RESEARCH

Following the April *P. ramorum*-positive stream baiting sample taken from the Sammamish River in King County, WA, Washington State University (WSU) and the Washington State Department of Agriculture (WSDA) collaborated with USDA Agriculture Research Service (ARS) to genotype 40 isolates sampled from 12 nurseries both within and outside the Sammamish watershed in an attempt to trace the origin of the Sammamish River isolate. The isolates were genotyped and compared with the Sammamish River isolate using four previously characterized microsatellite markers. These data were also used to estimate genotypic diversity within single nurseries to help infer the likelihood of multiple introduction events at retail nurseries in WA as well as to obtain a general overview of the distribution and abundance of the three *P. ramorum* lineages in WA nurseries.

Upon analysis, the Sammamish River isolate had a unique fingerprint that matched an isolate (of the 40 fingerprinted in WA nurseries) from a landscape supplier located outside of the Sammamish watershed. While not a conclusive study, the finding does suggest the Sammamish River isolate may have been introduced from nursery stock originating from this wholesaler.

Study results to date also indicate that only the North American lineages of the pathogen (NA1 & NA2) are currently found in Washington nurseries, suggesting that previous eradication efforts of the European lineage (EU1) in WA were successful. Fifteen percent of the isolates genotyped so far have been the NA2 lineage (found only in US nurseries), which is considered to be relatively uncommon. These isolates came from three of the 12 nurseries sampled; one nursery had both the NA1 (found in US nurseries and in CA and OR forests) and NA2 lineage.

Additionally, high heterozygosity coupled with the clonal population structure observed suggests that *P. ramorum* in WA nurseries has not undergone sexual recombination. The high level of genotypic diversity at three retail nurseries and lack of sexual recombination indicates multiple introduction events at these nurseries. The nurseries at which single genotypes were detected typically had smaller sample sizes which may explain why fewer genotypes were detected. Collaborative sampling efforts continue between WSU and WSDA in an effort to gain a clearer picture of long-term genotypic diversity at single nurseries and better infer the role reintroduction versus establishment plays in the repeat detections of *P. ramorum* at nursery sites.

Balci, Y.; Balci, S.; Eggers, J.; MacDonald, W.L.; Juzwik, J.; Long, R.P.; and Gottschalk, K.W. 2007. *Phytophthora* spp. associated with forest soils in eastern and north-central U.S. oak ecosystems. Plant Dis. 91:705-710.

Abstract: A survey of soils associated with oak species was conducted in 2003 and 2004 in Indiana, Illinois, Maryland, Michigan, Minnesota, Pennsylvania, Ohio, West Virginia,

and Wisconsin to investigate the occurrence of *Phytophthora* spp. Soils taken from around the base of healthy and declining oak trees were flooded with water and *Quercus robur* leaflets were used as bait for *Phytophthora* spp. From 829 soil samples collected near trees, 21% were positive for *Phytophthora* spp., with 55% of the 125 sites surveyed yielding a *Phytophthora* sp. *Phytophthora cinnamomi* was the most frequently isolated species, representing 69.4% of the *Phytophthora*-infested sites surveyed. Other species, in decreasing order of isolation frequency were *Phytophthora* sp. 2, *P. citricola*, *P. europaea*, *P. cambivora*, *P. quercina-like* isolates, and *Phytophthora* sp. 1. No significant association was found between the presence of *Phytophthora* organisms and site characteristics such as latitude, elevation, soil pH, or the crown condition of the trees. However, in *P. cinnamomi*-infested sites, a significant association was found with the deteriorating crown status of *Q. alba* and the presence of *P. cinnamomi*. The absence of *P. cinnamomi* above the 40ºN latitude range also was noteworthy.

Manter, D.K., Kelsey, R.G., and Karchesy, J.J. 2007. Photosynthetic declines in *Phytophthora ramorum*-infected plants develop prior to water stress and in response to exogenous application of elicitins. Phytopathology 97:850-856.

Phytophthora ramorum, causal agent of sudden oak death, is responsible for widespread oak mortality in California and Oregon, and has the potential to infect 100 or more species. Symptoms range from stem girdling and shoot blight to leaf spotting. In this study, we examined the physiological impacts of *P. ramorum* infection on *Rhododendron macrophyllum*. In stem-inoculated plants, photosynthetic capacity (*V*cmax) significantly declined by ≈21% 3 weeks after inoculation in visibly asymptomatic leaves. By 4 weeks, after the development of significant stem lesions and loss in water transport capacity, water stress led to stomatal closure and additional declines in photosynthetic capacity. We also report the isolation, characterization, and biological activity of two *P. ramorum* elicitins. Both elicitins were capable of inducing a hypersensitive-like response in one incompatible (*Nicotiana tabacum* SR1) and three compatible hosts (*R. macrophyllum, Lithocarpus densiflorus*, and *Umbellularia californica*). Infiltration of leaves from all three compatible hosts with both *P. ramorum* elicitins caused significant declines in chlorophyll fluorescence (*F*v/*F*m). For all four species, the loss of photosynthetic capacity was directly proportional to H+ uptake and ethylene production, two common components of the hypersensitive response. This is the first report of elicitins causing photosynthetic declines in compatible hosts independent of plant water stress.

Moralejo, E.; Muñoz, J.A. García; and Descals, E. 2006. Insights into *Phytophthora ramorum* sporulation: epidemiological and evolutionary implications. *OEPP/EPPO Bulletin* 36, 383–388.

Two aspects on the epidemiology of *Phytophthora ramorum* are discussed. Firstly, in order to elucidate its spatial scale of dispersal, its modes of asexual sporulation in culture and *in planta* are examined and compared to those of the also aerially dispersed *P. infestans*. On agar media, *P. ramorum* sporangia are slightly sticky, tend to aggregate in clusters, and are not shed into the air even when violently shaken or subjected to strong

air humidity changes. After inoculating the underside of leaves from an assortment of Mediterranean woody species, no evidence of any adaptations for wind dispersal was observed. On some hosts, it formed sporangiomata (clusters of sporangia) on the upper side. Our observations agree with previous field research in that *P. ramorum* is rain-splash rather than wind dispersed, as opposed to *P. infestans*. Secondly, in Petri dish cultures both species sporulate heavily. However, a steep reduction in sporangial density (sporangia cm^{-2}) was found on leaves of susceptible Mediterranean species for *P. ramorum* and a progressive one for *P. infestans* when this was inoculated on leaves of susceptible potato and other members of the Solanaceae. Furthermore, there was a negative correlation between sporangial density and virulence. We propose a similar pattern of a trade-off between spore production and virulence to that observed by Thrall & Burdon (2003) for the *Linum-Melampsora* plant-pathogen system. Epidemiological and evolutionary implications are discussed.

Prospero, S.; Hansen, E.M.; Grünwald, N.J.; and Winton, L.M. 2007. Population dynamics of the sudden oak death pathogen *Phytophthora ramorum* in Oregon from 2001 to 2004. Molecular Ecology. DOI: 10.1111/j.1365-294X.2007.03343.x.

Abstract: *Phytophthora ramorum* (Oomycetes) is an emerging plant pathogen in forests in southwestern Oregon (Curry County). Moreover, since 2003 it has been repeatedly isolated from plants in Oregon nurseries. In this study, we analyzed the genetic diversity of the *P. ramorum* population in Oregon from 2001 to 2004 by using microsatellites. A total of 323 isolates (272 from the infested forest; 51 from nurseries) were screened at 10 loci. The overall *P. ramorum* population in Oregon is characterized by low genetic diversity and has all the hallmarks of an introduced organism. All isolates within the A2 mating type belonged to the same clonal lineage and no recombinant genotypes were found. The forest population (24 genotypes) was dominated by a single multilocus genotype which persisted over years, indicating that eradication efforts in the forest have not completely eliminated inoculum sources. In contrast, genotypic evidence suggests that eradication was effective in nurseries. In 2003 and 2004, a total of 11 genotypes were found in the nurseries (one belonged to the European lineage of *P. ramorum*) but no genotype was recovered in both sampling years. Significant differentiation and low gene flow were detected between nursery and forest populations. Only two nursery genotypes were also found in the forest, and then at low frequency. Thus, the nursery infestation is not caused by the genotypes observed in Curry County, but likely resulted through introduction of novel genotypes from nurseries out-of-state. This highlights the continued importance of sanitation and quarantine in nurseries to prevent further introduction and spread of *P. ramorum.*

MONITORING

As a result of the Mississippi water baiting confirmation made earlier this year from a ditch draining a *P. ramorum*-positive nursery, follow-up surveys are underway. To date, all soil, vegetation, and stream baiting samples have been *P. ramorum*-negative. Survey activities will continue with additional sampling in the fall when weather

conditions are more conducive to pathogen activity. For more information on the survey, contact Steve Oak at soak@fs.fed.us.

NURSERIES

California had two *P. ramorum*-positive nurseries identified in June. The first was a San Diego County production nursery found PCR positive for *Pieris japonica* 'Amamiana' on 6/12/07. This facility is under compliance for *P. ramorum* and does ship interstate. Confirmed Nursery Protocol (CNP) activities began at the nursery on 6/13/07. Trace-forward investigations are underway, and include nurseries in seven western states and 10 CA counties. The second confirmation was made at an Alameda County production nursery found positive via PCR on 6/13/07. The positive *Magnolia grandiflora* was detected during their annual compliance agreement inspection. The nursery is under compliance for *P. ramorum* and does not ship interstate. CNP activities began on 6/14/07.

Nursery industry representatives met with National Plant Board (NPB), USDA Agricultural Research Service (ARS), and USDA Animal and Plant Health Inspection Service (APHIS) representatives in May to discuss draft industry-developed Best Management Practices (BMP). Suggested changes to the document have been sent back to the Industry BMP working group for review and action. A Steering Committee and a Funding Sub-Committee were also formed at the meeting. Committee members include representatives from CA, OR, WA, and GA Nursery Industries; CA, OR, and WA State Departments of Agriculture; USDA APHIS, ARS, and Cooperative State Research, Education, and Extension Service (CSREES); the American Nursery and Landscape Association/Horticultural Research Institute; and the NPB. The Steering Committee was created to help develop a voluntary Pilot BMP program for a sampling of nursery operations within the participating states, and the Funding Sub-Committee was created to identify funding sources to support the Pilot program. States participating in the program have been charged with submitting their budget proposal to the Steering Committee. The next scheduled meeting is set for July 10th. For more information on the meeting, contact Marc Teffeau at mteffeau@anla.org.

OTHER RESEARCH OF INTEREST

Hardham, A.R. 2007. Microreview: Cell biology of plant–oomycete interactions. Cellular Microbiology 9(1), 31–39. DOI: 10.1111/j.1462-5822.2006.00833.x.

Oh, E. and Hansen, E.M. 2007. Histopathology of infection and colonization of susceptible and resistant Port-Orford-cedar by *Phytophthora lateralis.* Phytopathology 97:684-693.

REGULATIONS

The USDA Animal and Plant Health Inspection Service (APHIS) Nursery Survey Protocol has been updated. The new protocol can be found at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/downloads/surveyplan/surveymanual.pdf.

USDA APHIS has revised the "Update on PPQ Diagnostics for SOD:
Diagnostics procedures approved by APHIS and diagnostic analyses performed by APHIS PPQ for *Phytophthora ramorum.*" Revisions to the "Culture/Morphology Protocol," "Nested PCR Protocol," and "Real Time PCR Protocol" have also been made. The updated information can be found at http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/protocols.shtml.

RESOURCES

Washington State University is excited to announce the posting of a "Virtual Oomycete Demonstration Nursery" where clients can tour a generic nursery and get ideas on how to improve management strategies for *Phytophthora* and *Pythium* at their particular nursery site. To visit the virtual nursery, go to http://www.puyallup.wsu.edu/ppo/nursery.html.

CALENDAR OF EVENTS

10/15 – 10/18 - XVI International Plant Protection Congress 2007, Glasgow, UK;
Full details on the recently announced call for papers can be found at: http://www.bcpc.org/IPPC2007/Call%5Ffor%5FPapers/. For more information, contact Dr. Slawson, PHSI DEFRA, at: david.slawson@defra.gsi.gov.uk.