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Potential susceptibility of Australian flora to a NA2 isolate of *Phytophthora ramorum* and pathogen sporulation potential

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Summary

Phytophthora ramorum is an invasive plant pathogen and the cause of considerable and widespread damage in nurseries, gardens and natural woodland ecosystems of the USA and Europe. It is considered to be a significant plant disease as it could cause biodiversity loss and severe economic losses in plant industries in areas where it is not yet known to exist, such as Australasia. Foliar susceptibility and sporulation potential were tested using detached-leaf assays for 70 Australian native plant species sourced from established gardens and arboreta in California using a NA2 isolate of *P. ramorum*. *Correa* 'Sister *Dawn*', *Eucalyptus regnans*, *Isopogon cuneatus*, *I. formosus*, *Leptospermum scoparium*, *L. lanigerum* and *Melaleuca squamea* were identified as potentially highly susceptible host species. *Hedycarya angustifolia*, *Olearia argophylla*, *Phyllocladus aspleniifolius, Pittosporum undulatum* and *Podocarpus lawrencei* were identified as potentially resistant. All 70 species were able to be infected with *P. ramorum*, as confirmed by reisolation. Putative sporulating hosts include five members of the Myrtaceae, *Agonis flexuosa*, *Corymbia ficifolia*, *Eucalyptus haemastoma, E. delegatensis* and *E. viminalis*. As a part of a precautionary strategy, the potentially highly susceptible species found in this study are suitable candidates for targeted surveillance programmes in high-risk incursion areas of Australia and within the global horticultural trade.

1 Introduction

Phytophthora ramorum is an invasive plant pathogen causing widespread damage in nurseries, gardens and natural woodland ecosystems of the USA and Europe (Werres et al. 2001; Rizzo et al. 2005; Brasier and Webber 2010). It is classified as a category 1 plant pest risk to Australian plant biosecurity (i.e. a pest that if not eradicated would cause major damage to both natural ecosystems and plant industries/amenity flora) (Plant Health Australia 2006) and is internationally recognized as a plant biosecurity threat. At least 68 countries, including South Korea, Canada, Mexico, Taiwan and New Zealand, have established quarantine policies and protocols against plant materials from areas known to have the pathogen (Sansford et al. 2009). Spread through the international nursery trade (Brasier 2008), *P. ramorum* can completely alter natural ecological landscapes and cause considerable economic losses (Rizzo et al. 2005; Dart and Chastagner 2007; Cobb et al. 2010). In the USA alone, it has caused extensive mortality of trees and shrubs in natural woodlands of California and Oregon (Meentemeyer et al. 2008), and its presence has imposed significant economic costs and hardships on nursery operators within quarantine areas affected by the disease (Dart and Chastagner 2007). It is of particular interest to Australian plant biosecurity as, like *P. cinnamomi*, another invasive *Phytophthora* species causing severe dieback in Australia (Environment Australia 2001; Shearer et al. 2007), it has the potential to become a major economic and ecological threat in areas with susceptible hosts and suitable climates.

The known worldwide host range of *P. ramorum* continues to grow, with more than 120 species of trees, shrubs and herbs (encompassing more than 25 plant families) affected in wildlands and nurseries of Europe and North America (RAPRA 2007; USDA-APHIS 2010), all of which must be managed according to their susceptibility and ability to drive potential epiphytotics. For example, more than a decade after it was first discovered in natural woodlands of the UK, two epidemiologically important sporulating hosts, *Vaccinium myrtillus* in heathlands (Sansford et al. 2010) and Japanese larch (*Larix kaempferi*) in plantations (Brasier and Webber 2010) have been discovered, increasing the mortality rates of susceptible plant species considerably and resulting in further management and quarantine efforts to contain the pathogen.

Two Australian host species, *Eucalyptus haemastoma* (Scribbly Gum) and *Pittosporum undulatum* (Sweet Pittosporum), have been listed as associated hosts of *P. ramorum*, based on field observations and pathogenicity tests in the USA and Europe (Hüberli et al. 2006; RAPRA 2007). In addition, *Eucalyptus gunnii* (Cider Gum) and *E. dalrympleana* (White Mountain Gum) have been found to be susceptible using artificial inoculation methods in the UK and Spain (Denman et al. 2005a; Moralejo et al. 2009). Similarly, *E. regnans* has been identified as a potential bole canker host, and a range of potential Australian branch hosts were identified in studies conducted in California (Ireland et al. 2011). Given the wide and increasing host range of *P. ramorum* and evidence of a multiple-host method of dispersal (Moralejo et al. 2006), it is expected that many more Australian native plant species are potentially susceptible and sporulating hosts.

Phytophthora ramorum causes three distinct diseases on susceptible plants: Ramorum Leaf Blight, Ramorum Shoot Dieback and Sudden Oak Death (characterized by lethal bole cankers) (Hansen et al. 2005). While all components of the disease are

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important when understanding potential impacts, foliar infection of some species has been found to play a crucial role in transmission of the pathogen in the UK (*Rhododendron* spp.) and California (*Umbellularia californica*, California Bay Laurel; and *Notholithocarpus densiflorus*, Tanoak) by providing key sources of inoculum that drive epiphytotics (Goheen et al. 2002; Brasier et al. 2004; Davidson et al. 2005). Detached foliar assays have been used by a number of authors to assess susceptibility and sporulation potential of a range of species to *P. ramorum in vitro* (Parke et al. 2002; Denman et al. 2005a; Hansen et al. 2005; Denman et al. 2006a; Hüberli et al. 2008). These methods have been confirmed as a good indicator of field susceptibility when compared with natural infection and other methods of inoculation (Hansen et al. 2005).

Australian quarantine restricts the entry of all materials that fall into known host genera of *P. ramorum*, from areas known to have the pathogen (Sansford et al. 2009). Given our incomplete knowledge of the host range and geographical origin of *P. ramorum*, research into the potential host range of Australian native species was undertaken to make an accurate assessment of the risk that it may pose to Australian and international plant biosecurity. Detached foliar assays were used to assess the susceptibility and sporulation production potential to *P. ramorum* of a range of Australian native species representative of climatic zones in Australia where the pathogen is predicted to survive and sporulate. Because of the quarantine status of the pathogen in Australia, all assays were conducted in Davis, California, on Australian plant material sourced from established gardens and arboreta throughout Northern California. The results of these assays are discussed and related to quarantine and management recommendations for Australian and international plant biosecurity.

2 Materials and methods

2.1 Experimental design

In vitro leaf inoculations of Australian native plants were used to determine potential foliar susceptibility to *P. ramorum* and sporangia production potential in 23 experiments between April 2008 and October 2009 at Davis, California, USA (Table 1). Potential foliar susceptibility was tested by examining measures of disease incidence, severity and infectivity over 16 experiments, eleven of which were conducted under 'summer' conditions and five of which were conducted under 'winter' conditions. Sporulation potential on the foliage was tested over four experiments in spring (May/June) 2009, and the influence of temperature on sporulation potential was tested in two experiments in October 2009.

Table 1. Details of detached foliage experiments used to test the susceptibility and sporulation potential of native Australian plant species to *Phytophthora ramorum*.

Experiment	Year	Month started	Location	Inoculation group/season	Non-wounded	Wounded	No. of species[1]
Susceptibility tested using leaf dip inoculation							(69)
F-01	2008	April	UCD	Summer	●	●	8
F-02	2008	May	SFBG	Summer	●	●	9
F-03	2008	May	SFBG	Summer	●	●	7
F-04	2008	May	SFBG	Summer	●	●	11
F-05	2008	June	UCD	Summer	●	●	6
F-06	2008	June	UCB	Summer	●	●	14
F-07	2008	June	UCSC	Summer	●	●	15
F-08	2008	June	UCSC	Summer	●	●	14
F-09	2008	July	UCSC	Summer	●	●	14
F-10	2008	Nov	UCD	Winter	●		14
F-11	2008	Nov	SFBG	Winter	●		24
F-12	2008	Dec	UCB	Winter	●		12
F-13	2009	Jan	UCSC	Winter	●		17
F-14	2009	Jan	UCSC	Winter	●		23
F-15	2009	May	UCSC	Summer	●	●	4
Susceptibility of lilioid monocot species tested using agar plug inoculation							(3)
A-01	2008	June	UCSC	Summer		●	3
Inoculum concentration study							(5)
I-01	2009	Jan	UCSC	Winter	●		5
Sporulation potential							(28)
S-01	2009	May	UCD	Spring	●		6
S-02	2009	May	SFBG	Spring	●		12
S-03	2009	May	UCSC	Spring	●		11
S-04	2009	June	UCSC	Spring	●		8
Temperature and sporulation potential							
T-01	2009	Oct	UCD	Autumn	●		3
T-02	2009	Oct	UCD	Autumn	●		3

SFBG, San Francisco Botanical Garden & Strybing Arboretum; UCB, University of California (UC) Berkeley Gardens; UCD, UC Davis Arboretum; UCSC, UC Santa Cruz Arboretum. (●) indicate which leaf treatments (non-wounded and/or wounded) were included in the experiment.

[1]Total number of species in brackets for each experiment type, species were replicated over inoculation groups and some had multiple individual plants tested per species. Positive control species *Rhododendron* 'Colonel Coen' was included in all experiments and *Umbellularia californica* was included in all sporulation experiments.

2.2 Isolate and inoculum production

Isolate Pr-510 (University of California (UC) Davis, D. Rizzo Laboratory Culture Collection) of the NA2 lineage, isolated from *Rhododendron* roots from a nursery in Sacramento in 2006, was used in all experiments. It was shown to be highly pathogenic on both *U. californica* and *Rhododendron* cultivar 'Colonel Coen' and fast growing on both one-third-strength clarified V8 juice agar (Campbell Soup Company, Camden, NJ, USA; 66 ml of clarified V8 juice and 17 g of agar/l) and the *Phytophthora*-selective medium, pimaricin–ampicillin–rifampicin–pentachloronitrobenzene agar (PARP) (Jeffers and Martin 1986), when compared with other isolates, including the commonly used NA1 genotype isolate Pr-52 (Hüberli et al. 2008) (data not shown). The isolate was passaged through detached *R.* 'Colonel Coen' leaves at the beginning of each inoculation group (i.e. 'summer' and 'winter') to maintain pathogenicity and maintained on PARP. Inoculum was cultured on one-third-strength clarified V8 juice agar. Zoospores were produced using a modified method of Parke et al. (2002). Briefly, plugs of mycelia were removed from 5-day-old cultures, transferred to a sterile soil water solution and incubated for 48 h at 20°C in the dark. Once sporangia were observed, zoospores were obtained by decanting plugs and soil water solution into a sterile beaker, cold shocking them in the refrigerator at 7°C for 1 h and then returning them to room temperature for 75–90 min to induce zoospore release. The resulting zoospore suspension was filtered through four layers of cheesecloth into a sterile beaker. A 1-ml subsample of inoculum was vortexed to initiate zoospore encystment, and the concentration of the zoospore suspension was determined with a haemocytometer. The concentration of each suspension was adjusted to approximately 2×10^4 zoospores/ml. To determine viability and possible dilution of inoculum because of continued leaf-dipping, three aliquots of 10 μl of the suspension in each beaker were spread onto PARP agar plates before, mid-way through and at the end of each leaf-dipping session. These plates were incubated at 20°C for 2–4 days in the dark, and the number of colony-forming units was counted.

2.3 Host plants and preparation of plant material

Seventy Australian native plant species within 24 families and 43 genera were sourced from mature healthy plants in established gardens and arboreta in Northern California: San Francisco Strybing Arboretum, University of California (UC) Berkeley Botanical Garden, UC Davis Arboretum and UC Santa Cruz Arboretum. Species were selected from areas in their natural Australian range considered to have climates suitable for *P. ramorum* survival, based on observations of suitable climate for the pathogen in the USA and Europe, and a preliminary CLIMEX (Sutherst et al. 2007) model was developed by E.A. Pinkard and I.W. Smith (personal communication) using the parameters published by Venette and Cohen (2006), as well as for their ecological and economic importance to Australian plant industries. Individuals of a species were duplicated where possible from different locations or accessions (plant material was limited by the extent of the botanical collections) to give a total of 135 individual plants tested. The known susceptible host *R.* 'Colonel Coen' (kept in controlled environment facilities and greenhouses at UC Davis) was used as a positive control species in all experiments to confirm pathogenicity of *P. ramorum*. Likewise, *U. californica* (sourced from a private garden in Davis, California) was included in all sporangia production experiments and in one foliar susceptibility experiment (F-15; Table 1) as a positive control species.

Branches of each individual were collected the day before inoculations were undertaken, and cut stems and branches were kept in deionized water overnight. Before inoculation, leaves were cut at the base of the petiole from branches, rinsed with deionized water and placed on paper towels to air-dry. Mature, fully-expanded leaves were used for all species. Juvenile-aged leaves were tested for 24 of the test species, as well as for *R.* 'Colonel Coen'. Juvenile leaves were included to account for overall susceptibility of the test plants and to test for differences in susceptibility between leaf ages.

Plants from the UC Santa Cruz Arboretum were visually inspected and treated with insecticide before shipping to UC Davis, in accordance with California's Light Brown Apple Moth (*Epiphyas postvittana*) quarantine regulations at the time. Insecticide treatments were made up in water with either DiPel (*Bacillus thuringiensis*; Abbot Laboratories, Chicago IL, USA) at 1.6–3.9 ml/l of water and Vegol (canola oil; Lilly Miller Brands, Clackamas OR, USA) at 3.9–19.5 ml/l or Sunspray Oil (Paraffinic Oil; Sun Refining & Marketing Co., Philadelphia PA, USA) at 6.5 ml/l during the 'summer' and with Conserve SC (Spinosad; Dow Agrosciences LLC, Indianapolis IN, USA) at 1.7 ml/l and Bonide All Seasons Spray Oil (Petroleum Oil, Oriskany, NY, USA) at 10 ml/l during the 'winter' inoculations. These species were rinsed well with deionized water upon arrival in Davis to remove the insecticides. A preliminary test (data not shown) showed that insecticide applications did not significantly influence host susceptibility to *P. ramorum* for *Agonis flexuosa*, *Corymbia ficifolia*, *Eucalyptus sideroxylon*, *E. viminalis* and *R.* 'Colonel Coen'.

2.4 Susceptibility testing

Foliage of 69 of the 70 Australian plant species studied was tested for susceptibility to *P. ramorum* using a detached-leaf dip assay adapted from a method of Parke et al. (2002) and modified by Denman et al. (2005a) over 15 of the 16 foliar susceptibility experiments (Table 1). Host plants were divided into three leaf categories, namely needle-like conifer, broad-leaf and odd-leaf species – according to foliage morphology so as to allow for different disease assessment methods; and two treatment groups, wounded and non-wounded. Odd-leaf species were those with asymmetrical (i.e. highly lobed species such as *Brachychiton populneus*) or small (often <1 cm in length) leaves, making inter-comparison with other species very difficult. Leaves were dipped in inoculum to an approximate mid-way point on each leaf for 1 min each. Two conifers, *Phyllocladus aspleniifolius* and *Podocarpus lawrencei*, were treated as broad-leaf species and the other two, *Callitris rhomboidea* and *Lagarostrobus franklinii*, were treated as needle-like conifers. The needle-like conifers were inoculated to the mid-way point of each needle, with wounded inoculations conducted by excising approximately 1 mm of the needle tips before inoculation. Broad-leaf-wounded inoculations were conducted by cutting off the petiole, making two v-shaped incisions in the basal half of

the leaf and inoculating the leaf from the basal end, while non-wounded inoculations were made by immersing the non-wounded distal half of the leaf. Odd leaves were inoculated in the same manner as broad-leaf species. Non-inoculated control leaves of each species and treatment group were dipped in sterile deionized water.

Xanthorrhoea australis, a perennial long-lived monocot species with long narrow leaves (2–4 mm diam), was tested by placing a *P. ramorum* colonized agar plug (2 mm diam) over a wound created by a 15 gauge (approximately 1.8 mm diam) hypodermic needle and attached to the leaf. *Lomandra longifolia* and *Xanthorrhoea preissii*, tested using the leaf dip method, were also inoculated in this manner to test the potential suitability of this inoculation method for the grass-like, lilioid monocot species.

Three to 24 hosts were tested in any one experiment, based on collection from common locations, plant family and easy management of material (Table 1). Experiments were conducted during two inoculation periods, defined as 'summer' and 'winter', as the material was collected in warmer or cooler months of the year, respectively (Table 1), and the inoculation chamber conditions were regulated to reflect these seasons. Broad-leaf and conifer inoculations were performed in both inoculation periods. Odd-leaf species and the effect of wounding were assessed only during the 'summer' inoculations. 'Summer' experiments were carried out from April to July of 2008 and in May 2009, while 'winter' experiments were carried out from November 2008 to January 2009. Ten to twenty leaves of each individual plant were inoculated in the summer studies and ten to fifteen leaves in the 'winter' studies. Inoculated material was placed on raised mesh trays in moist transparent plastic chambers and kept in temperature-controlled facilities (PGR15, 2002; Conviron Controlled Environment Ltd, Winnipeg, MB, Canada) with cyclic regimes of 20–25°C and 16 h photoperiod during 'summer' inoculations and 15–20°C and 12 h photoperiod during 'winter' inoculations. Lower temperatures occurred during dark periods overnight, to simulate natural conditions. Chambers were checked regularly throughout the experiment and sprayed when necessary with deionized water to ensure that they remained moist and humid. At the end of each experiment, all leaves were scanned using a flatbed scanner to obtain a digital record of lesion size, and two or more pieces of plant tissue (approximately 4–10 mm^2) per leaf were then plated onto PARP to confirm infection by *P. ramorum*. Leaves were surface sterilized in 70% ethanol for 30 s, rinsed in sterile deionized water and isolations were made from the margins of lesions when present; otherwise, pieces were selected randomly from the inoculated area.

Response of the hosts to *P. ramorum* was assessed by adapting the methods of Denman et al. (2005a). Three parameters were used to evaluate disease development 6–8 days after inoculation. Disease incidence (parameter 1) was a record of presence or absence of necrosis, based on visual inspection only. Disease severity (parameter 2) was recorded as a proportion of necrotic needles per shoot for coniferous hosts (*C. rhomboidea* and *L. franklinii*) and as a measure of the percentage necrotic surface area for odd- and broad-leaf hosts, calculated from the scanned digital images taken at the completion of the experiment using the image analysis software ASSESS v1.01 (APS Press, St Paul, MN, USA). For the three lilioid monocot species inoculated with an agar plug, lesion length along the length of the leaf was recorded as a measure of disease severity 7 days after inoculation. Leaf infection (parameter 3) was an indication of presence or absence of *P. ramorum* infection per leaf, as confirmed by reisolation, allowing for calculation of the proportion of infected leaves for all species.

2.5 Inoculum concentration study

During winter, January 2009, the effect of inoculum concentration was tested on five broad-leaf hosts (*Corymbia ficifolia*, *Correa reflexa*, *Eucalyptus denticulata*, *Isopogon cuneatus* and *Lomatia myricoides*; one plant each) sourced from the Santa Cruz Arboretum (Table 1), which were shown to be highly susceptible from previous experiments. The positive control *R.* 'Colonel Coen' was also tested. Inoculations were as described earlier, using only non-wounded leaves, with concentrations of inoculum made to 2×10^2, 2×10^3 and 2×10^4 zoospores/ml. Leaves were placed directly onto moist paper towels and kept in a moist chamber at room temperature under laboratory light conditions (approximately a 12-h photoperiod) in the laboratory for 6 days.

2.6 Sporulation potential study

Twenty-four broad-leaf and four odd-leaf Australian species (*Acacia dealbata*, *Dicksonia antarctica*, *Isopogon formosus* and *Leptospermum scoparium*) were selected for further studies of sporulation potential based upon their position in the canopy, prevalence in the nursery trade, previous susceptibility and their provenance from moist Australian environments suitable for *P. ramorum* spread (see Table 4). Mid- to upper-canopy species were preferentially selected as it was assumed, based on the Californian and UK epiphytotics, that rain-splash and wind-driven inoculum from these heights would be more likely to reach a wider range of hosts across a forest and present a significant ephiphytotic risk. Individuals of a species were duplicated where possible from different locations or accessions, to give a total of 37 individual plants tested. These leaves were collected, stored and prepared as previously described. Seven to twelve host plants were tested in any one experiment, forming four inoculation groups, from May to early June of 2009 (Table 1). The timing of the studies coincided with the end of the rainy season in Northern California, when high rates of sporangia production have been recorded (Davidson et al. 2005). The positive controls *R.* 'Colonel Coen' and *U. californica* were included in each experiment. An additional five juvenile leaves were collected from *Acmena smithii*, *A. flexuosa*, *C. ficifolia*, *Eucalyptus globulus*, *E. haemastoma*, *E. viminalis* and *P. undulatum* to test for potential effects of leaf age on these species.

Sporulation potential was tested using a method adapted from Denman et al. (2006a). Leaves were placed on racks in moist, transparent chambers, and up to 100 μl of inoculum (2×10^4 zoospores/ml) was applied as a drop of fluid close to the midrib on the abaxial surface of non-wounded leaves. Non-inoculated control leaves were treated with sterile water only. Leaves were incubated at a constant 20°C with a 16-h photoperiod in temperature-controlled facilities (PGR15, 2002; Conviron Controlled

Environment Ltd). The inoculum drop was removed after 24 h using a paper tissue. Chambers were kept moist by spraying regularly with deionized water. Nine days after inoculation, a large drop of sterile deionized water, sufficient to cover the lesion or inoculation area, was placed on each leaf for 24 h, after which the water droplets were removed from the leaves and transferred to 2-ml microtubes. The leaf surface below the droplet was gently scraped with a rounded scalpel blade (No. 24) to free sporangia from the leaf surface. A 200-μl drop of sterile water was placed on the inoculum spot to suspend the scrapings, then removed and added to the microtube using a pipette. This was repeated once more for the abaxial side and then once again for the adaxial side of the leaf to capture any additional sporulation on this surface. A 5-μl drop of cotton blue (5%; C.I. 42780) in lactophenol was added to each tube, and the tubes were placed in a refrigerator at 7°C until counting could take place, from 1 day to 6 months later. Leaves were then scanned using a flatbed scanner to record lesion size and returned to their moist chambers for a further 5 days. After 15 days, the leaves were scanned again, to capture any increase in lesion size during this time, and then destructively sampled to confirm infection by *P. ramorum*. Two pieces of tissue (approximately 4–10 mm^2) from the lesion or inoculation area per leaf were plated onto PARP to confirm infection by *P. ramorum*.

Sporangial suspensions were centrifuged at 1585 ***g*** for 3 min, and all excess liquid removed. The remaining 20–100 μl of liquid was agitated using a vortex stirrer for 30 s, and one to four drops of 20 μl each dispensed onto glass slides for counting. Sporangia were counted for each leaf using a compound microscope at 50 × or 100 × magnification, depending on the concentration of the suspension. Because of relatively high concentrations of sporangia on the positive control *R.* 'Colonel Coen', an approximate count of sporangia was made. This was performed by reducing the suspension to a 1-ml solution by centrifuging and pipetting, agitating the solution using a vortex stirrer and inversion, and then calculating an approximate sporangia count based on the average of three 20-μl aliquots of solution. Leaves were assessed based upon presence or absence of sporangia, as well as the number of sporangia per lesion, or inoculation point (if no lesion present) and the number of sporangia produced per cm^2 of lesion area per leaf.

2.7 Temperature and sporulation potential

During autumn, October 2009, the effects of temperature and incubation period were tested for three Australian native species (Table 1), known from our studies to produce sporangia: *Agonis flexuosa*, *Corymbia maculata* and *Eucalyptus viminalis*. All were sourced from UC Davis, and the study included the positive control species *R.* 'Colonel Coen' and *U. californica*. Five fully-expanded mature leaves of all species, including the positive controls, and three to five juvenile leaves of the Australian species were inoculated, incubated and harvested as described earlier for the sporangia potential study. Leaves were incubated at three constant temperatures (15, 20, and 25°C) and for three time periods (3, 6 and 9 days), with a 16-h photoperiod. All leaves were moistened with a large drop of sterile deionized water sufficient to cover the lesion or inoculation area that had already developed at least 24 h before harvesting the sporangia and at 2, 5 and 8 days. The experiment was repeated once.

2.8 Statistical analysis

Statistical analysis of susceptibility and sporulation potential was performed with SAS software (version 9.1; SAS Publishing, Cary, NC) using fixed effects multivariate methods. Disease incidence (parameter 1) and leaf infection (parameter 3) were analysed using a binomial generalized linear model with a logit link. Disease severity (parameter 2) was analysed using a log + 0.01 transformation and a general linear model. Use of these multivariate methods allowed for comparison across an unbalanced data set, utilizing all of the data in the one statistical run per parameter and weighting for significant results appropriately. Predictions of the means generated by the models are presented, as given that the experiment is so complex, we believe that they represent a more appropriate comparative point amongst the species, which were tested over a number of individual experiments. Predictions represent how the statistical model predicts each species would behave under each condition given the effects of season, experiment and location of host material (fixed effects) for each parameter.

Paired *t*-tests were used to test significance of leaf age on all parameters of susceptibility of 24 Australian species (34 individual plants in total) and *R.* 'Colonel Coen' (two individual plants), as leaf age was not found to be significant using the SAS models. 'Summer' non-wounded, 'summer' wounded and 'winter' non-wounded pairings were treated separately, and results of the *t*-test for unbalanced variances were used, as well as a Students *t*-test to compare means between juvenile and mature leaves. These same tests were conducted for all parameters measured for sporulation potential studies on seven species with juvenile leaves and to assess whether lesion size increased significantly from the sporangia (10 day) and chlamydospore (14 day) harvests. The Tukey Kramer test was used to compare differences amongst mean lesion lengths recorded in the agar plug inoculations for the lilioid monocot species *L. longifolia*, *X. australis* and *X. preisii*. Analysis of variance was used to test significance of inoculum dose on all parameters, and the Tukey Kramer test was used to compare differences between species across all parameters at different inoculum concentrations. Paired *t*-tests, Tukey Kramer test and analysis of variance (ANOVA) analyses were conducted using JMP software (version 8.0, SAS Publishing, Cary, NC).

2.9 Susceptibility rating

An overall susceptibility rating was calculated from the disease severity and leaf infection predictions of the statistical models. Firstly, disease severity was grouped into four classes, zero (0), >0–20% (low; 1), >20–35% (moderate; 2) and >35–100% (high; 3); while leaf infection was grouped into four classes of infection: none infected (zero; <0), >0–40% (low; 1), >40–75% (moderate; 2) and >75–100% infected (high; 3). These classifications were modified from classes defined by Denman et al. (2005b). Secondly, for each of the treatment combinations of 'winter' non-wounded (WN), 'summer' non-wounded (SN) and 'summer' wounded

(SW), the class value of disease severity (0–3) was multiplied by the class value for leaf infection (0–3), resulting in a value from 0 to 9. Finally, the overall rating of susceptibility was then calculated using the following equation: susceptibility rating = 3 × WN + 2 × SN + 1 × SW, with the rating ranging from 0 to 54 (weighted for non-wounded and 'winter' responses which are more reflective of likelihood of infection and severity of infection under natural conditions). Ratings were then classified as zero (0), low (1–18), moderate (19–36) and high (37–54). Where a species was inoculated in only one inoculation group (i.e. 'summer' or 'winter' only), the susceptibility rating was doubled to obtain a range from 0 to 54, for comparative purposes. Care was taken to indicate which of the species did not have a full complement of treatment combinations and this was taken into account when presenting results and discussing comparative susceptibilities. Therefore, broad-leaf, odd-leaf and needle-like conifers, while having some overlap for comparison, were considered as independent groupings.

3 Results

Viable inoculum was recorded in all experiments, without considerable dilution from the initial to the final leaf dip.

3.1 Foliar susceptibility

The foliar susceptibility rating derived from disease severity (parameter 2) and leaf infection (parameter 3) showed that potentially highly susceptible Australian hosts include *Correa* 'Sister Dawn', *Eucalyptus regnans*, *I. cuneatus*, *I. formosus*, *L. scoparium*, *L. lanigerum*, *Melaleuca squamea* and *Taxandria marginata* (Table 2). Moderately susceptible hosts included *A. flexuosa, Banksia attenuata*, *Correa reflexa*, *C. ficifolia*, *Eucalyptus delegatensis*, *E. denticulata*, *E. haemastoma* and *E. viminalis*. *Acacia melanoxylon*, *Atherosperma moschatum*, *Eucalyptus globulus*, *Billardiera heterophylla* and the conifer and Xanthorrhoeacea species tested showed consistently low susceptibility. The low-susceptibility hosts, *Hedycarya angustifolia*, *Olearia argophylla*, *Phyllocladus aspleniifolius, P. undulatum* and *P. lawrencei*, may potentially be resistant, as indicated by low levels of leaf infection, particularly when non-wounded in the 'summer' inoculations (Table 2).

All species in the susceptibility study became infected with *P. ramorum* (parameter 3), with some asymptomatic infection of individual leaves recorded. Discrete dark-brown lesions were characteristic of infection on symptomatic species, with paler lesions observed on leaves of *E. globulus*. Seventy-six per cent of all inoculated leaves developed some degree of necrosis (parameter 1), while 77% were infected with *P. ramorum* (parameter 3). Disease incidence (parameter 1) and severity (parameter 2) were less severe ($P < 0.0001$) on the non-inoculated control leaves than on the inoculated leaves and shoots, and *P. ramorum* was not isolated from any of these control leaves. Inoculated *Rhododendron* leaves were predicted to have 100% infection by the statistical models in all cases, and all *U. californica* leaves were infected and diseased under all treatment conditions, confirming the virulence of the isolate (Table 2).

Inoculation group (i.e. 'summer' and 'winter') did not affect disease incidence or leaf infection, but did increase ($P \leq 0.05$) disease severity, particularly when considering a species × inoculation group interaction (Table 2). Wounding did not affect disease incidence overall in the 'summer', and while inoculated wounded leaves had higher ($P < 0.0001$) rates of leaf infection overall, there were no significant species × wounding interactions in this inoculation group. Conversely, while disease severity was not affected by wounding overall in the 'summer', lesions were larger ($P < 0.0001$) for those species with a significant species × inoculation group interaction under these experimental conditions (Table 2). *Eucalyptus saligna* (0.028 during 'winter' and 0.034 during 'summer'), *Lomatia myricoides*, *I. formosus* and *Taxandria marginata* leaves had larger ($P \leq 0.05$) lesions under these 'summer' conditions (Table 2).

Analyses of leaf infection were conducted on inoculated material only, as *P. ramorum* was not isolated from any of the control leaves. Eleven Australian species, *Bauera rubioides*, *C. maculata*, *Eucalyptus cneorifolia*, *E. delegatensis*, *E. globulus*, *E. regnans*, *E. saligna*, *Hakea rostrata*, *I. cuneatus*, *Leptospermum grandiflorum* and *Pomaderris apetala*, as well as the positive control *U. californica* in which all leaves were infected when inoculated with *P. ramorum,* were excluded from further analyses of leaf infection as it is statistically impossible to give an estimate of the probability of a species not being infected if it was always infected in the original data set.

Phytophthora ramorum was isolated from 87% of symptomatic inoculated leaves and 44% of asymptomatic inoculated leaves. Asymptomatic infection was recorded for 48 of the 69 Australian hosts tested in the leaf dip inoculations (Table 2), predominantly on non-wounded leaves. High levels of asymptomatic infection (data not shown) were recorded for *Tasmannia lanceolata* (58%), *P. apetala* (50%), *Lomandra longifolia* (39%), *E. saligna* (35%), *Acmena smithii* (31%) and *E. leucoxylon* (30%). Disease incidence (parameter 1) and severity (parameter 2) were unable to be recorded for the *A. flexuosa* cultivar 'Jervis Bay After Dark' Owing to the dark colour of the leaves, and disease severity was not recorded for *Acacia dealbata* because of its small leaves.

The majority of species fell into the low (49/70) susceptibility category, followed by moderate susceptibility (13/70) and high susceptibility (8/70) (Table 1). Both needle-like conifers and the two broad-leaf conifers were of low susceptibility. The positive control hosts, *R.* 'Colonel Coen' and *U. californica*, were moderately susceptible hosts according to this categorization. Species that were predicted by the statistical models to have 100% leaf infection or for which all leaves were infected during the course of the experiments, fell predominantly into the moderate- and high-susceptibility categories. This included the two positive control species and two of the three highly susceptible Australian hosts, *E. regnans* and *I. cuneatus*. However, both *E. globulus* and *E. saligna*, while classified as low susceptibility, also expressed 100% leaf infection during the experiments. No non-wounded odd-leaf hosts in the high-susceptibility category were 100% infected.

Low-susceptibility hosts, *H. angustifolia*, *O. argophylla*, *P. aspleniifolius* and *P. undulatum,* were considered as potentially resistant hosts as they were not infected during at least one of the non-wounded treatments. This classification held up, even

Table 2. Potential susceptibility, disease severity and leaf infection of detached leaves of Australian plant species inoculated with *Phytophthora ramorum* and the effects of inoculation conditions ('summer' and 'winter') and wounding on disease severity.

Leaf category[1], susceptibility group[2], species	Plants (exps)[3]	Disease severity[4,5]					Leaf infection[4,6,7]			Susceptibility rating[2] (0–54)
		Winter	Summer		Summer		Winter	Summer	Summer	
		Non-wounded		Sig.[8,9]	Wounded	Sig.[8,10]	Non-wounded		Wounded	
Positive control hosts										
Moderate susceptibility										
Rhododendron 'Colonel Coen'	(All)	0.06	0.27	*	0.52	***	1.00	1.00	1.00	30
Umbellularia californica	1 (1)	...	0.14		0.34	*	…	All	All	24
Broad-leaf hosts										
High susceptibility										
Correa 'Sister Dawn'	1 (1)	...	0.23		0.37		…	0.80	1.00	42
Eucalyptus regnans	1 (1)	...	0.40		0.64		…	All	All	54
Isopogon cuneatus	1 (3)	0.49	0.56		0.51		All	All	All	54
Moderate susceptibility										
Adenanthos obovatus#	2 (1)	...	0.14		0.36	**	…	0.55	0.95	26
Banksia attenuata#	1 (2)	0.06	0.22	***	0.11		0.90	0.93	1.00	24
Correa reflexa	3 (5)	0.02	0.47	***	0.57		0.79	0.95	1.00	36
Corymbia ficifolia#	2 (5)	0.01	0.23	***	0.42	*	0.92	0.93	1.00	30
Eucalyptus delegatensis	1 (2)	0.11	0.22	*	0.55	*	All	All	All	30
Eucalyptus denticulata	1 (4)	0.13	0.20		0.24		All	1.00	1.00	27
Eucalyptus haemastoma#	2 (2)	0.02	0.17	***	0.54	**	All	0.95	1.00	24
Eucalyptus pauciflora	3 (2)	0.06	0.52	***	0.43		0.92	All	1.00	36
Eucalyptus sideroxylon#	2 (4)	0.01	0.11	***	0.48	***	0.85	1.00	1.00	24
Polyscias sambucifolia#	2 (1)	...	0.02		0.46	***	…	0.80	0.93	30
Low susceptibility										
Acacia melanoxylon	1 (1)	...	0.02		0.06	*	…	0.30	0.40	8
Acmena smithii#	2 (4)	0.00	0.00		0.01		0.29	0.92	0.96	12
Agonis flexuosa#	4 (6)	0.05	0.06		0.09		0.94	0.96	1.00	18
Atherosperma moschatum	1 (2)	0.00	0.04	***	0.11	*	0.20	0.33	0.87	8
Banksia marginata#	5 (6)	0.01	0.04	***	0.13	***	0.48	0.62	0.84	13
Billardiera heterophylla#	3 (4)	0.00	0.00		0.00		0.23	0.02	0.15	6
Bursaria spinosa	1 (2)	0.05	0.07		0.16	*	All	0.47	1.00	16
Ceratopetalum apetalum	1 (2)	0.00	0.03	**	0.03		All	All	0.95	18
Correa alba#	3 (2)	...	0.01		0.15	***	…	0.41	0.95	14
Correa backhouseana#	1 (2)	0.00	0.00		0.03	**	0.13	0.40	1.00	6
Correa decumbens#	2 (4)	0.00	0.01	**	0.04	**	0.12	0.43	0.95	10
Correa 'Ivory Bells'#	2 (2)	...	0.03		0.11	***	…	0.54	0.89	14
Corymbia maculata#	1 (2)	0.13	0.04		0.06		All	All	All	18
Dodonea viscosa#	2 (3)	0.04	0.02		0.04		0.60	0.94	0.90	15
Eucalyptus camaldulensis#	2 (2)	0.05	0.02	*	0.08	***	0.80	1.00	0.92	18
Eucalyptus cneorifolia#	1 (1)	0.10					All	…	…	18
Eucalyptus diversicolor	1 (3)	0.05	0.12	**	0.17		All^	All	All	18
Eucalyptus globulus	1 (2)	0.01	0.01		0.01		All	All	All	18
Eucalyptus laeliae#	1 (2)	0.02	0.07	*	0.06		All	0.93	1.00	18
Eucalyptus leucoxylon#	4 (2)	0.03	0.05	***	0.05		0.98	All	0.98	18
Eucalyptus saligna#	1 (2)	0.03	0.03	**	0.00	**	All	All	All	18
Eucalyptus viminalis#	2 (4)	0.04	0.10	***	0.19	*	0.97	1.00	1.00	18
Eucryphia lucida#	3 (2)	0.00	0.01	*	0.05	***	0.16	0.74	0.91	10
Hardenbergia violacea#	3 (4)	0.01	0.03	**	0.09	**	0.97	0.81	1.00	18
Hedycarya angustifolia	1 (2)	0.01	0.00		0.00		0.25	None	0.00	6
Lomatia myricoides#	2 (3)	0.01	0.20	***	0.06	**	0.80	0.70	0.60	15
Macadamia tetraphylla#	1 (1)	...	0.02		0.02		…	0.87	1.00	18
Nothofagus cunninghamii	1 (2)	0.00	0.03	**	0.10	**	0.20	0.73	0.80	10
Nothofagus moorei#	2 (2)	0.00	0.01		0.04	**	0.80	0.57	1.00	16
Olearia argophylla#	2 (4)	0.00	0.00		0.01	**	0.45	None	0.93	9
Phyllocladus aspleniifolius#©	1 (2)	0.00	0.06	***	0.15	*	0.15	None	0.55	5
Pittosporum undulatum#	2 (4)	0.00	0.00		0.00		0.54	None	0.11	7
Podocarpus lawrencei©	2 (2)	0.00	0.12	***	0.83	***	None	None	0.55	6
Prostanthera lasianthos	2 (4)	0.02	0.15	***	0.11		0.65	0.73	0.81	13
Senecio linearifolius#	1 (2)	0.01	0.03		0.08	*	All	0.53	0.87	16
Tasmannia lanceolata#	3 (4)	0.00	0.00		0.01		0.78	0.81	0.62	17
Tristaniopsis laurina#	2 (4)	0.00	0.02	**	0.10	***	0.81	0.88	0.84	18
Needle-like conifers										
Low susceptibility										
Callitris rhomboidea#©	2 (2)	0.01	0.01	*	0.01		0.60	0.19	0.15	9
Lagarostrobos franklinii#©	2 (3)	0.01	0.08	***	0.20	**	0.45	0.60	0.72	12

Table 2. Continued

Leaf category[1], susceptibility group[2], species	Plants (exps)[3]	Disease severity[4,5] Winter Non-wounded	Summer Non-wounded	Sig.[8,9]	Summer Wounded	Sig.[8,10]	Leaf infection[4,6,7] Winter Non-wounded	Summer Non-wounded	Summer Wounded	Susceptibility rating[2] (0–54)
Odd-leaf hosts										
High susceptibility										
Isopogon formosus	3 (2)	...	0.82		0.48	*	…	0.84	0.95	54
Leptospermum lanigerum#	4 (3)	...	0.39		0.75	**	…	0.91	0.91	54
Leptospermum scoparium	3 (3)	...	0.66		0.98		…	0.86	0.91	54
Melaleuca squamea#	2 (1)	...	0.41		0.94	**	…	0.90	1.00	54
Taxandria marginata	1 (1)	...	0.36		0.06	***	…	All	0.80	42
Moderate susceptibility										
Bauera rubioides#	2 (1)	...	0.06		0.31	***	…	All	All	24
Brachychiton populneus	3 (2)	...	0.09		0.20	**	…	0.92	1.00	24
Grevillea synapheae	2 (1)	...	0.13		0.23		…	0.91	0.90	24
Low susceptibility										
Acacia dealbata#	1 (1)	…	…		…		…	0.44	1.00	…
Dicksonia antarctica#	3 (2)	...	0.05		0.13	**	…	0.92	1.00	18
Hakea rostrata	1 (1)	...	0.08		0.12		…	All	All	18
Leptospermum grandiflorum#	2 (1)	...	0.01		0.13	***	…	All	All	18
Lomandra longifolia#●	4 (3)	...	0.01		0.01		…	0.48	0.98	14
Pomaderris apetala#	1 (1)	...	0.00		0.00		…	All	All	6
Stylidium graminifolium#	1 (1)	...	0.01		0.01		…	0.20	0.47	8
Viola hederaceae#	1 (1)	...	0.00		0.02	*	…	0.73	1.00	14
Xanthorrhoea australis●	1 (1)	...	...		...			...	...	...
Xanthorrhoea preissii#●	3 (2)	...	0.02		0.02		…	0.21	0.94	10

[1]Species grouped to compare disease severity: broad, odd (asymmetrical or exceedingly small) and needle-like conifers. Hosts with recordings of asymptomatic infection (#). Conifers (©). Additionally tested using agar plug inoculation (●). Positive control species are known to be naturally highly susceptible to *P. ramorum*.
[2]Calculated as a function of disease severity and leaf infection ratings, as outlined in methods: susceptibility rating.
[3]The number of individual plants (and experiments) for each species. Leaves were collected randomly from multiple plants (>20) of *R.* 'Colonel Coen.' from the greenhouse at UC Davis for inclusion in all experiments.
[4]Ten to twenty leaves of each individual plant of each species were tested for each combination of inoculation group and wounding. All results presented are the predicted means of statistical analyses of a general linear model (disease severity) and generalized linear model (leaf infection) with suitable error and link functions applied as appropriate. Where a combination of a particular inoculation group and wounding was not conducted for a particular host (…).
[5]Mean proportion of necrotic leaf area or necrotic needles per shoot for needle-like conifers.
[6]Mean proportion of leaves or shoots positively infected with *P. ramorum*, as confirmed by reisolation.
[7]Where all leaves were infected (all) and no leaves were infected (none), these species were removed from statistical analyses. Where leaf infection was predicted as approaching 100%, that is in cases where a species that was included in the analysis had a small proportion of observations that were not infected, the model was unable to make an estimate because of extremely large standard errors and are identified by 'all ^'.
[8]Asterixes denote significant statistical significance, $P \leq 0.05$ (*), $P \leq 0.01$ (**) and $P \leq 0.001$ (***).
[9]Significance of difference between 'winter' and 'summer' non-wounded inoculations.
[10]Significance of difference between non-wounded and wounded 'summer' inoculations.

when small lesions were present (Table 2). However, all of these species were able to be infected when wounded. A similar result was obtained for the broad-leaf conifer *P. lawrencei*, which indicated some measure of disease incidence and severity during the 'summer', with 55% of leaves infected when wounded (Table 2). However, leaves of *P. lawrencei* were not readily infected ($P < 0.001$) following non-wounded leaf inoculations and no infection or disease incidence was recorded for the control inoculated leaves for this species. *Hedycarya angustifolia* showed consistently low levels of leaf infection. No non-inoculated control leaves of *H. angustifolia* inoculated with water responded to wounding and only one control leaf during the 'winter' showed any sign of disease incidence or severity.

Lomandra longifolia, *X. australis* and *X. preisii* all became infected using the needle-agar plug inoculation method, with no infection of the controls. Mean lesion length of *L. longifolia* (3.8 ± 2.3 mm), *X. australis* (1.3 ± 0.4 mm) and *X. preisii* (5.9 ± 0.8 mm) was slightly, but not significantly, higher than the controls and were all positively infected with *P. ramorum*. *Xanthorrhoea australis* was putatively classified as a low-susceptibility host, given that lesions which developed on this species were smaller than those developed on the other two lilioid monocot species, which were both classified as low susceptibility in leaf dip inoculations.

3.2 Leaf age

For the 65 individual plant/inoculation group/wounding treatment combinations tested, only 24 had significant ($P \leq 0.05$) differences in disease severity between juvenile and mature leaves (Table 3). Of these, 19 had increased disease severity and

Table 3. Effect of leaf age on the disease severity[1] of individual plants of 24 broad-leafed Australian native plant species, and the positive control *Rhododendron* '*Colonel Coen*'[2] inoculated with Phytophthora ramorum.

		'Winter'			'Summer'					
		Non-wounded			Non-wounded			Wounded		
Species	Site[3]	Juvenile	Mature	Sig.[4]	Juvenile	Mature	Sig.[4]	Juvenile	Mature	Sig.[4]
Acmena smithii	UCSC	0 ± 0	0 ± 0		…	…		…	…	
	SFBG	0.34 ± 0.19	0 ± 0		…	…		…	…	
Agonis flexuosa	UCD	…	…		0.95 ± 0.03	0.01 ± 0	***	0.78 ± 0.1	0.01 ± 0	**
Atherosperma moschatum	UCSC	…	…		1 ± 0	0 ± 0	***	1 ± 0	0.12 ± 0.06	***
Banksia marginata	UCB	…	…		0.33 ± 0.09	0.89 ± 0.02	**	0.94 ± 0.03	0.56 ± 0.11	*
	UCSC	0 ± 0	0.07 ± 0.06		…	…		…	…	
Billardiera heterophylla	SFBG	0.03 ± 0.02	0 ± 0		…	…		…	…	
Brachychiton populneus	UCB	…	…		…	…		0.25 ± 0.05	0.38 ± 0.06	
Correa backhouseana	SFBG	0 ± 0	0 ± 0		…	…		…	…	
Correa decumbens	SFBG	0 ± 0	0 ± 0		…	…		…	…	
Corymbia ficifolia	SFBG	…	…		0.71 ± 0.09	0.37 ± 0.05	*	0.82 ± 0.07	0.52 ± 0.06	**
Dicksonia antarctica	UCB	…	…		0.72 ± 0.02	0.28 ± 0.07	**	1 ± 0	0.61 ± 0.08	**
Eucalyptus camaldulensis	UCD	0.6 ± 0.14	0.13 ± 0.03	*	0.62 ± 0.21	0.07 ± 0.03		0.75 ± 0.19	0.06 ± 0.01	*
	UCD	0.05 ± 0.03	0.07 ± 0.04		0.14 ± 0.13	0.01 ± 0		0.37 ± 0.13	0 ± 0	*
Eucalyptus denticulata	UCSC	…	…		0.44 ± 0.14	0.33 ± 0.06		0.25 ± 0.07	0.45 ± 0.05	*
Eucalyptus diversicolor	UCSC	0.52 ± 0.2	0.06 ± 0.03		…	…		…	…	
Eucalyptus haemastoma	UCSC	0.02 ± 0	0.04 ± 0.01		0.57 ± 0.17	0.29 ± 0.04		0.79 ± 0.07	0.62 ± 0.06	
	UCSC	0.03 ± 0.01	0.01 ± 0	*	0.44 ± 0.23	0.11 ± 0.08		0.91 ± 0.05	0.21 ± 0.03	***
Eucalyptus laeliae	SFBG	0.25 ± 0.14	0.01 ± 0.01		…	…		…	…	
Eucalyptus leucoxylon	UCD	…	…		0.26 ± 0.14	0 ± 0		0.09 ± 0.09	0 ± 0	
	UCD	0.11 ± 0.04	0.09 ± 0.04		1 ± 0	0.43 ± 0.05	***	0.96 ± 0.02	0.45 ± 0.04	***
	UCD	…	…		0 ± 0	0.07 ± 0.04		0.01 ± 0.01	0.02 ± 0	
	UCSC	0 ± 0	0.65 ± 0.04	***	…	…		…	…	
Eucalyptus sideroxylon	UCD	0 ± 0	0.13 ± 0.05		0.48 ± 0.16	0.06 ± 0.03		0.73 ± 0.15	0.31 ± 0.05	*
Eucalyptus viminalis	UCD	0.2 ± 0.11	0.12 ± 0.04		0.11 ± 0.06	0.21 ± 0.06		0.4 ± 0.14	0.17 ± 0.03	
	UCSC	0.01 ± 0	0.01 ± 0		…	…		…	…	
Eucryphia lucida	UCSC	0.02 ± 0.01	0 ± 0		…	…		…	…	
Hardenbergia violacea	UCD	0.62 ± 0.11	0 ± 0	**	…	…		…	…	
	UCD	…	…		0.4 ± 0.15	0.01 ± 0		0.83 ± 0.11	0.02 ± 0.01	**
	SFBG	…	…		0.16 ± 0.16	0.5 ± 0.19		0.23 ± 0.14	0.55 ± 0.17	
Hedycarya angustifolia	UCSC	0.01 ± 0.01	0.01 ± 0		0 ± 0	0.01 ± 0.01		0 ± 0	0 ± 0	
Isopogon formosus	UCSC	…	…		1 ± 0	0.98 ± 0.02		0.31 ± 0.04	0.79 ± 0.08	**
Leptospermum lanigerum	UCSC	…	…		0.95 ± 0.05	1 ± 0		0.75 ± 0.25	1 ± 0	
Pittosporum undulatum	UCSC	0.1 ± 0.07	0 ± 0		…	…		…	…	
Rhododendron 'Colonel Coen'	GH	…	…		0.17 ± 0.07	0.78 ± 0.06	***	0.6 ± 0.04	0.81 ± 0.06	*
	GH	0.17 ± 0.05	0.16 ± 0.04		…	…		…	…	

[1]Calculated as the mean proportion of necrotic leaf area. Values shown are means ± standard error.
[2]Species known to be naturally highly susceptible to *P. ramorum*.
[3]Plant collection sites: GH, Glasshouse; SFBG, San Francisco Botanical Garden & Strybing Arboretum; UCB, University of California (UC) Berkeley Gardens; UCD, UC Davis Arboretum; UCSC, UC Santa Cruz Arboretum; Where a combination of a particular host, leaf age and wounding was not conducted for a particular host (…).
[4]Asterixes denote significant statistical significance, where $P \leq 0.05$ (*), $P \leq 0.01$ (**) and $P \leq 0.001$ (***).

six had a reduction in disease severity for juvenile leaves. The vast majority of these were in the 'summer'-wounded treatment categories, eleven of which increased in severity and three of which decreased.

3.3 Inoculum concentration

No infection was recorded for the non-inoculated control leaves or for the lowest inoculum concentration of 2×10^2 zoospores/ml. With the exception of *E. denticulata*, leaf infection and some disease severity were found for all species at 2×10^3 zoospores/ml (Fig. 1a). Disease severity (per cent necrosis of leaf) increased from 44 to 100% amongst the species as inoculum concentration increased from 2×10^3 to 2×10^4 zoospores/ml. Similarly, leaf infection increased by 5–100% between these inoculum concentrations (Fig. 1b). Analysis of variance (ANOVA; excluding the controls) showed a concentration–response relationship ($P < 0.05$) for all species across all parameters, with symptom development consistently greatest at the highest zoospore concentration. Differences ($P < 0.0001$) were detected amongst species for leaf infection at 2×10^3 zoospores/ml, but not at either of the other inoculum concentrations. Disease severity was clearly different ($P < 0.0001$) at 2×10^3 zoospores/ml amongst the tested species, with lesions formed on *I. cuneatus* larger than all of those formed on other species at this concentration. At 2×10^4 zoospores/ml, *I. cuneatus* and *R.* 'Colonel Coen' were more susceptible ($P < 0.05$) than *C. ficifolia* and *E. denticulata*, while *C. reflexa* and *L. myricoides* were less susceptible ($P < 0.005$) than *I. cuneatus*, but as susceptible as *R.* 'Colonel Coen'.

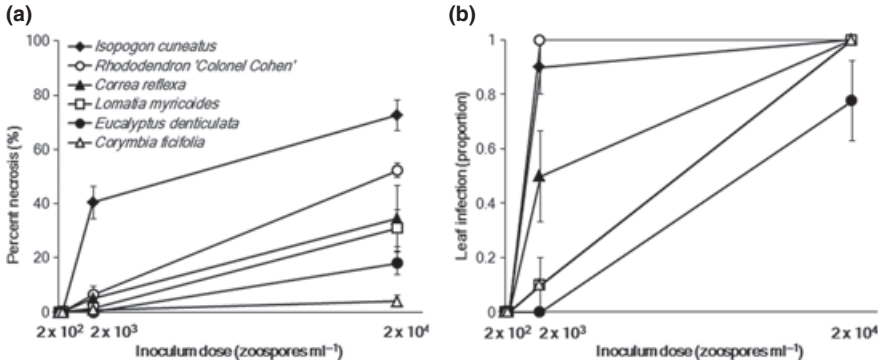

Fig. 1. Relationship between inoculum dose and per cent necrosis (a) and leaf infection (b) responses of five Australian native plant species and the known highly susceptible *Rhododendron* 'Colonel Coen' to leaf dip inoculation in suspensions of *Phytophthora ramorum* zoospores (0, 2×10^2, 2×10^3 and 2×10^4 zoospores/ml). Data points are means of ten leaves per plant species (except for three leaves for the controls, five leaves of *C. ficifolia* and nine leaves of *E. denticulata* at 2×10^4 zoospores/ml); bars indicate standard error of the means.

3.4 Sporulation potential

Rhododendron 'Colonel Coen' consistently had the highest proportion of leaves on which sporangia were produced, sporangia counts per leaf and number of sporangia per cm^2 of necrotic lesion (Table 4), with all infected leaves producing sporangia. *Eucalyptus haemastoma* was highest for these parameters of all the Australian hosts (Table 4). *Eucalyptus viminalis*, *I. formosus* and *N. cunninghamii* also produced sporangia consistently. These results indicate that reisolation methods for *I. formosus* were not reliable, given that sporangia presence was 80% and leaf infection was only 40% for these leaves. Hosts on which no sporangia were produced and lesions were small (<0.18 cm^2) were *Acacia melanoxylon*, *Atherosperma moschatum*, *Dicksonia antarctica*, *E. diversicolor*, *E. regnans*, *Hardenbergia violaceae* and *P. undulatum* (Table 4). No lesions or sporangia were observed for non-inoculated control leaves for all species.

The presence of sporangia was higher ($P < 0.0001$) for juvenile than mature leaves of *A. flexuosa* and *Corymbia ficifolia*. *Acmena smithii*, *E. haemastoma* and *E. viminalis* all had larger lesions on juvenile leaves ($P < 0.05$), but no difference in sporangia presence, when compared with lesions formed on mature leaves. Lesions formed 14 days after incubation were larger ($P < 0.0001$) than those formed after 10 days, with an overall increase of 75%. *Banksia attenuata*, *B. marginata*, *C. reflexa*, *D. antarctica*, *D. viscosa, E. denticulata*, *E. diversicolor, H. violaceae* and *I. formosus* had no significant increase in lesion size (data not shown).

3.5 Temperature and sporulation potential

Zero to very few sporangia were produced on the majority of leaves in the study across all temperatures and periods of time for all species (data not shown). The highest maximum sporangia counts occurred for *R.* 'Colonel Coen', being 8187 sporangia per leaf at 20°C after 6 days, 4500 at 15°C after 9 days and 3212 sporangia at 20°C after 9 days. Analysis of variance showed no significant differences between the proportion of leaves infected at any of the temperatures after 3 and 9 days, except for 25°C, which had lower incidence ($P < 0.0001$) of leaf infection after 6 days (8%, as compared to 34 and 54%, respectively, at 15 and 20°C). The presence of sporangia differed ($P = 0.0127$) only between 20 and 25°C, with a fourfold decrease from 28% of leaves producing sporangia to 6% of leaves producing sporangia as the temperature increased. There was no significant difference in the presence of sporangia between 15–20 and 15–25°C. While not statistically significant, the trend indicated that the lower temperatures of 15 and 20°C were more conducive to infection and sporangia production.

4 Discussion

A wide range of susceptibility and sporulation potential were recorded amongst the Australian species tested, with all 70 species screened capable of being infected with *P. ramorum*. High levels of susceptibility, measured as leaf infection and disease severity, were recorded for *E. regnans*, *I. cuneatus*, *I. formosus*, *L. scoparium*, *L. lanigerum*, *Melaleuca squamea* and *T. marginata*. Moderately susceptible hosts included *A. flexuosa, B. attenuata*, *C. ficifolia*, *C. reflexa, E. delegatensis*, *E. denticulata*, *E. diversicolor*, *E. haemastoma* and *E. viminalis*. The conifers and lilioid monocot species tested showed consistently low susceptibility, along with *A. melanoxylon*, *A. moschatum*, *E. globulus*, *B. heterophylla* and the remaining *Correa* species. (Denman et al. 2005a; Hansen et al. 2005). Potentially resistant hosts included *H. angustifolia*, *O. argophylla*, *P. aspleniifolius, P. undulatum* and *P. lawrencei*. While disease severity was low in many of the Australian species tested in the foliar dip studies (32/69), 47 of these species exhibited disease symptoms on more than 80% of their leaves during the 'summer' inoculations. As has been observed in other studies, disease levels varied within species (Dodd et al. 2005; Anacker et al. 2008), genera (Grünwald et al. 2008; Tooley and Browning 2009; De Dobbelaere et al. 2010) and families (Tooley and Browning 2009) of plants.

Our studies confirm the susceptibility of *E. haemastoma*, previously recorded as a natural host of *P. ramorum* in the United Kingdom (RAPRA 2007). On the other hand, we identified *E. globulus* as a potential host in our study, in contradiction to results

Table 4. Potential sporulating hosts, presented in order of sporangia-producing potential per leaf, of detached leaves of Australian plant species and the positive control *Rhododendron* 'Colonel Coen' inoculated with *Phytophthora ramorum*[1].

				Sporangia[1]				
Species[2]	Plants (exps)[3]	Leaf infection[4]	Lesion area (cm^2)	Presence[5,α]	Per leaf[β]	Max. count per leaf[β]	Per cm^2 of lesion area[β]	Max. per cm^2 of lesion area
Rhododendron 'Colonel Coen'^	(All)	1.00	7.8633	All	1882.2	33 967	190.06	2726
Eucalyptus haemastoma[#]	1 (1)	0.93	1.8665	0.93	210.7	1763	112.58	1055
Eucalyptus viminalis[#]	2 (2)	0.94	0.7329	0.62	9.875	3900	8.90	2829
Isopogon formosus	1 (1)	0.40	1.2911	0.80	1.691	210	1.42	114
Nothofagus cunninghamii	1 (1)	0.70	0.0025	0.70	1.366	29	0.07	1148
Umbellularia californica^	1 (4)	1.00	0.4759	<0.01	0.3421	1975	0.38	950
Eucalyptus denticulata	1 (1)	0.60	0.3515	0.40	0.3385	790	0.06	700
Corymbia ficifolia[#]	1 (1)	0.74	0.0925	0.37	0.1896	1053	0.06	309
Eucalyptus delegatensis	1 (1)	All	0.3442	0.30	0.1325	2157	0.14	145
Acacia dealbata	1 (1)	None	…	0.33	0.0366	1	…	175
Banksia marginata	2 (2)	0.55	0.0011	0.24	0.0340	9	<0.01	370
Correa reflexa	3 (2)	0.34	0.0235	0.20	0.0305	58	0.01	60
Dodonea viscosa	1 (1)	0.90	0.1926	0.30	0.0299	1	<0.01	601
Corymbia maculata	1 (1)	All	0.0628	0.20	0.0259	35	0.04	17
Leptospermum scoparium	1 (1)	0.90	0.0007	0.20	0.0213	9	<0.01	<0.01
Prostanthera lasianthos	1 (1)	0.10	0.0270	0.10	0.0144	74	0.02	<0.01
Pomaderris apetala	1 (1)	0.30	0.3336	0.10	0.0086	5	<0.01	<0.01
Agonis flexuosa[#]	5 (3)	0.88	0.0262	0.07	0.0084	571	0.01	<0.01
Banksia attenuata	1 (1)	0.30	<0.0001	0.10	0.0070	2	<0.01	<0.01
Eucalyptus pauciflora	1 (1)	0.85	0.0473	0.10	0.0059	1	0.01	<0.01
Nothofagus moorei	1 (1)	All	0.0027	0.10	0.0059	1	<0.01	<0.01
Eucalyptus globulus[#]	1 (1)	0.80	0.0029	0.07	0.0036	1	<0.01	<0.01
Acmena smithii[#]	2 (2)	0.55	0.0122	0.04	0.0021	4	<0.01	<0.01
Eucalyptus diversicolor	1 (1)	All	0.0906	None	None	None	None	None
Acacia melanoxylon	1 (1)	All	0.0015	None	None	None	None	None
Eucalyptus regnans	1 (1)	0.50	0.1702	None	None	None	None	None
Dicksonia antarctica	1 (1)	0.30	0.0007	None	None	None	None	None
Atherosperma moschatum	1 (1)	None	0.0291	None	None	None	None	None
Hardenbergia violacea	1 (1)	None	0.0030	None	None	None	None	None
Pittosporum undulatum[#]	1 (1)	None	0.0001	None	None	None	None	None

[1]All results presented are the predicted means of statistical analyses of generalized linear models (α) and general linear models (β), with suitable error and link functions applied as appropriate. Where data was unavailable or unattainable for a particular component of measuring sporangia-producing potential (…).
[2]Species known to be naturally susceptible to *P. ramorum* and which produce high numbers of sporangia (^). Species where juvenile leaves were tested (#).
[3]The number of individual plants (and experiments) for each species. Ten to fifteen leaves of each individual plant of each species were tested. Leaves were collected randomly from multiple plants (>20) of *R.* 'Colonel Coen.' from the greenhouse at UC Davis for inclusion in all experiments.
[4]Proportion of leaves positively infected with *P. ramorum*, as confirmed by reisolation. Where all leaves were infected (all) and no leaves were infected (none), these species were removed from statistical analyses.
[5]Proportion of inoculated leaves producing sporangia.

obtained by Hüberli et al. (2008). Similarly, our results for *Leptospermum scoparium*, examined in the same study by Hüberli et al. (2008), differed significantly. While Hüberli et al. (2008) observed no disease incidence or severity, we consistently observed symptoms, and similarly, they observed greater incidence and amount of sporulation on *L. scoparium*. In a similar fashion and in a separate study, Hüberli et al. (2006) described *P. undulatum* as a potential Australian host of *P. ramorum*. No lesions as described by Hüberli et al. (2006) were observed in the current study on *P. undulatum*, with a negligible disease severity of <0.5% (Table 2). Inoculations of the same *P. undulatum* plants used by Hüberli et al. (2006), sourced from the UC Berkeley campus, also failed to reproduce the same results (K.B. Ireland, unpublished data). The differences between these studies may be due to varied environmental conditions between years or the use of different isolates of *P. ramorum*, resulting in different susceptibilities. Alternatively, the different inoculation methods used by Hüberli et al. (2006, 2008), which involve agar plugs or immersion of the tip of the leaves in inoculum for 12 h, may induce a more severe response from the host as they are exposed to inoculum for an extended period of time. Under these conditions, leaves would be likely to undergo physiological changes that may exacerbate susceptibility or produce an abiotic necrotic response.

Putative sporulating hosts identified in the study included *E. haemastoma*, *E. viminalis*, *I. formosus* and *N. cunninghamii*, with lower levels of sporulation occurring in a number of other species such as *E. denticulata*, *C. ficifolia*, *L. scoparium* and *A. flexuosa*. Sporangia production was observed even on plants with low susceptibility to *P. ramorum* such as *N. cunninghamii*, on which only a few sporangia were observed per leaf. However, when one considers the relatively high levels of leaf infection that correlated with the presence of sporangia (70% of leaves), there exists the potential for large numbers of sporangia to be produced on infected *N. cunninghamii* plants during a rain event. Sporangia-producing, non-lethal foliar infections, such as those which occur on *U. californica*, in Northern California, are considered to be the most epidemiologically important

infections for the transmission of *P. ramorum* (Swiecki and Bernhardt 2002). Abundant sporulation on *U. californica* leaves during winter (Davidson et al. 2005; Maloney et al. 2005) and potential survival of the pathogen within leaves during dry summers are postulated to contribute greatly to epiphytotics and persistence of the disease within Northern California. Our study shows that *N. cunninghamii* may potentially fulfil this role in similar Australian ecosystems as it demonstrates high rates of infection, low levels of disease severity and consistent production of sporangia. This is of concern as *N. cunninghamii* and the other high sporulating Australian host identified in this study, *E. viminalis*, coexist with *E. regnans,* in the cool, moist highland areas of Victoria (Boland et al. 2006). This area has been identified as climatically suitable for *P. ramorum* growth and establishment (Ireland et al., unpublished), while *E. regnans* has been identified as a potential bole canker host in branch and bole canker studies conducted at the same time as the foliar studies presented in this paper (Ireland et al. 2011). All of the species identified in our study as putative sporulating hosts are important commercially in global forest and/or horticulture industries, or as keystone species in their native environments, and are therefore widespread in landscapes most at risk for the establishment and spread of *P. ramorum* worldwide. Species such as *A. flexuosa*, *C. ficifolia*, *Correa* and *Eucalyptus* species are planted and distributed widely as street trees and hardy garden plants throughout the world, including in areas where *P. ramorum* is already known to exist in California (K. B. Ireland, personal observation). These species have not been found naturally infected in these areas, and no comprehensive studies examining pockets of native Australian plants in high inoculum pressure zones and infested nurseries have taken place so far. Despite the lack of confirmation of host status by natural infection, many of the species identified here as susceptible and sporulating hosts are potential carriers for *P. ramorum* and should be treated with caution when being traded amongst regions known to have *P. ramorum* in the global forestry and horticulture industries.

The range of sporangial density on Australian hosts (0–113 sporangia per cm^2 of lesion), as well as *R.* 'Colonel Coen' (averaging almost 190 sporangia per cm^2 and up to 2726) are similar to those of other studies of common north-east American understory species (Tooley and Browning 2009), Mediterranean species (Moralejo et al. 2006), *Rhododendron* cultivars (De Dobbelaere et al. 2010) and New Zealand plant species (Hüberli et al. 2008). Under natural conditions during rainstorms, the mean number of zoospores produced from infected *U. californica* leaves was 1173.0 ± SE 301.48 zoospores per leaf, to as high as 5200 spores per leaf (which was comparative with laboratory trials), in studies by Davidson et al. (2005). Taking into account that the mean number of zoospores released from a single sporangium ranges from 13 to 32 (Moralejo et al. 2006; Widmer 2009), the number of sporangia found in nature (average of 27–113) are much less than those we observed on the Australian species in the present study. Similarly, sporangia production on *U. californica* in our studies (ranging from 0 to 1975 sporangia per cm^2 of lesion area) was lower than that recorded by Davidson et al. (2005). This may be associated with the phenological condition of the host, as the plant on which these studies were based was grown in the warmer and drier climate of Davis, California, or the experimental conditions we used. Given this, we urge caution when extrapolating these laboratory results to potential field sporulation capacities.

Zoospore concentrations of 1×10^2 zoospores/ml were not adequate for producing infection in any of the hosts tested in the inoculum concentration study, including the highly susceptible *R.* 'Colonel Coen'. Leaf infection occurred in all but *E. denticulata* at 2×10^3 zoospores/ml, with higher levels of infection occurring at 2×10^4 zoospores/ml, which was the concentration of inoculum we used across all of the susceptibility and sporulation potential studies. Turner et al. (2008) found that a single zoospore of *P. ramorum* was sufficient to produce lesions on susceptible species of *Rhododendron*, *Viburnum*, *Kalmia* and *Pieris*. In the same study, *Syringa* species required at least 100 zoospores, while *Camellia* and *Leucothoe* required a threshold of 10 000 zoospores before an infection was established. Likewise, under natural conditions in California, Tanoak (*N. densiflorus*) appears to have a much lower infection threshold than Coast Live Oak (*Quercus agrifolia*) (Davidson et al. 2011), and this may explain the high comparative susceptibility of the former species. These results may indicate lower sensitivity and increased tolerance to *P. ramorum* infection by particular species, with particular species-specific thresholds required to induce infection. The lack of infection of *E. denticulata* at 2×10^3 zoospores/ml may indicate a higher specific threshold for infection than the other species tested, and a range of tolerances may, therefore, exist within other Australian plant species as well. Likewise, the high susceptibility of *I. cuneatus* at lower inoculum concentrations may indicate that it is consistently a susceptible species, similar to results obtained for *Fuscia exortica* in a similar study by Hüberli et al. (2008), which may indicate that it has a high probability of being a naturally infected host under conducive environmental conditions. Our results and the results of Turner et al. (2008) support our decision to use an inoculum concentration of 2×10^4 zoospores/ml in this study. This relatively high concentration of zoospores is consistent with other *P. ramorum* susceptibility studies, which have used between 1×10^4 and 2×10^5 zoospores/ml (Denman et al. 2005a; Hansen et al. 2005; Hüberli et al. 2008; De Dobbelaere et al. 2010). In the future, species-specific responses to different inoculum concentrations may be able to be used as an additional measure of susceptibility and to select indicator plants for early detection in nurseries and natural ecosystems.

The susceptibility of leaves and their sporulation potential were affected by the season in which they were inoculated and chamber conditions in which they were kept (designed to coincide approximately with natural conditions of summer and winter). The pathogen was able to infect and cause disease under both of these climatic conditions, with greater disease expression during the 'summer' experiments. This is consistent with observations under natural conditions in California, where transmission and impact of the pathogen becomes apparent in the summer following spring rains (Davidson et al. 2005). Seasonality has regularly been highlighted as a contributing factor to the severity of infection and susceptibility of hosts to *P. ramorum* under controlled conditions (Dodd et al. 2008; Tjosvold et al. 2009; De Dobbelaere et al. 2010). Similarly, our studies on sporulation attempted during the autumn month of October were largely ineffective, while those conducted in the spring month of April were successful. Reduced sporangia production agrees with epidemiology studies that show infection is most successful during the spring and early summer months in both natural ecosystem (Davidson et al. 2005; Dodd et al.

2005) and laboratory-based (Denman et al. 2006b) studies. Therefore, conducting susceptibility studies during the spring and summer should be the most informative for biosecurity purposes. Further studies comparing host responses under the same chamber conditions across both seasons with a study similar to ours would be valuable in elucidating whether seasonal responses were a result of host phenology at the time of collection of plant material or a response of hosts and pathogens to chamber conditions alone.

Disease severity also increased for some species when leaves were wounded in the 'summer' experiments, as shown in other studies (Kaminski and Wagner 2008; De Dobbelaere et al. 2010). We agree with De Dobbelaere et al. (2010) that the results of inoculations of non-wounded leaves are the most informative and relevant when determining the levels of susceptibility amongst a range of species. However, identification of those species that become infected when wounded allows us to understand questions relating to the susceptibility or resistance of a species. Further research into the morphological and biochemical basis of higher levels of resistance by low-susceptibility hosts such as *A. moschatum*, *B. marginata* and *P. lawrencei* when non-wounded may be useful in selecting particular cultivars, species or incorporating particular resistance genes into new cultivars, to help manage the disease in the future. Examination of individual plants showed that the influence of leaf age on susceptibility was variable, indicating species or individual plant-specific responses, with generally higher levels of disease severity recorded for juvenile leaves when differences did occur. Our studies correspond with those of Hansen et al. (2005) and Denman et al. (2005b), who showed younger leaves were more susceptible for evergreen huckleberry (*Vaccinium ovatum*) and sweet chestnut (*Castanea sativa*), respectively. Additionally, our studies correspond with those of De Dobbelaere et al. (2010) who showed that younger leaves were consistently more susceptible to *P. ramorum* infection when they were wounded. Our results indicate that the phenological condition of the host at the time of transmission of the pathogen may affect its overall susceptibility and that this is likely to be variable amongst different species (Dodd et al. 2008).Those species with highly susceptible juvenile foliage would therefore be in a more vulnerable position for infection and increased disease severity during the spring, when pathogen spread is known to occur (Davidson et al. 2005; Dodd et al. 2005).

Asymptomatic infection was recorded in some species, with high levels (>30%) recorded for *Acmena smithii*, *E. saligna*, *E. leucoxylon*, *Lomandra longifolia*, *Pomaderris apetala* and *Tasmannia lanceolata*. Additionally, infection may not be readily apparent for species such as *A. dealbata*, which have particularly small compound leaves, and species such as *D. viscosa* and cultivars such as *A. flexuosa* 'Jervis Bay After Dark', which have particularly dark leaves. Asymptomatic infection and sporulation has been recorded by Denman et al. (2008) on fruit and foliage of *Rosa* species, on foliage of *Leptospermum scoparium* (Hüberli et al. 2008) and on root systems of *Rhododendron* (Fichtner et al. 2008; Riedel et al. 2009), *Camellia* (Shishkoff 2006) and *Lilac* (Shishkoff 2007) species. Asymptomatic plants may also be an issue for quarantine authorities where plant release is based on the visible expression of disease symptoms.

Susceptibility studies, particularly those conducted on detached plant material, are naturally fraught with difficulties, especially when it comes to interpretation of results. No standard methodology has been developed for susceptibility studies with *P. ramorum*. Past studies have used different inoculation techniques, incubation regimes and analyses of results, making comparisons between studies exceptionally difficult. The detached, *in vitro*, leaf inoculation method of Denman et al. (2005a) was used in the current study, as the method is well established and applied as a RAPRA (the European risk assessment for *P. ramorum*) protocol throughout Europe (Denman 2007). The use of whole-plant studies is generally preferable as they potentially predict the most comprehensive range of symptoms observed in natural ecosystems for known hosts (Hansen et al. 2005), while detached-leaf studies are more likely to indicate higher than natural susceptibility levels as the leaves have been removed from the plant and are under physiological stress when tested (Tooley and Browning 2009). We would recommend future work on Australian species incorporate whole-plant studies to elucidate a better understanding of their potential susceptibility. Inoculation methods used in this study were selected in an attempt to reflect the natural environment conducive to *P. ramorum* disease development. Zoospores were used as they have been recorded as being released naturally as infective propagates in natural ecosystems for *P. ramorum* (Davidson et al. 2005) and are generally believed to be the most important infection pathway in the disease cycle of *Phytophthora* species (Judelson and Blanco 2005). The temperatures used in our study were selected to reflect warmer ('summer') and cooler ('winter') conditions surrounding the optimum range for the growth and sporulation of *P. ramorum*. The majority of other studies have used a constant temperature, ranging from 17°C (Werres et al. 2001) to 24°C (Shishkoff 2007), with the majority of studies incubating material at approximately 20°C (Denman et al. 2005a; Shishkoff 2006; Hüberli et al. 2008; Kaminski and Wagner 2008). Studies by Hansen et al. (2005) on the other hand used a cyclic temperature regime ranging from 17 to 20°C. Cyclic regimes in our study were chosen to reflect natural conditions, where temperatures fluctuate diurnally. In our study, we used only one isolate of NA2 lineage (Grünwald et al. 2009). In a similar detached-leaf studies, isolates of NA2 and EU1 lineage have been found to be more aggressive than those of the NA1 lineage for *R.* 'Cunningham's White' (Elliott et al. 2011). While earlier studies demonstrated clear differences in aggressiveness amongst A1 (EU1) and A2 (NA1) mating-type isolates in log inoculations (Brasier 2003), many foliar inoculation studies with multiple hosts have found no significant differences in aggressiveness amongst isolates (Tooley et al. 2004; Denman et al. 2005a; Kaminski and Wagner 2008). Where multiple isolates are used, it may be necessary to use them independently as significant isolate–species interactions have been reported for disease severity measures (Linderman et al. 2007; Kaminski and Wagner 2008; Elliott et al. 2011; Hüberli and Garbelotto 2011) and sporulation potential (Denman et al. 2006a), which could be explored further in future work on Australian plant susceptibility. Together, the results of these other studies indicate that isolate selection is still a highly questionable and variable component of host range testing for *P. ramorum*. For the purposes of our study, we believe that the use of the one NA2 isolate is valid as it provides a preliminary assessment of potential Australian plant susceptibility and a starting point to explore any future nuances of the effects of *P. ramorum* genotype and isolate differences.

The work presented here is only a first step towards identification of potential Australian hosts of *P. ramorum*. Given the limitations of the study, the results presented here do not represent a definitive confirmation of any of the species presented here as hosts capable of being naturally infected by *P. ramorum*. Collection of small amounts of material and conducting the experiments outside all of the plants endemic range, while not ideal, were necessary to avoid any of the risks associated with importing the pathogen to Australia for experimentation and to adhere to current Australian quarantine for category 1 plant pathogens. Caution is advised when interpreting these results, particularly for those species with low levels of infection and degrees of susceptibility, which may represent an individual of that species which could be more tolerant or resistant to *P. ramorum,* given the conditions under which it has been grown. We do suggest that all species with high levels of infection and leaf necrosis should be accepted as putative hosts, pending more comprehensive studies, as concluded by Hüberli et al. (2008) in assays for NZ plants. As the plants were collected outside of their endemic ranges, it is possible that these plants have been selected for Californian growing conditions and their reactions to *P. ramorum* may not be representative of how they would respond to *P. ramorum* in their native ranges.

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