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Year: 2008

Source: Australasian Plant Pathology, 37 (6). pp. 615-625

Official URL: http://dx.doi.org/10.1071/AP08064

Susceptibility of New Zealand flora to *Phytophthora ramorum* and pathogen sporulation potential: an approach based on the precautionary principle

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Abstract. *Phytophthora ramorum*, the cause of sudden oak death in the western USA and a damaging pathogen in Europe, is a biosecurity threat of unknown magnitude to New Zealand and Australasia because of its presence in traded ornamental plants. Knowledge of potential hosts acting as carriers and of symptoms caused by the pathogen on such hosts will strengthen precautionary quarantine regulations to prevent inadvertent introductions of *P. ramorum* into the region. Also, the identification of potential hosts will permit determination of areas at risk within countries that do not have *P. ramorum*. Susceptibility of New Zealand plants including 17 endemic and three commercial species (*Eucalyptus globulus*, *Pinus radiata* and *Acacia melanoxylon*), as well as two known *Rhododendron* cultivar hosts, was determined by analysing the size of lesions on inoculated excised leaves and branches, while infectivity was determined by counting sporangia produced on leaves. In order to identify extremely susceptible hosts, seven species were inoculated using three concentrations of zoospores ranging from low ($1x10^2$ zoospores/mL) to high ($5x10^3$ zoospores/mL). In branch inoculations, *P. radiata* and *Nothofagus fusca* were as susceptible as the *Rhododendron* cultivars. *Pseudopanax arboreus*, *Fuchsia excorticata* and one *Rhododendron* cultivar were equally susceptible in leaf inoculations. However, *F. excorticata* was the only species with 100% infected leaves, high foliar sporulation and was highly susceptible at all three zoospore concentrations. *Leptospermum scoparium* was the only asymptomatic foliar host that had high reisolations of the pathogen. *F. excorticata*, *P. radiata*, *N. fusca*, *P. arboreus* and *L. scoparium*, should be added to the potential host list for *P. ramorum* and monitored for symptoms and sporulation in gardens and nurseries in the USA and Europe. As part of a precautionary strategy, these species are suitable candidates for targeted surveillance programs in high-risk incursion areas of New Zealand. Furthermore, the sympatry of foliar hosts with high infectivity and of highly susceptible stem hosts was identified: these areas may be at risk for the development of a forest epidemic.

Additional keywords: asymptomatic, oomycete, potential hosts, sporangia, sudden oak death, symptomatic.

Introduction

Increased international trade in plants and plant products heightens the risk of introduction of alien plant pathogens (Levine and D'Antonio 2003). Once introduced these pathogens normally cannot be eradicated, due to the fact that their recognition occurs years or decades after they have already become established and caused disease over significantly large zones of infestation (e.g. oomycete pathogens such as *Phytophthora ramorum*, *P. cinnamomi*, *P. kernoviae* and *P. lateralis*; Brasier 2007, Hansen 2008). International trade regulations are designed to prevent movement of known dangerous pathogens, but are of limited use in preventing the introduction of novel pathogens; moreover, because extensive biosecurity measures may interfere with vast and profitable international trade in plants or plant products, quarantine measures must be carefully targeted to maximise protection at a reasonable cost. The precautionary principle can be used as a guide in situations of high plausibility regarding the environmental risk associated with a potential pathogen introduction, but characterised by high uncertainty over the possible impacts. It states:

> 'Where there are threats of serious or irreversible damage, lack of full scientific certainty should not be used as a reason for postponing measures to prevent environmental degradation. In the application of the precautionary principle, public and private decisions should be guided by: (i) careful evaluation to avoid, wherever practicable, serious or irreversible damage to the environment; and, (ii) an assessment of the risk-weighted consequences of various options' (The Intergovernmental Agreement on the Environment, May 1992, quoted in Deville and Harding 1997, p. 13)

In applying the precautionary principle, it is necessary to establish if precautionary measures are needed and, if so, what measures are appropriate. Simultaneously, measures should be taken to reduce ignorance regarding both the risk and the extent of potential impact, in an attempt to move from precautionary to preventive actions (Deville and Harding 1997; for applications of the precautionary approach see Calver *et al*. 1999, Grayson and Calver 2004, Lilith *et al*. 2006).

The precautionary principle is particularly relevant to the possibility of the introduction of *P. ramorum* to Australasia, especially New Zealand. *P. ramorum* presents a significant and costly problem where it was recently introduced to western USA and Europe from an unknown origin (Werres *et al*. 2001; Rizzo *et al*. 2002) through the nursery trade (Ivors *et al*. 2006; Mascheretti *et al*. 2008). In Californian coastal forests, over 90 species are susceptible and the severity of the disease is mostly exemplified by the high levels of mortality of susceptible oaks (*Quercus agrifolia*, *Q. kelloggii*, *Q. parvula* var. *shrevei*, and *Q. chrysolepis*) and of the related *Lithocarpus densiflora* (tanoak), (Rizzo *et al*. 2002; Murphy and Rizzo 2003). The epidemic is driven largely by *Umbellularia californica* (bay laurel), a host that supports abundant foliar sporulation (Davidson *et al*. 2005). The ability of *P. ramorum* to disseminate aerially differentiates it from most forest *Phytophthora* pathogens and, together with its ability to disperse in soil and water, places it in a category of extreme potential risk in any moist temperate climate. It infects many ornamental plants routinely traded on the international market (Werres *et al*. 2002; Tooley *et al*. 2004; APHIS 2008; RAPRA 2008) and it has been reported to sporulate on some species with limited or no symptoms (Bienapfl *et al*. 2005; Denman *et al*. 2006), making it a severe quarantine risk. In New Zealand, all but a few of the 27 plant families containing known hosts of *P. ramorum* in the USA and Europe have representative genera that are either indigenous or naturalised. One known hosts is indigenous (*Griselinia littoralis*) and eight are naturalised (*Drimys winterii*, *Frangula purshiana*, *Fraxinus excelsior*, *Pseudotsuga menziesii*, *P. undulatum*, *Sequoia sempervierens*, *Syringa vulgaris* and *Taxus baccata*) (Allan Herbarium 2007; RAPRA 2008). Overall, we believe that the

demonstrated impacts of *P. ramorum* in the USA and Europe and the likelihood of susceptibility of New Zealand native flora argue for high levels of precaution.

One prompt and effective precautionary measure that could be applied to protect New Zealand is to identify susceptible New Zealand species. Knowledge of potential hosts acting as vectors of the pathogen may bolster quarantine guidelines to help prevent further inadvertent introductions of *P. ramorum* into uninfested countries. For example, the recent European isolations of *P. ramorum* from several tree species in the field previously identified as potential hosts from artificial inoculations (Brasier *et al*. 2004) highlights the importance in identification of potential hosts prior to establishment. In this paper, the susceptibility levels of a broad range of plant taxa from New Zealand to *P. ramorum* are compared to those of two known susceptible *Rhododendron* cultivars, using an excised inoculation test with leaves and branches. The potential of each host species to support sporulation and thus be infectious was examined by counting sporangia produced after inoculation of detached leaves. Finally, a subset of species comprising five New Zealand species and two "control" *Rhododendron* cultivars was exposed to three different levels of zoospore concentrations to determine whether inoculum dilution may affect predicted susceptibility levels.

Methods

Inoculation experiments

Two inoculation experiments using 20 different plant species from New Zealand (endemic or commercial) were conducted concurrently using excised branches (Experiment 1) and leaves (Experiment 2) in July 2005 (Table 1). Two *Rhododendron* cultivars, 'Cunningham's White' rhododendron and 'White Dwarf' azalea were included as positive controls in each inoculation, bringing the total number of plant species tested to 22. Both are known hosts to *P. ramorum*, with the azalea having somewhat lower susceptibility than the rhododendron (Steve Tjosvold, personal communication). In May 2006, a third experiment was conducted involving inoculations of leaves using three different zoospore concentrations on a subset of five New Zealand species from Experiment 2, and on the same two susceptible rhododendron cultivars used previously. This experiment was designed to identify plant species that may become infected even when pathogen inoculum may be low, a condition likely to occur initially when a pathogen has just been introduced in a new environment.

Phytophthora ramorum *isolate*

Isolate PR-52 (CBS 110537; ATCC MYC-2436) of the A2 mating-type was used in all experiments. It was isolated from a *Rhododendron* in 2000 from a nursery in California and was shown to be highly pathogenic compared to other isolates in previous inoculation studies of two hosts (Hüberli *et al*. 2006a). For branch inoculations, inoculum discs (3 mm dia.) were cut with a sterile cork borer from the margin of 14-day-old cultures grown at 20 ºC on vegetable-8 juice (Campbell Soup Company, Davis, CA, USA) agar (V8A). For foliar inoculations, zoospores were produced as described in Hüberli *et al*. (2003). After haemocytometer counts, zoospore concentrations were adjusted to $2x10^4$ zoospores/mL in Experiment 2 and to three concentrations, $1x10^2$, $1x10^3$ and $5x10^3$ zoospores/mL in Experiment 3. Prior to contact with zoospore solutions, lab-ware was acid washed (5 M hydrochloric acid) for 24 h and then washed three times with deionised water to reduce zoospore attraction to these surfaces so that zoospores remained in suspension.

Plant material

Plants were selected to represent common species in New Zealand, either native to the insular nation or abundantly traded and imported into that country. For Experiments 1 and

2, branches of 25-30 cm in length with eight to ten mature healthy leaves, and no lateral branches, were collected in July 2005 from a total of 20 New Zealand plant species and the two positive controls (*Rhododendron* cultivars) growing at the University of California Botanical Garden in Berkeley or on the university campus (Table 1 and 2). Two separate species of the 20, were not tested in Experiment 1 (*Melicytus ramiflorus* and *Pittosporum eugenioides*) and 2 (*Olearia bullata* and *Sophora microphylla*). Plant material was collected from one individual in most cases, or two to three individuals when these were available. All branches were kept in cooler-boxes with their basipetal-end in jars filled with sterilised water. One day after collection, branches (average 4 mm dia.) were trimmed to a length of 20 to 25 cm, with four to five leaves remaining, for inoculations in Experiment 1. Remaining branches provided the foliar material used for foliar inoculation in Experiment 2. Prior to inoculation, leaves were rinsed in sterile deionised water to remove any debris. In May 2006, plant material for Experiment 3 was collected and prepared for foliar inoculation as described above. Only a subset of five species was tested in this last experiment, namely *Dacrydium cupressinum*, *Fuchsia excorticata, Nothofagus fusca*, *Pinus radiata* and *P. eugenioides*. The same two *Rhododendron* cultivars used in Experiments 1 and 2 were inoculated as well, and served as controls.

Experiment 1: Branch inoculation

Inoculation and test conditions

To prevent excessive evaporation during the experiment, jars were sealed by wrapping Parafilm (American National Can, Chicago, U.S.) around their mouths, with branches protruding through a hole in the Parafilm. Using a sterile syringe needle, a wound was created through the bark, 10 cm from the acropetal end of the branch. An inoculum disc was placed mycelium-side-down on the outside of the wound and the inoculation point was then wrapped with a layer of Parafilm on top of which a layer of silver Nashua® tape (Tyco Adhesives, Massachusetts, U.S.) was superimposed. For each individual plant, four replicate branches were inoculated. In total, material from 27 plants was used in this experiment (including the different genotypes of some plant species) resulting in 108 inoculated branches. Since most material was available only from the botanical garden, we were restricted in the amount of branches we could remove from plants and hence replicate numbers were low. One branch from each individual plant was inoculated with a sterile V8A disc as a negative control. Branches in jars were arranged randomly in a misting chamber in a greenhouse with natural light of 12 h photoperiod, ambient temperatures (averages 16 - 22 ºC) and automatic misting three times daily for 30 min. The jars were given supplemental water as needed.

Measurements

Ten days after inoculation, the outer bark around the inoculation site was carefully scraped off with a scalpel and the entire lesion (if present) was exposed. Total lesion length extending above and below the site of inoculation was measured and a note was made to indicate whether the lesion had girdled the entire inoculated stem. In order to determine survival of the pathogen in inoculated stems, small pieces of tissue from the margins of the longitudinal lesion and from the site of inoculation were cut and plated onto pimaricin-ampicillin-rifampicin-PCNB agar (P_{10}ARP) selective medium, modified to contain one quarter of the original amount of PCNB used by Erwin and Ribeiro (1996).

Experiments 2 and 3: Foliar inoculations

Inoculation and test conditions

Shallow plastic trays (40 x 60 x 5 cm) were used to hold 1 L of zoospore inoculum which filled the tray to an approx. depth of 5 mm. Fourteen leaves of each species were

dipped simultaneously tip-first into the inoculum at a concentration of $2x10^4$ zoospores/mL for 5 min (Experiment 2). In order to do so, leaves were sandwiched between two Perspex strips (50 x 5 cm) held together with fold-back clips, the strips were then suspended above the inoculum such that the leaf tips were immersed. The leaves from the different plants were placed randomly across the tray to account for minor differences in zoospore distribution. In Experiment 3, three sets of 14 leaves from each of the selected species were dipped into inoculum concentrations of $1x10^2$, $1x10^3$ and $5x10^3$ zoospores/mL. One control leaf for each species for both experiments was dipped in sterile deionised water. After inoculation, all leaves were removed from the Perspex strips and placed, adaxial side down, into trays containing moist paper towels. The trays were incubated in a growth chamber at 19 ºC for 6 days in darkness.

Measurements

After incubation, all leaves were scanned and the size of lesions was recorded using the image analysis software ASSESS V1.01 (APS Press, Minnesota, USA). Presence/absence of lesions was also recorded to determine the ratio of successful inoculations. Prior to scanning leaves in Experiment 2, the region of the inoculation was gently scraped with a flat spatula on both surfaces into individual wells of a microtiter plate containing 300 µL sterile deionised water. To each well, 10 µL of phenol-cotton blue was added and sporangia per well were counted using a microscope at 40x magnification. Sporangia were not counted in Experiment 3.

After surface sterilisation of leaves with 70% ethanol, two segments from the lesion margin, or the region of inoculation if lesions were absent, were plated onto P_{10}ARP to determine the pathogen reisolation proportion out of the total leaves inoculated.

Statistical Analysis

All zero values for lesion length and area and sporangia counts were removed from the data set prior to calculations of means and the statistical analysis. All statistical analyses were done using the Statistica software V6.1 (Statsoft, Inc., Tulsa, OK, US). We used k-means clustering for branch and foliar inoculations and each of the three zoospore concentration foliar inoculations to determine whether any New Zealand plant species were equally susceptible to *P. ramorum* as the known highly susceptible *Rhododendron* cultivars. K-means clustering is a variation on conventional cluster analysis in which the investigators designate the number of groups into which the cases (in this study, the plant species) will be clustered. We specified two clusters, seeking to force a classification into two groups, using all the disease variables assessed. In this case, the results are useful if one group contains one or both of the susceptible *Rhododendron* cultivars. Any other species classified with them can be regarded as similarly susceptible and thus a potential host of *P. ramorum*. The analysis also calculates an F-statistic comparing each variable between the two groups.

Results

Experiment 1: Branch inoculation

P. radiata and *N. fusca* had large lesions that were in the range of the two susceptible *Rhododendron* cultivars, and k-means clustering grouped these species together (Table 1). The remaining 16 New Zealand species had very low susceptibility to *P. ramorum* in comparison to the susceptible *Rhododendron* cultivars and were clustered into a second k-means clustering group (Table 1). Furthermore, the two groups were significantly ($P<0.02$ in all cases) different for lesion length, symptomatic ratio and branch girdling, but not for pathogen reisolation.

Girdling of branches by *P. ramorum* occurred in *Pseudopanax arboreus, N. fusca, P. radiata, Prumnopitys spicata,* and *Rhododendron* cultivars (Table 1). Most severely affected were *N. fusca* and the *Rhododendron* cultivars.

Foliar inoculations

Experiment 2: Susceptibility and sporulation potential of 20 species

Five of the 18 New Zealand species formed foliar lesions when inoculated with *P. ramorum* zoospores (Table 2, Fig. 1). K-means clustering grouped the three species, *F. excorticata, P. arboreus* and 'Cunningham's White' rhododendron, into one group. For this particular group, lesion area, symptomatic ratio, and sporangia/leaf were significantly different ($P<0.002$) than equivalent values from the other species. *F. excorticata* was the only species among the three for which all leaves were infected; this species also supported the highest recorded foliar sporulation. Notably, one of the genotypes of *F. excorticata* produced twice as many sporangia than either *Rhododendron* cultivar. *P. arboreus* had the largest lesions, but only a few leaves (2/14) were infected and they both supported little sporulation. *P. ramorum* was recovered not only from all species with visible lesions, but also from nine New Zealand spp. that were asymptomatic (*Brachyglottis repanda, Corynocarpus laevigatus, Leptospermum scoparium, N. fusca, N. menziesii, Dacrycarpus dacrydioides, P. spicata, Pomaderris prunifolia, Melicytus ramiflorus*). In particular, *L. scoparium,* the species with the highest reisolation from asymptomatic leaves, was found to support sporulation at levels comparable to 'White Dwarf' azalea (Table 2).

Experiment 3: Susceptibility of seven *species at three different inoculum concentrations*

At the highest inoculum concentration ($5x10^3$ zoospores/mL), lesion size was significantly different among tested species ($P < 0.05$) and k-means clustering grouped *F. excorticata* and 'White Dwarf' azalea together (Figure 2, Table 3). For the remaining four species, which had previously been identified as resistant, and 'Cunningham's White' rhododendron, there was no detectable effect of inoculum concentration at the highest concentration. For *D. cupressinum* and *P. eugenioides*, disease incidence increased slightly at the higher inoculum levels.

Lowering the zoospore concentration below our standard inoculation concentration (1-$2x10^4$ zoospores/mL) did not change the high susceptibility of *F. excorticata* (Fig. 2). *D. cupressinum, P. radiata* and *P. eugenioides* always clustered together across the three zoospore concentrations. For both *Rhododendron* cultivars, susceptibility was lower when $1x10^2$ zoospores/mL was applied. In fact, they were clustered together with the other four species at the low zoospore concentration inoculation. At this concentration, lesions on *F. excorticata* were larger than those of any other species including the two susceptible Rhododendron cultivars and this species was clustered in its own group by k-means clustering analysis (Table 3).

Reisolation of *P. ramorum* increased with each successive increase in inoculum for all species except *P. radiata,* from which no reisolations were obtained (Fig. 2c). At the low inoculum concentrations recovery was also low except for *F. excorticata*. *P. ramorum* was consistently isolated from about 40% of *F. excorticata* leaves for all three inoculations.

Discussion

Are precautionary measures needed?

For an alien *Phytophthora* species to present a significant biosecurity problem, susceptible hosts are required, as well as favourable environmental conditions. We identified several potential hosts for *P. ramorum* from New Zealand, including symptomatic foliar (*F.*

excorticata and *P. arboreus*) and canker (*P. radiata* and *N. fusca*) hosts. Previous studies also found susceptibility of other *Fuchsia*, *Nothofagus* and *Pinus* spp. (Inman *et al*. 2002; RAPRA 2008). This expands the list of potential Australasian hosts for *P. ramorum*, highlighting the potential impacts of an incursion on the region (Hüberli *et al*. 2006b; RAPRA 2008).

Production of caducous sporangia on leaves of hosts such as *Umbellularia californica* and *Rhododendron spp.* drives the *P. ramorum* epidemic in the USA and European nurseries, respectively (Werres *et al*. 2001; Davidson *et al*. 2005). In our *in vitro* assay, at least one genotype of *F. excorticata* (the tree fuchsia) supported exceptionally high sporulation comparable to that produced by detached *U. californica* leaves (Hüberli *et al*. 2006b). *F. excorticata* is a tree (up to 15 m tall) common in lowland to lower montane forests throughout New Zealand, especially on forest margins. If inoculum dispersal is as liberal as it is for *U. californica*, also a tall forest tree, this New Zealand species could be the driving force of an epidemic similar to that observed in California. We have overlapped the occurrence of this particular species with *N. fusca*, a potential canker host, (Fig. 3), to demonstrate areas that have the highest potential risk for development of an epidemic similar to 'Sudden Oak Death'. The area characterised by sympatry of these two species is quite extensive and highlights portions of New Zealand that are indeed at risk, were *P. ramorum* to be introduced. In California, it is the areas of sympatry between the infectious *U. californica* and the dead-end hosts (oaks or tanoaks) that have witnessed the most destructive levels of the 'Sudden Oak Death' epidemic (Rizzo and Garbelotto 2003). Clearly, the potential role of *F. excorticata* as a potential vector for *P. ramorum* should be investigated further because sporulation varied among the genotypes tested. It is also possible that susceptibility of hosts may vary between individuals grown in North America and individuals growing in their native land.

We reisolated *P. ramorum* from nine of ten asymptomatic indigenous species after surface sterilisation. Sporangia were observed on all leaves, albeit at very low numbers, corroborating results from tests on UK and Mediterranean hosts (Denman *et al*. 2006). In particular, *L. scoparium*, with 64% (9/14) reisolation from asymptomatic tissue was of high concern. The global trade of plant and plant products places countries at extreme risk of inadvertent introductions of devastating pathogens, especially via unidentified hosts or asymptomatic hosts producing infectious inoculum. In New Zealand, the agricultural and horticultural industries rely on imported stock (Warmington *et al*. 1996). More than 13,000 vascular plants have been introduced into New Zealand, and almost 20% of these are now naturalised (Warmington *et al*. 1996). At such high importation levels, the chance of potentially infectious asymptomatic plant material entering into New Zealand is also much greater, further threatening the 2,287 endemic terrestrial plants, of which 20 are listed as "critical", 37 as "endangered", 62 as "vulnerable", and 79 as "rare" (Cameron *et al*. 1995).

P. radiata, which was highly susceptible in wound branch inoculations, comprises about 89% of the planted production forest resource in New Zealand (Anon. 2006). Another planted production forest species in New Zealand is *P. menziesii* (Douglas fir), also of the family Pinaceae, is a known host for *P. ramorum* (Davidson *et al*. 2003). The conifer, *D. cupressinum* (popular for quality furniture wood and an iconic indigenous species to New Zealanders), showed twig dieback in our foliar inoculation assay, with symptoms very similar to those described for *P. menziesii*. Reliable data on the susceptibility of these three species to *P. ramorum* and other alien *Phytophthora* spp. is crucial to New Zealand's timber industry, as well as to regions with *P. ramorum* where these species may be indigenous or planted in large numbers.

Further screenings using the methods employed here as well as other approaches (e.g. log inoculations described by Brasier and Jung (2006) are needed to identify plants and

ecosystems at risk of infestation by *P. ramorum*. Caution should be used in extrapolating data to infection in natural conditions using *in vitro* tests as such tests cannot fully reproduce natural conditions. Although a study like the one here described is only a first step towards the identification of potential host and geographic ranges favorable to this pathogen in New Zealand, it does have the obvious advantage of avoiding any of the risks associated with importing the pathogen to New Zealand for experimentation. The number of New Zealand species and the number of genotypes per species available at U. C. Berkeley was obviously limited. Under these constraints, we propose caution regarding species that were negative in foliar and branch inoculations, furthermore those species that were positive should be accepted as putative hosts pending a comprehensive study to determine whether resistance plays a role (see Tooley *et al*. 2004, Dodd *et al*. 2005, Hayden *et al*. 2005 and Hüberli *et al*. 2006a). Nonetheless the positive results obtained here are likely to be highly correlated to the genetic makeup of the tested species, due to the fact that all plants were from the U. C. botanical garden, and hence differential site effects on disease development were reduced to a minimum, as expected for a common garden experiment.

One approach we adopted to bypass the issue of artificial results caused by artificial conditions in the laboratory, was that of checking which plant species were susceptible *in vitro* when using low, but maybe more realistic, zoospore concentrations. At low inoculum concentrations, disease dropped markedly for all species including the known susceptible *Rhododendron* cultivars, but not for *F. excorticata*. Results from the low inoculum concentration inoculation thus identified *F. excorticata* as extremely susceptible. At higher inoculum, *F. excorticata* even surpassed the susceptible *Rhododendron* cultivars in susceptibility. Since disease was unrelated to inoculum load for *F. excorticata*, this potential host is probably extremely susceptible and we recommend it is used as an indicator for early detection of *P. ramorum* infection in high risk areas of New Zealand.

Previous studies found that detached foliar tests are often affected by host physiological factors, including leaf age and position in the canopy (Denman *et al*. 2005; Hansen *et al*. 2005). In our foliar assay, we only used mature leaves as these are less susceptible than juvenile leaves (Denman *et al*. 2005; Hansen *et al*. 2005). Additionally, leaves were always chosen from the sunny side of a plant where the potential for epidermis formation was highest. Incubation in darkness in our foliar assay was unnatural and may have contributed to lower resistance because leaves could not photosynthesize as stomatal opening was affected (see Tooley *et al*. 2004). Detached branch inoculations are widely used to assess susceptibility (Dodd *et al*. 2005). Despite the artificial nature of our inoculation assays, we showed susceptibility among species tested in excised branches and leaves. When available, whole plants exposed to natural inoculum in the field are the best substitute for detached leaves or branches as they provide holistic plant responses.

All our inoculations used the A2 mating type isolate, Pr-52. We chose this isolate because it had a known pathogenicity from our previous studies (Hüberli *et al*. 2006a) and also for comparability to other studies that used this isolate on several hosts with differing inoculation methods (Rizzo *et al*. 2002; Tooley *et al*. 2004). Whilst quarantine regulations prohibited using the A1 isolate in our inoculations, other foliar inoculation studies using both A1 and A2 mating types found no statistical difference in aggressiveness amongst isolates (Tooley *et al*. 2004; Denman *et al*. 2005). Clear differences in aggressiveness amongst the A1 and A2 mating type isolates occurred in log inoculations (Brasier 2003), but, as far as we are aware, never in foliar inoculations.

Overall, while our experiments do not definitively establish the extent of susceptibility of the New Zealand flora to *P. ramorum*, they do show clearly the potential for the spread of any introduction with possibly severe economic and conservation consequences. This argues for strong precautionary measures.

What precautionary measures should be applied?

New potential hosts were identified in this study that should be included in monitoring programs not only in New Zealand, but in all places where *P. ramorum* is regarded as a dangerous pathogen and regulated. Because some plants display no symptoms, the presence of *P. ramorum* should be screened not only by surveying for symptoms, but also by destructive sampling of a small number of plants to be assessed using the most sensitive available DNA-based assays (for instance Hayden *et al*. 2006, or Hughes *et al*. 2006). In addition, soil baiting of a subset of remaining plants could be used to identify the presence of the pathogen. Intensive screening of potential Australian hosts to *P. ramorum* is underway to identify hosts with possible high sporulation potential and develop incursion risk models (Hüberli *et al*. in press).

Our current study lists potential hosts, both foliar and stem, which may place New Zealand's native forest and the commercial plantations at risk of a *P. ramorum* incursion. While few indigenous plants were tested for susceptibility to *P. ramorum*, 12% (2/17) of them showed substantial susceptibility. Areas where *F. excorticata* and *N. fusca* are sympatric were further identified as zones extremely favorable to invasion by this pathogen; were this pathogen to be introduced in New Zealand, it is these areas that could witness the development of a forest epidemic comparable to Sudden Oak Death in California.

More species need to be screened to assess accurately the risk posed to New Zealand, and the movement of these foreign plant species within USA and Europe. But until such time, in accord with the precautionary principle, it is prudent to implement strict quarantine measures in an attempt to prevent an accidental introduction of *P. ramorum*.

Acknowledgements

We thank T. Harnik, K. Maeda, and A. Smith for technical assistance; G. Hardy, K. Howard and K. Ireland for comments and assistance with the manuscript; and two anonymous reviewers who made this paper stronger. Most plant material was kindly supplied by the University of California Botanical Garden at Berkeley. We acknowledge the use of Fig. 3. drawn from the National Vegetation Survey Database (NVS); © Manaaki Whenua - Landcare Research New Zealand Ltd 2004. The project was funded by Biosecurity New Zealand, the Betty and Gordon Moore Foundation, and the Pacific Southwest Research Station USDA Forest Service.

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Table 1. Disease parameters assessed on excised branches of 18 New Zealand (NZ) plant species (indigenous, exotic and commercial) and two *Rhododendron* cultivars (in bold; known susceptibles) wound inoculated with *Phytophthora ramorum* colonised agar discs and incubated in the greenhouse for 10 days

Species are sorted by lesion area in ascending order

Species[A]	Family	Plant status in NZ	Plants (n)	K-mean cluster groups[B]	Lesion length (mm) ±SE[C]	Symptomatic ratio	Pathogen reisolation	Branches girdled
Brachyglottis repanda	Asteraceae (Compositae)	Indigenous	1	1	2.3	3/4	3/4	0/4
Dacrydium cupressinum	Podocarpaceae	Indigenous	1	1	4.0	2/4	2/4	0/4
Olearia bullata	Asteraceae (Compositae)	Indigenous	1	1	4.0	3/4	1/4	0/4
Dacrycarpus dacrydioides	Podocarpaceae	Indigenous	2	1	4.3 ±0.8	1/4	1.5/4	0/4
Sophora microphylla	Fabaceae (Leguminosae)	Indigenous	1	1	4.8	2/4	2/4	0/4
Leptospermum scoparium	Myrtaceae[D]	Indigenous	1	1	5.5	2/4	2/4	0/4
Corynocarpus laevigatus	Corynocarpaceae	Indigenous	1	1	6.0	4/4	3/4	0/4
Prumnopitys spicata[E]	Podocarpaceae	Indigenous	3	1	6.3 ±3.7	3.5/4	3.5/4	0.5/4
Nothofagus menziesii	Nothofagaceae[D]	Indigenous	1	1	6.5	2/4	3/4	0/4
Eucalyptus globulus	Myrtaceae[D]	Exotic/ commercial	1	1	7.0	3/4	0/4	0/4
Fuchsia excorticata	Onagraceae	Indigenous	2	1	7.4 ±4.0	3.5/4	2.5/4	0/4
Coprosma robusta	Rubiaceae	Indigenous	1	1	8.0	2/4	3/4	0/4
Agathis australis	Araucariaceae	Indigenous	2	1	8.5 ±0.5	1/4	1.5/4	0/4
Pseudopanax arboreus	Araliaceae	Indigenous	1	1	14.3	3/4	2/4	1/4
Acacia melanoxylon	Fabaceae (Leguminosae)	Exotic/ commercial	1	1	15.0	2/4	4/4	0/4
Pomaderris prunifolia	Rhamnaceae[D]	Indigenous	1	1	17.5	2/4	4/4	0/4
Pinus radiata	Pinaceae[D]	Exotic/ commercial	1	2	22.8	4/4	3/4	1/4
***Rhododendron* cultivar ('White Dwarf' azalea)**	**Ericaceae[D]**	**Exotic**	**2**	**2**	**24.1 ±5.6**	**2.5/4**	**3/4**	**1/4**
Nothofagus fusca	Nothofagaceae[D]	Indigenous	1	2	26.6	4/4	3/4	3/4
***Rhododendron* cultivar ('Cunningham's White' rhododendron)**	**Ericaceae[D]**	**Exotic**	**2**	**2**	**51.1 ±2.1**	**4/4**	**3.5/4**	**2.5/4**

[A]*Pittosporum eugenioides* and *Melicytus ramiflorus* were tested leaf inoculations, but not in branch inoculations.

[B]K-mean cluster groups determined on all four disease parameters assessed; the variables lesion length, symptomatic ratio and branches girdled were significant ($P < 0.02$).

[C]SE presented only for species with more than one replicate plant.

[D]Family containing at least one known host for *P. ramorum* (RAPRA, 2008).

[E]For *Prumnopitys spicata*, one plant of three was not infected and the pathogen was not reisolated; it was not included in the data presented.

Table 2. Disease parameters assessed on excised leaves of 18 New Zealand (NZ) plant species (indigenous, exotic and commercial) and two *Rhododendron* cultivar (in bold; known susceptibles) inoculated with zoospores *Phytophthora ramorum* and incubated for 6 days at 19°C

Species are sorted by lesion area in ascending order

Species[A]	**Family**	**Plant status in NZ**	**Plants (n)**	**K-mean cluster groups**[B]	**Lesion area (mm^2) ±SE**[C]	**Symptomatic ratio**	**Pathogen reisolation**	**Leaves with sporangia**	**Sporangia /leaf ±SE**[C]
Acacia melanoxylon	Fabaceae (Leguminosae)	Exotic/ commercial	1	1	0	0/14	0/14	-[D]	-
Agathis australis	Araucariaceae	Indigenous	2	1	0	0/14	0/14	9/14	1.5 ±0.5
Brachyglottis repanda	Asteraceae (Compositae)	Indigenous	1	1	0	0	2/14	5/14	1.8
Corynocarpus laevigatus	Corynocarpaceae	Indigenous	1	1	0	0	2/14	12/14	6.1
Dacrycarpus dacrydioides	Podocarpaceae	Indigenous	2	1	0	0/14	4/14	7/14	1.9 ±0.3
Eucalyptus globulus	Myrtaceae[E]	Exotic/ commercial	1	1	0	0/14	0/14	-	-
Leptospermum scoparium	Myrtaceae[E]	Indigenous	1	1	0	0/14	9/14	9/14	12.0
Melicytus ramiflorus	Violaceae	Indigenous	1	1	0	0	3/14	13/14	4.5
Nothofagus fusca	Nothofagaceae[E]	Indigenous	1	1	0	0	4/14	9/14	3.6
Nothofagus menziesii	Nothofagaceae[E]	Indigenous	1	1	0	0	2/14	8/14	2.2
Pinus radiata	Pinaceae[E]	Exotic/ commercial	1	1	0	0/7	0/7	0/7	0.0
Pomaderris prunifolia	Rhamnaceae[E]	Indigenous	1	1	0	0	5/14	6/14	1.2
Prumnopitys spicata	Podocarpaceae	Indigenous	3	1	0	0	0.3/14	8.7/14	7.0 ±2.7
Dacrydium cupressinum	Podocarpaceae	Indigenous	1	1	10.8	3/14	12/14	10/14	2.8
Pittosporum eugenioides	Pittosporaceae[E]	Indigenous	1	1	22.5	5/14	7/14	101/4	3.8
***Rhododendron* cultivar ('White Dwarf' azalea)**	**Ericaceae[E]**	**Exotic**	**2**	**1**	**32.7 ±4.1**	**7/28**	**17/28**	**9/14**	**9.8 ±6.8**
Coprosma robusta	Rubiaceae	Indigenous	1	1	39.9	2/14	2/14	12/14	1.6
Fuchsia excorticata	Onagraceae	Indigenous	2	2	81.5 ±6.4	14/14	5.5/14	11/14	65.3 ±59.7
***Rhododendron* cultivar ('Cunningham's White' rhododendron)**	**Ericaceae[E]**	**Exotic**	**2**	**2**	**119.1 ±3.9**	**12.5/28**	**10.5/28**	**12.8/14**	**23.5 ±10.2**
Pseudopanax arboreus	Araliaceae	Indigenous	1	2	216.3	2/14	3/14	12/14	3.5

[A]*Olearia bullata* and *Sophora microphylla* were tested in branch inoculations, but not in leaf inoculations.

[B]K-mean cluster groups determined on all five disease parameters assessed; the variables lesion area, symptomatic ratio and sporangia/leaf were significant ($P < 0.002$ in all cases).

[C]SE presented only for species with more than one replicate plant.

[D]No data.

[E]Family containing at least one known host for *P. ramorum* (RAPRA, 2008).

Table 3. K-means clustering of five New Zealand species and two *Rhododendron* cultivars in foliar inoculations with *Phytophthora ramorum* zoospores at three different concentrations. For each concentration, the table shows: Cluster (which of two clusters the species belongs to), cluster means (the mean lesion area, symptomatic ratio and pathogen reisolation proportion) for the species in each cluster, and analysis of variance statistics testing for differences in these disease variables between the two clusters at each concentration.

Species	**Cluster** [A]	**Cluster means**		
		Lesion area	**Symptomatic ratio**	**Pathogen reisolation proportion**
$1x10^2$ zoospores/mL:				
Fuchsia excorticata	1	84.2	21.4	35.7
Dacrydium cupressinum	2			
Nothofagus fusca	2			
Pinus radiata	2			
Pittosporum eugenioides	2			
Rhododendron cultivar (‘White Dwarf’ azalea)	2			
Rhododendron cultivar (‘Cunningham’s White’ rhododendron)	2			
	Mean (Cluster 2) =	2.9	45.8	8.6
	Analysis of variance	$F_{(1,5)}$=2388.4; P<0.0001	$F_{(1,5)}$=6.9; P=0.05	$F_{(1,5)}$=8.0; P<0.04
$5x10^2$ zoospores/mL:				
Fuchsia excorticata	1			
Nothofagus fusca	1			
Rhododendron cultivar (‘White Dwarf’ azalea)	1			
Rhododendron cultivar (‘Cunningham’s White’ rhododendron)	1			
	Mean (Cluster 1) =	24.6	53.2	51.9
Dacrydium cupressinum	2			
Pinus radiata	2			
Pittosporum eugenioides	2			
	Mean (Cluster 2) =	3.6	50.0	5.2
	Analysis of variance	$F_{(1,5)}$=0.80; P=0.41	$F_{(1,5)}$=0.02; P=0.88	$F_{(1,5)}$=35.4; P=0.002
$5x10^3$ zoospores/mL:				
Fuchsia excorticata	1			
Rhododendron cultivar (‘White Dwarf’ azalea)	1			

	Mean (Cluster 1) = 2	96.8	69.6	57.1
Dacrydium cupressinum	2			
Nothofagus fusca	2			
Pinus radiata	2			
Pittosporum eugenioides	2			
Rhododendron cultivar (‘Cunningham’s White’ rhododendron)	2			
	Mean (Cluster 2) =	7.3	57.1	30.2
	Analysis of variance	$F_{(1,5)}$=17.1; *P*=0.009	$F_{(1,5)}$=0.38; *P*=0.56	$F_{(1,5)}$=1.5; *P*=0.27

[A]K-means clustering determined using lesion area, symptomatic ratio and pathogen reisolation proportion

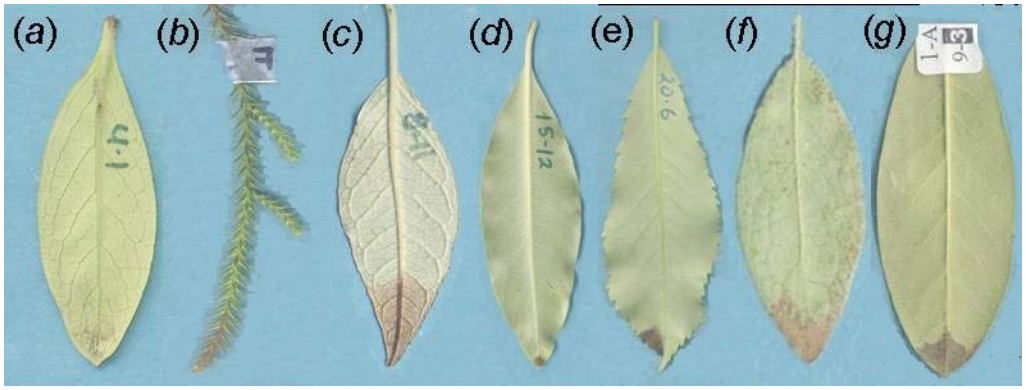

Fig. 1. Leaves of (*a*) *Coprosma robusta*, (*b*) *Dacrydium cupressinum*, (*c*) *Fuchsia excorticata*, (*d*) *Pittosporum eugenioides*, (*e*) *Pseudopanax arboreus*, (*f*) *Rhododendron* cultivar ('White Dwarf' azalea) and (*g*) *Rhododendron* cultivar ('Cunningham's White' rhododendron) showing lesions 6 days after incubation in the dark with zoospores of *Phytophthora ramorum*.

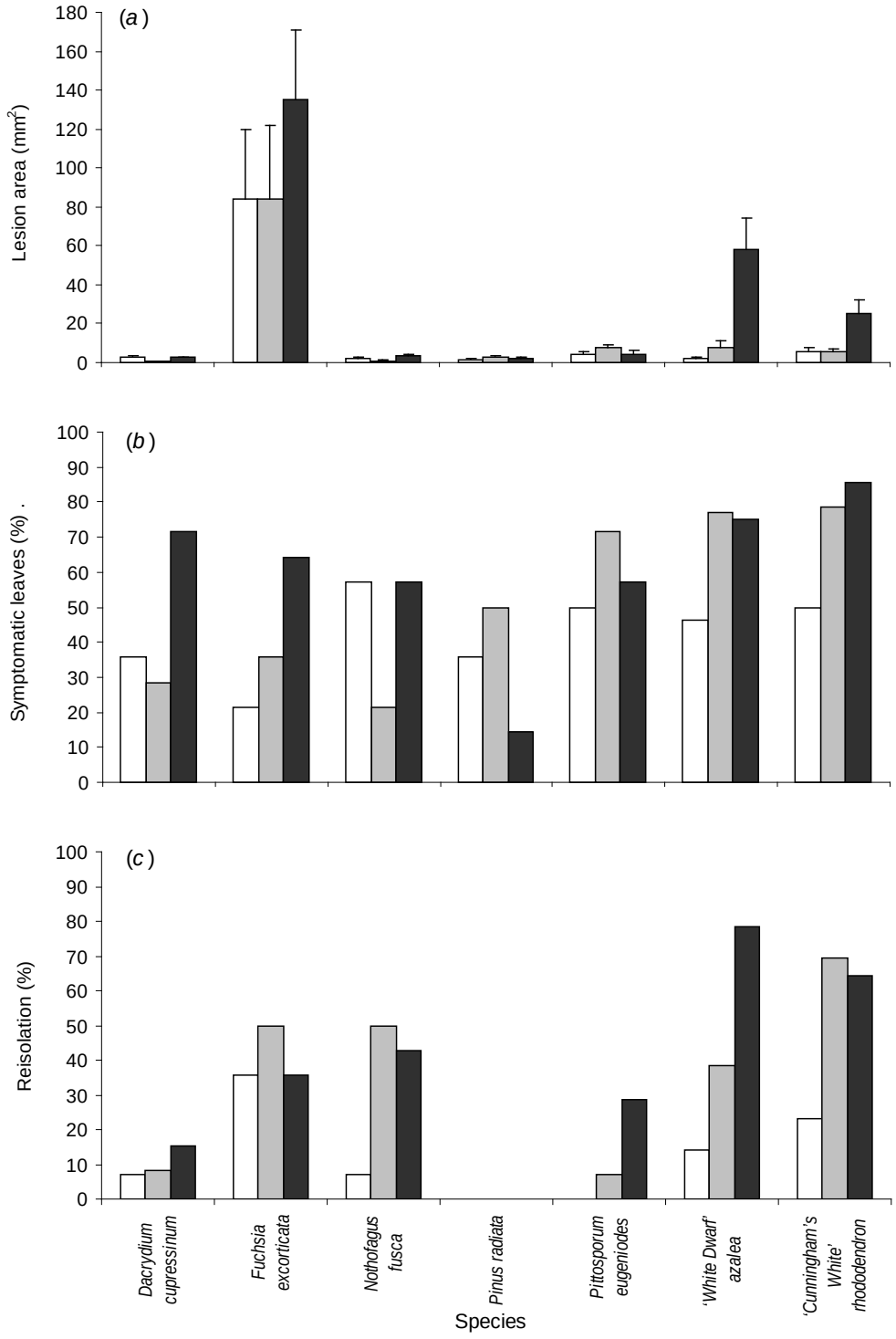

Fig. 2. (*a*) Mean lesion area (±SE), (*b*) Symptomatic leaves of total inoculated (%) and (*c*) Pathogen reisolation (%) of *Phytophthora ramorum* on selective agar from excised leaves of seven plant species inoculated with three zoospore concentrations (□ $1x10^2$, ▫ $1x10^3$ and ■ $5x10^3$ zoospores/mL) of *P. ramorum* after 6 days incubation at 19°C; N = 12-14.

Fig. 3. Distribution of *Nothofagus fusca* (●) and *Fuchsia excorticata* (●) in New Zealand.