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***Phytophthora ramorum* research at the National Ornamentals Research Site at the Dominican University of California†**

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Abstract

Phytophthora ramorum Werres, De Cock & Man in't Veld, causal agent of sudden oak death (SOD) and ramorum blight, has been detected in container-grown plants, soil and irrigation ponds in various United States' nurseries. *Phytophthora ramorum* has also been detected in runoff water from some nurseries and adjoining streams. Despite emergency regulatory actions, there is concern that *P. ramorum* infected nursery stock may further spread the disease in the United States of America (USA), particularly to previously unaffected wildlands. If established in the south-eastern USA, it could cause damage similar to that occurring in the coastal forests of California and Oregon. To develop solutions for nurseries that trade plants susceptible to *P. ramorum*, a quarantine nursery was established in Marin County, California, to investigate pathogen eradication and disease management. More than four years of collaborative efforts between the California Department of Food and Agriculture, California county Agriculture Commissioners, the California Oak Mortality Task Force, US National Plant Board, United States Department of Agriculture Animal and Plant Health Inspection Service Plant Protection and Quarantine and nursery industry resulted in locating a suitable site for developing the National Ornamentals Research Site (NORS) at the Dominican University of California (DUC). Funding to set-up and run the research nursery was awarded in 2008 through congressionally approved, Farm-bill (Section 10201) funding. The site is designed to perform research on quarantine pests and pathogens while safeguarding plant health and the surrounding natural environment. Research initiatives on *P. ramorum* have commenced at the NORS-DUC. Research grants are awarded to undertake research at the NORS-DUC and proposals can be submitted through www.dominican.edu/norsduc.

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Keywords: containment; disease management; eradication; plant health; *Phytophthora ramorum*; quarantine nursery; remediation.

Introduction

Phytophthora ramorum Werres, De Cock & Man in't Veld (2001), causal agent of sudden oak death (SOD) and ramorum blight, has been detected in container-grown plants in various nurseries in the the United States of America (USA) and Europe. (Goss et al., 2009; Palmieri et al., 2009; Animal and Plant Health Inspection Service (APHIS), 2010a). Inoculum from the infected plants has contaminated soil and irrigation ponds in nurseries in several US states (Jeffers et al., 2009). *Phytophthora ramorum* has occasionally been detected in runoff water from these nurseries and also in adjacent streams (Oak, 2010). There is concern that this pathogen may potentially become established in the natural ecosystems in the south-eastern USA where many susceptible plants (e.g. trees in the Fagaceae and shrubs in the Ericaceae family) are present (Brasier et al., 2002; Brasier et al., 2004; Linderman et al., 2007; Tooley and Kyde, 2007). Risk maps based on plant species susceptibility and climate models suggest that *P. ramorum* is capable of establishing in the south-eastern USA (Margarey et al., 2004; Cave et al., 2008; Cave et al., 2005).

Recognising a need for *Phytophthora ramorum* field research, USDA Agricultural Research Service and Oregon State University researchers proposed the development of a "mock nursery" at which *P. ramorum* research could be conducted under simulated, controlled conditions (Grünwald & Parke, 2006). The European Institute for Agricultural and Fisheries Research (ILVO) in Belgium has also developed a facility where investigations of within-field spread of *P. ramorum* on *Rhododendron* L. spp. have been conducted (Heungens et al., 2009). Within the USA, more than four years of collaborative efforts between California Department of Food and Agriculture (CDFA), Hines Nursery, California county Agriculture Commissioners, the California Oak Mortality Task Force, US National Plant Board, United States Department of Agriculture Animal and Plant Health Inspection Service Plant Protection and Quarantine (USDA-APHIS-PPQ) and nursery industry resulted in locating a suitable site. Subsequently, in 2009 funding via the 2008 Farm Bill (Section 10201) was obtained to develop a National Ornamentals Research Site, where nursery research could be performed on quarantine pests and pathogens while safeguarding plant health and the environment. The National Ornamentals Research Site at Dominican University of California (NORS-DUC) has initiated several research projects that address the treatment and prevention of *P. ramorum* in soil, the risk of asymptomatic infection in fungicide-treated plants, the effect of fungicides and biocontrol agents on inoculum production, and the affects of abiotic stress and ramorum blight in nursery ornamentals.

Mission of the NORS-DUC

The main objective of the NORS-DUC is to identify, prioritise, facilitate and conduct research related to pests and diseases of nursery stock while safeguarding plant health and the environment.

Governance of the NORS-DUC

The NORS-DUC's governance is structured to have an Executive Committee, Steering Committee, and several subcommittees (Table 1). The Executive Committee represents members from USDA APHIS PPQ Centre for Plant Health Science and Technology (CPHST), Dominican University of California and the US National Plant Board. Stakeholders and representatives hold positions in the Steering Committee. Each Steering Committee member is designated to chair separate subcommittees, such as the Nursery Industry, US Forestry, Research, State and Local Issues, the *Phytophthora ramorum* Working Group, Nursery Associations, National/International Interests and Communications.

Facility oversight is directed by an Executive Committee comprised of appointed representatives from the USDA authorised departmental officer's designated representative, Dominican University of California and the National Plant Board. The Executive Committee works closely with the Steering Committee, comprised of regulatory agencies, the nursery industry and *Phytophthora ramorum* research scientists, to establish overall priorities and provide general direction and management by identifying and implementing research that supports the general objective of the project. Engagement of stakeholders is further facilitated through subcommittees chaired by the Steering Committee members. These committees are designed to address stakeholder interests and operate under a defined mission (Table 1). All individuals in governance hold positions for a minimum of one year but can lengthen their service commitment to the NORS-DUC.

Location of the National Ornamentals Research Site

A suitable nursery site in California for *Phytophthora ramorum* research required that the location be in a *P. ramorum* quarantined county, have an appropriate micro-climate for disease development, have limited proximal *P. ramorum* infested host plants and have accessible scientific laboratory facilities. From 2005 – 2009, collaborative efforts resulted in locating a suitable site at Dominican University of California, in San Rafael, Marin County, California – the epicentre of sudden oak death in the USA.

TABLE 1: Missions of the NORS-DUC governance committees.

Committee	Mission
Executive Committee	Facility oversight is directed by an Executive Committee comprised of appointed representatives from USDA, Dominican University of California and the National Plant Board. The Executive Committee works closely with the Steering Committee, comprised of regulatory agencies, the nursery industry and <i>Phytophthora ramorum</i> research scientists to establish overall priorities and provide general direction and management by identifying and implementing research that supports the general purpose of the project.
Steering Committee	This committee will work with the Executive Committee to establish overall priorities and provide general direction and management by identifying and implementing research that supports the general purpose of the project. The primary role is to identify and prioritise research proposals recommended by the Research Committee and confirm conformity to safeguards and permit requirements. The Steering Committee will also provide recommendations for the oversight and direction of the site.
Nursery Industry Committee	This committee consists of members of the nursery industry and researchers who work on <i>P. ramorum</i> in the nursery setting. The participants will provide input to the development and management of the facility. Immediate responsibilities include: Set up the Forest Meadows Research Site, set objectives for the design of the Forest Meadow II Site, assist in planning the transitions to new pest research, serve as liaison to California Oak Mortality Task Force (COMTF) and provide feedback from COMTF.
Forestry Committee	This committee will: (1) serve as a liaison between forestry professionals and the NORS-DUC; (2) identify issues, research needs and educational opportunities for the wildland aspects of <i>Phytophthora ramorum</i> /sudden oak death or other emerging, damaging forest pathogen or insect concerns that could be best addressed at NORS-DUC; and (3) provide technical assistance to the NORS-DUC staff and collaborators related to sudden oak death and other wildland aspects of <i>P. ramorum</i> .
Research Committee	This committee will identify research priorities and review research proposals based on established criteria to insure that the objectives and mission of NORS-DUC are aligned with the chosen projects goals. The participants on the committee will represent regulatory agencies, the nursery industry and <i>Phytophthora ramorum</i> research scientists.
Federal State and Local Committee	This committee will address local and state concerns. They will develop and define permit conditions and safeguards, modifying as needed.
National Plant Board Working Group Committee	This is an established committee and will serve as a conduit of information from the National Plant Board. They will provide input on preferred or recommended research.
Nursery Association Committee	This committee will provide recommendations from nursery associations across the USA and Canada on the research they consider to be needed and their preferred proposals.
National Interests Committee	This committee will provide input related to national interests, including nursery, forest, conservation and other potential interests.
Communications Committee	This committee will serve as point of contact for public inquiries regarding NORS-DUC and all public or press releases of the project.

Subsequent site visits to the Dominican University campus were carried out with federal and state government officials, a nursery industry representative, Dominican University of California Facilities management and individuals from the Department of Natural Sciences and Mathematics. These visits resulted in formally identifying Forest Meadows and Deer Park as the two areas on the campus for development of the National Ornamentals Research Site (NORS). In late spring 2010, an additional third location 250 m from Forest Meadows was identified (<http://media.dominican.edu/maps/map.php>). Vegetation surveys in and around these sites were carried out in collaboration with the CDFA, Plant Pest Diagnostics Centre and the California Department of Forestry. Vegetation and soil samples were processed at the CDFA Plant Pest Diagnostics Centre in Sacramento, California following the *P. ramorum* diagnostic protocols established by the USDA APHIS PPQ (http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/protocols.shtml).

An established shade house constructed of laths existed at the Forest Meadows Research Site. Initial field inspections and subsequent area surveys revealed no *Phytophthora ramorum* at this location. The area landscape around Forest Meadows Research Site is marked by buildings on the northern and southern edges, a creek with *Umbellularia californica* (Hook. & Ann.) Nutt. (California bay laurel), *Quercus agrifolia* Née (coast live oak) and old growth *Eucalyptus globulus* Labill. (bluegum eucalyptus) trees on the eastern edge while the western edge of the site opens onto park-like surroundings scattered with established pines and *Eucalyptus globulus* Labill. An area of 0.4 km surrounding the Forest Meadows Research Site is under continuous monitoring for *P. ramorum*. More extensive sampling and surveying in the adjacent Black Canyon Creek and further up in the watershed has shown that water in the seasonal creek is positive for *P. ramorum* during periods of consistent rain but that only areas further upstream near the water source for Black Canyon creek remain positive once creek flow becomes intermittent. Creek monitoring was halted when water levels receded and water temperature conditions were not conducive for *P. ramorum* sporulation. Vegetation surveys have detected *P. ramorum* on bay laurels upstream from the creek and near the nursery site. Near the nursery site, selected tree removal of positive finds has been employed to eliminate movement of inoculum from the native landscape to the nursery site in accordance with recommended Nursery Industry Best Management Practices (Horticultural Research Institute (HRI) *P. ramorum* Industry Working Group, 2005).

Whereas, the Forest Meadows Research Site is situated in a moderately developed area, the undeveloped Deer Park site is located on a west-facing slope in otherwise dense *Eucalyptus globulus* woodland. The Deer Park

site was found to be dominated by mature *Cytisus monspessulanus* L. (french broom) with a surrounding forest understory of *Quercus agrifolia*, *Acacia* Mill. spp., *Umbellularia californica* and *Arbutus menziesii* Pursh (Pacific madrone). Surveys of the area for nursery use have revealed no *P. ramorum* infestations, however, a 20% coverage survey revealed positive detections on some *Umbellularia californica*. A more extensive survey of the surrounding vegetation will precede any development of the site. Prerequisites for development of the Deer Park site have included initial surveys and regulatory requirements to obtain the necessary permits. Further development of the Deer Park site will depend on several factors, including the ability to obtain the necessary capital to develop the site and the need for space to accommodate researchers.

The Forest Meadows Research Site

Nursery features

The following nursery safeguards were formally accepted by the NORS-DUC Steering and Executive Committees and incorporated in the design features of the Forest Meadows Research Site (Figures 1, 2, 3 and not shown):

1. physical separations, such as screened fencing, to prevent spread from plot to plot and into the surrounding area;
2. disinfecting foot baths at egress and ingress;
3. equipment cleaning and disinfecting apparatus;
4. ground barriers designed to capture all runoff from each study plot;
5. fencing to provide security from unauthorised entry and protection from deer;
6. special drainage systems that will allow runoff to be collected for study purposes, diverted to a holding tank, or to be modified for special studies;
7. an evaporation pond or holding tank to collect all water runoff from plots;
8. potting and storage areas; and
9. steam sterilisers outfitted for direct decontamination of research pads and disinfecting dipping tank for used pots.

Following these NORS-DUC Steering and Executive Committees guidelines, design plans were developed by the Dominican University of California Facilities management and NORS personnel. The Nursery Committee provided input on nursery functionality and

safeguards. Model construction for design review was carried out by members of the University's Facilities team. The University's Facilities management, Office of External Relations, Advancement Division and the Department of Natural Sciences and Mathematics with the City of San Rafael and CDFA worked together in communications with the neighbourhood association and the public. External Review was conducted by the City of San Rafael to demonstrate that engineering and site materials used for nursery development met the guidelines of the City of San Rafael. Site development was carried out by certified contractors and members of the Dominican University of California facilities team.

Highlighted features

The perimeter of the site is fenced with primary access through an office and secondary access for nursery-only movement of equipment and materials. Prior to site entry, shoe decontamination is mandatory and all visitors are required to log-in and put on appropriate shoe covers or boots supplied by the site. During periods of research activity, visitor access is restricted; however, non-research related visitors are given tours around the perimeter of the site.

Research plots

The Forest Meadows Research Site contains eight individually fenced-in research plots, four of which were developed on the existing nursery site (Figure 1A). Each research plot is securely fenced with a single point of entry and contains a 3.5 x 9 m. nursery bed that can be subdivided into two 3.5 x 4.5 m. beds. The 3.5 x 4.5 m. beds have separate drainage and water sampling areas (Figures 1A & 2A) as well as options for well water or municipal water through either drip or overhead irrigation (Figures 1B & 2B).

Nursery monitoring

In addition to research initiated at the nursery, on-site, continuous monitoring for the presence of *Phytophthora ramorum* is being conducted (Figure 3).

A triple layer, perimeter plant system was developed by the NORS-DUC Nursery Committee for monitoring *Phytophthora ramorum* inoculum movement, allowing inoculum source identification from either the native landscape or the nursery. Plants bought for use in the perimeter system were received from a nursery operating with a Compliance Agreement (APHIS, 2009), held in quarantine and inspected routinely for disease development. When demonstrated *P. ramorum*-free and disease-free, these plants were incorporated into the perimeter monitoring system.

To monitor and determine if inoculum has the potential to move from the native landscape towards the nursery, a layer of sentinel plants, susceptible to *P. ramorum*,

line the outside perimeter of the nursery (Figure 3A). A layer of sentinel plants lining the inside perimeter of the nursery will be used to monitor if inoculum has the potential to move away from the nursery into the landscape (Figures 2C & 3B). The inner and outer perimeter plant layers are separated by a barrier of non-susceptible plants, completing the triple layer, perimeter plant system (Figure 3).

Monthly nursery inspections of perimeter sentinel plants have been carried out in collaboration with the California Primary State Pathologist and the Plant Pest Diagnostics Centre of CDFA. Tissue samples were collected during nursery inspections prior to research activity (spring 2010) and also once research initiated (summer 2010). These samples were assayed by approved APHIS diagnostic protocols (APHIS, 2011) and found to be negative for *Phytophthora ramorum*.

As part of water management at the nursery, water monitoring is carried out at each water access point in the nursery (Figures 3C-H), using water filtration (APHIS, 2010b) and water baiting methods for *Phytophthora ramorum* (Davidson et al., 2005; APHIS, 2010b).

Additionally, using methods recommended by the Forestry Committee, water-bait buckets were installed within each nursery plot prior to an established perimeter plant system (Figures 1, 2D & 3C) and have served as a transitional monitoring system. These water-bait buckets help monitor movement of windblown inoculum from the native landscape into the nursery. To assay for the presence of *P. ramorum*, modified mesh, leaf-bait bags containing three *P. ramorum* susceptible leaves from *Rhododendron* L. spp. and *Camellia japonica* L. 'Korean fire' were floated on the water surface of buckets at two week intervals and tissue from leaf baits was plated into PARP and PAR(PH)-V8 media for culturing (Davidson et al., 2005; APHIS, 2010b). After nine months of monitoring (spring, summer and autumn (fall), 2010), no positive detections have occurred, suggesting that no *P. ramorum* inoculum from the native landscape has moved into the nursery. Eventually these buckets will be phased out or may be employed at select locations within the nursery.

For each nursery bed within a research plot, two independent plot sampling areas, positioned in front of each plot, are available for water sampling (Figures 1, 2A & 3D). This allows both research-related and site-related monitoring of *P. ramorum* movement off an active research plot. Furthermore, in the case of on-site monitoring, post-clean-up of a research plot can be assessed to determine whether complete disinfection has been achieved.

Accessible above ground, *Phytophthora ramorum* susceptible leaves from *Rhododendron* spp. and

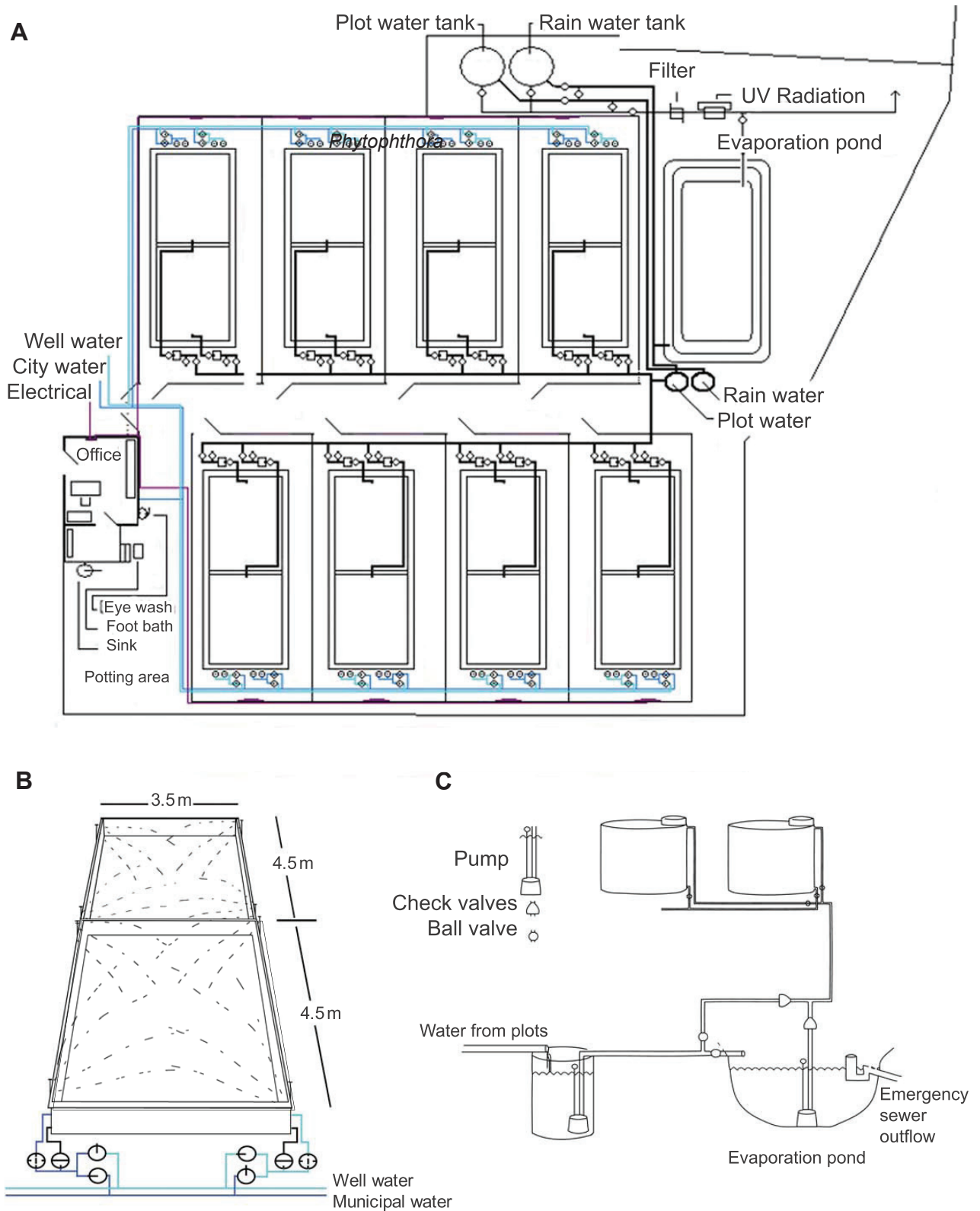


FIGURE 1: Design schematics for the Forest Meadows Research Site.

- A. Layout of the Forest Meadows Research Site.
- B. Schematic for the irrigation available for a respective research plot. Overhead irrigation is depicted and the plumbing options for municipal or well water are shown. Drip or alternative irrigation options are not shown.
- C. Schematic for the collection and movement of water from research plots. Walkway water collection not shown.



FIGURE 2: Nursery features of the Forest Meadows Research Site.

- A. Example of a plot sampling area to access water flowing off a research plot.
- B. Research plot with overhead irrigation setup. Drip irrigation not shown.
- C. Inside layer of perimeter plants along the nursery fence-line are part of a triple layer, plant perimeter system designed to assess *P. ramorum* movement into or out of the nursery.
- D. Transitional leaf baiting in water buckets to assess inoculum movement from the native landscape prior to an establish nursery perimeter system.
- E. Example of the top of a drainage collection area that is accessible for water monitoring.
- F. Evaporation pond collects seasonal rain water for monitoring or can be used in further water remediation studies.
- G. Above-ground storage tank.
- H. Water filtration system that filters water from the above-ground storage tank prior to ultra-violet (UV) sanitation.
- I. UV sanitation system takes filtered water and exposes it to a UV wavelength for sanitation of the water prior to release from the nursery.
- J. Non-permeable liner spread across the graded slope of the nursery site during the initial phase of development.

Camellia japonica 'Korean fire,' similar to bait buckets, are used to assay for the presence of *P. ramorum* in both of the in-ground tanks that collect either irrigation water from the plots or walkway water from rain and windblown irrigation spray (Figures 1, 2E & 3E-F). Prior to research, no *P. ramorum* has been detected from baiting assays, further supporting that no inoculum has moved from the native landscape into the nursery. Since it is expected that *P. ramorum* will be present in the in-ground tank while the nursery is active, water collection and filtration will be employed instead of baiting to determine the level of inoculum moving off the plots. Given the potential for high inoculum loads in the nursery, any positive detection in the walkway drainage basin will trigger additional survey and monitoring to determine whether the source of the inoculum may indicate that *P. ramorum* has moved from a research plot and established in the gravel of the walkway or affected the inside perimeter system. Likewise if any perimeter plant is identified with a positive detection, monitoring the walkway water from the in-ground tank will serve to monitor whether there is further movement of the pathogen to the walkway.

The evaporation pond holds seasonal rain and allows untreated *Phytophthora ramorum* infested water to be diverted from the plot in-ground tank for research in water remediation (Figures 1, 2F & 3G). Seasonal rain water in the evaporation pond has been monitored for over 11 months using *P. ramorum* susceptible *Rhododendron* spp. and *Camellia japonica* 'Korean fire' leaf baits to assay at two week intervals for the presence of *P. ramorum* (Davidson et al., 2005; APHIS, 2010b). No *P. ramorum* was detected, further supporting that no inoculum has moved from the native landscape into the nursery.

To periodically confirm that all ultra-violet (UV)-sanitised water used within the nursery or draining from the nursery into the creek is *Phytophthora ramorum* free, a post-UV-sanitised collection point allows for sampling and monitoring (Figure 3H).

Water sterilisation and emergency overflow

When water fills each in-ground drainage tank or if a heavy rain event should occur, an interior float valve is triggered and water automatically pumps into a dedicated above ground storage tank (Figure 2G) or can be manually diverted from the plot drainage into the evaporation pond (Figures 1A & C). Once water levels trigger the interior float valve in the above ground tanks, water is automatically diverted into the filtration system prior to entering the UV system for sanitation and subsequent release (Figures 1A, C & 2G-I). Depending on the experiments currently underway, untreated or treated water can be recycled back into the nursery for experimental use.

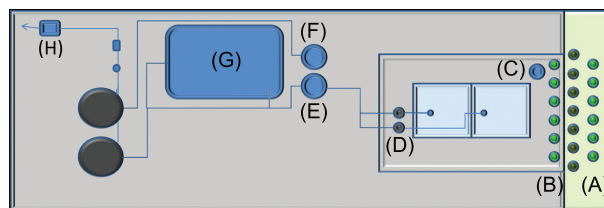


FIGURE 3: Diagram of monitoring locations in NORS-DUC Forest Meadows Research Site.

- A. Outside-perimeter sentinel plants.
- B. Inside-perimeter sentinel plants.
- C. Water bucket for baiting within a research plot.
- D. Plot sampling areas in front of the research bed.
- E. Plot in-ground tank.
- F. Walkway in-ground tank.
- G. Evaporation pond.
- H. Post UV-sanitisation collection point.

If an unforeseen failure should occur and all power is lost, high water levels from the nursery will automatically divert through an emergency overflow system. Above-ground tanks are equipped to passively overflow into the evaporation pond if water capacity exceeds maximum levels while emergency sewer outflows in both the in-ground drainage tanks and the evaporation pond direct water into the sanitary sewer system of the San Rafael Sanitation District for treatment (Figures 1A & C).

Containment

Below the rock surface of the Forest Meadows Research Site floor, is a durable, non-permeable liner that covers the entire nursery site and separates the nursery from the native landscape (Figure 2J). It provides a containment barrier and facilitates collection of water across the sloped grade of the nursery toward the walkway drainage area for further monitoring (Figure 3F).

Research at the NORS-DUC

The Research Committee is composed of members from: United States universities; the United States Department of Agriculture; the Horticultural Research Institute; the nursery industry; United States Forestry; the United States Department of Agriculture; and the UK Food and Environmental Research Agency, Department for Environment, Food and Rural Affairs (Fera Defra) It has developed research priorities and criteria for research at the NORS-DUC and submitted these to the Steering Committee (Table 2). Upon approval by the Steering Committee these research priorities and criteria were forwarded to the Executive Committee for final approval.

TABLE 2: Priorities for *Phytophthora ramorum* research at the NORS-DUC.

Area of Interest	Priorities
Stopping the Spread	<p>Effective, fast, reliable field diagnostic tools and methods for early detection and screening in plants, water, and soil.</p> <p>Options for treating <i>Phytophthora ramorum</i> in soil.</p> <p>Research on water contamination, causes of repeat infested nurseries, potential pathways, and pathogen movement.</p> <p>Applied science and methods (i.e. soil and soil treatments) for control and eradication.</p> <p>Disease indexing and other testing protocols that can be implemented to verify and assure propagative material entering the US is disease free.</p> <p>Efficacy of the 40 sample minimum protocol to detect <i>P. ramorum</i> in the <i>P. ramorum</i> US federal regulation.</p> <p>Causes/pathways of repeat infested nurseries – sources of inoculum.</p> <p>Soil contamination testing and mitigation measures.</p> <p>Water contamination (i.e. genotyping of pathogen in water and nurseries).</p> <p>Potential movement of pathways into, and within, nurseries and environment.</p> <p>Efficacy of the destruction lot size (Confirmed Nursey Protocol).</p> <p>Validation of block buffer distance to control pathogen movement.</p> <p>Relationship and/or distribution of the pathogen in water and soil and its potential to infect plant material in nurseries and the environment.</p>
Symptomless Hosts and Root Infections	<p>Conditions and frequency when infections may be latent and symptoms not visible.</p> <p>Symptomless carriers and frequency and importance of root infection in horticultural crops.</p>
Repeat/Recurrent Nurseries	<p>Distinguish between repeat and recurrent nurseries. Genotype isolates for epidemiological studies, sources and pathways, modelling approaches and critical soil sampling focal points and distribution in nurseries.</p> <p>Proper nursery layout, procedures and protocols. Physical location, soil and water treatment options.</p>
Buffers and Fungicides	<p>Cross contamination between healthy and diseased plants in nursery settings.</p> <p>Evaluate registered materials, biocontrol options, efficacy and management options.</p> <p>Sanitation methods for disinfection of nursery beds, propagation facilities, pruning shears.</p>

The NORS-DUC is a secure site modelled to replicate a working production nursery for the purpose of performing studies on nursery stock in a “real world” environment while ensuring high level safeguards to prevent the escape of pests that can affect plant health. The NORS-DUC operates under a plant pest permit issued by the California Department of Food and Agriculture and all approved research is conducted in accordance with CDFA permit regulations

and applicable federal permit regulations. The NORS-DUC aligns with US Farm Bill, Section 10201, goal (4) Safeguarding Nursery Production (http://www.aphis.usda.gov/plant_health/plant_pest_info/pest_detection/farm_bill_background.shtml).

Scientists from the national and international research community will initially conduct studies focused on understanding and controlling the long-range spread of



FIGURE 4: Activities at the National Ornamentals Research Site.

- A. Outreach activities at NORS-DUC engage Dominican University of California undergraduates. Here undergraduates from the Department of Natural Sciences and Mathematics learn how to prepare baiting traps for use in the nursery. From left to right, Arianna Anies, Jose Hernandez, Jose Contreras, and Luis Loza.
- B. Steven Jeffers and Inga Meadows from Clemson University inspect *Camellia japonica* L. 'Korean fire' plants for use in their research at NORS-DUC.
- C. Nursery inspection training for the nursery staff of NORS-DUC. Tomas Pastalka (right) from the California Department of Food and Agriculture explains how CDFA nursery inspections of California Nurseries are carried out. Glenn Copeland (middle) and Miguel Gonzalez (left).

Phytophthora ramorum through infested nursery stock shipments. In addition to supplying data to control the long range spread of *P. ramorum* and other pests affecting plant health, the NORS-DUC will: provide validation of established Best Management Practices (BMPs) and develop new BMPs for nursery stock production; will identify new treatment or remediation options for soil, media, water and plant materials to exclude, contain, and eradicate *Phytophthora ramorum* and other plant pathogens and pests affecting plant health; will develop and assess sterilisation and water disinfection systems; will produce valuable epidemiological data on new and emerging species affecting ornamentals; and will provide outreach through reporting the results of studies and by engaging students in research activities (Figure 4A). Specific research priorities are detailed in Table 2.

Currently, five research projects are underway that address the development of new treatment or remediation options for soils (Figure 4B), the development and assessment of incorporation of BMPs for nursery production, and the collection of epidemiological data about *Phytophthora ramorum* on ornamentals. Published abstracts for all research at the NORS-DUC are available through the NORS-DUC website at www.dominican.edu/norsduc under the Research at the NORS-DUC link. For those researchers interested in collaborating with the NORS-DUC, information about Request for Proposal announcements and submission deadlines are available at the above mentioned NORS-DUC website, including research with international collaborators.

Conclusions

Situated in a *Phytophthora ramorum*-quarantined county of California, the National Ornamentals Research Site was developed to incorporate the necessary safeguards for protecting the native landscape. Management operations employ continuous environmental monitoring, combining area vegetation and stream surveys with practical applications of Best Management Practices (HRI *P. ramorum* Industry Working Group, 2005) and *P. ramorum* regulations in the nursery (Figures 3 & 4C). Current research at the NORS-DUC will provide practical solutions to local, national and international issues associated with the potential movement of *P. ramorum* from a nursery to native wildlands, benefiting stakeholders beyond the nursery industry.

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References

Animal and Plant Health Inspection Service (APHIS). (2009). *Compliance agreement for nurseries*. (PPQ-519). Retrieved 1 August 2011, from

http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/compliance/ppq519.pdf

Animal and Plant Health Inspection Service (APHIS). (2010a). *Phytophthora ramorum program updates*. Retrieved 30 September 2010, from http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/updates.shtml

Animal and Plant Health Inspection Service (APHIS). (2010b). *2011 Phytophthora ramorum Nursery Survey Manual Appendix I, Water Sampling Protocol*. Retrieved 1 August 2011 from http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/surveyplan/appendixl.pdf

Animal and Plant Health Inspection Service (APHIS). (2011). *Phytophthora ramorum/Sudden Oak Death Protocols* Retrieved 1 August 2011 from http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/protocols.shtml

Brasier, C. M., Rose, J., Kirk, S. A., & Webber, J. F. (2002). Pathogenicity of *Phytophthora ramorum* isolates from North America and Europe to bark of European Fagaceae, American *Quercus rubra* and other forest trees. *Sudden Oak Death Science Symposium, Monterey, California, USA, 15-18 December 2002*. (pp. 30-31). Berkeley, CA, USA: University of California and Albany, CA, USA: USDA Forest Service. Retrieved 30 September 2010 from <http://www.suddenoakdeath.org/?bibliography=pathogenicity-of-phytophthora-ramorum-isolates-from-north-america-and-europe-to-bark-of-european-fagaceae-american-quercus-rubra-and-other-forest-trees>

Cave, G. L., Randall-Schadel, B. L., & Redlin, S. C. (2005). *Risk analysis for Phytophthora ramorum Werres, de Cock & Man in't Veld, causal agent of Phytophthora canker (Sudden Oak Death), ramorum leaf blight, and ramorum dieback*. 5 May 2005. USDA, APHIS, PPQ, Centre for Plant Health Science and Technology, Plant Epidemiology and Risk Analysis Laboratory. Raleigh, North Carolina, USA. Retrieved 30 September 2010, from http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/pramorumpra05-05-05.pdf

Cave, G. L., Randall-Schadel, B. L., & Redlin, S. C. (2008). *Risk Analysis for Phytophthora ramorum Werres, de Cock & Man in't Veld, causal agent of Sudden Oak Death, ramorum leaf blight, and ramorum dieback*. 25 January 2008. USDA, APHIS, PPQ, Centre for Plant Health Science and Technology, Plant

- Epidemiology and Risk Analysis Laboratory. Raleigh, North Carolina, USA. Retrieved 30 September 2010 from http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/pram-cphst-08.pdf
- Davidson J. M., Wickland, A. C., Patterson, H. A., Falk, K. R., & Rizzo D. M. (2005). Transmission of *Phytophthora ramorum* in mixed-evergreen forest in California. *Phytopathology*, 95(5), 587-596. DOI: 10.1094/PHTO-95-0587. Retrieved 30 September 2010, from <http://ddr.nal.usda.gov/bitstream/10113/2258/1/IND43694558.pdf>
- Goss, E. M., Larsen, M., Chastagner, G.A., Givens, D. R., & Grünwald N. J. (2009). Population genetic analysis infers migration pathways of *Phytophthora ramorum* in US nurseries. *PLoS Pathogens*, 5(9), e1000583. doi:10.1371/journal.ppat.1000583. Retrieved 30 September 2010, from <http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1000583>
- Grünwald, N., & Parke, J. (2006). *Phytophthora ramorum biology and management*. (Research Progress Report). Floriculture and Nursery Research Initiative: Second floral and nursery crops researchers workshop. 12-15 June 2006, Doubletree Hotel and Executive Meeting Centre – Lloyd Centre Portland, Oregon, USA. Retrieved 30 September 2010, from [http://www.ars.usda.gov/sp2UserFiles/Program/301/FNRIDocs/2005/Day1/Grunwald-Parke Floriculture Nursery Initiative.ppt](http://www.ars.usda.gov/sp2UserFiles/Program/301/FNRIDocs/2005/Day1/Grunwald-Parke%20Floriculture%20Nursery%20Initiative.ppt)
- Heungens, K., De Dobbelaere, I., Gehesquière, B., Vercauteren, A., & Maes, M. (2009). Within-field spread of *Phytophthora ramorum* on Rhododendron in nursery settings. In S. J. Frankel, J. T. Kliejunas, & K. M. Palmieri, (Tech. Coords), *Proceedings of the Sudden Oak Death Fourth Science Symposium*, (pp. 72-75). General Technical Report PSW-GTR-229. Albany, CA, USA: US Department of Agriculture, Forest Service, Pacific Southwest Research Station. Retrieved 30 September 2010, from http://www.fs.fed.us/psw/publications/documents/psw_gtr229/psw_gtr229.pdf
- Horticultural Research Institute (HRI) *P. ramorum* Industry Working Group. (2005). *Nursery Industry Best Management Practices for Phytophthora ramorum – to prevent the introduction or establishment of California nursery operations. Version 1.0*. Retrieved 30 September 2010, from http://www.suddenoakdeath.org/pdf/cangc_bpm_FINAL.pdf
- Jeffers, S. N., Hwang, J., Wamische, Y. A., & Oak, S. W. (2010). Detection of *Phytophthora ramorum* at retail nurseries in the southeastern United States. In S. J. Frankel, J. T. Kliejunas, & K. M. Palmieri, (Tech. Coords), *Proceedings of the Sudden Oak Death Fourth Science Symposium*, (pp. 69-71). General Technical Report PSW-GTR-229. Albany, CA, USA: US Department of Agriculture, Forest Service, Pacific Southwest Research Station. Retrieved 30 September 2010, from http://www.fs.fed.us/psw/publications/documents/psw_gtr229/psw_gtr229.pdf
- Linderman, R. G., de Sá, P. B., & Davis, E. A. (2007). Comparative susceptibility of plants native to the Appalachian range of the United States to inoculation with *Phytophthora ramorum*. *Plant Health Progress* doi:10.1094/PHP-2007-0917-01-RS. Retrieved 30 September 2010, from <http://www.plantmanagementnetwork.org/pub/php/research/2007/native/>
- Magarey, R., Garrett, L., & Fowler, G. (2004). *Risk Model for Phytophthora ramorum (Sudden Oak Death) in Nursery Shipments*. *Phytophthora ramorum* Science Panel, Centre for Plant Health Science and Technology. 29-30 June 2004. Raleigh, North Carolina, USA.
- Oak, S. (2010). *P. ramorum in Waterways Nationwide*. Proceedings of the California Oak Mortality Task Force 2010 Annual Meeting. 8-11 June 2010, San Rafael, California, USA. Retrieved 30 September 2010, from <http://www.sod2010.net/news-and-events/past-events/2010-annual-meeting/>
- Palmieri, K., Alexander, J., Lee, C., & Frankel, S. J. (2009). *Sudden Oak Death and Phytophthora ramorum 2009 Summary Report - a Compendium of monthly newsletters, California Oak Mortality Task Force*. Retrieved 30 September 2010, from <http://nature.berkeley.edu/comtf/pdf/2009YearEndSummary.pdf>
- Tooley, P. W., & Kyde, K. L. (2007). Susceptibility of some eastern forest species to *Phytophthora ramorum*. *Plant Diseases* 91, 435-438. doi:10.1094/PDIS-91-4-0435. Retrieved 30 September 2010, from <http://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS-91-4-0435>
- The 2008 Farm Bill: Section 10201. (2008). Retrieved 1 August 2011 from http://www.aphis.usda.gov/plant_health/plant_pest_info/pest_detection/farm_bill_background.shtml
- Werres, S., Marwitz, R., Man in't Veld, W. A., de Cook, A. W. A. W., Bonants, P. J. M., de Weerd, M., Themann, K., Ilieva, E., & Baayen, R. P. (2001). *Phytophthora ramorum* sp. nov., a new

pathogen on *Rhododendron* and *Viburnum*.
Mycological Research, 105(10), 1155-1165.
Retrieved 30 September 2010, from [http://
nature.berkeley.edu/comtf/pdf/Bibliography/
werres2001a.pdf](http://nature.berkeley.edu/comtf/pdf/Bibliography/werres2001a.pdf)