Determining the Risk of *Phytophthora ramorum* Spread From Nurseries Via Waterways[1]

Marianne Elliott,[2] Gary Chastagner,[2] Katie Coats,[2] and Gil Dermott[2]

Abstract

Phytophthora ramorum, the fungus-like pathogen which causes sudden oak death, is a threat to the Pacific Northwest nursery industry. Because this is a quarantine organism, the destruction of plants and mitigation treatments resulting from a positive *P. ramorum* detection has caused millions of dollars in losses to the commercial nursery industry in California, Oregon, and Washington. There is concern about movement of the pathogen to nurseries and forests in the eastern United States. An increase has been seen in the NA2 and EU1 lineages from nursery samples in Washington in recent years, so a study of the relative fitness of *P. ramorum* isolates in the Washington State University culture collection was undertaken. Eighty-five isolates were screened for sensitivity to the fungicide mefenoxam and for relative pathogenicity on detached rhododendron leaves. Most isolates of *P. ramorum* were sensitive to the fungicide with the exception of some EU1 isolates from one nursery and its trace-forwards. A strong relationship between phenotypic characteristics such as fungicide sensitivity and pathogenicity, and the originating nursery, was seen. Since *P. ramorum* is moving from nurseries into streams, a method for exposing plants to contaminated stream water was tested. Further studies will include measuring inoculum levels in irrigation water from streams to determine whether this pathway is of importance in disease spread.

Key words: *Phytophthora ramorum*, nursery industry, mefenoxam sensitivity

Introduction

Long-distance spread of *Phytophthora ramorum* occurs mainly by movement of infected nursery stock, and some countries have imposed quarantines to reduce further spread (EPPO 2006). *Phytophthora ramorum* was first detected in a California nursery in 2001, and in nurseries in California, Oregon, Washington, and British Columbia in 2003. In 2004, a shipment of camellias from a California nursery resulted in the spread of *P. ramorum* to nurseries across the United States and in British Columbia, Canada. Since then, infected ornamental nursery stock has been detected in 48 nurseries in Washington State, as well as throughout the United States and British Columbia. Three of the four genetic lineages of *P. ramorum* have been found in Washington nurseries. When *P. ramorum* is detected in a nursery, measures are taken to eradicate the pathogen. This can result in a financial burden to the nursery and some have gone out of business (Dart and Chastagner 2007).

In some cases, *P. ramorum* is not completely eradicated from a nursery, and soil and water becomes contaminated. The pathogen has spread from nurseries to adjacent waterways in several locations in Washington. Once infested, streams remain positive for *P. ramorum*, even after mitigation steps have been taken at the nursery and the pathogen can no longer be detected at the nursery site. The movement of an isolate from the NA2 lineage of *P. ramorum* to salal (*Gaultheria shallon* Pursh) and soil outside of a Washington nursery via contaminated water in 2009 and 2010 illustrates the importance of this pathway as a means of spreading *Phytophthora* pathogens from nurseries to plants and soil in the landscape. In another case, *P. ramorum* was detected once on riparian willow plants outside of a nursery in Mississippi, although subsequent sampling failed to recover the pathogen (Jeffers 2011). Other than the single incidents in Washington and Mississippi,

[1] A version of this paper was presented at the Sudden Oak Death Fifth Science Symposium, June 19-22, 2012, Petaluma, California.

[2] Washington State University, Puyallup Research and Extension Center, 2626 W. Pioneer, Puyallup, WA 98371. Corresponding author: melliott2@wsu.edu.

the pathogen has not spread from streams to vegetation, even in areas with forest infestations in California and Oregon.

Mefenoxam (Metalaxyl-M®, Subdue MAXX®) is one of the most commonly used chemicals for controlling *Phytophthora* species on ornamentals and other crops. It has been shown to be effective when used preventively against *P. ramorum* and is inhibitory to both mycelial growth and sporulation. Unfortunately, resistance to this chemical can develop in *Phytophthora* spp., including *P. ramorum* (Wagner et al. 2007). In some studies, it has been shown that metalaxyl resistant (MR) isolates of *Phytophthora* are more fit than metalaxyl sensitive (MS) isolates. These isolates tended to be more aggressive on host material, have a higher infection rate, and increased sporangia and oospore production than MS isolates (Chycoski and Punja 1996, Hu et al. 2008, Mukalazi et al. 2001). Exposure to sublethal doses of mefenoxam and other fungicides induces a switch in mating type, which could lead to sexual recombination and the production of new genotypes (Groves and Ristaino 2000).

In recent years, the EU1 and NA2 lineages of *P. ramorum* have been found more frequently during Washington nursery inspections, and the NA1 lineage less often. In this study, we examined some isolates of *P. ramorum* to determine whether there was a difference in fitness among isolates that might explain this trend. We also tested a method for determining whether inoculum levels in streams were sufficient to cause infection on plants irrigated with stream water.

Examining Fungicide Resistance and Pathogenicity Among Clonal Lineages in *Phytophthora ramorum*

The objectives of this study were to examine 85 isolates of *P. ramorum* in the Washington State University (WSU) culture collection for resistance to mefenoxam and to evaluate the fitness of sensitive and resistant isolates. Lesion size on wounded rhododendron leaves was used to examine relative pathogenicity of *P. ramorum* isolates.

Isolates of *P. ramorum* were collected from 12 nurseries in Washington, one in Oregon, and one Christmas tree farm in California. Isolates from Washington nurseries included those collected from symptomatic plant material, soil and stream baits, and trace-forward sites where plant material purchased at the nursery was planted into a landscape. Isolates from the NA1, NA2, and EU1 lineages were tested, in addition to four isolates that were a co-mingling of NA2 and EU1. The co-mingled isolates were acquired from several rhododendron plants at an infested nursery and, although they contain *P. ramorum* of both lineages, do not appear to be hybridizing or sexually reproducing. All isolates of *P. ramorum* were grown on media amended with varying concentrations of mefenoxam.

Isolates were considered to be sensitive to the fungicide if there was scant or no growth at 1 ppm active ingredient and resistant if there was significant growth at 1 ppm. Only a few of the isolates tested showed resistance to mefenoxam, and these belonged to the EU1 lineage and originated from Nursery #41 and its trace forwards.

When the relationship between pathogenicity and fungicide sensitivity was examined, three groups were observed: isolates showing some resistance to the fungicide, isolates with low pathogenicity, and the remaining isolates not having characteristics of the other two groups (fig. 1). The fungicide-resistant group was composed entirely of isolates from the EU1 lineage and originated from Nursery #41. The isolates of low pathogenicity were mostly of the NA1 lineage, with two from the co-mingled EU1/NA2 samples and one EU1 isolate from Nursery #44. Most of the weak NA1 isolates originated from Nursery #35. A positive relationship between pathogenicity and fungicide sensitivity was seen when the first two groups were removed from the analysis ($R^2 = 0.3877$), suggesting that there may be some trade-offs between fungicide resistance and pathogenicity. However, the EU1 isolates showing resistance were some of the most aggressive. Preliminary spore counts show that the NA2 isolate taken from the salal plant outside of Nursery #45 and an EU1 isolate from Nursery #41 are the most prolific sporulators, and this may explain their persistence in soil and water and ability to

spread. Likewise, there has not been spread from Nursery #35, although it has been found positive multiple times. NA1 isolates from this nursery were less fit than NA1 isolates collected from other nurseries.

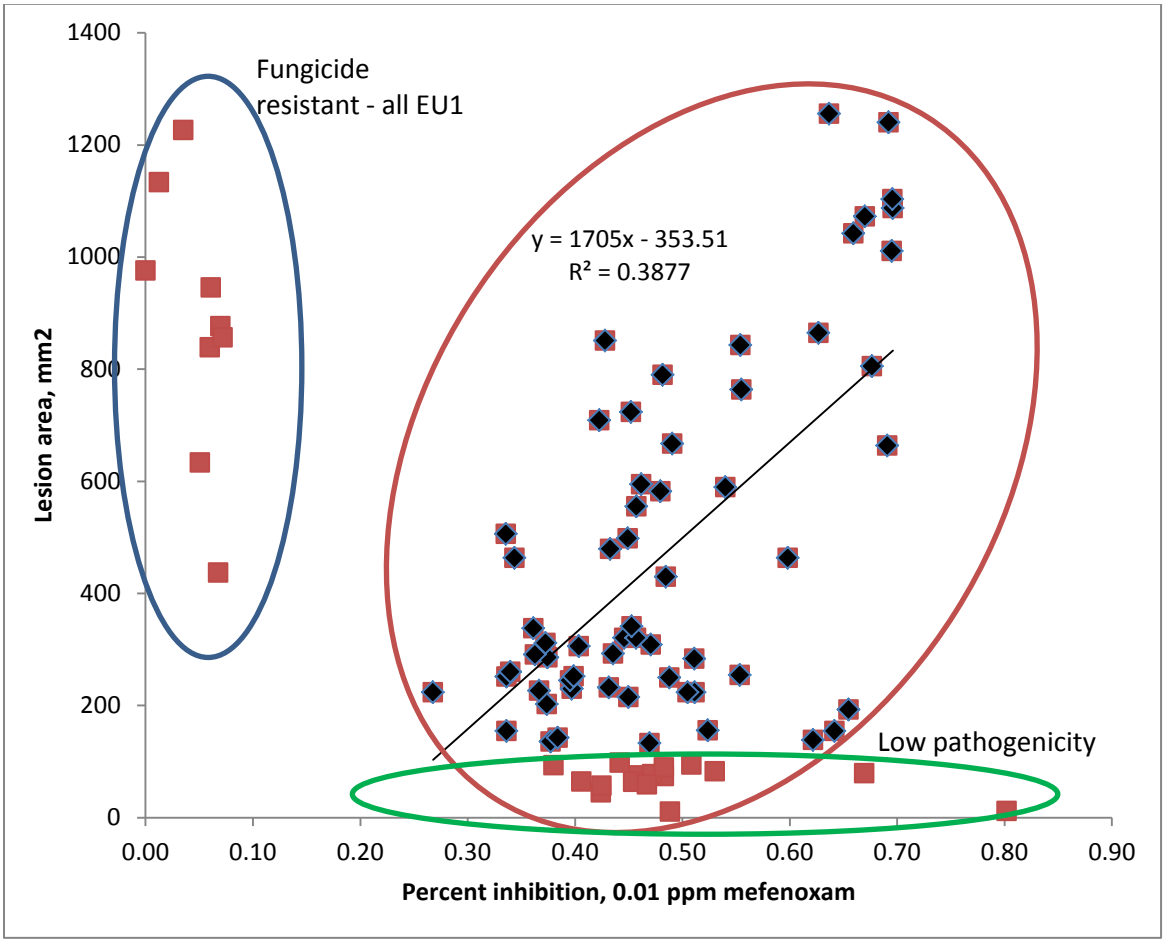

Figure 1—Scatter plot showing relationship between pathogenicity (lesion area on rhododendron leaves) and fungicide tolerance (percent inhibition on 0.01 percent mefenoxam) for 85 isolates of *Phytophthora ramorum* collected from Washington nurseries and other locations.

A Technique for Determining Inoculum Threshold for the Spread of *Phytophthora ramorum* in Irrigation Water

Nationally, there is concern about the potential risk of spreading *P. ramorum* via the irrigation of plants with infested water. Although research in California and Europe has shown that plants in nurseries can be infected when overhead irrigated with water infested with *P. ramorum*, it is unknown what levels of inoculum are present in streams in Washington and how much is needed for infection of plants or infestation of soil to occur. Being able to quantify inoculum levels in waterways and understand the inoculum threshold necessary for infection will assist the nursery industry and regulatory agencies in making decisions about the level of risk in using *P. ramorum* infested water for irrigation in Washington State.

To test whether plants overhead-irrigated with contaminated stream water would become infected, a “shower” apparatus was constructed (fig. 2). A bilge pump was attached to a floating platform and pumped water through a sprinkler onto potted rhododendron plants. Humidity was maintained by

enclosing the plants in the fiberglass "shower stall." A timer was included to allow for irrigation at predetermined intervals, and the apparatus was powered using a car battery.

The "shower" was first tested in the biocontainment unit at WSU-Puyallup. Potted rhododendron seedlings were placed in the chamber and overhead-irrigated with a zoospore suspension of *P. ramorum* at a concentration of 1 x 10^4 zoospores/ml. After 4 weeks, the plants were sampled for *P. ramorum* by culturing and by qPCR. A small amount of *P. ramorum* DNA was present on three samples, one sample was positive for *P. ramorum* in culture, and only one plant had symptoms of *P. ramorum* infection. More than 80 percent of soil and root baits were positive for *P. ramorum*. In this small study, soil and roots had very high levels of colonization by *P. ramorum* after overhead irrigation. Plants had very low levels of infection. The apparatus was then deployed at a site where leaf baiting has been positive for *P. ramorum* in 2009 and 2010. Rhododendron plants were placed into the shower and bait bags were deployed upstream from the pump for three, 1-week intervals in June 2011. After exposure, plants were taken to WSU-Puyallup and placed in the biocontainment unit for 4 to 6 weeks. All samples were negative for *P. ramorum* using culturing methods and qPCR. *Phytophthora ramorum* was found in the stream earlier in the year by the Washington State Department of Natural Resources, but possibly June was too late for detection. Several other Oomycetes were isolated from leaf baits and identified using DNA sequence analysis. Based on these preliminary results, it appears that inoculum levels of *P. ramorum* were very low or nonexistent in the stream that was sampled, or that it was too late in the season to detect it. Water temperature in the ditch was 15 ˚C at deployment of the shower, which is in the range for *P. ramorum* growth. However, other Oomycete species present may be more competitive than *P. ramorum* under these conditions. Plans are underway to use the shower apparatus in addition to baiting and PCR methods for quantifying *P. ramorum* inoculum in streams and to determine whether these inoculum levels are sufficient to cause disease on plants.

Figure 2—"Shower" apparatus for exposing plants to *Phytophthora ramorum* inoculum in stream water. Potted rhododendron plants were placed inside the shower and overhead irrigated with water, which was pumped from the stream at three 1-minute intervals programmed into the timer. Plants were exposed for 1 week, after which time they were taken to the biocontainment facility at Washington State University-Puyallup for incubation.

Conclusions

From this study, we have determined that there are considerable differences in fitness of *P. ramorum* isolates both within and between clonal lineages. Pathogenicity and fungicide screening of additional isolates collected from Washington nurseries is ongoing, as are further tests of fitness, including sporulation potential of selected isolates.

Determining the critical level of *P. ramorum* inoculum to cause infection from irrigation water is also underway. In the laboratory study, very little aboveground infection was seen at the high-inoculum level used, but root and soil baiting was almost 100 percent positive. The lack of *P. ramorum* found in the small field test could be due to a number of factors, such as absence or extremely low inoculum concentration, competition by other organisms, and water quality parameters.

Acknowledgments

The authors wish to thank Washington State Department of Agriculture (WSDA), Washington State Department of Natural Resources (WADNR), and the USDA Forest Service for their cooperation and financial support; and our students, volunteers, and technicians for their help in the lab and field.

Literature Cited

Chycoski, C.I.; Punja, Z.K. 1996. Characteristics of populations of *Phytophthora infestans* from potato in British Columbia and other regions of Canada during 1993-1995. Plant Disease. 80: 579–589.

Dart, N.L.; Chastagner, G.A. 2007. Estimated economic losses associated with the destruction of plants due to *Phytophthora ramorum* quarantine efforts in Washington State. Online. Plant Health Progress doi: 10.1094/PHP-2007-0508-02-RS.

European and Mediterranean Plant Protection Organization [EPPO]. 2006. *Phytophthora ramorum*. http://www.eppo.org/QUARANTINE/Alert_List/fungi/PHYTRA.htm. (19 August 2012).

Groves, C.T.; Ristaino, J.B. 2000. Commercial fungicide formulations induce in vitro oospore formation and phenotypic change in mating type in *Phytophthora infestans*. Phytopathology. 90: 1201–1208.

Hu, J.H.; Hong, C.X.; Stromberg, E.L.; Moorman, G.W. 2008. Mefenoxam sensitivity and fitness analysis of *Phytophthora nicotianae* isolates from nurseries in Virginia, USA. Plant Pathology. 57: 728–736.

Jeffers, S. 2011. Challenges of repeat nurseries. California Oak Mortality Task Force 2011 Meeting. June 1, 2001 online webinar. http://www.suddenoakdeath.org/news-and-events/comtf-2011- meeting/. (23 November 2011).

Mukalazi, J.; Adipala, E.; Sengooba, T.; Hakiza, J.J.; Olanya, M.; Kidanemariam, H.M. 2001. Metalaxyl resistance, mating type, and pathogenicity of *Phytophthora infestans* in Uganda. Crop Protection. 20: 379–388.

Wagner, S.; Kaminski, K.; Werres, S. 2007. Evaluation of fungicides for control of *Phytophthora ramorum.* In: Frankel, S.J.; Kliejunas, J.T.; Palmeri, K.M., tech. coords. Proceedings of the sudden oak death third science symposium. Gen. Tech. Rep. PSW-GTR-214. Albany, CA: U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station: 481.