Phytophthora ramorum: Impacts on Forest, Conservation and Native Plant Nurseries

by Thomas D. Landis

This article was written with the help of many experts who were gracious enough to share their knowledge and experience: Gary Chastagner and Marianne Elliott, Washington State University; Susan Frankel and Ellen Goheen, USDA Forest Service; Prakash Hebbar, USDA, APHIS; Jennifer Parke, Oregon State University; and Jane Alexander, University of California.

Phytophthora ramorum (PRAM) is a fungus-like pathogen that, although it was originally identified on ornamental plants in a German nursery (Werres and others 2001), has become a destructive forest pest in the coastal forests of California, Oregon and in other locations in Europe. Because more than 100 species of trees and shrubs from 36 different families are susceptible (Chastagner and others 2012), PRAM has the potential to become the most serious forest pest since white pine blister rust and chestnut blight. Disease symptoms on nursery stock are relatively minor and, what's most worrisome, is that many infected plants show no visible symptoms at all (Vercauteren and others 2013). Genetic testing has proven that long-range spread can be attributed to the shipping of infected nursery stock, and that PRAM can then be transmitted from nurseries to surrounding forests (Mascheretti and others 2008).

Although PRAM has not proven to be a disease with severe symptoms in nurseries, it can still have serious economic impacts due to plant quarantine regulations. At one ornamental nursery in Southern California, more than 1 million camellias worth $9 million had to be destroyed because of a PRAM infestation (Alexander 2006). PRAM has only been positively identified on ornamental nursery stock as of the current date, but it is only a matter of time until infections are discovered on forest, conservation and native plant species. Because they ship their plants directly into forests and other natural settings, forest and native plant nurseries represent a serious transmission threat. Unfortunately, this has already happened in the United Kingdom where nursery stock has been shown to be the cause of a devastating forest disease outbreak in Japanese larch (Larix kaempferi) plantation where 3 million trees have been killed (Brasier 2012).

Figure 1 - Phytophthora ramorum (PRAM) is a new and aggressive pest that affects plants in nurseries, but is much more destructive in plantations and natural forests. So far, 3 clones (EU1, NA1, NA2) have been identified (modified from Grunwald 2011).
1. A New and Complicated Pest

*Phytophthora* species resemble true fungi because they grow by hyphae and produce spores, but they are actually more closely related to brown algae. Disease symptoms caused by *Phytophthoras* include blights, cankers, dieback, wilts, root rots, and decline. To make diagnosis even more challenging, some species cause multiple symptoms on a single host, or different symptoms on different hosts (Forest Phythophthoras of the World 2012). In nurseries, *Phytophthora* root rot has been a serious but well known nursery pest for decades, where the most common symptoms were root decay and lower stem canker (Cram and Hansen 2012).

What makes PRAM unusual and interesting is that nobody is exactly sure where it originally came from. Although PRAM has been identified only in North America and Europe, it is not considered to be native to either of these continents (Grunwald 2011). PRAM was first detected on ornamental nursery stock in Europe in the early 1990s (Figure 1). The first evidence that this pathogen had reached the US was the sudden oak death (SOD) epidemic in the coastal forests of northern California and southern Oregon where trees with bleeding stem cankers were dying at an alarming rate (Goheen and others 2006). The first detection of PRAM in a US nursery was on ornamental rhododendron container plants in Santa Cruz, California in December, 2000 (Alexander 2006). Based on microsatellite laboratory analysis, researchers determined that PRAM made its first appearance in California forests at 2 separate sites in northern California. Because the genetics of the forest strains were identical to those from local nurseries, this is strong evidence that PRAM entered California via the nursery trade (Mascheretti and others 2008).

Another unusual aspect of PRAM is its genetic makeup. *Phytophthora* genetics are discussed in terms of “clades”, which are a group of organisms with similar features that are derived from a common ancestor. As of 2011, researchers had identified 3 clades for PRAM that were named for where they were first identified (Grunwald 2011). The European clade (EU1) was first identified on ornamental nursery plants in the early 1990s but has since been found on ornamental plantings and in the forest (Table 1). The first North American clade (NA1) was responsible for the SOD epidemic that was identified in the mid 1990s in northern California, and was subsequently confirmed in ornamental nurseries in the area. The NA2 clade was first identified on nursery stock in Washington State (Chastagner 2013) where, by 2005, the NA1 and EU1 clades were also discovered (Figure 1). Just last year, a fourth, genetically distinct clade of PRAM (EU2) was identified as the cause of an epidemic stem canker disease of Japanese larch in the United Kingdom (Brasier 2012). The European clades are of mating type A1 and the North American clades of type A2. The fact that PRAM clades of both mating types were identified in Washington State gives cause for concern but, so far, no evidence of mating has been discovered although it has been accomplished in the laboratory (Garbelotto and others 2006).

2. Symptoms

The symptoms of PRAM vary considerably in both type and intensity between different plant species and between plants in nurseries and forests; as we will discuss, this latter fact is a major concern.

2.1 Forests

Sudden oak death (SOD) is the most common disease caused by PRAM in the US, but it only affects woody plants in forests (Table 2). An unusual die-off of tanoaks (*Lithocarpus densiflorus*) in Marin County, California in early 1995 was the first evidence of SOD and the

<table>
<thead>
<tr>
<th>Clade</th>
<th>Year Discovered</th>
<th>Distribution</th>
<th>Habitat</th>
<th>Mating</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA1</td>
<td>Early 1990s</td>
<td>North America</td>
<td>Forest, nurseries</td>
<td>A2</td>
</tr>
<tr>
<td>NA2</td>
<td>Early 2000s</td>
<td>Washington State, California &amp; British Columbia</td>
<td>Nurseries</td>
<td>A2</td>
</tr>
<tr>
<td>EU1</td>
<td>Early 1990s</td>
<td>Europe &amp; North America</td>
<td>Forests, nurseries, ornamental plantings</td>
<td>A1</td>
</tr>
<tr>
<td>EU2</td>
<td>2011</td>
<td>United Kingdom</td>
<td>Forests</td>
<td>A1</td>
</tr>
</tbody>
</table>

Modified from Grunwald (2011); Brasier (2012)
In addition, host species are noticeably different in nurseries compared to forests (Table 2; Figure 2). Although ornamental cultivars of *Rhododendron, Camellia, Viburnum, Pieris,* and *Kalmia* are most commonly infected, most of these genera have native species somewhere in the US. Even more worrisome is that the “Others” category in Figure 2A contains *Aesculus, Pseudotsuga, Acer,* and *Quercus.* As far as I’ve been able to find out, no plants in forest, conservation, or native plant nurseries have been positively identified for PRAM as of the present date but Douglas-fir and true fir Christmas trees have been infected (Figure 2B). Considering the rapid spread of this pathogen so far and the extensive host list, all nursery workers should be vigilant and employ the latest phytosanitary procedures.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Symptoms</th>
<th>Host Examples</th>
<th>Forest Problem</th>
<th>Nursery Problem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sudden oak death (SOD)</td>
<td>Bleeding stem cankers, tree death</td>
<td>Oaks, tanoak, larch</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>PRAM shoot blight</td>
<td>Shoot tip dieback</td>
<td>Redwood, Douglas-fir, white fir, red fir</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>PRAM leaf blights</td>
<td>Spots and necrosis on leaf edges &amp; tips</td>
<td>Rhododendron, viburnum, camellia, Oregon myrtlewood</td>
<td>YES</td>
<td>YES</td>
</tr>
</tbody>
</table>

Modified from Goheen and others (2006); Chastagner and others (2012)

Disease symptoms consisted of scattered patches of dying trees with their entire crowns dead due to bleeding basal cankers. A couple of years later, other trees including coast live oaks (*Quercus agrifolia*) exhibited similar symptoms. The rapid spread of the disease in an urban-wildland interface in a highly populated area caused public concern, and all the dead trees caused a severe fire hazard. New PRAM hosts included California bay laurel (*Umbellularia californica*) and coastal redwood (*Sequoia sempervirens*) and by 2009 the host list included 109 plant species (Kliejunas 2010). Even more concerning was that PRAM was discovered in remote locations in the coastal forests of southwestern Oregon in 2001 (Goheen and others 2006).

### 2.2 Nurseries

Disease symptoms on nursery stock are much less severe than those of SOD, and generally consist of leaf and shoot blights (Chastagner and others 2012).

Figure 2 - Most of the nursery plants commonly infected with *Phytophthora ramorum* are not produced by forest and native plant nurseries (A). However, many woody natives have been shown to be susceptible, and infections of native Christmas trees has been documented (B) (A, from USDA - APHIS 2011; B, from Chastagner 2013).
3. Disease Spread

*Phytophthora ramorum* has proven to be an aggressive pathogen both in the nursery and in the natural stands. One of their unusual but operationally relevant characteristics is that all Phytophthoras produce zoospores which are able to swim in water (Figure 3A). PRAM also produces two other types of spores (Forest Phytophthoras of the World 2012). Chlamydospores are asexual structures that form in organic matter such as leaves and function as resting spores that allow the pathogen to survive periods of stress (Figure 3B). Oospores are sexual spores produced by the pairing of 2 opposite mating types (A1 & A2 in Figure 3C), but oospore formation has not been observed in nurseries where both mating types have been detected (Grunwald and others 2008). This is lucky because sexual recombination would create new challenges for controlling these pathogens.

PRAM can be spread from nursery to nursery and within nurseries in 2 different ways: on plant material or in water.

3.1 Plant material

Up until now, PRAM has spread both from nursery to nursery and from nursery to forest on infected nursery stock. This has occurred because plants infected with PRAM may or may not show visible symptoms; these latent infections have been shown to be responsible for long distance spread of this pathogen (Mascheretti and others 2008). PRAM could be transmitted between nurseries on transplants or cuttings and, because the pathogen can subsist in soil or growing media as chlamydospores (Figure 4A), could be spread on contaminated containers or even equipment. As of the present time, PRAM has not been positively identified on forest, conservation, or native plant nursery stock but, because so many plant species are susceptible, it’s probably only a matter of time.

3.2 Water

Due to the ease with which the zoospores can move in water, this pathogen can easily move from plant to plant whenever free water is allowed to persist (Elliott 2012). In nurseries, this would account for most short distance spread. In research trials with container-grown *Rhododendron*, aerial dispersal of PRAM was minimal whereas spread in surface water between containers could occur over several meters (Huengens and others 2010). Another worrisome fact about PRAM is that the pathogen is able to escape nurseries in surface runoff water, presumably as

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*Figure 3 - Phytophthoras produce 3 types of spores: motile zoospores, which can actively disperse in water (A), chlamydospores (B), which can survive long periods in plant tissue or even organic matter, and thick walled oospores (C) that are sexually produced by the combination of the two mating types (modified from Phytophthoras of the World 2012).*

*Figure 4 - Phytophthora ramorum is spread between nurseries and from nurseries to forests in two ways: 1) On nursery stock as latent infections or chlamydospores in organic matter (A), or 2) In nursery runoff; for example, this pathogen has been detected in waterways around nurseries in 8 states (B) (A from Elliott 2012, and B from Chastagner and others 2010).*
4. Diagnosing *Phytophthora ramorum* in the Nursery

Many nursery diseases can be diagnosed by their unique signs and symptoms but this is not the case with PRAM. Signs and symptoms are extremely variable between hosts and are impossible to distinguish from other plant pathogens (including other *Phytophthora* species), insect damage or abiotic injury (Kliejunas 2010). The presence of the pathogen can only be confirmed through laboratory culturing on artificial media, or by molecular tests (Figure 5).

4.1 Culturing on selective media

PRAM can be isolated on selective artificial media and its identity confirmed by its unique morphological

<table>
<thead>
<tr>
<th>County</th>
<th>Waterway</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>King</td>
<td>Sammamish River</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ditch by Nursery 34</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Little Bear Creek</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Woodin Creek</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Cottage Lake Creek</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Pierce</td>
<td>Rosedale Stream</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Ditch by Nursery 45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Thurston</td>
<td>Ditch by Nursery 41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
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<td>Lewis</td>
<td>Mill Creek</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Clark</td>
<td>Ditch by Nursery 44</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
characteristics. However, culturing from symptomatic plant material is time consuming and success may vary with the host plant. Differentiating PRAM from other Phytophthora species can sometimes be difficult (Kliejunas 2010).

4.2 Serological tests

The enzyme-linked immunosorbent assay (ELISA) test that uses antibodies and color change to identify a substance. An ELISA test that is specific to PRAM is not yet available, due to cross reaction with other Phytophthora and Pythium spp. (Avila and others 2010). If a large number of samples are to be processed for PRAM, ELISA can be used as a low-cost, prescreening to reduce the number of samples that will need to be processed for subsequent tests (Kliejunas 2010).

4.3 Molecular tests

Several different DNA-based molecular techniques have been used to diagnose PRAM infections, and are new variations are continually being developed (Kliejunas 2010). Both real-time and nested polymerase chain reaction (PCR) based molecular diagnostic assays have proven useful for detecting PRAM from leaf baits, and greatly reduce the turnaround time (Colburn and Jeffers 2011).

When the various diagnostic techniques were tested on camellia (Camellia spp.) plants at a California nursery, all the procedures were highly correlated with disease symptoms. The PCR test had the correlation, followed by ELISA, and finally culturing on selective media (Bullock and others 2006).

The diagnostic protocols approved by the USDA APHIS-PPQ are explained in detail on their website: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/pdf_files/DiagnosticsTable.pdf

5. Assessing the Threat

So, for us, the important question is: How big a threat is PRAM to forest and native plant nurseries? We don't have a ready answer, but looking back at past epidemics gives cause for concern (Table 4). Chestnut blight and white pine blister rust were devastating epidemics that are still affecting our forests, but these fungal diseases only affected one plant genus.

The host range for PRAM is currently at 36 plant families so the threat is potentially much greater (USDA-APHIS 2011). Pathologists consider PRAM as a generalist pathogen whose hosts include hardwood and conifer trees, shrubs, herbaceous plants, and ferns (Kliejunas 2010). Some hosts are native plants from forest environments but many of the most susceptible species as common landscape and ornamental plants. Five species of common shrubs comprise almost 95% of the confirmed PRAM infections (Figure 2), and disease

Table 4 - Comparison between previous disease epidemics and Phytophthora ramorum

<table>
<thead>
<tr>
<th>Name of Pest</th>
<th>Scientific Name</th>
<th>Date Introduced into US</th>
<th>Plant Hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chestnut blight</td>
<td>Cryphonectria parasitica</td>
<td>Early 1900s</td>
<td>1 Genus: Castanea</td>
</tr>
<tr>
<td>White pine blister rust</td>
<td>Cronartium ribicola</td>
<td>Early 1900s</td>
<td>1 Genus: Pinus</td>
</tr>
<tr>
<td>Sudden oak death, Ramorum shoot or leaf blight</td>
<td>Phytophthora ramorum</td>
<td>Early 1990s</td>
<td>36 Families (and counting)</td>
</tr>
</tbody>
</table>
surveys showed a large variation in disease incidence among genera and specific cultivars within a genus (Tubajika and others 2006).

The explosive potential of this pest can be seen in the APHIS annual reports of the number of PRAM detections in US nurseries (Table 5). Since the initial detection in central California, the disease spread relatively slowly until 2004 when 2 large southern California ornamental nurseries shipped millions of infected container plants to other nurseries in 39 states (Frankel 2008). Inspections later that year revealed 176 nursery-related detections in 21 different states (Garbelotto and Rizzo 2005). As a result, the USDA Animal and Plant Health Inspection Service (APHIS) issued an order to inspect 1,400 nurseries that ship host plants or associated plants in California, Oregon, and Washington (Jones 2006). Even more recently, a nursery in Washington State shipped potentially infected Gaultheria procumbens nursery plants to customers in 30 states (Chastagner 2013).

### Table 5 - Annual detection of Phytophthora ramorum in US nurseries

<table>
<thead>
<tr>
<th>Year</th>
<th>No. Of Positive Detections</th>
<th>No. Of States</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>1</td>
<td>1 (CA)</td>
</tr>
<tr>
<td>2002</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2003</td>
<td>20</td>
<td>3 (CA, OR, WA)</td>
</tr>
<tr>
<td>2004</td>
<td>176</td>
<td>21</td>
</tr>
<tr>
<td>2005</td>
<td>99</td>
<td>7</td>
</tr>
<tr>
<td>2006</td>
<td>62</td>
<td>11</td>
</tr>
<tr>
<td>2007</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>2008</td>
<td>28</td>
<td>8</td>
</tr>
<tr>
<td>2009</td>
<td>26</td>
<td>11</td>
</tr>
<tr>
<td>2010</td>
<td>34</td>
<td>13</td>
</tr>
<tr>
<td>2011 (through Sept)</td>
<td>25</td>
<td>5</td>
</tr>
</tbody>
</table>

Modified from Kliejunas (2010) and Alexander (2012)

6. Quarantine Considerations

As we discussed, the transport of nursery stock has been proven to be the primary means of long-distance spread of PRAM, and is also implicated in how the pathogen moves from the nursery to the forest. APHIS has adopted an interim federal quarantine to prevent the spread of PRAM to other parts of the U.S. Other states and countries such as Canada have also issued quarantines. APHIS maintains a website that contains the most current list of affected plant species (USDA-APHIS 2013), and has identified 3 categories of susceptibility to PRAM (Kliejunas 2010).

#### 6.1 Regulated hosts

These are plants in which infections have been verified by Koch’s postulates, which is the traditional test to confirm the a pest is the cause of the disease. Examples include: California maidenhair fern (*Adiantum aleuticum*), manzanita (*Arctostaphylos* spp.), false Solomon's seal (*Maianthemum racemosum*), Douglas-fir (*Pseudotsuga menziesii*), California black oak (*Quercus kelloggii*), and evergreen huckleberry (*Vaccinium ovatum*).

#### 6.2 Associated plants

In this case, the plants have been naturally infected with PRAM and confirmed by culture or with PCR tests, but the infections have not been confirmed with Koch's postulates. Examples include: white fir (*Abies concolor*), vine maple (*Acer circinatum*), blue-blossom (*Ceanothus thyrsiflorus*), California wood fern (*Dryopteris arguta*), Oregon grape (*Mahonia nervosa*), northern red oak (*Quercus rubra*), and Pacific yew (*Taxus brevifolia*).

#### 6.3 Experimental hosts

These plants have been infected with PRAM in laboratory screening, but no actual infections have been documented in nature.

The issue of quarantines is complicated and frequently changing so check with your local forest pest experts or go to the following websites:

- For the latest national information on PRAM: http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/
- For the latest PRAM information in Oregon: http://www.oregon.gov/ODA/CID/PLANT_HEALTH/Pages/sod_index.aspx
- For the latest PRAM information in California: http://www.cdfa.ca.gov/plant/PE/interiorexclusion/SuddenOakDeath/
- For the latest PRAM information in Washington: http://agr.wa.gov/plantsinsects/diseases/sod/
7. Implications for Forest, Conservation, and Native Plant Nurseries

Nurseries in the quarantine areas of the western states are already being impacted by PRAM, but all nurseries and nursery customers have an obligation to help stop this disease. Phytosanitation is the key to controlling the spread of any nursery pest, and can most simply be viewed as an input-output model. The basic idea is to prevent pests from entering your nursery as well as making certain that your plants are not carrying pests when they leave your nursery for sale or outplanting.

Two major approaches to phytosanitation can be employed. The systems approach is based on a Hazard Analysis of Critical Control Points and comprehensive programs that have been developed for ornamental nurseries can easily be modified for forest, conservation and native plant facilities (Parke and Grunwald 2012). A second approach based on target pests might be easier for smaller nurseries with limited funds and manpower. Here, the idea is to learn as much as possible about pests that are already found in your nursery or ones, like PRAM, that could threaten it. The following is a brief example of the target pest approach to phytosanitation.

7.1 Type of Pest

PRAM a fungus-like pathogen that produces relatively minor symptoms in nursery stock, but research has shown that it can persist on plant material or even organic matter.

7.2 Method of Spread

This pest produces 3 types of spores: motile zoospores, which can actively disperse in water; chlamydospores, which can survive long periods in plant tissue or even organic matter (Figure 4a); and thick walled oospores that are sexually produced by the combination of 2 mating types (Chastagner and others 2012).

7.3 Critical Control Points

Due to its many spore types, PRAM has multiple modes of transmission. It is most commonly spread through any type of plant material shared between nurseries including cuttings and transplants. Seed transmission has not been proven so far. Zoospores can spread through any form of water such as rain splash and surface runoff, and has been shown to persist in waterways around nurseries (Chastagner and others 2012).

By focusing on the type of pest and its methods of spread, nurseries can adapt their scouting and cultural practices to minimize adverse affects. Because their stock is outplanting directly into forests and other wildland plant communities, nursery managers should be especially vigilant to make sure that PRAM isn’t spread to or from their operation.

8. References


