tree
 - Arctostaphylos columbiana*- Manzanita
 - Arctostaphylos uva-ursi*- Kinnikinnick, bearberry
 - Ardisia japonica*- Ardisia
 - Berberis diversifolia* (= *Mahonia aquifolium*) (Oregon grape)
 - Calycanthus occidentalis*- Spicebush
 - Ceanothus thyrsiflorus*- Blueblossom
 - Cercis chinense* (Chinese redbud)**
 - Cinnamomum camphora*- Camphor tree
 - Clintonia andrewsiana*- Andrew's clintonia bead lily
 - Cornus kousa* x *Cornus capitata*- Cornus Norman Haddon
 - Corylopsis spicata*- Spike winter hazel
 - Corylus cornuta*- California hazelnut
 - Drimys winteri*- Winter's bark
 - Dryopteris arguta*- California wood fern
 - Euonymus kiautschovicus*- Spreading euonymus
 - Fraxinus latifolia*- Oregon ash
 - Garrya elliptica*- Silk tassel tree
 - Gaultheria shallon*- Salal, Oregon wintergreen
 - Hamamelis mollis*- Chinese witch-hazel
 - Hamamelis* x *intermedia* (*H. mollis* & *H. japonica*)- Hybrid witchhazel
 - Ilex purpurea*- Oriental holly
 - Leucothoe axillaris*- Fetter-bush, dog hobble

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Leucothoe fontanesiana- Drooping leucothoe
Loropetalum chinense- Loropetalum
Magnolia denudata x salicifolia (magnolia)
Magnolia ernestii (= *Michelia wilsonii*) (Michelia)
***Magnolia figo* (= *Michelia figo*) (banana shrub)**
Magnolia grandiflora- Southern magnolia
Magnolia kobus (kobus magnolia)
Magnolia liliiflora (= *M. quinquepetala*) (purple magnolia)
Magnolia maudiae (= *Michelia maudiae*) (Michelia)
Magnolia salicifolia (= *M. proctoriana*) (anise magnolia)
Magnolia stellata- Star magnolia
Magnolia x loebneri- Loebner magnolia
Magnolia x soulangeana- Saucer magnolia
Magnolia x thompsoniana (*M. tripetala* and *M. virginiana*) (magnolia)
Manglietia insignis- Red lotus tree
Nerium oleander- Oleander
Nothofagus obliqua- Roble beech
Osmanthus decorus (*Phillyrea decora*; *P. vilmoriniana*)-
Osmanthus
Osmanthus delavayi- Delavay Osmanthus, Delavay tea olive
Osmanthus fragrans- Sweet olive
Osmanthus heterophyllus- Holly olive
Osmorhiza berteroi- Sweet Cicely
Parakmeria lotungensis- Eastern joy lotus tree
Physocarpus opulifolius- Ninebark
Prunus laurocerasus- English laurel, cherry laurel
Prunus lusitanica- Portuguese laurel cherry
Pyracantha koidzumii- Formosa firethorn
Quercus acuta- Japanese evergreen oak
Quercus petraea- Sessile oak
Quercus rubra- Northern red oak
Rosa (specific cultivars)-
 Royal Bonica (tagged: "MEImodac")
 Pink Meidiland (tagged: "MElpoque")
 Pink Sevillana (tagged: "MElgeroka")
Rosa rugosa- Rugosa rose
Rubus spectabilis- Salmonberry
Schima wallichii- Chinese guger tree
Taxus brevifolia- Pacific yew
Taxus x media- Yew
Torreya californica- California nutmeg
Toxicodendron diversilobum- Poison oak
Vancouveria planipetala- Redwood ivy

Sec. 301.92-3 Quarantined and regulated areas

(A) **Quarantined areas.** Except as otherwise provided in paragraph (A)(2) of this section, the Administrator will list as a quarantined area in paragraph (A)(3) of this section each State, or each portion of a State, in which *Phytophthora ramorum* has been confirmed by an inspector to be established in the natural

environment, in which the Administrator has reason to believe that *Phytophthora ramorum* is present in the natural environment, or that the Administrator considers necessary to quarantine because of its inseparability for quarantine enforcement purposes from localities in which *Phytophthora ramorum* has been found in the natural environment. Less than an entire State will be designated as a quarantined area only if the Administrator determines that:

- (i) The State has adopted and is enforcing restrictions on the intrastate movement of the regulated, restricted, and associated articles that are substantially the same as those imposed by this subpart on the interstate movement of regulated, restricted, and associated articles; and
- (ii) The designation of less than the entire State as a quarantined area will prevent the interstate spread of *Phytophthora ramorum*.

2. The Administrator or an inspector may temporarily designate any nonquarantined area in a State as a quarantined area in accordance with paragraph (A)(1) of this section. The Administrator will give a copy of this regulation along with a written notice for the temporary designation to the owner or person in possession of the nonquarantined area. Thereafter, the interstate movement of any regulated, restricted, or associated article from an area temporarily designated as a quarantined area will be subject to this subpart. As soon as practicable, this area will be added to the list in paragraph (A)(3) of this section or the designation will be terminated by the Administrator or an inspector. The owner or person in possession of an area for which designation is terminated will be given notice of the termination as soon as practicable.
3. The following areas are designated as quarantined areas:

CALIFORNIA

Alameda County. The entire county.
Contra Costa County. The entire county.
Humboldt County. The entire county.
Lake County. The entire county.
Marin County. The entire county.
Mendocino County. The entire county.
Monterey County. The entire county.
Napa County. The entire county.
San Francisco County. The entire county.
San Mateo County. The entire county.
Santa Clara County. The entire county.
Santa Cruz County. The entire county.
Solano County. The entire county.
Sonoma County. The entire county.

OREGON

Curry County. That portion of the county as follows: In T. 39 S., R. 13 W., secs. 32, 33 and 34; T. 40 S., R. 13 W., secs 3, 4, 5, 8, 9, 10, southeast quarter of sec 11, southwest quarter of sec. 12, northwest quarter of sec. 13, northeast quarter of secs. 14, 15, 16, and 17, east half of sec. 18, east half of secs. 19, 20, 21, 22, 28, and 29, northwest quarter of secs. 30, 32, 33 and 34; T. 40 S., R. 14 W., southeast quarter of sec. 23, southwest quarter of 24, northwest quarter of sec. 25, and northeast quarter of sec. 26.

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(B) Regulated areas. The following areas are designated as regulated areas:

CALIFORNIA

All counties in the State not listed in paragraph (A) of this section as quarantined areas.

OREGON

All areas in the State not listed in paragraph (A) of this section as quarantined areas.

WASHINGTON

The entire State.

Sec. 301.92-4 Conditions governing the interstate movement of regulated, restricted, and associated articles, and non-host nursery stock from quarantined and regulated areas.

Regulated, restricted and associated articles and non-host nursery stock may be moved interstate from quarantined and regulated areas only if moved in accordance with this section.

(A) Interstate movement of regulated and associated articles from quarantined areas. Regulated and associated articles may be moved interstate from a quarantined area only in accordance with this subpart.

1. With a certificate. Any regulated or associated article may be moved interstate from a quarantined area if accompanied by a certificate issued and attached in accordance with 301.92-5 and 301.92-8, and provided that the regulated or associated article is moved through the quarantined area without stopping except for refueling, rest stops, emergency repairs, and for traffic conditions, such as traffic lights or stop signs.
2. Without a certificate.
 - (i) The regulated or associated article originated outside the quarantined area and the point of origin of the article is indicated on the waybill of the vehicle transporting the article; and
 - (ii) The regulated or associated article is moved from outside the quarantined area through the quarantined area without stopping except for refueling or for traffic conditions, such as traffic lights or stop signs, and the article is not unpacked or unloaded in the quarantined area.

(B) Interstate movement of restricted articles from quarantined areas. Restricted articles may be moved interstate from a quarantined area only in accordance with this section.

1. With a permit. Any restricted article may be moved interstate from a quarantined area only if the article is moved pursuant to a permit issued by the Administrator in accordance with part 330 of this chapter.
2. Without a permit.
 - (i) The restricted article originated outside the quarantined area and the point of origin of the article is indicated on the waybill of the vehicle transporting the article; and
 - (ii) The restricted article is moved from outside the quarantined area through the quarantined area without stopping except for refueling or for traffic conditions, such as traffic lights or stop signs, and the article is not unpacked or unloaded in the quarantined area.

(C) Interstate movement of nursery stock from nurseries in quarantined areas.

1. Regulated articles of nursery stock and associated articles. Regulated articles of nursery stock and associated articles may only be moved interstate from nurseries in quarantined areas in accordance with paragraph (A) of this section
2. Non-host nursery stock. Any nursery stock of taxon not listed in 301.92-2 as a regulated or associated article may only be moved interstate from nurseries in quarantined areas as follows:
 - (i) With a certificate. If the non-host nursery stock originates from a nursery in a quarantined area that contains regulated or associated articles, the nursery stock must be accompanied by a certificate issued and attached in accordance with 301.92-5 and 301.92-8, and be moved through the quarantined area without stopping except for refueling, rest stops, emergency repairs, and for traffic conditions, such as traffic lights or stop signs.
 - (ii) Without a certificate. If the non-host nursery stock originates from a nursery in a quarantined area that does not contain regulated or associated articles, the nursery stock may be moved interstate without a certificate, provided that:
 - (a) The nursery from which plants originate has been inspected and found free of evidence of *Phytophthora ramorum* in accordance with 301.92-11(B)(3), and
 - (b) The nursery stock is not rooted in soil or growing media.

(D) Interstate movement of nursery stock from nurseries in regulated areas.

1. Regulated and associated articles of nursery stock. Regulated articles of nursery stock and associated articles may only be moved interstate from nurseries in regulated areas if accompanied by a certificate issued and attached in accordance with 301.92-5 and 301.92-8, and provided that, if moved through a quarantined area en route to another State, the regulated articles of nursery stock or associated articles are moved through the quarantined area without stopping except for refueling, rest stops, emergency repairs, and for traffic conditions, such as traffic lights or stop signs.
2. Non-host nursery stock. Any nursery stock of a taxon not listed in 301.92-2 as a regulated or associated article may only be moved interstate from nurseries in regulated areas as follows:
 - (i) With a certificate. If non-host nursery stock originates from a nursery in a regulated area that contains regulated or associated articles, the nursery stock must be accompanied by a certificate issued and attached in accordance with 301.92-5 and 301.92-8, and provided that, if moved through a quarantined area en route to another State, the nursery stock is moved through the quarantined area without stopping except for refueling, rest stops, emergency repairs, and for traffic conditions, such as traffic lights or stop signs.
 - (ii) Without a certificate. If non-host nursery stock originates from a nursery in a regulated area that does not contain regulated or associated articles, the nursery stock may be moved interstate without a certificate, provided that the nursery from which plants originate has been inspected and found free of evidence of *Phytophthora ramorum* in accordance with 301.92-11(D)(3).

Sec. 301.92-5 Issuance and cancellation of certificates.

(A) Movements from quarantined areas.