Research Note

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Phytophthora ramorum infection of coast live oak leaves in Californian forests and its capacity to sporulate *in vitro*

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Abstract. Coast live oak (*Quercus agrifolia*) is a known host for *Phytophthora ramorum*, the casual agent of sudden oak death in California, with symptoms expressed as necrotic stem cankers. In the forest, leaves on two saplings in California were found to be infected with *P. ramorum* and these were associated with infected bay laurel (*Umbellularia californica*) trees. Coast live oak leaves supported sporulation and produced chlamydospores *in vitro*. This is the first report to identify foliage of coast live oak as a source of infection of *P. ramorum* in the forest and its confirmation in *in vitro* inoculations.

Phytophthora ramorum Werres, De Cock & Man in't Veld (Werres *et al*. 2001) is the agent of sudden oak death (SOD), a disease that has killed large numbers of tanoak (*Lithocarpus densiflorus* (Hook & Arn.) Rehd.) and coast live oak (*Quercus agrifolia* Neé) in California, USA. The pathogen causes a broad range of symptoms, which can be confused with those of other pathogens, on different hosts (Rizzo 2007). Foliar lesions have not been reported in descriptions of the disease occurring on oaks (Rizzo *et al*. 2002; Tooley and Kyde 2007). On coast live oak, the disease is manifested by stem cankers that are usually fatal, with no sporulation observed (Davidson *et al*. 2005). In California, these stem cankers are highly correlated with the presence of infected bay laurel leaves (*Umbellularia californica* (Hook. & Arn.) Nutt) (Kelly and Meentemeyer 2002; Swiecki and Bernhardt 2002).

During sampling of symptomatic leaf and stem material in several locations in California in July and September 2005, necrotic lesions were observed on leaves of two coast live oak saplings in Samuel P. Taylor, Marin County. Bay laurel trees with known *P. ramorum* infections were closely associated with these foliar symptoms (Hüberli *et al*. 2006). Necrosis on coast live oak appeared to develop on the petiole, spreading from the main vein to the leaf tissue (Fig. 1). Sometimes the entire leaf was necrotic. There was no evidence of extensive defoliation or bleeding stem cankers on the saplings.

Symptomatic leaves were plated onto pimaricin–ampicillin–rifampicin–PCNB agar (P_{10}ARP) containing 25 mg PCNB, a selective medium for *Phytophthora* spp. (Erwin and Ribeiro 1996).

A *Phytophthora* sp. was consistently isolated and identified as *P. ramorum* by its morphological and molecular traits (Werres *et al*. 2001; Rizzo *et al*. 2002). This result was also confirmed by ELISA analysis (Agdia Inc. Indiana USA) and real-time PCR with SyberGreen and Taqman (Hayden *et al*. 2006). The region of the ITS rDNA sequences of *P. ramorum* isolates Pr-420 (American Type Culture Collection, Manassas, VA, ATCC MYA-3678) and Pr-421 obtained from the saplings were deposited in the NCBI database (GenBank accession numbers DQ168872 and DQ168873, respectively).

To test for pathogenicity to coast live oak leaves, V8 agar mycelium plugs of isolate Pr-420 were placed onto freshly cut petioles of 20 asymptomatic leaves collected from one tree growing at the University of California Botanical Garden at Berkeley. Control leaves were inoculated with sterile V8 agar plugs. All leaves were maintained in a moist chamber at 19°C in the dark for 11 days. Symptoms, close to those observed in the field, started to develop after 4 days. Necrosis was mainly present

Fig. 1. Foliar symptoms on coast live oak (*Quercus agrifolia*) leaves collected from the forest in California from which *Phytophthora ramorum* was isolated.

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Fig. 2. Symptoms on coast live oak (*Quercus agrifolia*) leaves inoculated with *Phytophthora ramorum* after 11 days incubation in a moist chamber.

along the petiole and the main vein, covering ~30% of the leaf length and spreading into 2.5% of the tissue (Fig. 2). Reisolation of *P. ramorum* on P_{10}ARP was successful for all inoculated leaves and negative for control leaves. At harvest, inoculated leaves had on average 19 ± 2.8 sporangia and 14 ± 3.3 chlamydospores, while no spores were present on the control leaves.

To our knowledge this is the first report of foliar lesions on coast live oak caused by *P. ramorum* in the field and sporangia production *in vitro*. Recent artificial inoculations of coast live oak foliage have also confirmed its susceptibility (Tooley and Kyde 2007). The epidemiological role of coast live oak leaf infections is not known. However, unlike infected bark (Davidson *et al*. 2005), we showed that foliage has the potential to produce sporangia and chlamydospores and may serve as a source of inoculum for other *P. ramorum* hosts and introduction into new sites. It is not known if inoculum produced on oak leaves could result in oak cankers, but this seems unlikely, since the concentration produced is very low compared with the thousands of spores produced on bay laurel leaves (Davidson *et al*. 2005). In addition, this study contributes to our understanding of SOD with the description of new symptoms on coast live oak being essential for accurate and rapid diagnosis of the disease.

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