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Pathogenicity and infectivity of *Phytophthora ramorum* vary depending on host species, infected plant part, inoculum potential, pathogen genotype, and temperature

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Abstract

A total of 25 ornamental plant species representing 10 families were inoculated using three genotypes, each representing one of the genetic lineages NA1, NA2, and EU1 of the pathogen *Phytophthora ramorum*. Leaves were inoculated using suspensions with two zoospore concentrations and exposure at three temperatures, while stems were inoculated using agar plugs colonized by mycelia. Susceptibility was determined by measuring either the success of pathogen reisolation or lesion length caused by the pathogen. Infectivity was determined by counting sporangia in washes of inoculated leaves or stems. Results from all three pathogen genotypes combined were used to rank each of the 25 plant species for susceptibility and infectivity, while pooled results per genotype from all 25 hosts combined were employed for a preliminary comparison of pathogenicity and infectivity among genotypes. Statistical analyses showed that leaf results were affected by the concentration of zoospores, temperature, plant host, pathogen genotype, and by the interaction between host and pathogen genotype. Stem results were mostly affected by host and by the interaction between host and pathogen genotype. Hosts ranked differently when looking at the various parameters, and differences in rankings were also significant when comparing stem and leaf results. Differences were identified among the 25 hosts and the three pathogen genotypes for all parameters: results can be used for decision-making regarding regulations or selection of plants to be grown where infestations by *P. ramorum* are an issue.

KEYWORDS

host infectiousness, host susceptibility, pathogen infectivity, pathogen virulence, temperature effects

1 | INTRODUCTION

Although formally described only in 2001 (Werres et al., 2001), the oomycete plant pathogen *Phytophthora ramorum* has emerged as one of the most interesting model organisms of our times in the field of plant pathology (Garbelotto and Hayden, 2012; Grünwald et al., 2019; Rizzo et al., 2005). The interest around *P. ramorum* stems from a variety of reasons. First, it is an incredibly destructive forest pathogen in world regions where it has been introduced (Brasier and Webber, 2010; Rizzo and Garbelotto, 2003; Rizzo et al., 2002). Secondly, it is a generalist pathogen, present both in natural ecosystems and in the ornamental plant industry, making regulations for its control rather complex (Grünwald et al., 2012). Thirdly, although a generalist, *P. ramorum* can cause different

types of disease, infecting only stems, only leaves, or both, depending on the plant host (Grünwald et al., 2008). Fourthly, sporulation occurs only on some infectious hosts, while other hosts only support minimal sporulation and are regarded as epidemiological dead-ends (Garbelotto and Hayden, 2012; Grünwald et al., 2008). Fifthly, the species includes multiple lineages that are genetically and phenotypically distinct from one another (Ivors et al., 2006; Van Poucke et al., 2012), each containing mitotically generated genotypes. The four lineages known outside the putative native range of the pathogen, although distinct, have an estimated divergence time of only 165,000 to 500,000 years; thus in a strict evolutionary sense, these lineages can be regarded as being rather "young" (Goss et al., 2009a). Finally, there is no known sexual reproduction of the pathogen in areas where it has been introduced, and, consequently, *P. ramorum* has been employed as a model organism to study clonal evolution of a plant pathogen (Croucher et al., 2013; Dale et al., 2019; Elliott et al., 2018; Malar et al., 2019; Serrano et al., 2020).

P. ramorum has been unwittingly introduced into both Europe and North America by the trade and sale of infected ornamental plants (Croucher et al., 2013; Goss et al., 2009b, 2011). While infestations in commercial nurseries and in landscaped settings have been manageable using traditional disease management approaches (Tjosvold et al., 2005), infestations in natural ecosystems have been impossible to control due to their scale and intensity (Cunniffe et al., 2016; Rizzo et al., 2005). Infestations in larch plantations have been controlled, but with a huge cost and accepting significant losses (O'Hanlon et al., 2018). *P. ramorum* was the first generalist pathogen to be regulated at many national and international levels (Garbelotto and Rizzo, 2005). Regulations have targeted hosts in both natural ecosystems and in the ornamental trade (Anonymous, 2007, 2019); however, in both cases, such regulations have been drafted mostly from data available only for a few hosts. The sporulation potential of this pathogen on most native Californian plants is still poorly understood (Davidson et al., 2005; Fichtner et al., 2012). Despite the fact that the pathogen has been isolated from over 100 ornamental plant species (Anonymous, 2007, 2019), disease susceptibility and especially transmission potential are known only for a handful of plant species traded and sold as ornamentals (Kaminski and Wagner, 2008; Linderman and Davis, 2007; Parke et al., 2004; Shishkoff, 2007; Tooley et al., 2004; Widmer, 2010).

The mechanisms behind generalism, also known as polyphagy, have not been well studied for plant pathogens compared to herbivore insects (Via, 1984a, 1984b). Furthermore, our inability to rank hosts properly for susceptibility and infectivity has made regulations less effective. A further complication has been added by the presence of at least four genetically distinct lineages of the pathogen affecting ornamental stock (Ivors et al., 2006; Van Poucke et al., 2012), which reportedly differ in their aggressiveness on plant hosts (Elliott et al., 2011; Eyre et al., 2014; Manter et al., 2010; O'Hanlon et al., 2017). The relative abundance of lineages in the ornamental plant industry varies with time and location (Grünwald et al., 2019), making long-term studies on the impact each lineage may have, particularly complex. Forests of the US West Coast and larch plantations in the UK and Ireland were initially infested by a single lineage, NA1 in North America and EU1 in Europe (Garbelotto and Hayden, 2012). However, more recently, outbreaks in European plantations have also been caused by an additional lineage, later named EU2 (Van Poucke et al., 2012), and the EU1 lineage has emerged in forests of southwestern Oregon (LeBoldus et al., 2018). Predictions based on data from studying the spread and impact of a single lineage may not apply when more than one lineage is present, and while interactions between *P. ramorum* and other co-mingling *Phytophthora* species have been studied (Kozanitas et al., 2017), the outcome of interactions among genotypes belonging to different lineages is still widely unknown, with the exception of a few studies on their sexual reproductive potential (Boutet et al., 2010; Vercauteren et al., 2011). A few studies have compared lineages using inoculation trials (Elliott et al., 2011; Eyre et al., 2014; Manter et al., 2010; O'Hanlon et al., 2017); however, these were carried out on a rather small number of hosts, thus a true understanding of the differences among these lineages is still lacking.

Generalism and the presence of distinct and clonally reproducing lineages do provide an excellent opportunity to study adaptive evolution of a pathogen. Genomic studies have provided ample evidence of multiple encounters between *P. ramorum* and a large number of hosts, by identifying a very large number of genes involved in plant–microbe communication (Goss et al., 2013; Tyler et al., 2006). Results of genomic analyses could greatly benefit from a better understanding of the outcome of the interaction between *P. ramorum* and each one of its hosts. For instance, comparative virulence and genomic analyses of pathogen genotypes from transmissive (i.e., with sporulation) and dead-end (i.e., without sporulation) hosts (Hüberli and Garbelotto, 2012) have resulted in the discovery of rapid evolution associated with chromosomal rearrangements, gene copy number variations, and transposon derepression almost exclusively present in pathogen populations from dead-end hosts (Kasuga et al., 2012, 2016).

The first aim of this study was to investigate variability in susceptibility and transmission potential of a large number of plant hosts, many commercially traded, when infected by *P. ramorum*. This was achieved by studying infections of stems and leaves of each species, and was further investigated using different inoculum loads. The second aim of the study was to identify the effect of temperature on disease development and transmission potential of the pathogen. The third aim of this study was to provide a comparative assessment of differences in pathogenicity and sporulation among three genotypes, each representing one of the three lineages of the pathogen commonly present in North American commercial plant nurseries.

Knowledge of the susceptibility and transmission potential of a large number of hosts will greatly assist both regulators and the ornamental plant industry in drafting better regulations, trade policies, and best management practices in plant production facilities. Finally, the information from investigating infections by three genotypes representing the three lineages of *P. ramorum* and from combined plant host and pathogen genotype information may be used

by evolutionary biologists to design better comparative studies for understanding the evolution of plant–microbe interactions.

2 | MATERIALS AND METHODS

2.1 | Plant species and pathogen isolates

A total of 25 plant species from 10 plant families were selected to be inoculated based on the United States Department of Agriculture, Animal and Plant Health Inspection Service (USDA APHIS) list of proven and associated hosts for *P. ramorum* of relevance in the ornamental trade (Anonymous, 2007, 2019). Associated hosts are plant species from which *P. ramorum* has been isolated, but Koch's postulates or even pathogen inoculations have not yet been performed, at least at the time the present study was initiated. Availability on the market of these ornamental plant species was often a limiting factor in the selection of hosts to be tested. The plant species used in the study are listed in Table 1. Plants were in 1-gallon pots and bought from facilities that did not use fungicides, to ensure symptoms of *Phytophthora* infection would not be masked by chemical treatments. To check whether plants were already cryptically infected, they were kept on raised beds at the U.C. Berkeley greenhouse with abundant overhead watering for 3–12 months, ambient relative humidity of about 60%, and temperatures of 20–25°C to facilitate the development of any ramorum blight symptoms. Inoculations were performed using the following three genotypes: 1461 (A genotype, NA1 lineage, isolated from California bay laurel in San Mateo County, California), MR-88 (RHCC31 genotype, NA2 lineage, isolated from rhododendron cv. Colonel Coen in Sacramento County, California), and SI556 (baited from a stream in Humboldt County, California, near an ornamental plant nursery). Each of them grew and sporulated well in vitro, had a colony morphology typical of wild-type phenotypes (Kasuga et al., 2012), and the NA1 and NA2 genotypes had performed well in prior inoculations (Eyre et al., 2014; Serrano et al., 2020). To ensure their vitality, the genotypes were inoculated on rhododendron leaves before being used in this experiment, and lesion sizes were evaluated to ensure all isolates were performing adequately.

TABLE 1 Abbreviations or plant codes, scientific binomial names, common names, and plant family of the 25 plant species used in this study

Plant code	Scientific binomial name	Common name	Family	Used in temperature study
Cilo	*Citrus × limon*	Lemon	Rutaceae	No
Coco	*Corylus cornuta*	Beaked hazel	Betulaceae	No
Gapr	*Gaultheria procumbens*	Winterberry	Ericaceae	No
Gash	*Gaultheria shallon*	Salal	Ericaceae	Yes
Hain	*Hamamelis intermedia*	Hybrid witch hazel	Hamamelidaceae	Yes
Ilaq	*Ilex aquifolium*	Holly	Aquifoliaceae	No
Ilco	*Ilex cornuta*	Chinese holly	Aquifoliaceae	No
Lano	*Laurus nobilis*	Bay laurel	Lauraceae	Yes
Lefo	*Leucothoe fontanesiana*	Highland doghobble	Ericaceae	No
Loch	*Loropetulum chinense*	Chinese fringe flower	Hamamelidaceae	No
Maaq	*Mahonia aquifolia*	Oregon grape	Berberidaceae	Yes
Magr	*Magnolia grandiflora*	Southern magnolia	Magnoliaceae	No
Mast	*Magnolia stellata*	Star magnolia	Magnoliaceae	Yes
Osde	*Osmanthus delavayi*	Delavay osmanthus	Oleaceae	Yes
Osfr	*Osmanthus fragrans*	Sweet olive	Oleaceae	No
Oshe	*Osmanthus heterophyllus*	Holly-leaf osmanthus	Oleaceae	No
Prla	*Prunus laurocerasus*	Cherry laurel	Rosaceae	No
Prlu	*Prunus lusitanica*	Portugal laurel	Rosaceae	No
Rhca	*Rhododendron catawbiense*	Rhododendron	Ericaceae	Yes
Roca	*Rosa californica*	California wildrose	Rosaceae	No
Rogy	*Rosa gymnocarpa*	Wood rose	Rosaceae	No
Syme	*Syringa meyeri*	Korean lilac	Oleaceae	No
Sypa	*Syringa pubescens* subsp. *patula*	Manchurian lilac	Oleaceae	No
Syvu	*Syringa vulgaris*	Lilac	Oleaceae	Yes
Trja	*Trachelospermum jasminoides*	Star jasmine	Apocynaceae	No

2.2 | Leaf inoculations

The first set of inoculation experiments was run on all 25 hosts at 20 °C, between May 2013 and May 2016. A total of 60 leaves were selected from 20 plants per species and were inoculated in six treatments of 10 leaves each, with each leaf coming from a different plant and randomly assigned to each treatment. Treatments were as follows: leaf tips mock inoculated using 1% soil tea (Erwin and Ribeiro, 1996), petiole-side of leaves mock inoculated using 1% soil tea, leaf tips inoculated using a soil tea suspension of 2×10^4 zoospores/ml (high inoculum load), petiole-side of leaves inoculated using a 1% soil tea suspension of 2×10^4 zoospores/ml, leaf tips inoculated with a 1% soil tea suspension of 5×10^2 zoospores/ml (low inoculum load), and petiole-side of leaves inoculated using a soil tea suspension of 5×10^2 zoospores/ml.

Leaves were inoculated as follows. Each leaf was first sterilized by submerging it in 70% ethanol for 30 s, followed by a rinse in deionized water for 30 s. Exposure to the inoculum was performed by dipping the top or bottom 5 mm of the leaves in soil tea (controls) or in soil tea zoospore suspensions for 5 min. Leaves were then placed in black trays on a plastic grid rack overlaid with a sheet of absorbent paper previously wetted with 140 ml deionized water. Leaves were quickly misted with deionized water (8–10 sprays depending on the type of leaf), before a similar tray was put on top of the bottom tray. The two trays were then sealed together using aluminium tape pieces. At that point, each tray "sandwich" was put into a plastic bag that was carefully closed and secured to the trays using binder clips. Trays were then placed in the dark at 20 °C constant temperature for 7 days, checking every 2 days to see if additional misting was needed. Additional misting was done with a spray bottle filled with deionized water, employing 8–12 sprays per tray, depending on leaves' size and physical attributes (e.g., waxy, thick, soft, hairy), to ensure all leaves were equally wetted. At the end of the experiment, 10 leaves were first carefully removed for sporangia and chlamydospore counts. Leaves were either all with symptoms or, if there were no leaves with symptoms, they were randomly selected. For each leaf, the entire lesion (or 1 cm from the tip and the base of the petiole, if lesions were not visible) was washed using 700 µl sterile water on the front and 700 µl on the back, holding the leaf over a 1.5 ml Eppendorf tube. After the first wash per side, the surface of the leaf being washed was gently scraped with a spatula and then the wash was repeated twice more using the same 700 µl. Finally, a total of 1.4 ml from the front and back washes were collected in the Eppendorf tube. A droplet of trypan blue was added to each tube using a dropper and the spore solution was stored at 5 °C, until sporangia and chlamydospores were counted as follows. Eppendorf tubes containing spores were centrifuged at a low speed of 12,300 × *g* for 3 min (Fitchner et al., 2012). The supernatant was removed (from the top down) to leave 0.1 ml, from which two 9 µl drops were assessed using a haemocytometer at 100× magnification using a compound microscope. The entirety of the haemocytometer plate was used for spore counts, taking 10 counts per haemocytometer, in 10 preselected cells along one of the 10 lines, five on each side of the haemocytometer.

After collecting spore washes, the leaves used for spore counts and the remaining leaves were all transferred onto 8.5 × 11 inch blotting paper to remove water, covered with a glass sheet to flatten all leaves and photographed on a camera stand using a Nikon Coolpix P7800 camera fitted with a 45 mm lens. Lesion area and length, as well as leaf area and length were measured using the program Photoshop (Adobe), although, based on previous studies (Hayden et al., 2011, 2013), further analyses were performed using only lesion length and leaf length values.

After surface sterilization with 70% ethanol, two sample discs from each leaf were taken using a hole-punch and placed onto PARPH agar, a selective medium containing five antibiotics, to isolate any *Phytophthora* present (Solel and Pinkas, 1984). If there was a lesion, the disc was taken from the lesion margin. If no lesion was observed, discs were taken from the area that had been initially submerged in the inoculum (leaf tip or petiole). Plates were incubated in the dark at 20 °C for 30 days, and inspected every 5 days for the presence of *P. ramorum* colonies.

2.3 | Stem inoculations

A total of 15 stem inoculations per plant species and pathogen genotype were performed between November 2013 and January 2017 using the same three genotypes of *P. ramorum* and the same plant species used for the leaf inoculations described above (Table 1). Plants were grown in 1-gallon pots and three stems were inoculated on each of five plants. Inoculation points were chosen to be approximately halfway along the length of each branch and the diameter of each branch at the inoculation point was measured using a caliper. Inoculation points were then wounded by pinpricking them ten times. Round plugs, 4 mm in diameter, were obtained from the edges of 15-day-old *P. ramorum* colonies growing on V8 agar, using a cork borer (Erwin and Ribeiro, 1996). One inoculum plug, with the mycelium side facing the wounded bark, was placed on each wound, and the inoculation point was then wrapped tightly with Parafilm and further covered by a strip of aluminium foil. Mock inoculations were performed in the same way, using uninoculated V8 agar plugs. Plants were kept at 20 °C and watered every 3–4 days. Twenty-one days after inoculation, inoculated branches were removed from plants, the foil and Parafilm were removed and any visible agar remains were gently scraped away or pulled off. Spore washes were similar to those used for leaves, except the stem length washed was constant at 15 mm, extending from 7.5 mm above the inoculation point to 7.5 mm below it. A total of 1.4 ml deionized water was used to wash the stem area and collect spores into 1.5-ml Eppendorf tubes. The entire area was then gently scraped and the wash water was repipetted twice over the area back into the tube. Washes were treated as described above for leaves. The bark was then scraped away and the lesion length, if any, was measured using a ruler. Finally, each branch was surface sterilized with 70% ethanol and isolations were made by

taking tissue 1.5 cm above inoculation point or at the lesion margin, and 1.5 cm below the inoculation point or at the lesion margin and placing such plant tissue onto PARPH agar plates. Plates were kept and inspected as described above.

2.4 | Effect of temperature on foliar infection

A final set of leaf inoculations was performed between November 2015 and September 2016 on a subset of eight hosts selected to represent plant species of high and intermediate susceptibility (Table 1). The protocol followed was identical to the one described above for leaf inoculations at 20 °C except that only leaf tips were used for inoculations and controls. Each treatment consisted of 20 leaves placed at 12, 20, or 25 °C, to investigate the effect of temperature on infection success, lesion development, and sporulation.

2.5 | Analysis

The following data sets were generated in the course of the experiment, all using the three pathogen genotypes. (a) A 20 °C leaf infection data set for 25 plant hosts using low pathogen inoculum (5×10^2 zoospores/ml) or soil tea for controls. (b) A 20 °C leaf infection dataset for 25 plant hosts using high pathogen inoculum (2×10^4 zoospores/ml) or soil tea for controls. (c) A 20 °C stem infection data set for 25 plant hosts using a plug of agar colonized by the pathogen as inoculum or a plug of agar for controls. (d) Eight plant hosts were also inoculated in an additional experiment using high inoculum (2×10^4 zoospores/ml) and placing one full set of inoculated leaves at three different temperatures: 12, 20, and 25 °C.

Although there were slight variations in parameters studied in each data set, in general, the variables recorded may have included success (%) of pathogen reisolation, lesion length, lesion length as a percentage of leaf length, number of sporangia in lesion washes, and number of chlamydospores in lesion washes.

In no case was the pathogen ever reisolated from mock-inoculated controls (Table 2); therefore, given the large scale of this experiment, data from controls were simply omitted from the analyses. Analysis focused on (a) identifying significant variation in parameters associated with host susceptibility and infectiousness among the 25 hosts tested when pooling data of the three pathogen genotypes together; and (b) highlighting differences in pathogenicity and infectivity among the three genotypes of the pathogen. Likewise, model-fitting analyses were performed for a few key select variables, such as success of pathogen reisolation, lesion length, and number of sporangia, to identify whether pathogen genotype, host species, and the interaction between the two had a measurable effect on the results.

The first of such groups of broad analyses was to compare success of pathogen reisolation from leaves and average leaf lesion length for each of the three genotypes and especially for all three genotypes combined when using low and high inoculum suspensions.

TABLE 2 Effect of *Phytophthora ramorum* inoculum load on success of pathogen reisolation and on lesion length from leaves that had been inoculated using suspensions of either 5×10^2 zoospores/ml (low inoculum) or 2×10^4 zoospores/ml (high inoculum)

	Success of reisolation (%)		Average lesion length (mm; *SE*)	
Genotype	Low inoculum	High inoculum	Low inoculum	High inoculum
NA1	59.7	84.8***	10.9 (0.62)	14.3 (0.57)***
NA2	55.0	62.8***	8.8 (0.54)	10.2 (0.64)
EU1	67.3	81.5***	13.3 (0.67)	16.8 (0.70)***
All three	60.6	76.5***	11.1 (0.35)	14.1 (0.38)***
Control	0.0	0.0	0.0	0.0

***$p < .0001$.

For pathogen reisolation success, low and high inoculum data were compared using chi-square analysis, while a Student's *t* test was used instead to compare lesion length data.

The second set of analyses included the following comparisons among the three pathogen genotypes: percentage of successful pathogen reisolation and leaf lesion length in the low inoculum dataset, percentage of successful pathogen reisolation, leaf lesion length, and leaf sporangia counts in the high inoculum test and in the stem data sets, analysed separately. Numerical continuous variables such as lesion length and number of sporangia were analysed using analyses of variance (ANOVAs) and means from individual genotypes were compared using Tukey's tests. Ordinal 1/0 (presence/absence) reisolation data were analysed using chi-square tests. Differences among the three genotypes were determined running Pearson's test in all three possible pairwise combinations.

The third set of analyses consisted of model-fitting tests to identify whether dependent variables such as successful pathogen reisolation from leaves, successful pathogen reisolation from stems, leaf lesion length, stem lesion length, number of sporangia in leaf washes, and number of sporangia in stem washes each significantly varied depending on genotype inoculated, on host species tested, and on the interaction between genotype and plant host. Numerical continuous variables such as lesion length and sporangia counts were investigated using general linear models, with standard least squares selected in the "Personality" box. Models for binary ordinal reisolation data were fitted using the nominal logistic selection in the "Personality" box.

A full group of analyses was performed to compare the susceptibility and infectiousness on 25 host species selected for this study, and to provide information on the type of disease each may develop when infected by *P. ramorum*. A suite of metrics was analysed for each host infected by each genotype, and summary statistics of most metrics are presented by host and genotype in supplementary data files; however, our main comparisons among species were done using data from all three genotypes combined. This approach was chosen for a variety of reasons. First, it tripled

the number of replicates, providing more solid data. Secondly, each host was inoculated on multiple dates, and tests using rhododendron leaves and stems indicated significant differences among test dates (data not shown). However, when using data from all genotypes, data for each and all hosts were generated in multiple tests executed in different times, thus overall decreasing the effect of trial time on the results. Thirdly, although most model testing identified differences among genotypes, and a significant interaction among genotypes and host species, these differences were only occasionally noteworthy or of a magnitude deserving special attention. Additionally, genotype-level results were more difficult to interpret because each genotype was a single representative of the three different pathogen lineages.

Binary data sets such as those of success of pathogen reisolation were analysed using chi-square tests and contingency tables, identifying significant differences for the entire data set, but avoiding the investigation of presence and composition of statistically homogeneous groups. All other data were numerical and continuous and thus were instead analysed overall by ANOVAs, using lesion length or percentage of leaf area with a lesion as the dependent variables, and host species as the independent variable. Statistically homogeneous groups were identified using Tukey's tests.

Susceptibility of the 25 plant species to low pathogen inoculum was studied on detached leaves by comparing percentages of successful pathogen reisolation and lesion length values from leaves that yielded positive reisolations. We also ranked species using a leaf disease index (LDI) obtained by multiplying the percentage of successful pathogen reisolation for each plant species by its average lesion length. The same analyses above were performed using leaf data obtained using high pathogen inoculum, and stem data. Because leaf length may possibly be a factor limiting lesion development when inoculating leaves (i.e., small leaves may only develop equally small lesions), we also present data for leaf lesions expressed as percentage of leaf length for this data set. For the high inoculum leaf data, the LDI was calculated as explained above by multiplying the percentage of leaves from which the pathogen was successfully reisolated by the average lesion length from leaves that yielded positive reisolations. For the stem data, the stem disease index (SDI) was calculated by multiplying the percentage of stems from which the pathogen was successfully reisolated for each host by the average stem lesion measured in that host from stems that yielded positive reisolations. The total disease index (TDI), an overall index representing the overall susceptibility of a host plant affected by aerial infections only, that is, excluding potential root infections, was then calculated as follows: TDI = LDI + SDI.

The sporulation potential among the 25 host plants was studied by comparing the number of sporangia in leaf and stem washes inoculated using high inocula, whether 5×10^4 zoospore suspensions for foliar tests or pathogen inoculum plugs for stem tests. ANOVAs were employed to study the effect of host (independent variable) on sporangia counts (dependent variable), and hosts were further ranked and grouped using Tukey's tests. Leaf and stem analyses were performed independently. A leaf sporulation index (LSI) was calculated for each host by multiplying the average number of sporangia in leaf washes by the percentage of leaves from which the pathogen was successfully reisolated. A stem sporulation index (SSI) index was calculated multiplying the average number of sporangia in stem washes for each host by its percentage of successful stem pathogen reisolations. Finally, a total sporulation index (TSI) for each host was calculated as follows: TSI = LSI + SSI.

Two additional parameters are presented for the high inoculum leaf and the stem inoculation datasets analysed by pooling data from the three pathogen genotypes together: the percentage of successful reisolations from leaf tips versus leaf petioles, and the average number of chlamydospores in leaf and stem washes. These parameters are presented but only minimally analysed. Leaf versus petiole data were compared using chi-square analyses to identify plants that may develop one of three different type of foliar disease: disease may preferentially start at the petiole and develop along the mid vein, disease may preferentially begin at the tips and the margins of leaves, or disease may affect any part of the leaf. Chlamydospore data are presented, but counts were highly variable, thus those data are not analysed and should be interpreted with caution. Finally, ad hoc comparisons were performed to identify interesting results in the data sets including the presence of hosts with low pathogen reisolation success but large lesion lengths; the presence of hosts with small stem and/or leaf lesions but large number of sporangia in washes; and hosts characterized by large foliar lesions and small stem lesions or vice versa.

A final group of analyses was performed to compare lesion length values and sporangia counts at three different temperatures (12, 20, and 25 °C). Overall, these analyses were performed on leaves of a subset of eight host plants. Specific comparisons were performed as follows: (a) for each one of the eight plants tested using data pooled for all three genotypes, (b) for each one of the three genotypes using data pooled from all eight plant species, and, (c) for all three genotypes combined using data pooled from all eight plant species. ANOVAs were employed to perform these comparisons, and statistically homogeneous groups were identified using Tukey's tests.

In order to meet the assumptions of normality and equal variances of data distribution, data transformations were necessary, including the square root of foliar lesion length at both inoculum levels, and squaring the number of sporangia for both foliar datasets. Lesion length values were cubed for the stem inoculation data. Sporangia data from stems and from leaves in the temperature studies met the assumptions and thus were analysed without transformation. Leaf lesion data in the temperature study were also analysed without transformation for analyses regarding individual hosts. Because transformations of the overall foliar lesion data set for analyses of the effect of temperature on all the three genotypes combined and for each individual genotype did not significantly improve normality or equality of variances, Welch's test was employed to perform ANOVAs and nonparametric comparisons among all pairs were performed using the Steel–Dwass method. All analyses were performed using JMP v. 14 (2019).

3 | RESULTS

3.1 | Effect of inoculum load on infection and lesion development

Percentage of successful pathogen reisolation and lesion length values were compared between leaf inoculations performed using inocula with different zoospore concentrations (Table 2). Comparisons were made for each genotype separately and for all three genotypes together. Both parameters were significantly ($p < .001$) higher when using high zoospore inoculum levels than when using low inoculum levels. The only exceptions were lesion length values for the genotype belonging to the NA2 lineage, where the results using the two different inoculum levels were comparable. It should also be noted that, overall, foliar lesion length values were lower for the NA2 genotype than the other two genotypes at both inoculum concentrations.

3.2 | Foliar inoculations using low inoculum

When success of pathogen reisolation from leaves and foliar lesion length values obtained using low inoculum were compared, significant differences were identified among genotypes for both parameters (Table 3). Percentage of successful pathogen reisolation was highest and lowest for the EU1 and NA2 genotypes, respectively, while NA1 genotype values were intermediate. Lesion length values were highest and comparable for the EU1 and NA1 genotype, and lowest for the NA2 genotype.

Percentage of successful reisolation and lesion length values were compared among hosts for each genotype and for all genotypes together for the low inoculum data set. Both parameters were found to differ among host species for individual pathogen genotypes (Table S1) and for all genotypes together (Table S2; Figure 1). Percentage of successful pathogen reisolation considering all genotypes together ranged between 0% and 100% and was found to be different among hosts based on a chi-square analysis (Pearson chi-square = 1,042, $p < .0001$; Figure 1a). A nominal logistic fit model indicated a significant interaction between pathogen genotype and plant host species ($p = .000$; Table S3). Lesion length considering data from all three pathogen genotypes together ranged between 0 and 28 mm (Table S2; Figure 1b) and was also found to be different among hosts based on an ANOVA ($df = 25$, F ratio = 43.9, $p < .0001$). Figure 1b presents mean leaf lesion length by each host for all three pathogen genotypes together, including the assignment of each host to a statistically different group based on Tukey's test with $\alpha = 0.05$. A general linear model (GLM) based on standard least squares identified a significant interaction between pathogen genotype and host ($p < .0001$; Table S4). Figure 1c shows the low inoculum LDI calculated using only *P. ramorum*-positive replicates. A group of hosts (see Table 1 for host names' abbreviations) including Syme, Sypa, Hain, Syvu, Osde, and Magr had LDI values larger or equal to that of Rhca when exposed to low levels of inoculum. Prlu, Coco, Lano, Prla, Oshe, Gapr, Ilaq, Trja, Lefo, Ilco, and Cilo had extremely low LDI values when leaves were challenged with low inoculum levels.

When comparing percentage of successful reisolations from leaf tips and petioles (Table S2), the following species had a larger

TABLE 3 Comparison of disease parameters among three genotypes of *Phytophthora ramorum*

Comparison	Pathogen genotype			p
	NA1	NA2	EU1	
Leaf % successful pathogen reisolation; low inoculum	59.7 b	55.0 c	67.3 a	<.001
Leaf mean lesion length in mm (*SE*); low inoculum	13.7 (0.7) a	10.9 (0.6) b	13.4 (0.7) ab	<.02
Leaf % successful pathogen reisolation; high inoculum	62.8 b	84.8 a	81.5 a	<.001
Leaf mean lesion length in mm (*SE*); high inoculum	15.4 (0.6) a	11.4 (0.7) b	16.5 (0.8) a	<.001
Leaf mean number of sporangia (*SE*); high inoculum	177.0 (34.6) a	21.2 (4.7) b	32.7 (6.2) b	<.001
Stem % successful pathogen reisolation	58.8 b	76.1 a	58.4 b	<.001
Stem mean lesion length in mm (*SE*)	31.7 (4.1) a	21.7 (2) b	27.5 (3.3) ab	.009
Stem mean number of sporangia (*SE*)	6.1 (0.8) a	5.9 (0.7) a	5.1 (0.7) a	.6

Note: Results were obtained in three experiments described in this study: leaf inoculations using low inoculum, leaf inoculations using high inoculum, and stem inoculations using agar plugs colonized by the pathogen. Chi-square tests were used to analyse % pathogen reisolation data, while continuous numerical variables such as lesion length and number of sporangia were analysed by analyses of variance. Homogeneous groups, indicated by a letter, were determined running Pearson's test in all three possible pairwise combinations for % pathogen reisolation data, and by Tukey's tests for all other variables. Values in the same row followed by different letters were significantly different at the p value indicated.

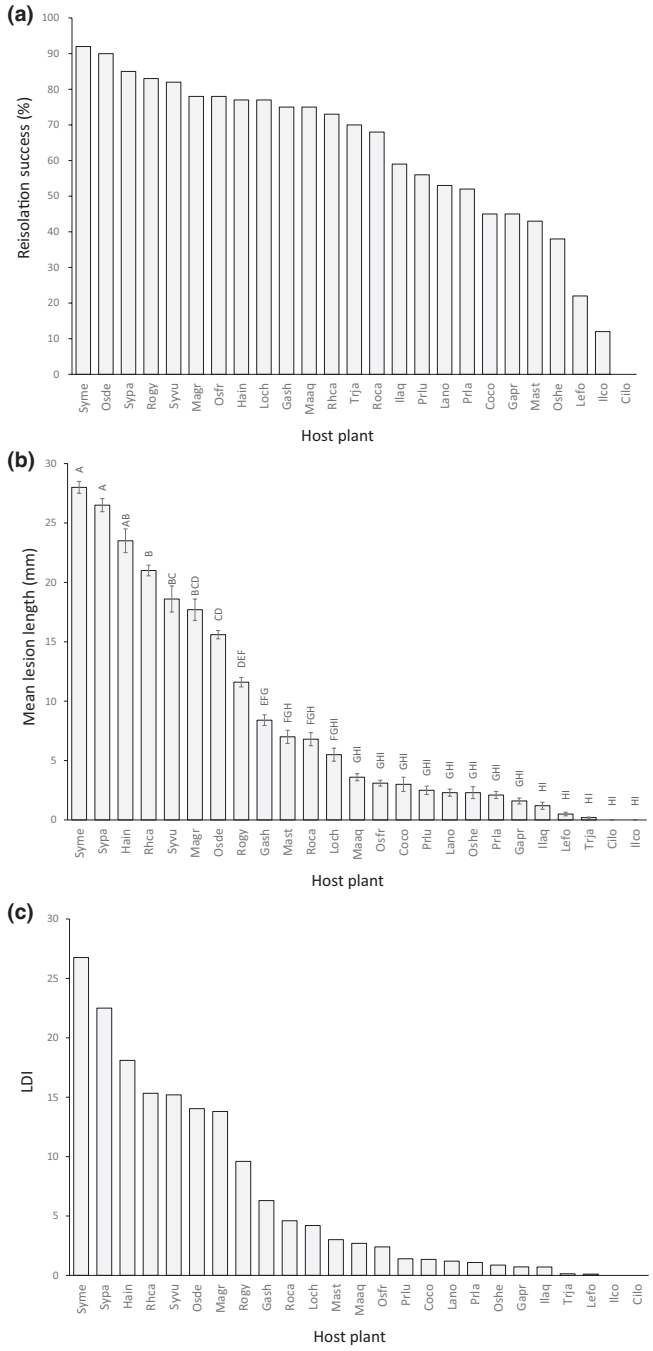

FIGURE 1 Results of inoculations of 60 leaves from each of 25 plant species, using a low level of inoculum consisting of soil tea suspensions containing 5×10^2 zoospores/ml of the pathogen *Phytophthora ramorum*. (a) The percentage of leaves of each species that yielded positive pathogen reisolations from necrotic tissue a week after inoculation. (b) The mean foliar lesion length caused by *P. ramorum* on each species calculated only from plants with a positive pathogen reisolation result. (c) The leaf disease index (LDI; percentage successful pathogen reisolation × average lesion length) for each species. Where appropriate, species with indistinguishable results based on Tukey's tests with $\alpha = 0.05$ are identified by the same letter. Data were obtained by pooling results from all three genotypes of *P. ramorum* used in this study

success from leaf tips: Lano, Mast, and Roca. Conversely, reisolations were more successful from leaf petioles than from leaf tips for Gapr, Loch, Oshe, and Prla.

3.3 | Foliar inoculations using high inoculum

When success of pathogen reisolation, lesion length values, and mean number of sporangia obtained using high inoculum on leaves were compared, significant differences were identified among genotypes for all three parameters (Table 3). Percentage of successful pathogen reisolation was highest for the EU1 and NA2 genotypes, and lowest for the NA1 genotype. Lesion length values were highest for the EU1 genotype but statistically undifferentiated from those caused by the NA1 genotype, while they were significantly lower for the NA2 genotype. Numbers of sporangia were highest for the NA1 genotype, and much lower for the NA2 and EU1 genotypes.

Percentage of successful reisolation, lesion length values, lesion length expressed as percentage of leaf length with a lesion, and number of sporangia were compared among hosts for each genotype and for all genotypes together for the foliar high inoculum dataset. The four parameters were found to be different among host species for individual genotypes (Table S5) and for all genotypes together (Table S6; Figures 2 and 3). Percentage of successful pathogen reisolation from leaves considering all genotypes together ranged between 35% and 100% and was found to be different among hosts based on a chi-square analysis (likelihood ratio = 595; $p < .0001$; Figure 2a). A nominal logistic fit model indicated a significant interaction between pathogen genotype and plant host species ($p < .0001$; Table S3). Lesion length considering all pathogen genotypes together was found to range between 0 and 41.6 mm (Table S6) and to be different among hosts based on an ANOVA ($df = 24$, F ratio = 87, $p < .0001$). Figure 2b presents the mean low inoculum lesion length by each host for all genotypes together, including the assignment of each host to a statistically different group based on Tukey's test with $\alpha = 0.05$. A GLM based on standard least squares identified a significant interaction between pathogen genotype and host ($p < .0001$; Table S4). Lesion length expressed as percentage of leaf length with lesion, and considering all genotypes together, ranged between 0% and 85% (Table S6) and was found to be different among hosts based on an ANOVA ($df = 24$, F ratio = 105, $p < 0.0001$). Figure 2c presents mean percentage of leaf length with lesion by each host for all genotypes together, including the assignment of each host to a statistically different group based on Tukey's test with $\alpha = 0.05$. A comparison between Figure 2b and 2c indicates Syme, Osde, and Sypa were more significantly affected by the pathogen in terms of percentage of leaf length that can be colonized. Figure 2d shows the high inoculum LDI; a group of hosts (see Table 1 for abbreviations of host names) including Hain, Sypa, Syme, and Syvu developed foliar lesions larger than or equal to Rhca when exposed to high levels of inoculum.

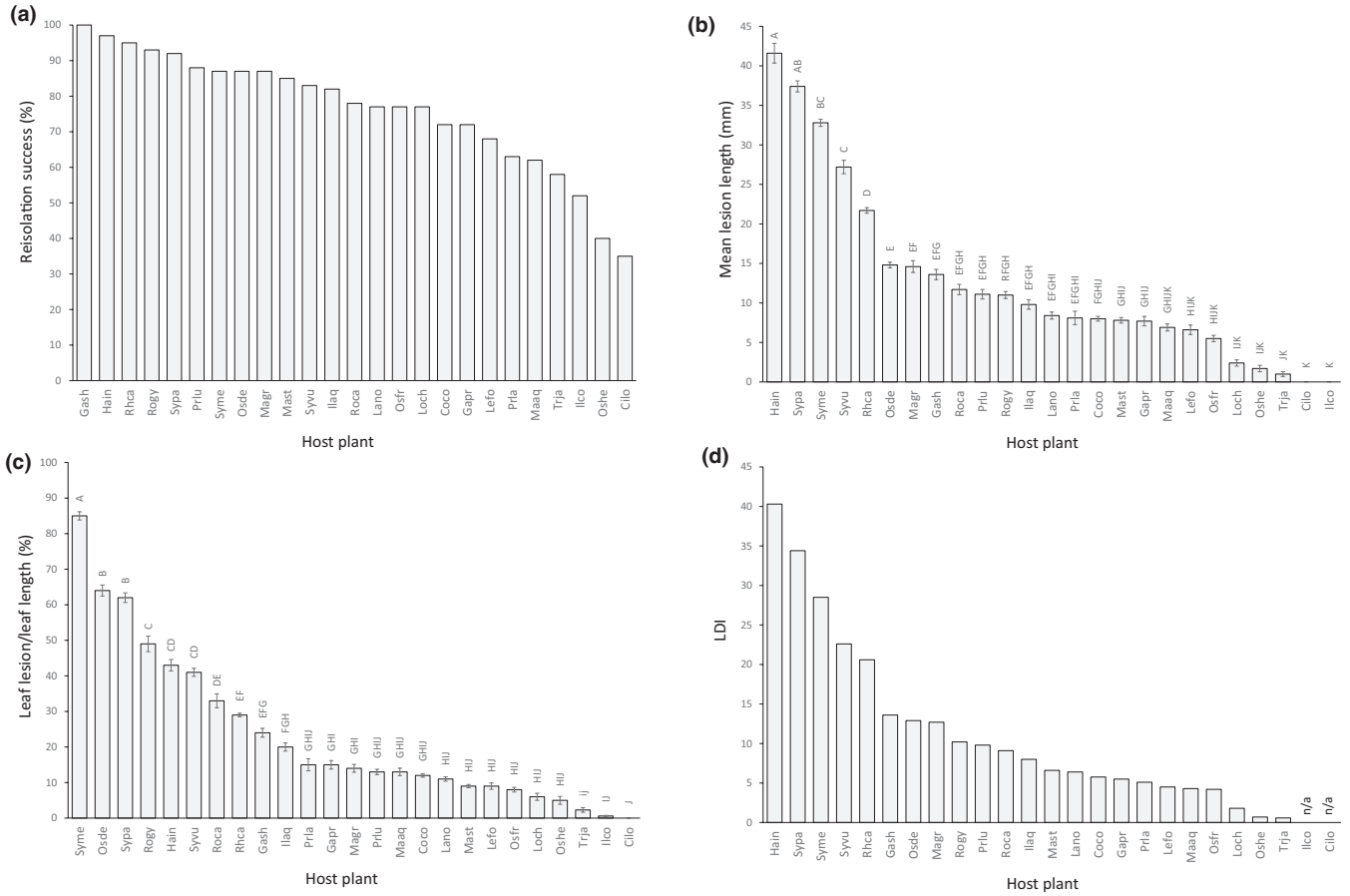

FIGURE 2 Results of inoculations of 60 leaves from each of 25 plant species, using a high inoculum level consisting of soil tea suspensions containing 2×10^4 zoospores/ml of the pathogen *Phytophthora ramorum*. (a) The percentage of leaves of each species that yielded positive pathogen reisolations from necrotic tissue a week after inoculation. (b) The mean foliar lesion size caused by *P. ramorum* on each species calculated for all plants with a positive pathogen reisolation result. (c) Results expressed as mean percentage of leaf length blackened by *P. ramorum* on each species, again calculated excluding all plants without a positive pathogen reisolation result. (d) The leaf disease index (LDI; percentage successful pathogen reisolation × average lesion length) for each species. Where appropriate, species with indistinguishable results based on Tukey's tests with $\alpha = 0.05$ are identified by the same letter. Data were obtained by pooling results from all three genotypes of *P. ramorum* used in this study

Loch, Oshe, Trja, Ilco, and Cilo had extremely low LDI values when leaves were challenged with high inoculum levels.

Mean number of sporangia produced on leaves considering all three genotypes together ranged between 0.8 and 518 (Table S6) and was found to be different among hosts based on an ANOVA ($df = 24$, F ratio = 6.1, $p < .0001$; Figure 3a). A GLM based on standard least squares identified a significant interaction between pathogen genotype and host ($p < .0001$; Table S7). A comparison of the LSI of each species showed that Syvu, Hain, Syme, Rogy, and Sypa had a greater LSI than Rhca (Figure 3b). The values from Lano and Lefa were comparable to the value from Rhca, while all other species had a very small or nil LSI. There was a good match between species rankings when comparing mean number of leaf sporangia with LSI.

When comparing percentage of successful reisolation from leaf tips and petioles (Table S6), the following species had a larger success from leaf tips: Cilo, Mast, and Roca. Conversely, reisolations were more successful from leaf petioles for Gapr, Osde, Oshe, Sypa, and Syvu.

When comparing lesion length results of foliar inoculations using low and high inoculum, the rankings of individual species were fairly similar whichever level of inoculum was used. Two exceptions were notable: Loch appeared to be more susceptible in the low inoculum dataset, while Prlu had small lesions in the low inoculum data set, but was of intermediate to high susceptibility in the high inoculum data set. Oshe, Trija, Ilco, Cilo displayed extremely small lesions in both data sets. When comparing LDI and LSI results, species displaying higher LDI values also had higher LSI values.

3.4 | Stem inoculations

When success of pathogen reisolation, lesion length values, and mean number of sporangia obtained by inoculating stems were compared among genotypes, significant differences were identified both for success of pathogen reisolation values and for stem lesion sizes (Table 3). Percentage of successful pathogen reisolation was highest

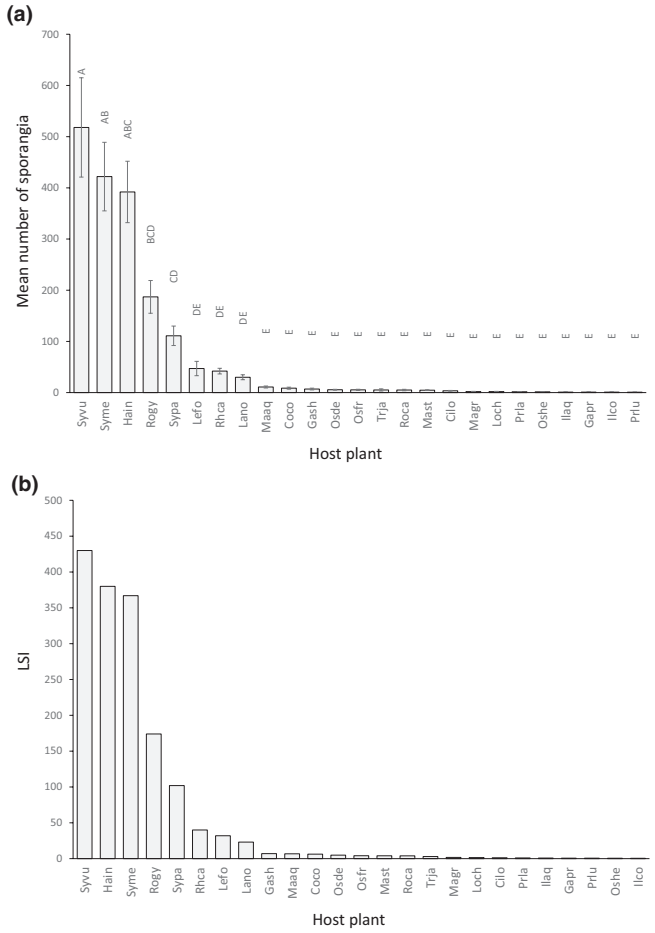

FIGURE 3 Results of inoculations of 30 leaves from each of 25 plant species, using a high inoculum consisting of soil tea suspensions containing 2×10^4 zoospores/ml of the pathogen *Phytophthora ramorum*. (a) The mean number of sporangia that were washed from leaves that yielded a positive pathogen reisolation result out of a total of 10 inoculated. (b) The leaf sporulation index (LSI; average number of sporangia in leaf washes × percentage successful pathogen reisolation) for each species. Data were obtained by pooling results from all three genotypes of *P. ramorum* used in this study

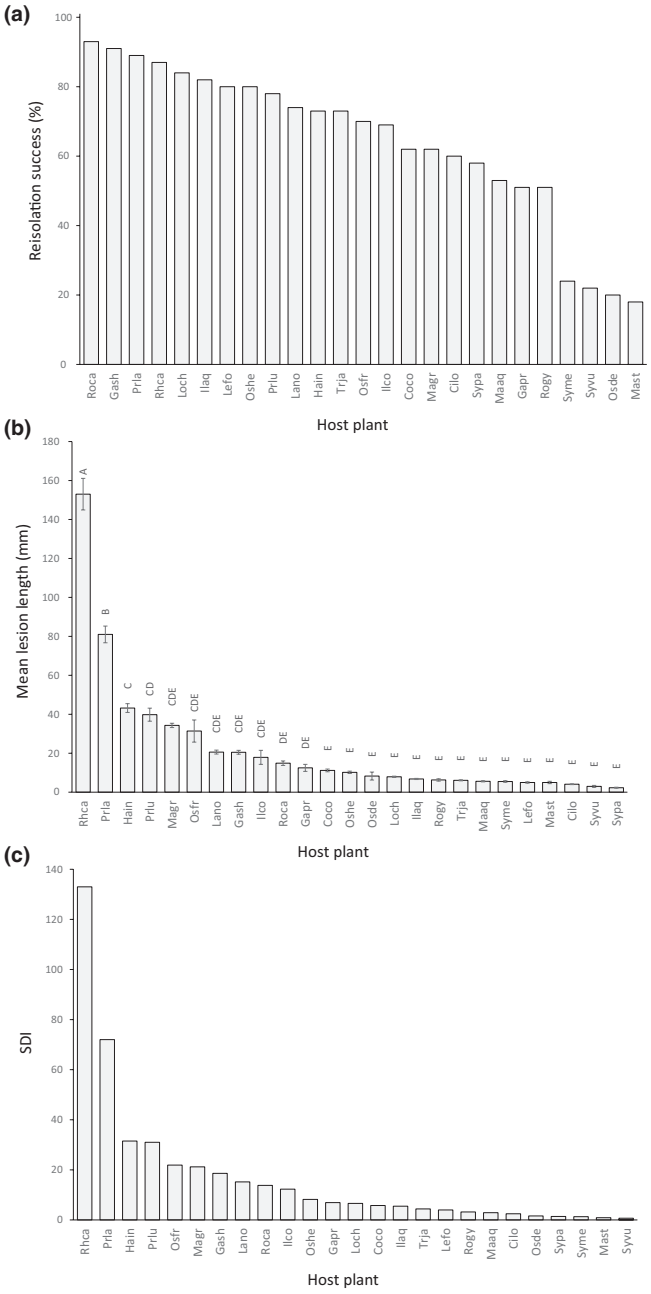

FIGURE 4 Results of inoculations of 45 stems of each of 25 plant species using agar plugs colonized by the pathogen *Phytophthora ramorum*. (a) The percentage of stems of each species that yielded positive pathogen reisolations from necrotic tissue 3 weeks after inoculation of 15 stems per species. (b) The mean stem lesion size caused by *P. ramorum* on each species calculated from all stems with a positive pathogen reisolation result. (c) The stem disease index (SDI; percentage successful pathogen reisolation × average stem lesion length from those stems) for each species. Where appropriate, species with indistinguishable results based on Tukey's tests with $\alpha = 0.05$ are identified by the same letter. Data were obtained by pooling results from all three genotypes of *P. ramorum* used in this study

for the NA2 genotype, while values for the NA1 and EU1 genotypes were lower and comparable to one another. Stem lesions were largest for the NA1 genotype, intermediate for EU1 genotype, and smallest for NA2 genotype. It is interesting to note that the average size of stem lesions ranged between 21.7 mm for the NA2 genotype and 31.7 mm for the NA1 genotype, while lesions on leaves inoculated with high inoculum were smaller and ranged between 10.16 mm for the NA2 genotype and 16.8 mm for the EU1 genotype. Conversely, average numbers of sporangia on stems ranged between 5 (EU1 genotype) and 5.9 (NA2 genotype), while average numbers of sporangia on leaves inoculated using high inoculum were much larger, and ranged between 20.9 for the NA2 genotype and 176.3 for the NA1 genotype.

Percentages of successful pathogen reisolation, lesion length values, and number of sporangia were compared among hosts for each genotype and for all genotypes together for the stem inoculation data set. The three parameters were found to be different among host species for individual genotypes (Table S8) and for all

genotypes together (Table S9; Figure 4). Percentages of successful pathogen reisolation from stems considering all genotypes together ranged between 18% and 91% and were found to be different among hosts based on a chi-square analysis (likelihood ratio chi-square = 253, $p < .0001$; Figure 4a). A nominal logistic fit model indicated a significant interaction between pathogen genotype and plant host species ($p < .0001$; Table S3). Lesion length values on stems considering all genotypes together were found to range between 2.3 and 153 mm (Table S9) and to be different among hosts based on an ANOVA ($df = 24$, F ratio = 37, $p < .0001$). Figure 4b presents mean stem lesion length by each host for all genotypes together, and a GLM based on standard least squares identified a significant interaction between pathogen genotype and host ($p < .0001$; Table S4). Figure 4c shows the SDI for each host, calculated using only *P. ramorum*-positive replicates. Rankings of species were identical for stem lesion results and SDI, hence we briefly discuss the presence of different groups of species based on the stem lesion length analysis. Rhca and Prla developed the largest and the second largest stem lesions, respectively. These two species were followed by a group of species developing intermediate stem lesions, comprising Hain, Prlu, Magr, Osfr, Lano, Gash, and Ilco. All the other species tested developed small or very small stem lesions.

The mean number of sporangia produced on stems considering all genotypes together ranged between 1.1 and 14.5 (Table S9; Figure 5a) and was found to be different among hosts based on an ANOVA ($df = 24$, F ratio = 3.45, $p < .0001$). Figure 5a presents mean number of sporangia by each host for all genotypes together and a GLM based on standard least squares identified a significant interaction between pathogen genotype and host ($p < .0001$; Table S7); the SSI for each host species is shown in Figure 5b. Roca had by far the highest SSI value, while Hain, Osfr, Ilco, Lano, and Sypa had SSI values in between those of Roca and Rhca. Gash, Rogy, Oshe, and Maaq had values comparable to the value from Rhca, while all other species had smaller SSI values. There was a good match between species rankings when comparing mean number of stem sporangia with SSI.

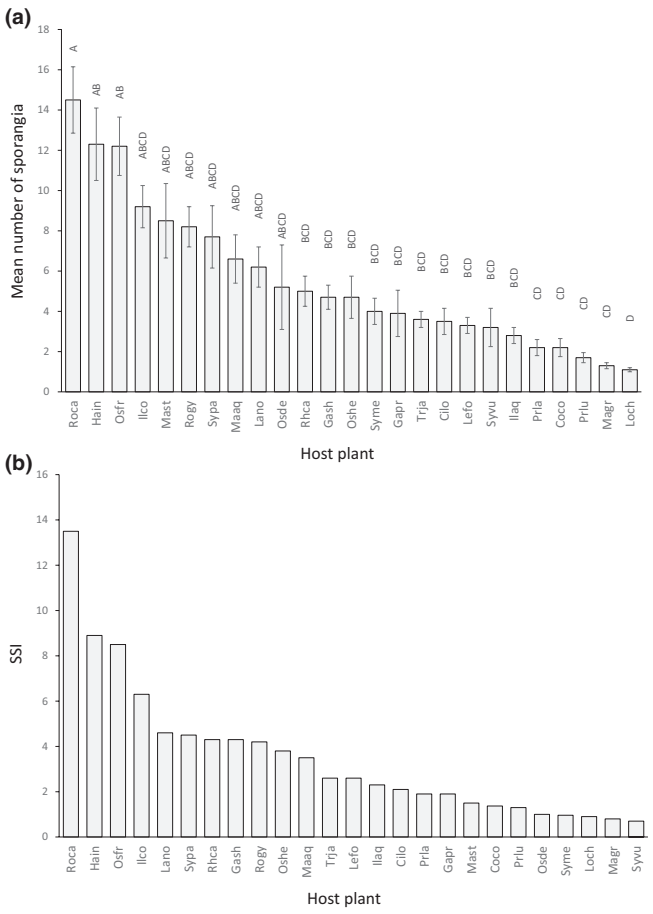

FIGURE 5 Results of stem inoculations of 25 plant species using agar plugs colonized by the pathogen *Phytophthora ramorum*. (a) The mean number of sporangia that were washed from stems that yielded a positive pathogen reisolation result out of 15, 3 weeks after inoculation. (b) The stem sporulation index (SSI; average number of sporangia in stem washes × percentage of successful stem pathogen reisolations) for each species. Where appropriate, species with indistinguishable results based on Tukey's tests with $\alpha = 0.05$ are identified by the same letter. Data were obtained by pooling results from all three genotypes of *P. ramorum* used in this study

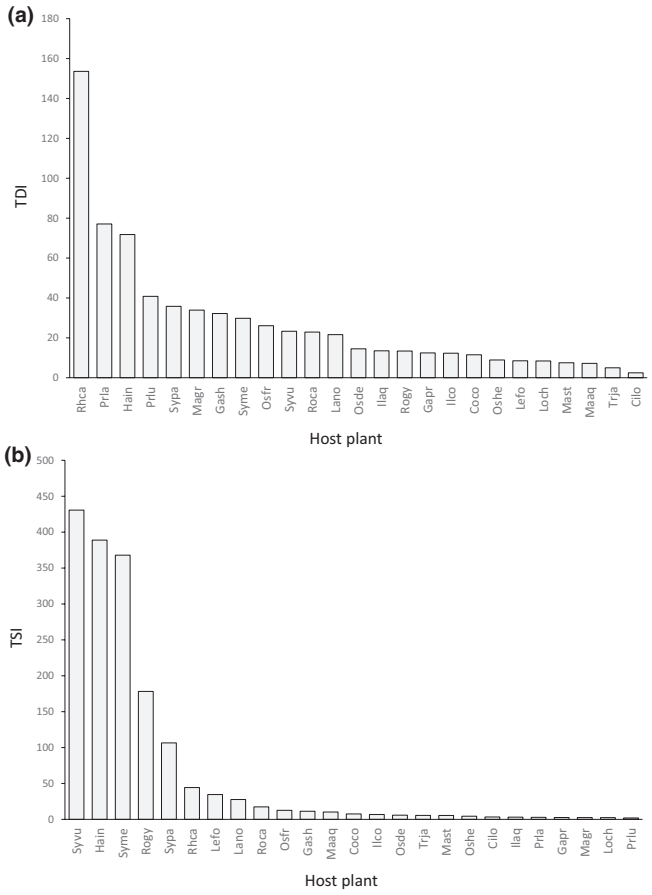

FIGURE 6 (a) The total disease index (TDI = leaf disease index + stem disease index) for each of 25 host species inoculated with *Phytophthora ramorum*. (b) The total sporulation index (TSI = leaf sporulation index + stem sporulation index) for the same set of plants. Data were obtained by pooling results from all three genotypes of *P. ramorum* used in this study

When comparing SDI and SSI values, the two datasets were conspicuously incongruent. Rhca, Prla, Prlu had high SDI values but ranked much lower for SSI. Conversely, Roca, Lano, and Ilco had relatively low SDI values but ranked high for SSI.

3.5 | The total disease and sporulation indices, TDI and TSI

Figure 6a shows the ranking of each species for overall susceptibility obtained by adding LDI and SDI to give the TDI. The 25 species tested can be divided in three groups as follows. Highly susceptible species, given their high TDI values, included Rhca, Prla, and Hain. Intermediately susceptible plants, characterized by intermediate TDI values, included Prlu, Sypa, Magr, Gash, Syme, Osfr, Syvo, Roca, and Lano. Species of low susceptibility, with low TDI values, included Osde, Ilaq, Rogy, Gapr, Ilco, Coco, Oshe, Lefo, Loch, Mast, Maaq, Trija, and Cilo.

Figure 6b shows the ranking of each species for overall levels of sporulation, obtained by adding LSI to SSI to obtain the TSI. Syvu, Hain, and Syme had high TSI values. Rogy and Sypa had medium-high TSI values. Rhca, Lefo, Lano, and Roca had medium-low TSI values. All other species had low or very low values. An interesting lack of congruency can be observed when comparing TDI and TSI results. Rhca had the highest TDI score but only had a medium-low TSI spore. Prla and Prlu had high and medium TDI scores, respectively, but scored very low for TSI. Conversely, Syvu and Syme had medium TDI values but some of the highest TSI values. Rogy had the fourth highest TSI values, but ranked 15th for TDI. Hain was the only species that scored high for both indices.

3.6 | Effect of temperature on leaf lesion length and sporulation

Results of leaf inoculations on a subset of eight plant species inoculated with an isolate each from the three pathogen genotypes and kept at three different temperatures (12, 20, and 25 °C) are summarized below. Lesion length values and number of sporangia are presented in Tables 4 and 5, respectively. For both parameters, data are reported for each individual species and for all hosts together when pooling the data from the three pathogen genotypes. This way of presenting data helps understanding of how temperature affects infection for each species individually and in general for all hosts. Results obtained when pooling data from all host species inoculated with each pathogen genotype are also reported in order to show how infection by individual genotypes may be affected by temperature.

Lesion length (Table 4) was of intermediate size and unaffected by temperature only in two species, namely Hain and Mast. Lano, Osde, and Syvu developed larger lesions when inoculated at 20 °C, but developed smaller lesions both in colder (12 °C) or in warmer (25 °C) conditions. Gash developed the largest and smallest lesions at 25 and 12 °C, respectively. Lesions in Maaq and Rhca were comparable in size at 20 and 25 °C, but were significantly smaller at 12 °C. When looking at each one of the three genotypes, the NA1 genotype performed equally well at 20 and 25 °C, but underperformed at 12 °C, while NA2 and EU1 genotypes both performed best and worst at 20 and 12 °C, respectively.

TABLE 4 Average lesion size caused on 30 leaves of each of eight plant species inoculated using a high inoculum level of *Phytophthora ramorum* zoospore suspension and kept at three different temperatures for 7 days

Host	Pathogen genotype	Average lesion length (mm; *SE*)		
		12 °C	20 °C	25 °C
Gash	All 3	12.6 (0.7) c	21.1 (0.9) b	27.6 (1.0) a
Hain	All 3	10.0 (0.9) a	11.6 (1.4) a	11.7 (1.7) a
Lano	All 3	7.1 (0.6) b	12.0 (1.0) a	7.2 (0.4) b
Maaq	All 3	2.8 (0.4) b	7.3 (0.6) a	5.4 (0.5) a
Mast	All 3	8.8 (0.6) a	9.6 (0.7) a	8.4 (0.8) a
Osde	All 3	13.9 (0.6) c	20.4 (0.6) a	17.5 (1.0) b
Rhca	All 3	10.5 (0.4) b	16.2 (0.6) a	16.4 (0.6) a
Syvu	All 3	14.6 (0.7) b	23.0 (1.8) a	8.2 (1.5) c
All hosts	All 3	10.6 (0.2) c	15.7 (0.4) a	13.6 (0.4) b
All hosts	NA1	11.7 (0.4) b	14.8 (0.6) a	15.2 (0.7) a
All hosts	NA2	8.6 (0.5) c	14.6 (0.7) a	11.5 (0.7) b
All hosts	EU1	11.0 (0.4) bc	17.7 (0.7) a	13.7 (0.8) b

Note: Data are presented pooling results from each of the three genotypes of the pathogen employed in this study. Data for individual genotypes are shown by pooling results for all plant hosts. Tukey's tests with $\alpha = 0.05$ were used to identify differences. Significantly different means are identified by different letters.

The number of sporangia (Table 5) was in general highest at 20 °C. However, sporangia were also produced in large numbers on Lano and Mast at 12 °C, and good sporulation was also obtained on Gash and Hain at 25 °C. When looking at the data by genotype, 20 °C was ideal for maximum sporulation by each genotype. However, the NA1 genotype was a relatively good sporulator at 12 °C, while the NA2 genotype was a relatively good sporulator at 25 °C.

4 | DISCUSSION

The study presented here has important implications for the understanding of complex pathosystems including those caused by generalist pathogens such as *P. ramorum*, further characterized by substantial subspecific genetic structure. The results of this study provide critical information on which ornamental hosts may suffer the most when infected and on which ones may most contribute to the spread of the pathogen, due to the abundant sporulation they support. Because we selected species to be tested from those that are important in the ornamental plant trade, and because *P. ramorum* is highly regulated worldwide, the results published

TABLE 5 Average number of *Phytophthora ramorum* sporangia collected in leaf washes from 30 leaves of each of eight plant species inoculated using a high inoculum of zoospore suspension and kept at three different temperatures for 7 days

Host	Pathogen genotype	Average number of sporangia (*SE*)		
		12 °C	20 °C	25 °C
Gash	All 3	1.2 (0.3) b	137.7 (44.6) a	48.0 (16.3) ab
Hain	All 3	3.5 (1.0) b	52.5 (19.0) a	40.4 (14.7) ab
Lano	All 3	55.1 (18.0) a	38.5 (8.7) ab	12.1 (3.3) b
Maaq	All 3	1.9 (0.6) a	135.0 (67.0) a	15.3 (3.5) a
Mast	All 3	15.0 (6.0) ab	34.0 (9.5) a	2.0 (0.6) b
Osde	All 3	17.1 (7.8) b	106.5 (33.8) a	9.2 (5.8) b
Rhca	All 3	20.7 (8.8) b	81.0 (16.0) a	19.1 (9.4) b
Syvu	All 3	0.6 (0.2) b	178.0 (56.0) a	8.5 (6.5) b
All hosts	All 3	15.7 (3.0) b	96.4 (11.7) a	18.3 (3.2) b
All hosts	NA1	23.3 (6.4) b	73.3 (15.7) a	10.4 (3.2) b
All hosts	NA2	8.7 (1.6) b	82.3 (12.9) a	36.4 (9.4) b
All hosts	EU1	13.5 (5.5) b	133.0 (27.7) a	11.8 (3.5) b

Note: Data are presented pooling results from each of the three genotypes of the pathogen employed in this study. Data for individual genotypes are shown by pooling results for all hosts tested. Tukey's tests with $\alpha = 0.05$ were used to identify differences. Significantly different means are identified by different letters.

here may be invaluable for the development of more effective, and possibly less costly, policies aimed at controlling the spread of the pathogen in the ornamental plant industry. Nonetheless, the results presented here may also have implications for the spread of *P. ramorum* in those natural ecosystems that have been invaded by the pathogen and in which its transmission is driven mostly by infection of foliar hosts (Brasier and Webber, 2010; Davidson et al., 2005; Fichtner et al., 2012). While knowledge on susceptibility and transmission potential of most species tested here was little known at the time of experiment, the inclusion in all trials of a species of known epidemiological significance, *Rhododendron catawbiense*, provides a practical benchmark to draw useful and intuitive comparisons among plant species. The various disease and sporulation indices offer an additional easy way to rank plants tested for susceptibility and sporulation potential, in part bypassing the analytical complications of a large and extremely variable data set.

Although not universally true, congeneric plants have often been regarded as having similar susceptibility to *P. ramorum*; thus, it may be believed that the information about one species may be used to provide preliminary assessments at the genus level. However, results of this study caution against this approach, given that the study included several congeneric species. The study included two species belonging to each of the following four genera, *Rosa*, *Gaultheria*, *Ilex*, and *Prunus*, and three species belonging to the two genera *Osmanthus* and *Syringa*. Results were fairly similar for species within the genera *Rosa*, *Prunus*, and *Syringa*, but quite different for species within the genera *Ilex*, *Gaultheria*, and *Osmanthus*. Thus, it is possible our results may be expanded to congeneric species at least for the three genera in the first group, but this approach cannot be generalized.

The experiment involved the testing of three genotypes of the pathogen, each representing a different lineage, on 25 plant host species from 10 families. When designing the study, a choice was made to focus on a broad host range, while keeping pathogen lineage representation to a minimum of one genotype per lineage to reduce the amount of testing required. That choice was justified as reproduction within each pathogen lineage is exclusively clonal, and there are no large differences in virulence among pathogen genotypes within a lineage (see Moralejo et al., 2009). The only exceptions are those of non-wildtype genotypes that are normally associated with significant genomic aberrations; these can be easily identified by inspecting the morphology of the pathogen in vitro (Kasuga et al., 2012). The three pathogen genotypes used grew well in vitro, had a wild-type morphology, had been used previously in inoculation trials, and were all passaged through rhododendron leaves prior to use as inoculum. Even so, the scale of the experiment required multiple inoculation trials performed on different dates in the course of 2 years. Our decision to focus mostly on results obtained by pooling data from the three genotypes of the pathogen was not just for the sake of brevity, but also because combining results from the three genotypes over several experiments reduced the bias that may arise when comparing smaller data sets, each generated at a different time. Genotype-level results are presented in the supplementary materials but are not explicitly discussed in this paper given that species' ranks were rather similar for the three genotypes, even if absolute values of the parameters measured were often different, and given that the use of one genotype per lineage is insufficient to draw conclusions about an entire lineage. In addition, more descriptive results, such as whether infection may be more likely to occur on the leaf tips or leaf bases or on both, are presented as

they may help understanding of the type of disease each host may suffer (e.g., when leaf infection on the petiole side is significant, one may expect pathogen colonization into the twigs), but are not discussed here for the sake of brevity.

The importance of subspecific variation within pathogens when dealing with virulence, irrespective of how the variation may be generated, is a relatively accepted concept for viruses and bacteria, mostly because of the importance these pathogens hold as agents of human, animal, and zoonotic diseases. Viral strains and bacterial pathovars are normally regulated as separate taxa (Andino and Domingo, 2015; Dye et al., 1980; Lwoff, 1959); however, subspecific variability is not as widely accepted when dealing with plant diseases caused by fungi or oomycetes. In part, this reluctance is justified by the presence of "fuzzy" species borders in unresolved fungal and oomycete taxonomy. For instance, much of what was once believed to be subspecific genetic variation in fungi and oomycetes turned out to be in reality species-level variation, when former subspecies or biological species belonging to so called "species complexes" were formally described as individual species (Taylor et al., 2000), and they were often proven to be extremely divergent genetically, despite their quasi-identical morphology (see Sillo et al., 2015). Formae speciales have been instituted to identify subspecific variability associated with different host specificity (Lievens et al., 2008), but when such host specificity is not present, subspecific variability has been mostly ignored at the regulatory level. This is the case for genetically distinct units in several truly important plant pathogens, including for instance the clonal lineages of *Phytophthora cinnamomi* (Socorro Serrano et al., 2019) and the subspecies of *Ophiostoma novo-ulmi* (Brasier and Kirk, 2001). We believe that the results of this study support the presence of significant phenotypic variability within the species, although, given that each genotype represented a different lineage, it is difficult to disentangle the genotype-specific (see Manter et al., 2010; Søndreli et al., 2019) from the lineage-specific effects (see Eyre et al., 2014).

Not surprisingly, foliar inoculations using low inoculum resulted in lower disease incidence (expressed as percentage of successful pathogen reisolations) and severity (expressed as linear lesion length) than results obtained using high inoculum. Nonetheless, these results support the importance of limiting inoculum loads to curtail disease. This is particularly relevant in light of recent evidence indicating large outbreaks can become the source of further outbreaks (Croucher et al., 2013; Della Rocca et al., 2019). When comparing the three genotypes, the EU1 genotype emerged as the most aggressive in terms of disease incidence (% positive pathogen reisolations) and it also ranked highest, although statistically comparable to the NA1 lineage, in terms of disease severity (lesion length). This information, although interesting, can only be regarded as preliminary given that only one genotype per lineage was employed.

Results identified eight plant species as being highly susceptible to infection even in the presence of relatively limited inoculum sources. These species should be monitored carefully for the early detection of novel infestations in plant production facilities or even in environments where they grow naturally. It is important to note that, based on the LDI, three out of the eight species are potentially more susceptible to *P. ramorum* infection than *R. catawbiense*. Similarly, in a study of New Zealand flora, some species were found to be more susceptible than those known in North America and Europe (Hüberli et al., 2008). Six additional species displayed medium LDI values and should also be monitored for the early detection of *P. ramorum* outbreaks, while four species with extremely low LDI values can be disregarded as significant hosts of *P. ramorum* with confidence. Planting or growing these four species represents a minimal risk when dealing with *P. ramorum*.

Higher inoculum loads matching those used as standard in the literature were employed to generate a second foliar data set. Although less relevant for prediction of early outbreaks, the use of a higher inoculum level resulted in higher disease incidence, thus generating data sets with fewer zeros that could be confidently used for a variety of comparative analyses. Significant differences were identified among genotypes. Once again, the EU1 genotype was the most aggressive in terms of both disease incidence and severity, although, as in the low inoculum data set, lesion length was not statistically different between the EU1 and NA1 genotypes. Conversely, the NA1 genotype was clearly the most infectious based on the number of sporangia it produced on infected hosts. These lineage-level results, although only based on a single genotype per lineage, give a preliminary indication that the co-mingling of genotypes belonging to the EU1 and NA1 lineages may result in a highly destructive forest disease, as both virulence and transmission potential would be maximized. It should also be noted the two lineages have different mating types, and, even if *P. ramorum* progeny has been reported as being potentially unstable (Vercauteren et al., 2011), it may still be viable and able to backcross with one of the parents, allowing for genetic exchange between the two lineages. We have already been facing a similar scenario with Dutch elm disease since *Ophiostoma ulmi* started being co-mingled with *Ophiostoma novo-ulmi* (Brasier, 2001), and with late blight of potatoes when both mating types of the pathogen *Phytophthora infestans* emerged in the same production areas (Gisi and Cohen, 1996). It has also recently been suggested that the co-mingling of different genotypes belonging to the same *P. cinnamomi* lineage could result in a different disease type as some genotypes are better at infecting roots and others are better at infecting stems (Serrano et al., 2020).

When comparing hosts for foliar susceptibility using high inoculum, five species emerged as being the most susceptible, four of them being more susceptible than *R. catawbiense*. Based on LDI, a total of 16 species were intermediate in susceptibility, and the four species identified as not susceptible to low inoculum were also not susceptible to the high inoculum level. LDI is an index generated using lesion length: leaf size may affect lesion size, in particular for highly susceptible species with limited leaf size. When we compared lesions expressed as length of necrotic tissue with lesions

expressed as percentage of leaf size that had a lesion, Osde ranked significantly lower when the first parameter was used, suggesting lesion length underestimates the susceptibility of this host. Thus, Osde should be regarded as displaying high and not intermediate foliar susceptibility.

When comparing hosts for potential for foliar sporulation, three species supported large numbers of sporangia and two species supported intermediate numbers. Although species rankings were not identical when comparing LDI and LSI, they were relatively similar. These five species, if present during an outbreak, could lead to a significantly faster disease spread due to higher transmission rates coupled with higher susceptibility rates. Interestingly, *R. catawbiense* is not included in the group of five large species with the highest foliar sporulation.

Foliar infections have been shown to be pivotal for disease transmission but to have a limited impact on the overall health and physiology of infected plants. Nevertheless, when ramorum blight affects stems or branches, the disease leads to progressive dieback and even mortality of infected hosts (Davidson et al., 2003). However, it is unknown what epidemiological role stem infections may play in most hosts, with twig infection of tanoak being a notable exception (Fichtner et al., 2012). Our results suggest that lesion size is significantly larger in stems than in leaves, but stem infection is less likely than leaf infection, as observed for hosts in natural systems (Davidson et al., 2003). Overall these results suggest leaves play a more important epidemiological role than stems. Other noteworthy results were (a) the only significant difference between genotypes when inoculated on stems was that NA2 caused a higher disease incidence; (b) a comparison between LDI and SDI identified Syme, Syvu, Osde, and Mast as extremely poor stem hosts, while being excellent or good foliar hosts; (c) rankings of stem and leaf susceptibility were quite different: of the five top species for LDI, only two, Rhca and Hain, are present in the top five species for SDI, suggesting disease may quickly progress from a foliar blight to a branch anthracnose in these two species; (d) the number of sporangia produced on "attached" stems was much lower than the number of sporangia produced on detached leaves. This result is extremely important for understanding the epidemiology of diseases caused by *P. ramorum*.

Because *P. ramorum* is mostly an aerial *Phytophthora*, host species can be ranked for overall susceptibility and overall transmission by adding the results from stem and foliar inoculations. The TSI can be interpreted rather easily because it provides an estimate of the average production of sporangia produced on the stem and leaves together. However, the TDI should be interpreted more cautiously because lesions on stems and leaves are different both in size and in impact on plant health. A comparison of the datasets shows that stems lesions are about two to three times larger than leaf lesions, hence the TDI calculations performed in this study without any rescaling put more weight on stem lesions, a desirable result based on our understanding of disease dynamics. Besides providing an important list of the most susceptible hosts and those supporting the most sporulation among the 25 tested in this study, a comparison of TDI and TSI indicated several species appeared less diseased, but supported larger amounts of sporangia (e.g., Syvu and Rogy), Conversely, other species were more diseased but supported less sporulation (e.g., Prla and Prlu). Therefore, a visual assessment of these species may under- or overestimate the role they may play in spread of the disease. A similar result has been reported for foliar infections of some hosts by *P. ramorum* in the UK (Denman et al., 2009). Likewise, underbark lesion size by *P. cinnamomi* has been deemed unreliable for gauging disease severity in some host species (Hüberli et al., 2000). The lack of visible symptoms in infected tissue may be due to a more diffuse plant tissue colonization process by hyphae without visible necrosis, or, in the case of foliar infection, to epiphytic pathogenic growth.

The temperature study was meant to assess how disease caused by *P. ramorum* in general and at the genotype level may be affected by different temperatures on a subset of eight species shown to be good hosts for the pathogen in the earlier experiments. This information may be useful for modelling purposes when comparing regions of the world characterized by different climates. In terms of susceptibility expressed as the development of foliar lesions, the pathogen (i.e., data from all three genotypes pooled and considering all hosts tested) was most pathogenic at 20 °C, had intermediate pathogenicity at 25 °C and lowest pathogenicity at 12 °C. This is in agreement with previous studies (Eyre et al., 2014; Hüberli et al., 2012). However, when looking at the performance of individual genotypes, the NA1 genotype was the only one to be equally proficient at 20 and 25 °C, while lesion development was depressed at 12 °C for all three genotypes, and in particular for the NA2 genotype. These results indicate disease development may vary depending on genotype and possibly lineage when comparing world regions characterized by different climates. Individually, most of the eight hosts developed disease at a comparable rate when comparing leaf lesion sizes at 20 and 25 °C. Only disease in Lano and Osde was negatively affected by the higher temperature: these are hosts that may be less affected by infection in warmer climates. Disease slowed significantly in leaves of six out of eight hosts tested at 12 °C; however, foliar lesions in Hain and Mast were equal across all three temperatures, suggesting disease may progress well in these species even if growing in cooler areas.

Disease transmission expressed as number of sporangia produced on inoculated leaves was also affected by temperature. All three pathogen genotypes fared best at 20 °C, while numbers of sporangia produced at 12 and 25 °C were lower, albeit undistinguishable from one another. These results confirm that areas with temperatures around 20 °C may be ideal for spread of the pathogen, if the other necessary requirements, such as rainfall, high relative humidity, and abundance of infectious hosts, are available. The NA2 genotype produced a larger number of sporangia at 25 °C than the other two genotypes, suggesting NA2 genotypes may spread better in warmer regions. The results divided the individual host species into three groups: Gash and Hain produced large numbers of sporangia at 25 °C and so should be inspected carefully in warmer regions. Lano and Mast produced relatively large numbers

of sporangia at 12 °C, suggesting they may spread the disease efficiently in cooler climates. Lesions on Osde, Rhca, and Syvu were negatively affected both at 12 and 25 °C, indicating their transmission potential is closely regulated by temperature, with a preference for mild temperatures around 20 °C. Mast was both highly susceptible and highly infectious at 12 °C and thus its use in the landscape or in growing facilities should be limited in colder areas infested by *P. ramorum*. Gash, Hain, and Maaq were both highly susceptible and infectious at 25 °C, thus their use should be limited in warmer areas infested by the pathogen.

In general, the results from individual pathogen genotypes in the temperature trials matched those obtained in the other two leaf inoculation trials, with one notable exception. In the temperature trials, and in contrast to the larger foliar inoculation tests, the EU1 genotype produced a larger number of sporangia than the NA1 genotype. This difference was caused by testing a smaller subset of plants, and highlights how the selection of hosts may affect the outcome of an experiment testing pathogenicity and transmission potential of a generalist pathogen.

This study has identified hosts that are particularly susceptible or almost resistant to infection by *P. ramorum*, thus laying the foundations for further work aimed at understanding the mechanisms regulating virulence and host–pathogen interactions. This would enable the drafting of better rules and regulations aimed at curtailing the spread of this pathogen. Our investigation indicated the presence of significant ($p < .0001$) pathogen genotype × host species interactions for every parameter in each test performed. This is probably a consequence of the different history and genetic isolation of the three genotypes studied here, as each belonged to a different lineage. Genomic analyses have identified a large number of genes in *P. ramorum* responsible for plant pathogen molecular communication (Goss et al., 2013; Tyler et al., 2006), proving that the history of host encounters has selectively driven the evolution of this pathogen. We have demonstrated the importance of genotype when investigating the epidemiology of ramorum blight; however, studies employing multiple genotypes per lineage on a large number of hosts are needed to confirm the presence of epidemiologically relevant differences among lineages.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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