

Susceptibility of Australian Plant Species to *Phytophthora ramorum*¹

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Abstract

Phytophthora ramorum is an invasive plant pathogen causing considerable and widespread damage in nurseries, gardens, and natural woodland ecosystems of the United States and Europe, and is classified as a Category 1 pest in Australia. It is of particular interest to Australian plant biosecurity as, like *P. cinnamomi*; it has the potential to become a major economic and ecological threat in areas with susceptible hosts and conducive climates. Research was undertaken in California to assess pathogenicity of *P. ramorum* on Australian native plants. Sixty-eight test species within 24 families were sourced from established gardens and arboretums. Foliar and branch susceptibility were tested using detached leaf and branch assays. The experiment was repeated to account for seasonality. Initial results indicate the majority of species tested were susceptible to varying degrees. Of particular interest are the high levels of variability within the eucalypts, low levels of susceptibility within the Pittosporaceae family, and a concerning number of latent or asymptomatic infections.

Introduction

Phytophthora ramorum, the cause of sudden oak death in California, is an invasive plant pathogen causing considerable and widespread damage in nurseries, gardens, and natural woodland ecosystems of the United States (U.S.) and Europe (Werres and others 2001, Rizzo and others 2002, Brasier and others 2004). Classified as a Category 1 pest in Australia (Plant Health Australia 2006), it is of particular concern to Australian plant biosecurity as, like *P. cinnamomi*, it has the potential to become a major economic and ecological threat in areas with susceptible hosts and conducive climate.

Success of invasive organisms like *P. ramorum* in novel environments such as Australia relies on establishment in areas with conducive climates and susceptible hosts. The known worldwide host range of *P. ramorum* continues to grow, presently incorporating more than 100 species covering a diversity of trees, shrubs, and herbaceous species found in wildlands and nurseries (RAPRA 2007a, 2007b; USDA APHIS 2007). Two Australian host species, *Eucalyptus haemastoma* (scribbly gum) and *Pittosporum undulatum* (sweet pittosporum) have been listed as natural hosts of

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P. ramorum based on field observations and pathogenicity tests in the U.S. and Europe (Hüberli and others 2006, RAPRA 2007a, USDA APHIS 2007). In addition, *Leptospermum scoparium*, *E. gunnii* (cider gum) and *E. dalrympleana* (white mountain gum), have been found to be susceptible using artificial inoculation methods in the U.S., the United Kingdom (U.K.), and Spain respectively (Denman and others 2005, Huberli and others 2008, Moralejo and others 2009). A number of natural and associated host species of *P. ramorum* have been introduced to Australia. Douglas-fir, *Pseudotsuga menziesii*, for example, exists as a naturalized species (Lefoe 2002).

Given the wide and increasing host range of *P. ramorum*, including Australian native species, and evidence of a multiple-host method of dispersal (Moralejo and others 2006), it is expected that many Australian native plant species are susceptible. Research into the potential host range of Australian native species was undertaken in California to make an accurate assessment of the risk that it may pose to Australian plant biosecurity. Detached foliar and branch assays were used to assess the susceptibility of a range of key Australian native species to *P. ramorum*.

Methods

A total of 68 test species within 24 families were sourced from established gardens and arboretums in northern California. Species were selected based upon provenance from areas of climatic suitability for *P. ramorum*, as well as ecological and economical importance to Australian plant industries. Species were duplicated where possible from different locations or accessions. A total of 135 individual plants were tested. The known susceptible host *Rhododendron* ‘Colonel Cohen’ was used as a positive control across all experiments.

Detached leaf and branch assays were used to test for susceptibility, following the protocols of Denman and others (2005) and Hüberli and others (2008) respectively, and using isolate Pr510 from the culture collection of the Rizzo lab (University of California, Davis, Davis, California). Experiments were conducted in the summer of 2008 and winter of 2008/2009. Wounded foliar inoculations were only done during the summer. All plant material were kept in moist chambers at 20 to 25 °C and 16 hours daylight during summer, and 15 to 20 °C and 12 hours daylight during winter. The *Phytophthora* selective medium PARP was used for both foliar and branch studies to confirm infection.

Preliminary analysis of the results was undertaken to compare the species amongst one another, based only on averages of the recorded data. Foliar susceptibility was grouped based upon disease severity (confirmed by re-isolation) into non-hosts (0 percent), low susceptibility (<15 percent), moderate susceptibility (15 to 30 percent), high susceptibility (30 to 45 percent), and very high susceptibility (>45 percent). For branch susceptibility, species were ranked from smallest to largest mean lesion length.

Results and Discussion

Foliar inoculation results indicate that the majority of species tested are susceptible to *P. ramorum* in varying degrees. Only *Hedycarya angustifolia* tested completely

negative in both wounded and unwounded inoculation rounds. No trends were found to indicate particular plant families or genera to be more susceptible than another. Of note is that both *Isopogon* species tested were highly susceptible, and as they are important species in the international cut flower and nursery industries, could prove to be important in disease spread or epidemiology in Australia and abroad. *Agonis flexuosa* tested moderately to highly susceptible depending on their provenance. *A. flexuosa* is a common West Australian native planted widely as a street tree both on the east coast of Australia and West Coast of California, making it an ideal candidate for spread of the disease through native and nursery settings. Many eucalypt species tested highly susceptible, including *E. paucifolia* (snow gum), *E. sideroxylon* (red ironbark), and *E. nitens* (shining gum), an important native forest and plantation species.

Results of summer branch inoculations indicate less highly susceptible species than those with foliar inoculations. *Isopogon formosus*, *E. nitens*, and *E. sideroxylon*, all highly susceptible in foliar inoculations, were again highly susceptible on branches. Interestingly, *Hedycarya angustifolia*, not susceptible at all in the foliar trials, was highly susceptible in branch inoculation trials. In general, many of the eucalypts appear to be susceptible to *P. ramorum* branch dieback. No correlation between foliar and branch inoculation was detected.

Some basic trends have been observed so far. In both foliar and branch inoculations, higher susceptibility was observed in juvenile foliage and branches, while wounding increased susceptibility in foliar studies. The conifers and grasses tested were all low to moderate in susceptibility. Much variability was observed within species, genera and family levels, indicating *P. ramorum* is a generalist pathogen, and negating the possibility of predicting host range possibilities without testing a number of species, and individuals within those species. Some hosts, such as *E. leucoxylon*, *E. sideroxylon*, and *I. formosus*, appear to be both foliar and branch hosts of the pathogen, and as such, populations of these species may be at risk in the wild or in the nursery. Many species on the other hand, while being foliar hosts to some degree, were far less susceptible on their branches (*N. cunninghamii*, *O. argophylla*). On the other hand, *H. angustifolia* was highly susceptible in branch inoculations, with no observed foliar susceptibility. Initial results also indicate seasonal variability, as has been shown in previous inoculation studies with *P. ramorum* (De Dobbelaere and others 2007, Tjosvold and others 2007).

These results clearly indicate the potential for *P. ramorum* to infect and colonize a range of Australian native plant species from different families. Inoculum concentration, foliar sporulation, and log susceptibility will be investigated shortly. These results will provide the basis for climate and spread models to predict the pathogen's spread and impact on Australian plant industries and natural ecosystems should an incursion occur. Risk predictions generated by the models, and an understanding of the pathogen's host range, will allow the targeting of high-risk areas for early detection surveillance and protection, and assist regulators in developing appropriate quarantine protocols.

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