MYCOLOGICAL RESEARCH 113 (2009) 298–307

British Mycological Society promoting fungal science

journal homepage: www.elsevier.com/locate/mycres

AFLPs detect low genetic diversity for *Phytophthora nemorosa* and *P. pseudosyringae* in the US and Europe

Rachel E. LINZER[a,*], *David M. RIZZO*[b], *Santa Olga CACCIOLA*[c], *Matteo GARBELOTTO*[a]

[a]*Department of Environmental Science, Policy and Management – Ecosystem Science, University of California, Berkeley, 137 Mulford Hall, Berkeley, CA 94720, USA*
[b]*Department of Plant Pathology, University of California, One Shields Avenue, Davis, CA 95616, USA*
[c]*Department of Biochemistry, Medical Chemistry and Molecular Biology, University of Catania, Viale Andrea Doria, 6 – 95126 Catania Edificio, Italy*

ARTICLE INFO

Article history:
Received 1 May 2008
Received in revised form
14 October 2008
Accepted 3 November 2008
Published online 21 November 2008
Corresponding Editor:
David E. L. Cooke

Keywords:
Forest *Phytophthoras*
Homothallic
Invasive
Phytophthora ramorum
Sudden oak death
Umbellularia californica

ABSTRACT

In California and Oregon, two recently described oomycete forest pathogens, *Phytophthora nemorosa* and *P. pseudosyringae*, overlap in their host and geographic ranges with the virulent *P. ramorum*, causal agent of "sudden oak death." Epidemiological observations, namely broader geographic distribution and lack of landscape-level mortality, led to the hypothesis they are native to this region, whereas multiple lines of evidence indicate *P. ramorum* is exotic to North America. We used AFLP analysis to measure genetic variability in the homothallic *P. nemorosa* and *P. pseudosyringae* and to evaluate the hypothesis of endemism. We analysed 39 *P. nemorosa* and 48 *P. pseudosyringae* isolates (29 American and 19 European) from throughout their geographic and host ranges. In the US, both *P. nemorosa* and *P. pseudosyringae* have a dominant AFLP clone with several closely related variants. There is no evidence that genetic diversity is partitioned by host or location in *P. nemorosa*, but the US *P. pseudosyringae* clonal lineage is largely nested within a more genetically variable European group. Though the absence of highly variable sampled source populations does not allow us to determine whether each species is native or introduced in the western US with certainty, the results are most consistent with the hypothesis that both are introduced — *P. pseudosyringae* perhaps from Europe. Invasive *Phytophthora* species are increasingly being implicated in emergent forest diseases, highlighting the need to identify and characterize both native and previously unknown introduced forest *Phytophthoras*.

Introduction

Although biotic surveys of ecosystems damaged by invasive pathogens often uncover previously undescribed microbes, the shortage of comprehensive baseline data on endemic microbial community composition makes it extremely challenging to determine whether newly identified microbes are native or introduced (Desprez-Loustau *et al.* 2007). The limited knowledge of the impact of invasive microbes on endemic microbes highlights the importance of recognizing and monitoring previously unknown native species (Rizzo 2005). Conversely, it is important to identify invasive microbes that cause less visible symptoms and might otherwise go undetected in order to interrupt unknown pathways of introduction.

Long known in agriculture for causing destructive crop diseases, such as the Irish potato blight, invasive oomycetes of

---

* Corresponding author.
E-mail address: rwarner@nature.berkeley.edu

0953-7562/$ – see front matter © 2008 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.mycres.2008.11.004

the genus *Phytophthora* are receiving increasing attention worldwide as emergent forest pathogens (Erwin & Ribeiro 1996; Hansen 2008). *P. cinnamomi* is destroying Australian jarrah forest and many other forest and crop trees internationally (reviewed in Hardham 2005); *P. ramorum* causes widespread mortality of oaks and tanoaks in California and Oregon; *P. lateralis* causes a lethal disease of American Port Orford cedars; the *P. alni* species complex is responsible for the dieback of European alders; and multiple *Phytophthora* species are contributing to European oak decline, European beech dieback and to outbreaks of chestnut ink disease (Balci & Halmschlager 2003; Biocca *et al.* 1994; Brasier *et al.* 2004; Gibbs *et al.* 2003; Hansen *et al.* 2000; Jönsson *et al.* 2005; Jung 2006; Jung & Blaschke 2004; Jung *et al.* 1999; Jung *et al.* 2000; Rizzo *et al.* 2002; Tucker & Milbrath 1942; Vettraino *et al.* 2001; Vettraino *et al.* 2005). The causal agents of most of these diseases are hypothesized to be introduced, but confirmation has been provided in only a few cases, as locating microbial source populations is difficult and information on endemic *Phytophthoras* is sparse. Often, limited genetic and phenotypic variability among populations of these emergent pathogens is presumed to be the result of a recent bottleneck and used as evidence to support their exotic origin (Cooke *et al.* 2005; Hansen *et al.* 2000; Ivors *et al.* 2004; Zentmyer 1988). Environmental surveys in conjunction with these emerging diseases have isolated many previously unidentified forest *Phytophthora* species and, due to the scarcity of baseline data, it is often extremely difficult to determine whether these too are native or introduced (Balci *et al.* 2007; Belbahri *et al.* 2006; Brasier *et al.* 2003; Brasier *et al.* 2004; Brasier *et al.* 2005; Cooke *et al.* 2007; Davidson *et al.* 2002; Hansen & Delatour 1999; Jung *et al.* 1999; Jung *et al.* 2000; Jung *et al.* 2002; Jung & Nechwatal 2008; Reeser *et al.* 2007; Vettraino *et al.* 2002).

P. nemorosa and *P. pseudosyringae* were first identified in the western US while sampling for *P. ramorum*, the causal agent of a complex of plant diseases including 'sudden oak death', and the two were initially grouped under the name '*P. ilicis*-like' (Davidson *et al.* 2002; Hansen *et al.* 2003; Murphy & Rizzo 2006; Rizzo *et al.* 2002; Rizzo *et al.* 2005; Werres *et al.* 2001; Wickland *et al.* 2008). Molecular phylogenetic analyses from a variety of nuclear and mitochondrial loci show the homothallic *P. nemorosa* and *P. pseudosyringae* are closely related but distinct taxa situated in a clade with *P. ilicis* and *P. psychrophila;* both are rather distantly related to *P. ramorum* (Blair *et al.* 2008; Ivors *et al.* 2004; Martin & Tooley 2003; Schena & Cooke 2006). All three of these species can be aerially dispersed and have overlapping host and geographic ranges, and although they appear to have different environmental limits, combinations of the three species can be found on the same sites and even on the same naturally infected host (Hansen *et al.* 2003; Jensen *et al.* 2006; Murphy & Rizzo 2006; Wickland & Rizzo 2006; Wickland *et al.* 2008). Both *P. nemorosa* and *P. pseudosyringae* alone cause symptoms similar to those of *P. ramorum* on shared hosts, including foliar lesions of California bay laurel trees, *Umbellularia californica* (Hook. & Arn.) Nutt., which have been shown to be key hosts in *P. ramorum* sporulation and disease spread (Davidson *et al.* 2005; Hansen *et al.* 2003; Jensen *et al.* 2006; Reeser *et al.* 2008; Wickland *et al.* 2008). However, mortality associated with *P. nemorosa* and *P. pseudosyringae* appears much more limited than the landscape-scale mortality due to *P. ramorum* (Hansen *et al.* 2003; Wickland & Rizzo 2006). Outside of nurseries, North American *P. ramorum* is currently confined to counties along the central California coast and a single location in Oregon, but the distributions of both *P. nemorosa* and *P. pseudosyringae* in this region are wider. The range of *P. pseudosyringae* begins farther south in San Luis Obispo County, and both species are isolated continuously from Monterey County in California through southern Oregon and disjunctly from interior sites in California's Sierra Nevada Mountains (Wickland *et al.* 2008). *P. nemorosa* is currently only known to occur in California and Oregon, but *P. pseudosyringae* has also been isolated from streams in the eastern US, and it is associated with declining oaks, beeches, and alders in Europe, where it has been described as a root and stem pathogen (Diana *et al.* 2006; Hwang *et al.* 2007; Jung *et al.* 2003). When compared with *P. ramorum*, the broader geographic distribution and reduced virulence of *P. nemorosa* and *P. pseudosyringae* led to the hypothesis that they were native to the western US (Garbelotto & Rizzo 2005; Hansen *et al.* 2003).

The aim of this study is to provide an assessment of the genetic structure of *P. nemorosa* from western North America and of *P. pseudosyringae* from western North America and Europe using AFLP genetic markers. Analysis of genetic diversity and the relationship between genetic and spatial data should assist in addressing whether these two recently described forest *Phytophthora* species are native to the western US. This study will also provide one of the first analyses of the genetic structure of homothallic *Phytophthoras* in forest ecosystems [see Cooke *et al.* (2005) for the first such study].

Materials and methods

Isolates and DNA extraction

Thirty-nine *Phytophthora nemorosa* isolates from California and Oregon and 48 *P. pseudosyringae* isolates from California, Germany, and Italy were analysed. All isolates were collected between 1997 and 2004. Although most North American isolates were collected along a latitudinal gradient in forests of the Pacific Coast mountain range, one *P. nemorosa* and two *P. pseudosyringae* isolates came from forests in Mariposa County, on the slopes of the Sierra Nevada mountain range. The two mountain ranges are separated by a significant extent of lowlands, currently and historically characterized by vegetation assemblages distinct from those found in the mountains. The known US geographic range for these two *Phytophthora* species is thus constituted by two disjunct areas, each represented by one of the two mountain ranges sampled in this study. The selected isolates represented most of the known host and geographic ranges at the time the study was conducted, though expanded ranges for both have subsequently been identified. *Phytophthora nemorosa* has currently only been isolated in California and Oregon, while *P. pseudosyringae* is also present in Europe; hence, 19 *P. pseudosyringae* isolates from four sites in Europe were also included in this study for comparison. One isolate of the closely related *P. ilicis* was included as an outgroup. Isolate details are listed in Table 1. *P. nemorosa* and *P. pseudosyringae* samples were treated together and randomized irrespective of species during the extraction and AFLP processes.