SCION

New Zealand Journal of Forestry Science

41S (2011) S133-S141

www.scionresearch.com/nzjfs

published on-line:
11/11/2011

Tracking populations of *Phytophthora ramorum* within trees and across the South-western Oregon tanoak (*Notholithocarpus densiflorus*) forest with DNA fingerprinting and the relative fitness of dominant and rare individuals.†

Jennifer Britt and Everett Hansen*

Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA.

(Received for publication 27 October 2010; accepted in revised form 26 October 2011)

*corresponding author: hansene@science.oregonstate.edu

Abstract

Since the discovery of *Phytophthora ramorum* Werres, De Cock & Man In't Veld in south-western Oregon forests in 2001, newly infected areas are detected each year. Yet, there are still gaps in our knowledge about how the pathogen spreads or where new infections come from. Our study aims to track the spread of *P. ramorum* in Oregon forests and within individual trees using DNA fingerprinting. We examined the genetic diversity of 1589 samples collected from 2001 to 2008 on several temporal and spatial scales. We identified 60 novel multilocus genotypes (MGs) with 9 to 44 MGs found in each year. While the majority of MGs were present in very low numbers (< 1%) one MG was dominant in all years representing 39 to 73% of isolates. The dominance of one MG was not attributable to higher fitness by any measure examined. Frequency of the dominant MG declined with time. This supports the hypothesis that it represents the founder genotype, and is being progressively diluted by new genotypes that arise through mutation. Our data also demonstrate that *P. ramorum* populations in Oregon forest are genetically distinct from those in nurseries and in California forests.

Keywords: epidemiology; microsatellites; multilocus genotypes.

† *Based on a paper presented at the fifth meeting of the IUFRO working party S07-02-09, Phytophthora Diseases in Forests and Natural Ecosystems, 7 – 12 March 2010, Auckland and Rotorua, New Zealand.*

Introduction

Phytophthora ramorum Werres, De Cock & Man In't Veld, is a recently established invasive pathogen found in the mixed hardwood forests of central coastal California (Rizzo et al., 2002; 2005) and south-west Oregon (Goheen et al., 2002). It is also found in horticultural nurseries and amenity plantings in Europe (Werres et al., 2001; Brasier et al., 2004) and in horticultural nurseries on the west coast of North America (Goss et al., 2009, Hansen et al., 2003), where it is subject to State and Federal quarantine and eradication programmes.

The cause of sudden oak death disease, *Phytophthora ramorum* is a pathogen that affects the aerial parts of primarily woody plants. It produces deciduous sporangia on infected leaf and twig tissue (Werres et al., 2001; Moralejo et al., 2006) and large girdling bark lesions on the trunks (boles) of tanoak (*Notholithocarpus densiflorus* ((Hook. & Arn.) Manos, Cannon & S.H.Oh)) trees. It is present in rainwater beneath diseased trees (Davidson et al., 2008; Hansen et al., 2008), and is also found in streamwater and soil in infected areas (Davidson et al., 2005; Fichtner et al., 2007; Hansen et al., 2008; Rizzo et al., 2005; Sutton et al., 2009). The processes by which *P. ramorum* infects and

ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)

spreads through individual trees and across forests are not completely understood. Affected trees often have multiple phloem lesions on twigs in the crown, along branches and on the main stem (Hansen et al., 2008). Sometimes the lesions appear to be connected through the cambium or wood but more often this is not the case (Parke et al., 2007).

We are studying the spread of *Phytophthora ramorum* in Oregon tanoak forests. Sudden oak death disease was first detected in Oregon in 2001 when *P. ramorum* was isolated from tanoak, wild rhododendron (*Rhododendron macrophylum* D.Don), and huckleberry (*Vaccinium ovatum* Pursh). The disease was then confined to a small area in Curry County in the southwest corner of the State. A 24 km^2 quarantine area was defined within which an eradication programme based on herbicide treatment, felling and burning of diseased tanoak trees and associated host plants was initiated (Goheen et al., 2002). The infected area included nine separate sites near Brookings (Hansen et al., 2008). New infections have been found each year since 2001, 79% of the more recently affected trees being located within 300 m of a tree previously known to exhibit disease symptoms. Dispersal up to 4 km has been recorded occasionally (Hansen et al., 2008). The pattern of spread is consistent with that associated with turbulent air dispersal of sporangia. Initial infection at sites beyond the range of splash dispersal is apparent in the upper crowns of tanoak trees (Hansen et al., 2008).

Worldwide, three distinct, asexually reproducing genetic (clonal) lineages of *Phytophthora ramorum* have been identified with the aid of microsatellite and amplified fragment length polymorphism markers (Ivors et al., 2004, 2006; Prospero et al., 2004, 2007). Microsatellites are polymorphic loci present in nuclear and organellar deoxyribonucleic acid (DNA). They consist of simple repeating units, 1 – 6 base pairs in length. The repeated sequence often comprises two, three or four nucleotides that may recur 10 to 100 times. Microsatellites mutate at a higher rate than other DNA regions most frequently through slipped strand mis-pairing during replication. They often present high levels of inter- and intra-specific polymorphism which offer useful information in population studies. The aim of this project was to use microsatellite markers in tracking the spread of *P. ramorum* in Oregon forests.

All isolates examined from Europe to date belong to Lineage EU1 and nearly all are A1 mating type. All forest isolates in North America belong to Lineage NA1 and A2 mating type. Representatives of Lineages EU1, NA1, and NA2 (which is also A2 mating type) are found in North American nurseries. No evidence of sexual recombination has been detected in either forest or nursery populations (Prospero et al., 2007).

A total of 272 *Phytophthora ramorum* isolates from Curry County, Oregon, were collected and genotyped between 2001 and 2004. All of these were found to be closely related (Ivors et al., 2006; Prospero et al., 2007). No differentiation in population structure in terms of year of collection or source (trees, soil, or streams) was detected. Isolates from the forest sites represented distinct populations to those from nurseries and, there was no evidence that genotypes from nurseries had entered the forest sites (Prospero et al., 2007). Genotype analysis of isolates collected between 2001 and 2004 supported estimates of the time of origin of the Oregon infestation (a few years before initial detection in 2001); the clustered distribution of infected trees (identified by culture of infected tissues); and dispersal distances.

In 2001, 13 multilocus genotypes (MGs) were detected. New genotypes were identified each year from 2001 to 2004. During this period, a total of 24 MGs were identified. All isolates belonged to the NA1 clonal lineage and 60 – 73% belonged to the MG PrOR1 (Prospero et al., 2007).

The objectives of the study presented here included:

- updating earlier studies on the population structure of *P. ramorum* in Oregon tanoak forests by examining isolates collected through to 2008;
- using microsatellite analysis to track and explain spread of individual genotypes in the Oregon forest;
- analysing the population structure and patterns of spread within individual trees; and
- ascertaining whether the dominance of the PrOR1 MG is maintained through epidemiological "fitness"or if its numerical dominance was a consequence of a "Founder Effect."

Materials and Methods

A combination of annual aerial and ground disease detection surveys, baiting in streams, isolation from tree tissues and microsatellite genotype analysis was used to follow the occurrence and dispersal of *Phytophthora ramorum* in Curry County, Oregon across the landscape from 2001 through to 2008. We collected 1589 isolates from 41 sites. *P. ramorum* forest isolates were identified on the basis of both morphological and molecular characters (Winton & Hansen, 2001). The location of each infection was spatially referenced using global positioning system (GPS) coordinates, and genotyped using the methods described in Hansen et al. (2008).

Isolates were genotyped at four microsatellite loci: PrMS39, PrMS43a, PrMS43b, and PrMS45. Details of the development and characterisation of microsatellite

ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)

loci are given by Prospero et al. (2004). Alleles were sized on an ABI Prism 3100 sequencer and results were analysed using GeneScan and Genotyper software (Applied Biosystems). Isolates giving ambiguous results were re-analysed

To investigate genetic diversity of *Phytophthora ramorum* within individual trees, we took samples from multiple locations within the crown and the trunk of each of 54 tanoak trees at ten sites.

We examined the relative fitness of the most common and the least common *Phytophthora ramorum* MGs collected from 2001 through 2008 in two experiments. In both experiments, the isolates represented the full geographic range of the pathogen in Curry County.

Experiment 1

We set up a genotype x environment interaction (G X E) experiment using five isolates from each of eight genotypes, including the four most abundant MGs and the four rarest MGs that included at least five isolates (Table 1). In the first experiment, all isolates were grown in the dark at four temperatures (10, 15, 20, and 25 °C) on either nutrient-rich carrot agar (Werres et al., 2001) or nutrient-poor cornmeal agar with ß-sitosterol (Hansen et al., 2008). We examined two replicates of each isolate. We measured colony growth rate and chlamydospore production, and observed colony morphology. Radial growth (mm) was measured on days 3 and 7, and chlamydospores were collected for counting on day 7.

TABLE 1: Multilocus genotypes (MGs) used in the "fitness" experiments. Five isolates of each MG were included in each test. Isolates were obtained from trees, soil, or streams in south-western Oregon tanoak forests affected by sudden oak death disease.

MG	Years MG detected	Frequency of detection 2001 – 2008, (%)
PrOR1	2001 – 2008	59.0
PrOR61	2001 – 2008	0.3
PrOR2	2002 – 2004, 2005 – 2008	7.9
PrOR5	2001, 2003, 2006 – 2008	3.5
PrOR7	2001, 2004, 2008	6.5
PrOR12	2001 – 2008	2.9
PrOR14	2003 – 2008	2.3
PrOR19	2008	1.2

Experiment 2

Eight hundred stump sprout tips, each 30 cm long, were collected from 34 different tanoak trees located outside the quarantine area in Curry County Oregon. We compared zoospore production and aggressiveness in the same 40 isolates used in the first experiment. Zoospores were collected by flooding 7-day-old cultures to dislodge sporangia, chilling the sporangial suspension and returning to room temperature to trigger zoospore release. Spores were counted by haemocytometer. Forty mL of zoospore suspension were collected for each isolate. A 2 mL aliquot, containing at least 10^3 zoospores, was transferred to a 15 mL-test tube, 20 replications being prepared for each isolate. The cut end of a sprout tip was immersed in each tube and the liquid level marked. Sterile water was added every other day to maintain the level. Lesion length was measured after 14 days (Figure 1).

Data from both experiments were analysed by one- or two-way ANOVA, usingJMP®, Version 7. SAS Institute Inc., Cary, NC, 1989-2007.

FIGURE 1: *Phytophthora ramorum* lesion on a tanoak sprout tip after 14 days' immersion of the cut end in a zoospore suspension.

Results

Population Structure

Among the 1589 forest isolates, 9 – 44 MGs were found in each year. Over the entire eight-year collection period (2001 – 2008), 60 novel MGs were identified. Four MGs were found in all years, and 28 in only one year. The majority of MGs were present in very low numbers (< 1% of total). One MG (PrOR1) was dominant in every year and represented 39 – 73% of all isolates investigated (Figure 2). The proportion of isolates containing PrOR1 each year declined from 67% in 2005 to 39% in 2008 (Figure 3).

In all years, MGs from Oregon tanoak forests, irrespective of whether they were isolates from tanoak trees, streams or soil were closely related. All MGs identified represented either a single mutational change from either PrOR1 or a MG recovered from Oregon tanoak forest in a previous year. We compared our results to data reported previously from similar work in

ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)

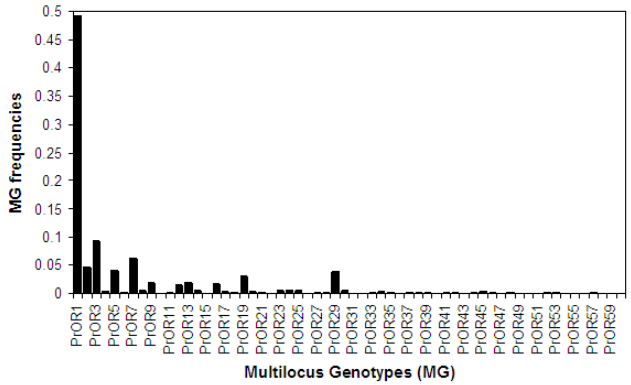


FIGURE 2: Total frequency of occurrence of 60 multilocus genotypes identified between 2001 and 2008.

nurseries in Oregon, Washington and California, and forests in California (Goss et al., 2009; Mascheretti et al., 2008; Prospero et al., 2007). Based on use of Nei's (1972) genetic distance of multilocus genotype variation, the phylogenetic relationships apparent in our data indicated that *Phytophthora ramorum* populations in Oregon forests were genetically distinct from any of these other populations (Figure 4). There was no genetic evidence of new introductions to southwest Oregon since 2001.

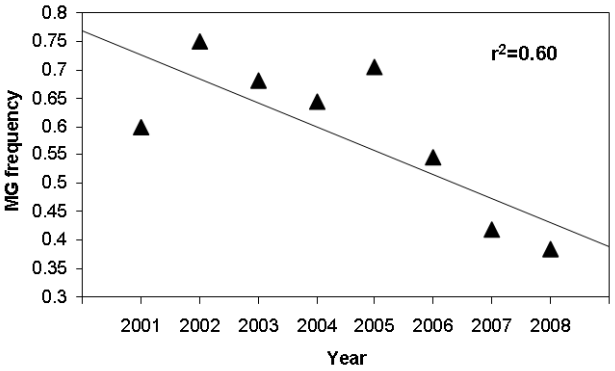


FIGURE 3: Frequency of occurrence of PrOR1, the most numerous multilocus genotype (MG) in the southwest Oregon tanoak forest quarantine area.

Results for isolates grouped by site (clusters of neighbouring isolates collected over the 8-year period) reflected the localised distribution of disease symptoms. In 2001, the presence of *P. ramorum* had been confirmed at nine sites containing clusters of a few infected tanoak trees. Isolates from that year contained 13 MGs. Seven isolates were PrOR1. This dominant genotype was present at 8 of the 9 sites at that time. The disease spread in succeeding years, with 32 clusters of one or more diseased trees being recognised in 2008. The MG PrOR1 was present at 73% of the sites sampled during the 8-year period.

The widespread distribution of the most common genotypes, the abundance of rare genotypes represented by only one or two isolates, and the close relationship between MGs precluded the tracing of origins of inoculum for new infections. Most of the newly-affected sites contained more than one MG from the year of first detection, and the number of MGs increased each year after first detection. The distribution of some MGs suggested discrete dispersal events. For example, in two trees at one site, PrOR19 was first detected in 2003 but was not detected again until 2007 when it was detected in three trees at a site approximately 5.5 km to the west. In 2008, it was present again at the 2007 site, and also at a new site approximately 8 km east of the original location.

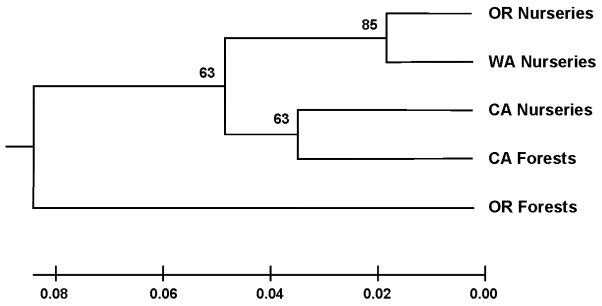


FIGURE 4: Population structure of *Phytophthora ramorum* from nurseries in Oregon (OR), Washington (WA) and California (CA), and forests in Oregon and California. Unweighted Pair Group Method with Arithmetic Mean-diagram of phylogenetic relationships based on use of Nei's (1972) genetic distance of multilocus genotype variation. California forest data taken from Mascheretti et al. (2008); nursery data from Goss et al. (2009).

Analysis of molecular variability through determination of the parameter PhiPT (proportion of variance among populations relative to the total variance) showed no significant difference in genetic population structure among *P. ramorum* isolates collected in different years. Only 2 % of the variance was associated with year of collection (Table 2). Moderate genetic variation (24% of total: Table 3) was apparent between sites represented by five or more isolates. Most of the site samples had similar mixtures of the most common MGs as well as a few unique or rare genotypes.

TABLE 2: Analysis of molecular variance of multilocus genotypes by year among 1589 isolates of *Phytophthora ramorum* collected in the eight years between 2001 and 2008 in Curry County, Oregon.

Source of variation	df	SS	Variance components	Variation (%)	PhiPT[1]
Among years	7	76	0.050	2	
Within years	1581	3314	2.096	98	
Total	1588	3390	2.146		0.023 (p<0.01)

[1] PhiPT = proportion of variance among populations relative to total variance.

ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)

TABLE 3: Analysis of molecular variance of multilocus genotypes by site among 1578 isolates of *Phytophthora ramorum* collected between 2001 and 2008 in Curry County Oregon. Only sites with 5 or more available isolates were included in the analysis.

Source of variation	df	SS	Variance components	Variation (%)	PhiPT[1]
Among years	40	844	0.525	24	
Within years	1537	2505	1.630	76	
Total	1577	3350	2.155		0.244 (p<0.01)

[1] PhiPT = proportion of variance among populations relative to total variance.

Samples from some of the sites contained distinctive combinations of MGs (Figure 5). For instance, samples from two sites located within 1 km of each other (Bean Creek and Bean Creek North) were comprised of distinctive combinations of MGs that suggested separate infection episodes from different sources. Interestingly, the Bean Creek North isolates were genetically indistinguishable from those derived from two other sites (1000 BLM and Bernhard) located several km away, and very similar to those from most of the other sites (Figure 5). Samples from the Bean Creek site, by contrast, showed a more distinctive genetic makeup than any of the other 41 sites investigated. Twelve MGs were identified, including three specific to this site. This is noteworthy because more than 100 trees in a single stand at Bean Creek appeared to have died in a single year. The Borax-site samples also had a distinctive genetic makeup. This was the most westerly location and represented an apparent dispersal distance of about 4 km when first detected. Ten MGs were present, but only one was site-specific.

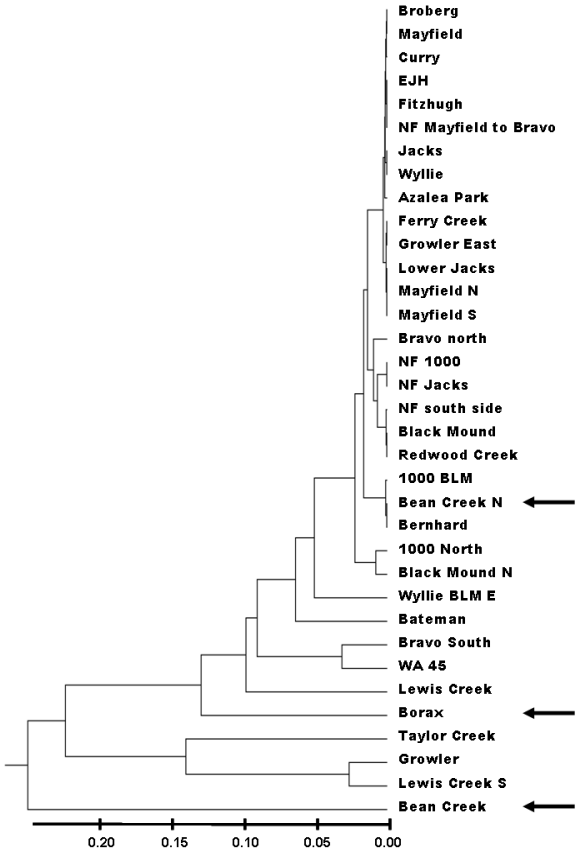


FIGURE 5: Phylogenetic relationships (Unweighted Pair Group Method with Arithmetic Mean) of *Phytophthora ramorum* isolates collected between 2001 and 2008 from 35 sites in tanoak forests in Curry County Oregon. Diagram based on microsatellite similarities, according to Nei's (1972) genetic distance of multilocus genotype variation. Arrows indicate sites discussed in text.

Within-tree sampling

Of the 54 tanoak trees from which more than one isolate was obtained, 29 showed evidence of more than one MG. In one tree (No. 4622; Figure 6; Table 4) eight MGs were detected among 43 isolates. The number tended to increase with the number of samples per tree. Ten or more isolates were obtained from

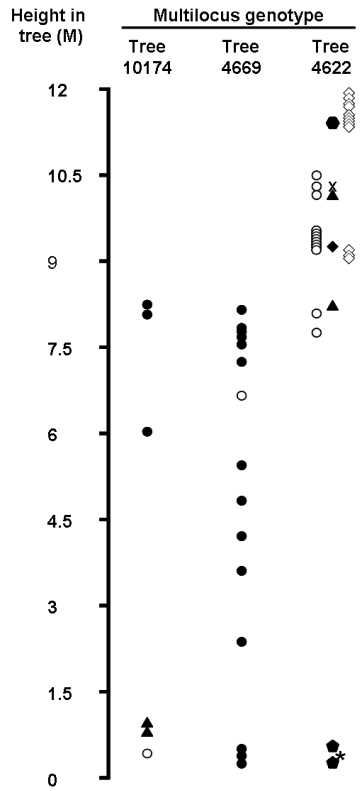


FIGURE 6: Stem sampling position and multilocus genotype (MG) designation of *Phytophthora ramorum* isolates collected from three tanoak trees in Curry County, Oregon. ○=PrOR1, ▲=PrOR3, **X**=PrOR5, ◇=PrOR7, ⬟=PrOR9, ⬣=PrOR16, ✶=PrOR27, ●=PrOR45.

ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)

TABLE 4: Multilocus genotypes (MGs) identified among *P. ramorum* isolates from 11 Curry County, Oregon tanoak trees from which 10 or more isolations were made.

Site	Year	Tree No.	Number of isolates	Number of MGs	MG	Location within tree
1000 BLM	2007	4669	15	2	PrOR1 PrOR45	Twigs, branches, stem
1000 BLM	2007	10176	46	4	PrOR1 PrOR20 PrOR45 PrOR49	Twigs, branches, stem
Bean Creek	2007	4738	24	2	PrOR29, PrOR34	Twigs, branches, stem
Bean Creek	2007	4740	10	1	PrOR29	Twigs, branches, stem
Curry	2006	4612	24	2	PrOR2 PrOR 8	Stem only
Curry	2006	4615	16	3	PrOR1 PrOR3 PrOR 26	Stem only
Mayfield	2006	4622	43	8	PrOR1 PrOR2 PrOR3 PrOR5 PrOR7 PrOR9 PrOR16 PrOR27	Twigs, branches, stem
Wyllie	2006	4606	16	3	PrOR1 PrOR4 PrOR 14	Twigs, branches, stem
Wyllie	2006	4656	10	1	PrOR2	Twigs, branches, stem
Wyllie	2006	4663	12	1	PrOR1	Twigs, branches, stem
Wyllie	2007	4670	11	5	PrOR1 PrOR3 PrOR7 PrOR13 PrOR 20	Twigs, branches, stem

11 trees (Table 4); these contained an average of 2.7 MGs. All of the MGs present at the site were identified in samples from this tree. Only one MG was recovered from the other 25 trees, including those from which 10 or 12 isolates were collected (Table 5).

Several of the isolates from some trees were derived from samples taken at closely spaced intervals up the stem (Figure 7). Of the 23 stem isolates made from Tree No. 4612 for example (Figure 8), 22 contained PrOR3, and one PrOR9, an MG considered to represent a single mutational step from PrOR3. In this tree, infection was apparently recent and there were no visible connections between lesions in the cambium or xylem. Although the upper crown of this tree was not sampled, the observed pattern is consistent with initial infection high in the tree by PrOR3, followed by downward stem flow of sporangia or zoospores in a rain event, leading to secondary infection of the stem. Tree No. 4669 (Figure 6) illustrates this scenario, a single MG being identified in separate lesions located on the stem from ground line to a height of 8 m. In other trees, however, MGs identified in the lower stem did not match the genotypes recovered from the upper canopy (e.g. Tree No. 4622 and Tree No. 10174; Figure 6).

Fitness

There was little evidence of difference between MGs in terms of any of the selected measures of fitness (Figure 9; Figure 10). No significant temperature x genotype interaction effect was apparent in terms of growth rate or chlamydospore production, regardless

ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)

TABLE 5: Tanoak trees in Curry County, Oregon from which multiple isolations of *P. ramorum* showed only a single multilocus genotype (MG).

Site	Year	Tree No.	No. of isolates	MG
Black Mound	2001	4119	3	PrOR1
EJH	2003	2611	4	PrOR1
EJH	2003	2617	3	PrOR1
EJH	2005	9158	3	PrOR1
EJH	2005	9160	4	PrOR1
Ferry Creek	2008	10353	5	PrOR1
Jacks	2003	2647	3	PrOR1
Jacks	2003	2336	5	PrOR1
Jacks	2003	2622	3	PrOR1
Jacks	2003	2641	2	PrOR1
Jacks	2003	2644	4	PrOR1
Mayfield	2006	4621	3	PrOR1
Mayfield	2006	4647	2	PrOR1
Wyllie	2006	4610	5	PrOR1
Wyllie	2006	4661	4	PrOR1
Wyllie	2006	4663	12	PrOR1
Wyllie	2006	4656	10	PrOR2
Black Mound	2006	2735	2	PrOR3
Black Mound	2007	4672	6	PrOR3
Wyllie	2006	4601	2	PrOR3
Wyllie	2007	4671	4	PrOR3
Mayfield	2006	4642	4	PrOR7
EJH	2005	4581	3	PrOR16
Bean Creek	2007	4739	9	PrOR29
Bean Creek	2007	4740	10	PrOR29

of the nutrient status of the culture medium (data not shown) The effects of temperature and media type on isolates with a common MG were highly variable. There was some evidence that isolates containing PrOR61 differed from others in being less “fit” by most measures. This MG was the least abundant, even though it was present in each of the sampling years. The MG PrOR1, dominant between 2001 and 2008, did not appear to confer more “fitness” than any of the others.

Discussion

Microsatellite markers are powerful tools for studies of the population structure of plant pathogenic fungi

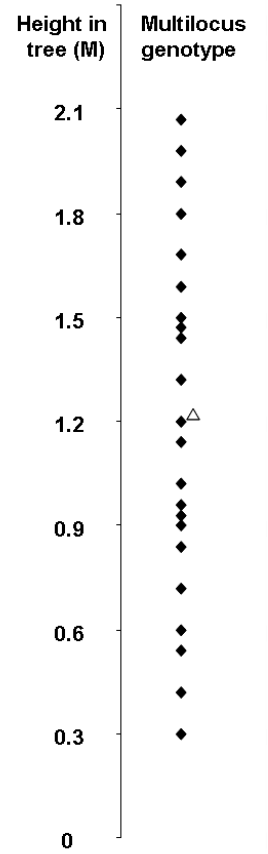
FIGURE 7: Stem of tanoak tree No. 4612 with outer bark removed to reveal lesions from which *Phytophthora ramorum* was isolated.

including *Phytophthora ramorum*. The present analysis confirmed and extended the work of Prospero et al. (2007) and provided useful insight into the local epidemiology of *Phytophthora ramorum* in Oregon tanoak forests. Importantly, our results provided no evidence of new *P. ramorum* introductions into the

FIGURE 8:

Location of tanoak stem tissue samples and multilocus genotypes found in *Phytophthora ramorum* isolates obtained from the lower 2.1 m of tanoak tree No. 4612.

◆=PrOR3, △=PrOR9.



ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)

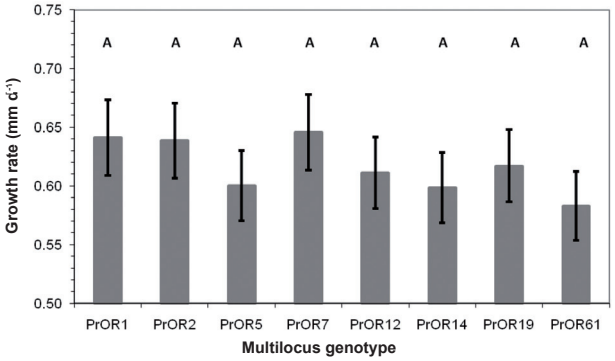


FIGURE 9: Radial growth rate (mm d^{-1}) of *Phytophthora ramorum* isolates on cornmeal agar with ß-sitosterol. Each value is the mean for five isolates having a common multilocus genotype. The multilocus genotypes were selected to represent the most and least abundant genotypes identified in Curry County tanoak forests. There was no significant difference in growth among the different genotypes as indicated by Student's t multi-comparison test. Multilocus genotypes connected by the same letter are not significantly different at the 0.05 probability level. Vertical bars indicate standard error.

southwest Oregon epidemic area up to 2008 following the original incursion in 2001.

The continued but decreasing dominance of a single multilocus genotype, PrOR1, is consistent with the suggestion that the current population is derived from a single introduction event. All MGs detected in the Oregon forest appeared to differ from PrOR1 only by single-step mutations. In contrast, a similar study in California provided evidence of several separate introductions of *P. ramorum* to forests from nursery material (Mascheretti et al., 2008).

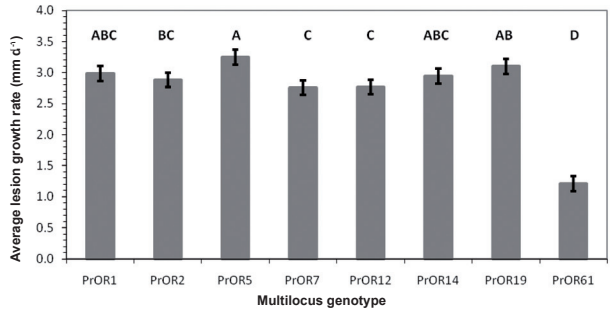


FIGURE 10: Lesion extension rates (mm d^{-1}) on tanoak sprouts inoculated with *Phytophthora ramorum*. Each value is the mean for five isolates having a common multilocus genotype. The multilocus genotypes were selected to represent the most and least abundant genotypes identified in Curry County tanoak forests. Multilocus genotypes not connected by the same letter are significantly different at the 0.05 probability level. Vertical bars indicate standard error.

The proportion of Oregon forest *P. ramorum* isolates containing PrOR1 declined between 2001 and 2008 while the total number of novel MGs increased. This indicates that the genetic diversity of the Oregon forest population is increasing. Low genetic diversity is considered to be a characteristic of an introduced pathogen. If observed trends continue, it is likely that the distinctive genetic nature of the initial source of the Oregon population will be masked.

The numerical dominance of PrOR1 is apparently not due to any superior fitness conferred by this MG. To our knowledge, the microsatellite markers observed in this study are genetically "neutral," i.e. they are not coding for genes and their effects are unlikely to be correlated with "fitness" traits. Our tests have shown that PrOR1 is only an average contributor to the selected fitness indicators.

The microsatellite markers employed in this study proved to have limited usefulness for tracking the spread of this clonal population of *Phytophthora ramorum* in the forest. Systematic analysis of the spread of the disease was precluded by the abundance of PrOR1, the apparent rapid generation of new, closely-related genotypes, and the overall low sampling efficiency. Most of the new infection sites contained multiple MGs dominated by PrOR1. Only in isolated cases could a distinctive population be traced back to a particular donor population. Collectively, our results are supportive of the idea that "clouds" of sporangia released simultaneously from several trees at a site are transported aerially in discrete events. Dispersal direction and distance are controlled by the microclimate prevailing at the time of release.

Acknowledgements

Financial support was provided by various programs of the USDA Forest Service, including the Pacific Southwest Research Station, and the Forest Health Protection groups in Oregon and California. Essential logistical support came from the field crews of the Oregon Department of Forestry.

References

Brasier, C., Denman, S., Brown, A., & Webber, J. (2004). Sudden Oak Death (*Phytophthora ramorum*) discovered on trees in Europe. *Mycological Research, 108,* 1108-1110.

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,* 587-596.

ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)

Davidson, J. M., Patterson, H. A, & Rizzo, D. M. (2008). Sources of inoculum for *Phytophthora ramorum* in a redwood forest. *Phytopathology, 98*, 860-866.

Fichtner, E. J., Lynch, S. C., & Rizzo, D. M. (2007). Detection, distribution, survival, and sporulation of *Phytophthora ramorum* in a California redwood-tanoak forest soil. *Phytopathology, 97*, 1366-1375.

Goheen, E. M., Hansen, E. M., Kanaskie, A., McWilliams, M. G., Osterbauer, N., & Sutton, W. (2002). Eradication of sudden oak death in Oregon. *Phytopathology, 92,* S30.

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,* e1000583. doi:10.1371/journal.ppat.1000583.

Hansen, E., Kanaskie, A., Prospero, S., McWilliams, M., Goheen, E. M., Osterbauer, N., Reeser, P., & Sutton, W. (2008). Epidemiology of *Phytophthora ramorum* in Oregon tanoak forests. *Canadian Journal of Forest Research*, *38*, 1133-1143.

Hansen, E. M., Reeser, P. W., Sutton, W., & Winton, L. M. (2003). First Report of A1 Mating Type of *Phytophthora ramorum i*n North America. *Plant Disease, 87,* 1267.

Ivors, K. L., Garbelotto, M., De Vries, I., Ruyter-Spira, C., Te Hekkert, B., Rosenzweig, N., & Bonants, P. (2006). Microsatellite markers identify three lineages of *Phytophthora ramorum* in US nurseries, yet single lineages in US forest and European nursery populations. *Molecular Ecology*, *15*, 1493-1505.

Ivors, K. L., Hayden, K., Bonants, P. J. M., Rizzo, D. M., & Garbelotto, M. (2004). AFLP and phylogenetic analyses of North American and European populations of *Phytophthora ramorum. Mycological Research, 108,* 378-392.

Mascheretti, S., Croucher, P. J. P., Vettraino, A., Prospero, S., & Garbelotto, M. (2008). Reconstruction of the Sudden Oak Death epidemic in California through microsatellite analysis of the pathogen *Phytophthora ramorum*. *Molecular Ecology, 17*, 2755-2768.

Moralejo, E., Puig, M., Garcia, J. A., & Descals, E. (2006). Stromata, sporangiomata and chlamydosori of *Phytophthora ramorum* on inoculated Mediterranean woody plants. *Mycological Research, 110*, 1323-1332.

Nei, M. (1972). Genetic distance between populations. *American Naturalist, 106*, 283-292.

Parke, J. L., Oh, E., Voelker, S., Hansen, E. M., Buckles, G., & Lachenbruch, B. (2007). *Phytophthora ramorum* colonizes tanoak xylem and is associated with reduced stem water transport. *Phytopathology, 97*, 1559-1567.

Prospero, S., Black, J. A., & Winton, L. M. (2004). Isolation and characterization of microsatellite markers in *Phytophthora ramorum*, the causal agent of sudden oak death. *Molecular Ecology Notes*, *4*, 672-674.

Prospero, S., Hansen, E. M., Grunwald, N. J., & Winton, L. M. (2007). Population dynamics of the sudden oak death pathogen *Phytophthora ramorum* in Oregon from 2001 to 2004. *Molecular Ecology, 16*, 2958-2973.

Rizzo D. M., Garbelotto, M., Davidson, J. M., Slaughter, G. W., & Koike, S. T. (2002). *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. *Plant Disease, 86*, 205-214.

Rizzo, D. M., Garbelotto, M., & Hansen, E. (2005). *Phytophthora ramorum*: Integrative research and management of an emerging pathogen in California and Oregon forests. *Annual Review of Phytopathology*, *43*, 309-335.

Sutton, W., Hansen, E. M., Reeser, P. W., & Kanaskie, A. (2009). Stream monitoring for detection of *Phytophthora ramorum* in Oregon tanoak forests. *Plant Disease, 93*, 1182-1186.

Werres, S., Marwitz, R., Man in't Veld, W. A., de Cock, A W. A. M., Bonants, P. J. M., de Weerdt, M., Themann, K., Ilieva, E., & Baayen, R. P. (2001). *Phytophthora ramorum* sp. nov., a new pathogen on *Rhododendron* and *Viburnum. Mycological Research, 105*, 1155-1165.

Winton, L. M., & Hansen, E. M. (2001). Molecular diagnosis of *Phytophthora lateralis* in trees, water, and foliage baits using multiplex polymerase chain reaction. *Forest Pathology, 31*, 275-283.

ISSN 0048-0134 (print)
ISSN 1179-5395 (on-line)