Research

Monitoring Using a Sentinel Plant System Reveals Very Limited Aerial Spread of *Phytophthora ramorum* From Infected Ornamental Plants in a Quarantine Research Nursery

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Abstract

The potential aerial spread of *Phytophthora ramorum*, causal agent of sudden oak death and Ramorum blight, from infected plants in a quarantine research nursery at the National Ornamentals Research Site at Dominican University of California (NORS-DUC) to the environment was monitored weekly for five years (2011 to 2016) using a sentinel system. *Phytophthora ramorum* was never detected on any of the sentinel plants (*Rhododendron*, *Viburnum*, and *Loropetalum* spp), indicating very limited aerial spread under sub-optimal meteorological and environmental conditions. An infection

experiment with host plants placed in the immediate vicinity of symptomatic plants proved the potential for short-distance (1 to 2 m) aerial transmission of *P. ramorum*. Other *Phytophthora* spp. causing symptoms similar to *P. ramorum* were detected during the rainy season (January to May) on the sentinel plants, among them potentially two novel species. These data reveal how sentinel monitoring at NORS-DUC allows for seasonal assessments of disease incidence and provide longitudinal data to assess the threat of *P. ramorum* movement in nurseries.

Invasive plant pathogens can cause severe diseases of forest trees, eventually leading to significant economic and environmental damage and possibly even the ecological extinction of native tree species (e.g., Chestnut blight, caused by *Cryphonectria parasitica*, and Dutch Elm disease, caused by *Ophiostoma ulmi*). The invasive forest pathogen *Phytophthora ramorum* Werres, De Cock & Main in't Veld was detected in the mid-1990s in coastal California (Santa Cruz and Marin counties), and spread quickly through the mixed hardwood-conifer forests of coastal California and southern Oregon, causing extensive mortality of tanoak (*Notholithocarpus densiflorus*) and coast live oak (*Quercus agrifolia*) (Rizzo et al. 2002). Airborne sporangia, produced mainly on leaves of tanoaks and California bay laurel (*Umbellularia californica*) under moist conditions, are responsible for short-distance spread of the disease. *Phytophthora ramorum* can also infect numerous ornamental plants, among them *Rhododendron* spp. and *Camellia* spp., causing foliar symptoms and occasionally branch dieback (Ramorum blight); the nursery trade of infected plants is considered a major pathway for the introduction and long-distance spread of the pathogen. The National Ornamentals Research Site at Dominican University of California (NORS-DUC) in San Rafael, CA, is a field research nursery designed to study quarantine pests and pathogens of ornamentals, with a main focus on *P. ramorum* and related *Phytophthora* species, with the mission to examine, develop, and implement control strategies (Johnson-

Brousseau et al. 2011). Federal and state regulations require the regular monitoring of potential *P. ramorum* spread through airborne inoculum from the research site to the environment. We developed a sentinel system, consisting of a triple layer of host and nonhost plants laid out along the perimeters of the nursery and within the nursery, to detect symptoms caused by *P. ramorum* and study environmental parameters which might play a role in the spread of the disease.

This project had the following aims: (i) to detect possible movement of *P. ramorum* from the research nursery into the environment (“outbound” movement); (ii) to detect possible movement of *P. ramorum* from the environment into the research nursery (“inbound” movement); (iii) to monitor the development of other biotic diseases which could infect research plants; and (iv) to identify climatic conditions favorable for disease spread.

Layout of a Quarantine Research Site for *P. Ramorum*

NORS-DUC was developed to study quarantine pathogens of ornamental plants, particularly *P. ramorum*, under field conditions in a research facility mirroring a commercial nursery. NORS-DUC contains two research sites: site SOUTH is trapezoid-shaped (40 × 36.5 × 47 × 21 m); and site NORTH is rectangular (65 × 16 m). The perimeters of both sites are fenced with a single entry point which is equipped with a footbath containing a quaternary ammonium solution for disinfection (AL-SAN 5, Nelson-Jameson, Marshfield, WI) to prevent pathogen movement via contaminated shoes. Each site has six individually contained research plots; within each research plot is a research bed for the experiments. Most research beds are 3.5 × 9 m and can be subdivided into two 3.5 × 4.5 m

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half-beds. Four research beds are filled with soil (originating from San Rafael, CA) for in-soil experiments, the other eight research beds are used for experiments with potted plants. All research beds are lined with a waterproof pond liner (30 mm thick) to maintain and collect water. The used water is filtered, UV-treated, and monitored for the presence of *P. ramorum* before being released from the research site. Research beds and infected experimental plants are disinfected by steam treatments of 50°C for 30 min after the completion of the project (Schweigkofler et al. 2014).

NORS-DUC is located in San Rafael, Marin Co., CA, an area with a Mediterranean climate characterized by warm, dry summers and mild, rainy winters. Precipitation is restricted mainly to the period from October through April with the wettest months being December through February; the total annual rainfall averages 90 cm. Temperatures below freezing are rare; the average low in the coldest month (December) is 6°C, and the average high in the warmest month (August) is 27°C. Prevailing wind direction is SW with wind speed rarely exceeding 30 km/h (Anonymous 2016).

The possible aerial movement of *P. ramorum* from the research beds toward the environment (and vice versa) was monitored using a triple-layered sentinel plant system (Fig. 1). *Phytophthora ramorum* host plants ($n = 145$; primarily *Rhododendron* cv. 'Cunningham White', 'Boursault', and 'Purple Splendour', but also *Camellia* spp., *Loropetalum chinense*, and *Viburnum* spp. were planted along the outside perimeter of both nursery sites. Leyland cypress ($\times$ *Cuprocyparis leylandii*) ($n = 159$), a fast-growing evergreen hybrid conifer, were planted outside the fence and behind the host plants to serve as a wind barrier. *Cuprocyparis leylandii* is susceptible to *Phytophthora* root rot (caused by *P. cinnamomi*), but is not a proven or associated host of *P. ramorum*. Inside the research plots, a total of 370 potted host plants (mainly *Rhododendron* and a few *Camellia* spp.) were used as the third sentinel layer, arranged in groups of five to eight at a distance of 2 to 5 m to the research bed used for infection experiments. Plants used in the sentinel system

were purchased from a nursery operating with a SOD Compliance Agreement and held in quarantine and inspected for disease development over a 6-week period. Only disease-free plants were included in the sentinel system. Healthy extra plants, which were kept for later use (e.g., to replace infected sentinels) were kept at a holding area 0.3 km east of the research nursery and also inspected weekly for symptoms. The sentinel and barrier plants were not treated with fungicides to allow for any potential disease development and were drip-irrigated and fertilized following best management practice.

NORS-DUC is a federally funded research facility for researchers across the United States working on *P. ramorum* and other soil-borne pathogens under field conditions not allowed in their home research centers due to federal and state regulations. Between 2010 and 2015, numerous projects were carried out at NORS-DUC using infected potted host plants (*Rhododendron* spp., *Camellia* spp., *Viburnum* spp., *Quercus agrifolia*, *Q. rubra*, and *Umbellularia californica*) as well as soil experiments using *P. ramorum*-inoculated *Rhododendron* leaf disks or host-free chlamydospores as inoculum. The experiments focused on a number of scientific questions, including chemical, thermal, and biological control; host-pathogen interaction; and genetic plasticity of *P. ramorum* (Schweigkofler et al. 2014; Funahashi and Parke 2016; Rollins et al. 2015; Kasuga et al. 2016).

Several experiments were conducted by two of our research partners to study the spread of airborne *P. ramorum* inoculum from infected plants. In both cases, *Rhododendron* plants with *P. ramorum* leaf symptoms were used as inoculum source. In one experiment, healthy *Rhododendron* leaf disks were used as baits in plastic cups placed at a distance of 15 and 40 cm to the inoculum source. In the second experiment, four infected plants were placed in the corner of a research bed, and noninfected *Rhododendron* plants were placed in several rows around the inoculum source. Whereas the first experiment did not result in any detectable *P. ramorum* spread, the second experiment showed some very limited aerial spread not further than approximately one meter from the inoculum source (B. Knaus and

FIGURE 1

Layout of the quarantine nursery at NORS-DUC (side NORTH) showing the position of the sentinel plants and research plots with plants infected with *Phytophthora ramorum*.

M. Garbelotto, *personal communication*). In both experiments, the research beds were irrigated overhead to simulate extensive rainfall.

Leaf Symptoms Detected on Sentinel plants

Leaf symptoms similar to those caused by *P. ramorum* were detected on sentinel plants, mainly from January through May (Fig. 2). Symptomatic leaves were tested for presence of *Phytophthora* using Agdia Immunostrips (Agdia, Inc., Elkhart, IN); in addition, leaf tissue was placed on *Phytophthora* selective medium (PARPH-V8; Ferguson and Jeffers 1999) and growing cultures were evaluated microscopically. Selected samples were identified using PCR sequencing of the ITS1, 5.8S rDNA, and ITS2 regions by the California Department of Food and Agriculture Plant Pest Diagnostics Lab (CDFA-PPDC) in Sacramento, CA (White et al. 1990).

Phytophthora ramorum was not detected on any sentinel plants during the five-year monitoring period. The most common fungal group was *Pestalotia* sp. and its related allies (*Pestalotiopsis* sp.), ascomycetous fungi belonging to the Xylariales. *Pestalotia* sp. is considered to be a nonaggressive pathogen of *Rhododendron* spp., only occasionally causing plant death. *Pestalotia* sp. was the only pathogen isolated throughout most of the year, including the dry, hot summer months (with the exception of August and October) and was also the only pathogen frequently isolated from sentinels inside the research plots. Young *Rhododendron* plants developed symptoms caused by *Pestalotia* commonly on lower leaves and after transplanting and moving to the research plots, potentially a result of being weakened by water stress. *Pestalotia* sp. was isolated less frequently from well-established *Rhododendron* sentinels, and also from *Eucalyptus* trees surrounding the research nursery, which might be a source of inoculum.

Several *Phytophthora* species were isolated from a total of 55 sentinel plants: *P. syringae* (n = 32; 58.2%); *P. multivora* (n = 10; 18.2%); *P. cf. fallax* (n = 3; 5.5%); *P. hibernalis* (n = 2; 3.6%); *P. cf. boehmeriae* (n = 1; 1.8%); and a few *Phytophthora* that were not identified to species level (n = 7; 12.7%). *Phytophthora syringae* was detected each year from 2012 to 2016, while *P. multivora* was detected in 2013, 2015, and 2016. A species closely related to *P. fallax* was detected in 2013 and 2014 and a species closely related to *P. boehmeriae* was detected in 2016. *Phytophthora hibernalis* was found in 2011 and 2013.

Phytophthora syringae was the most commonly detected species and belongs to *Phytophthora* clade 8, which also includes *P. ramorum* and *P. hibernalis*. *Phytophthora syringae* has a rather wide host range and is known to cause foliar spots and blights as well as root and stem rot on *Rhododendron*, mainly during the winter months. *Phytophthora multivora* belongs to *Phytophthora* clade 2 and was originally isolated from *Eucalyptus* and other tree species in western Australia, but is known also to induce leaf spots on *Rhododendron* spp. *Phytophthora hibernalis* is a common species in many nurseries and also causes leaf spots and twig blights on *Rhododendron*.

Phytophthora boehmeriae (clade 10) is found in Asia, Europe, South Africa, and South America, and is primarily associated with diseases of cotton, citrus, and *Eucalyptus* spp. There are no reports of it in North America. Although most closely related to *P. boehmeriae*, our isolate differs by 1 nucleotide in the ITS region and 8 nucleotides in the COX-2 gene, indicating that it might be a novel species. Studies on growth pattern, host range, and molecular taxonomy of the *P. cf. boehmeriae* isolate are ongoing in our lab. The *P. cf. fallax* isolate might also be a new, undescribed species. *Phytophthora fallax* was originally isolated from crown dieback from several nonnative *Eucalyptus* spp. in New Zealand (Dick et al. 2006). It belongs to

FIGURE 2

Symptoms caused by phytopathogenic oomycetes and fungi on *Rhododendron* spp.: (A) *Phytophthora ramorum* on an inoculated plant in the research plot; (B) *P. multivora*; (C) *P. cf. fallax*; and (D) *Pestalotia* sp., on sentinel plants outside the nursery.

Phytophthora clade 9, a group of mainly aquatic species often found in riparian eco-systems and rarely causing severe plant diseases. The ITS sequences of our isolates differ in four nucleotide positions from that of the *P. fallax* type strain, and therefore the exact taxonomic position is still unclear. *Phytophthora* cf. *fallax* seems to be a weak pathogen as inoculation experiments on detached leaves of *Rhododendron* sp., *Camellia* sp., *Q. agrifolia*, *U. californica*, and *Eucalyptus globulus* (Blue Gum) did not result in foliar symptoms (data not shown).

The seasonal appearance of leaf symptoms caused by the *Phytophthora* isolates (January through May) confirms their need for wetness and moderate temperatures (Fig. 3). The number of *Phytophthora* detections (all species combined) per rainy season from 2011 to 2016 varied from 2 to 20 with the most detections in winter 2015-2016, an El Nino year characterized by significantly higher precipitation than the preceding years. Leaf symptoms appeared predominantly on *Rhododendron* sentinel plants, and to a lesser extent on *Camellia* spp.. *Viburnum* sp. showed occasional symptoms caused by *Phoma exigua* and *Erysiphe viburni* (powdery mildew). No leaf symptoms were detected on *Loropetalum chinense*.

The location of the sentinel plants (within the research plot or lining the outside perimeter) had a clear effect on *Phytophthora* detection: 49 (89.1%) *Phytophthora* detections were made on the

145 plants along the perimeters, and only one (1.8%) detection was made on the 370 plants within the research plot, indicating that most pathogens originated from areas outside the research nursery (the remaining five isolates were detected in the holding area). Sentinel plants were removed and exchanged with new plants after appearance of symptoms, which might explain the lack of secondary spread from infected “external” sentinel plants to “internal” sentinel plants. Details on the timing and location of symptom detection are shown in Table 1.

Spread of *P. ramorum* from Naturally Infected Bay Laurel Trees to Rhododendrons

The lack of any detections of *P. ramorum* on the sentinel plants at NORS-DUC could be explained by a number of factors including low inoculum titer or suboptimal, local microclimatic conditions. Using a “natural infection” experiment, we tried to evaluate if different inoculum sources and geographic locations have an effect on transmission. Three potted *Rhododendron* plants were placed under known *P. ramorum*-infected California bay laurel trees at two different locations. One location was on the campus of Dominican University (DUC), approximately 150 m east of the research nursery, and the other location was a mixed hardwood/conifer forest in Mill Valley, in an area within the coastal

FIGURE 3

Seasonal appearance of symptoms on sentinel plants at NORS-DUC. Numbers indicate how often a *Phytophthora* species was detected each month (data from 2011 to 2016 were combined). Presence of *Pestalotia* sp. was not quantified. Meteorological data show the long-term average for San Rafael, CA.

“fog-belt” heavily infected with *P. ramorum* (Marin Co.; ~10 mi south west of NORS-DUC). The two sites differ significantly in annual average rainfall (San Rafael, 90 cm; Mill Valley, 120 cm). The California bay laurel trees and the potted *Rhododendron* plants were monitored weekly during the rainy season for appearance of *P. ramorum* symptoms; symptomatic leaves were sampled and (if positive for *Phytophthora* using Immunostrips) plated on PARP-V8 for morphological identification. The experiment was repeated during two rainy seasons (2014-2015 and 2015-2016), starting October 1 in both years.

The results are shown in Fig. 4. In Mill Valley, leaf symptoms caused by *P. ramorum* were present on California bay laurel leaves throughout the monitored period in both years. During the 2014-

2015 season, symptoms on California bay laurel at DUC appeared in early December while they appeared at the end of December during the 2015-2016 season. On *Rhododendron*, the first symptoms appeared in Mill Valley (on all three plants) and DUC (one out of three plants) on December 15 during the 2014-2015 season. However, during the 2015-2016 season, the first foliar symptoms appeared on *Rhododendron* in both Mill Valley (on one out of three plants) and DUC (one out of three plants) on January 19. On March 10, a second *Rhododendron* showed *P. ramorum* symptoms at DUC. Based on these results we conclude that direct transmission of *P. ramorum* from naturally infected bay trees to *Rhododendron* is possible under microclimatic conditions typical for the winter months at Dominican University, though the time in which symptoms are

TABLE 1
Detection of *Phytophthora ramorum*-like leaf symptoms on sentinel plants at two research sites (SOUTH and NORTH) at NORS-DUC from 2011 to 2016

Detection date	Species	Detection number	Sentinel plant	Location within NORS-DUC ^a
2011				
2/15	<i>P. hibernalis</i>	1	<i>Rhododendron</i>	HA ^b
5/23	<i>Phytophthora</i> sp.	1	<i>Rhododendron</i>	HA
2012				
5/28	<i>P. syringae</i>	3	<i>Rhododendron</i>	HA
2013				
1/02	<i>P. hibernalis</i>	1	<i>Rhododendron</i>	NORTH-P
1/14	<i>P. syringae</i>	2	<i>Rhododendron</i>	SOUTH-I
1/14	<i>Phytophthora</i> sp.	1	<i>Rhododendron</i>	SOUTH-P
2/19	<i>P. cf. fallax</i>	1	<i>Rhododendron</i>	NORTH-P
2/19	<i>P. multivora</i>	1	<i>Rhododendron</i>	SOUTH-P
4/6	<i>Phytophthora</i> sp.	1	<i>Rhododendron</i>	SOUTH-P
2014				
2/26	<i>P. syringae</i>	5	<i>Rhododendron</i>	NORTH-P
3/10	<i>P. cf. fallax</i>	1	<i>Rhododendron</i>	NORTH-P
3/10	<i>P. syringae</i>	3	<i>Rhododendron</i>	NORTH-P
3/13	<i>P. syringae</i>	2	<i>Rhododendron</i>	NORTH-P
3/13	<i>P. cf. fallax</i>	1	<i>Rhododendron</i>	NORTH-P
4/15	<i>P. syringae</i>	1	<i>Rhododendron</i>	NORTH-P
4/21	<i>Phytophthora</i> sp.	2	<i>Rhododendron</i>	NORTH-P
2015				
1/26	<i>P. multivora</i>	2	<i>Rhododendron</i>	NORTH-P
1/26	<i>P. multivora</i>	1	<i>Rhododendron</i>	SOUTH-P
1/26	<i>Phytophthora</i> sp.	1	<i>Rhododendron</i>	NORTH-P
3/2	<i>P. syringae</i>	1	<i>Rhododendron</i>	NORTH-P
3/2	<i>P. multivora</i>	2	<i>Rhododendron</i>	NORTH-P
3/2	<i>Phytophthora</i> sp.	1	<i>Rhododendron</i>	NORTH-P
2016				
2/1	<i>P. syringae</i>	4	<i>Rhododendron</i>	NORTH-P
2/8	<i>P. syringae</i>	1	<i>Rhododendron</i>	NORTH-P
2/25	<i>P. multivora</i>	1	<i>Rhododendron</i>	NORTH-P
2/25	<i>P. syringae</i>	4	<i>Rhododendron</i>	NORTH-P
3/7	<i>P. syringae</i>	3	<i>Rhododendron</i>	NORTH-P
3/7	<i>P. syringae</i>	1	<i>Rhododendron</i>	SOUTH-P
4/5	<i>P. multivora</i>	1	<i>Rhododendron</i>	SOUTH-P
4/5	<i>P. cf. boehmeriae</i>	1	<i>Rhododendron</i>	NORTH-P
4/5	<i>P. syringae</i>	2	<i>Rhododendron</i>	NORTH-P
4/11	<i>P. multivora</i>	2	<i>Rhododendron</i>	SOUTH-P

^a SOUTH-P and NORTH-P = sentinel plants along the outside perimeters of the two research sites; SOUTH-I = sentinel plants inside the research site.

^b HA = holding area.

expressed varies yearly depending on environmental parameters. We did not measure the inoculum load of *P. ramorum* on California bay laurel leaves, however, under favorable conditions, a significant proportion of the leaves showed symptoms.

Transmission Experiment Using “Naturally” Infected *Rhododendrons*

The spread of *P. ramorum* from symptomatic *Rhododendron* plants to healthy *Rhododendron* plants within the research nursery was studied using plants naturally infected in Mill Valley and on the DUC campus (see above). The experiment was repeated during two rainy seasons (winters 2014–2015 and 2015–2016). For the first experiment, three infected plants were moved to the research nursery on 23 December 2014. Nine healthy *Rhododendrons* were placed around the source of inoculum (0.5- to 1.5-m distance) to simulate conditions at commercial nurseries, where large numbers of conspecific plants are often kept in one big continuous block. After 13 days, *Phytophthora* symptoms appeared on one *Rhododendron* plant and after 24 days, symptoms occurred on a second plant (22.2% transmission rate). *Phytophthora ramorum* was isolated from both symptomatic leaves on PARP-V8. For the second experiment, three infected plants were moved to the research nursery on 22 January 2016, and 12 healthy *Rhododendrons* were placed around them (0.5- to 1.5-m distance). Fifty-nine days later, foliar symptoms were detected on five of the 12 *Rhododendron* plants. After 67 days, symptoms developed on two additional *Rhododendron* plants and after 87 days, on one additional plant (total of 8 infected plants, 75% transmission rate). For both experiments, plants were monitored until the following summer (for a total of approximately 200 days) to detect possible delayed spread of *P. ramorum*.

The differences in transmission rates between the two experiments might be related to climatic conditions; the 2014–2015 winter was characterized by some early-season rainfall (October till December), followed by very dry conditions, whereas the 2015–2016 winter (an El Niño year) showed significant rainfall between November and April, but with a month-long dry phase in February. No spread of *P. ramorum* was detected from the infected rhododendron plants in the research bed to the surrounding sentinel plants (minimum distance of 5 m).

Aerial Spread of *P. ramorum* Seems to Act Only on Very Short Distances

While the geographic origin and exact time of intercontinental spread of *P. ramorum* is still unknown, symptoms caused by the

pathogen have been identified since the mid-1990s in forests in coastal California and nurseries in northern Europe (Germany and The Netherlands) (Grünwald et al. 2012). Since then, *P. ramorum* has been spread in European nurseries and gardens by the movement of infected plant material and has not caused significant damage in forests, with the exception of a severe outbreak of *P. ramorum* on commercial plantations of the nonnative Japanese larch (*Larix kaempferii*) in the United Kingdom in 2009 (Brasier and Webber 2010), and Ireland in 2010. The spread of *P. ramorum* in North America is quite complex and involves multiple introductions in native forests in California through infected plants or soil, long-distance spread from California to other U.S. states and as well as a few cases of introduction of European isolates into the U.S. (Grünwald et al. 2012). Short-distance spread of *P. ramorum* is mainly caused by airborne sporangia, which are produced in high numbers on leaves of California bay laurels and tanoaks under moist and mild weather conditions.

Multiyear surveys from the California SOD Blitz, a citizen scientist project involving hundreds of volunteers to sample SOD symptoms each spring since 2008 and organized by researchers at UC Berkeley (Meentemeyer et al. 2015), support the idea that *P. ramorum* spread is very limited during years with average and below-average rainfall in coastal California, and that the main driver for the expansion of the disease range is wetter than average winters. The rather large sporangia size (length $\times$ width = 25 to 97×14 to $34 \mu\text{m}$, mean 46 to 65×21 to $28 \mu\text{m}$) indicates that *P. ramorum* might not be carried very well through air currents. Rain droplets (splash) or running water may play a more important role in short-distance transmission. *Phytophthora ramorum* is routinely baited from creeks and rivers in infested landscapes and forests; recirculated irrigation water in nurseries has also been shown to play a role in the infection process of healthy container plants (Werres et al. 2007). Our work has shown that *P. ramorum* can be detected from recirculated water in the NORS-DUC site as well as a seasonal creek on the DUC campus (data not shown).

Nurseries often are characterized by the presence of high plant diversity on a small area and conditions that are favorable for pathogen introduction and spread (e.g., movement of plant material between nurseries, over-head and extensive irrigation, use of recycled water), making them an ideal habitat for many *Phytophthora* species (Junker et al. 2016, Parke et al. 2014). Different niches within the nursery (soil, water, plants) can be colonized by different *Phytophthora* species, and both species composition and abundance can undergo seasonal fluctuations.

FIGURE 4

Symptom expression of *Phytophthora ramorum* on California bay laurel (green bars) and *Rhododendron* spp. (blue bars) at two locations (Mill Valley and Dominican University campus).

NORS-DUC is the only quarantine research nursery in the United States established to conduct mock field experiments with *P. ramorum* under the oversight of USDA-APHIS. Inoculation experiments at the research site impose the risk of pathogen escape into the environment. To mitigate the risks, NORS-DUC was built in Marin Co., an area with many mixed hardwood/conifer forests already heavily infected with *P. ramorum*. In addition, the sentinel system was established to monitor possible aerial movement of *P. ramorum* from the site. Sentinel plants have been used to monitor aerial spread of pollen of genetically modified crops (Watrud et al. 2004), pesticides (Felsot et al. 1996), and invasive plant pathogens (Sanatkar et al. 2015). An international project was initiated recently to use botanic gardens and arboreta worldwide to detect potential invasive pathogens and pests before they spread to new environments (Britton et al. 2010), and a similar approach was used to forecast potential invasions of alien plant pathogens from China to Europe with the planting of European hardwood trees (*Quercus* sp.) in southeastern China (Vettraiano et al. 2015). To our knowledge, our study is the longest-running sentinel monitoring program of a quarantined forest pathogen.

During a five-year period (2011-2016), we did not detect any *P. ramorum* infections in our sentinel plants within and outside the two research sites. Due to the highly diverse experiments conducted at NORS-DUC from 2011 to 2015 by different researchers (e.g., use of different host plants; inoculation of potted plants and soil; different inoculum type) it was not possible to estimate the amount of aerial *P. ramorum* inoculum present at the research site during that period. However, infected and symptomatic plants were present throughout most of the reported time, including the rainy winter months when most *P. ramorum* spread occurs in natural environments. *Phytophthora ramorum* requires a moist period of ~24 h for sporulation on *Rhododendron* 'Cunningham's White', and spore production is highest at temperatures between 15 and 20°C. While the environmental requirements of *P. ramorum* are similar to other *Phytophthora* species, *P. ramorum* produces sporangia more slowly than some of them (Tooley and Browning 2015). The local microclimatic conditions at NORS-DUC, with considerably less fog and more sun hours than the coastal areas mainly affected by SOD, are suboptimal for high inoculum production during the California drought years, but still not preventive for limited disease spread, as shown by regular outbreaks of *P. ramorum* symptoms on California bay laurel trees on campus. The results of our transmission experiment indicate that horizontal aerial spread of *P. ramorum* might be limited to a few meters under our local conditions, and support existing best management practice guidelines developed to minimize the risk of *P. ramorum* spread in commercial nurseries (Anonymous 2008), such as the creation of a physical barrier or a 2-m break between high-risk plants and all other crops as well as breaking up long sections of host and associated plants (HAP) with non-HAP material.

Based on cumulative results over a five-year period we consider the risk of aerial spread of *P. ramorum* from infected plants used at NORS-DUC into the environment as low. The usefulness of the sentinel system for early detection of pathogens was confirmed by the repeated isolation of multiple other *Phytophthora* spp., including *P. cf. fallax* and *P. cf. boehmeriae*, two likely novel species with unknown host ranges. The observed effect of seasonality on pathogen detection, resulting in *Phytophthora* detection only in late winter and spring, can be used to develop more efficient strategies for nursery surveys by timing them accordingly.

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