Pathogenicity and sporulation of *Phytophthora pinifolia* on *Pinus radiata* in Chile

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Abstract *Phytophthora pinifolia* causes the needle and shoot disease of *Pinus radiata* known as Daño Foliar del Pino (DFP) in Chile. The first pathogenicity trials with this organism utilized mycelial plugs placed on stem wounds. These resulted in lesions in the tissue, but did not reproduce the resinous bands on the needles, which are the most characteristics symptoms of the disease under natural conditions. In this study, stem inoculations were repeated, but to complete Koch's postulates fully, and to confirm that *P. pinifolia* causes the symptoms observed on naturally infected trees, zoospore/sporangial suspensions were used to inoculate pine foliage. This method produced the same symptoms observed on needles infected naturally. These results confirm that *P. pinifolia* is the causal agent of the Daño Foliar del Pino on *P. radiata* in Chile and successfully completed Koch`s postulates for the first time. Pathogenicity tests on different *Pinus* spp. and hybrids showed a wide range of responses to inoculation with *P. pinifolia* mycelial plugs, from highly susceptible to resistant. Monitoring of sporulation revealed that the

sporangia commonly remain on the needles for extended periods of time and their frequency of occurrence and dispersal appear to increase during the rainy season.

Keywords Daño Foliar del Pino, *Phytophthora pinifolia*, *Pinus radiata*, needle disease, sporulation monitoring

Introduction

Phytophthora pinifolia is a recently described pathogen that causes the disease known as Daño Foliar del Pino (DFP) on *Pinus radiata* in Chile (Durán et al. 2008). DFP has caused significant damage to the foliage of *P. radiata* growing in plantations in the coastal area of the Biobío and Los Ríos Regions (Durán et al. 2008). Symptoms of the disease were first observed on the Arauco coast (37º17'S; 73º36'W) in July 2004 on trees including a range of age classes. This was followed by mortality of one and two-year-old plantations in October 2004 (Durán et al. 2008). Annual monitoring showed a clear increase in the area affected over time, from 3,300 ha in 2004, to 30,000 ha in 2005 and 54,000 ha in 2006, with varying levels of severity across the region. The level of infection and damage decreased substantially in the subsequent years to 21,000 ha in 2007, 1,500 ha in 2008 and less than 1,000 ha between 2009 and 2011 (R. Gómez, pers. comm.).

Daño Foliar del Pino is characterized by relatively rapid death of infected needles followed by the defoliation of trees (Durán et al. 2008). Infection is normally observed from autumn to late spring, which is also the main rainfall season. Infected needles typically display distinct resinous bands on their laminas. When the infections reach the needle bases, resin exudes from the points of attachment causing death of the cambial cells surrounding the

fascicles and generating small cankers at the contact point between the needles and the stem. The dead needles remain attached to the stems until the late spring when they fall from the trees and branches. While much is known about the development of the disease caused by *P. pinifolia*, there is little knowledge regarding the biology of the pathogen including issues such as its sporulation, dispersal and how it infects trees.

Pinus radiata plantations in Chile have in the past been affected by shoot and needle diseases caused by *Dothistroma septosporum*, *Diplodia pinea* and *Cyclaneusma minus* (Toro and Gessel 1999; Ahumada 2003; Barnes et al. 2004). Damage has also been caused by European pine shoot moth, *Rhyacionia buoliana*, which has been the most important constraint to plantation forestry in the past (Lanfranco 2000; Ahumada 2003). However, the increase of areas infested by the woodwasp, *Sirex noctilio* and the emergence of DFP are by far the most important problems currently facing *P. radiata* forestry in Chile.

Phytophthora spp. are amongst the most serious and invasive plant pathogens on both agricultural and forestry crops (Fry and Goodwin 1997; Rizzo et al. 2002). In terms of forestry, the recent emergence of species such as *Phytophthora ramorum* and *P. austrocedrae* (Rizzo et al. 2002; Greslebin et al. 2007) has reinforced the fact that these pathogens are a serious threat to forestry worldwide, both in natural ecosystems and plantations (Davidson et al. 2003; Garbelotto et al. 2003; Greslebin and Hansen 2010). The appearance of a needle blight of *Pinus* spp. caused by a *Phytophthora* sp., as has occurred with *P. pinifolia* was, however, unprecedented and unexpected.

The first pathogenicity tests conducted with *P. pinifolia* using mycelial plugs showed clearly that *P. pinifolia* was able to infect and cause disease in the shoots in a period of less

than 30 days (Durán et al. 2008). However, the typical DFP symptoms observed in the field have never been artificially reproduced. In this study, we confirm the results from previous mycelial inoculation studies regarding the pathogenicity of *P. pinifolia*, and extend these to additional *Pinus* spp. that are of importance as potential plantation species in Chile and other parts of the world. The primary aim was, however, to produce sporangia and zoospores of *P. pinifolia* and to attempt to reproduce the characteristic resinous bands on the foliage of *P. radiata*, to re-isolate the organism from the infected needles, completing Koch's rules of proof for the organism. The focus on infection propagules also provided a parallel opportunity to study the means of dispersal of *P. pinifolia,* by monitoring sporangial production and the rainfall across different areas.

Materials and methods

Isolates and inoculum production

The three isolates used throughout the study were selected from a previous investigation where six isolates were inoculated into 180 1-year-old *P. radiata* plants (30 per isolate) and where it was shown that they were all pathogenic and did not differ in their relative levels of aggressiveness (R. Ahumada, unpublished). One isolate (CMW33986) of *P. pinifolia* was used in the first experiment with mycelial plugs and three isolates (CMW33983, CMW33986 and CMW34012) in the second experiment using suspensions of sporangia and zoospores. All the isolates were obtained from needles of *P. radiata* with the resinous bands characteristic of infection by *P. pinifolia*. The three isolates selected for the inoculations were chosen because they were similar in relative aggressiveness in a previous study (R. Ahumada, unpublished).

Isolates were identified based on culture morphology on the selective medium CARP (Hansen and Hamm 1996) and using the species-specific primers developed by Durán et al. (2009). Emerging colonies were transferred to Carrot Agar (CA) (Erwin and Ribeiro 1996) and maintained between 18–22°C for 20 days until they could be identified. All three isolates have been maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

For inoculum production, 20 replicates of each isolate were grown in V8 agar at 22ºC for two weeks. From the edges of actively growing cultures, 5 discs (7 mm diam.) were transferred to a 60 mm Petri dish containing 25 ml of 10% V8 broth and incubated for 24 h at 22ºC (Erwin and Ribeiro 1996). The agar discs were washed twice with autoclaved cold distilled water, and then immersed in filtered pond water for 48 h at 22ºC under continuous cool white fluorescent light (6,600 lux). Isolates were examined for the presence of sporangia and those bearing these structures were chilled at 4 ºC for 2 h to induce the release of zoospores. The primary zoospore suspension was poured into a sterile beaker and maintained at 4ºC until inoculation. Three aliquots of 10 µL were taken from the beaker and used to determine the zoospore concentration using a hematocytometer. The final zoospore suspension was prepared by adding autoclaved distilled water and adjusting this to approximately 5 x 10^4 zoospores ml^{-1}. The zoospore suspension was maintained at 4 ºC and transported to the field for inoculation on the same day. To determine the viability of the zoospores, three aliquots of 30 µL of the suspension were sampled in the field after the inoculation and spread onto CARP, transported to the laboratory at 4 ºC, incubated at 22ºC for two weeks and evaluated for growth.

Inoculation experiments

The inoculations were performed in two different experiments, under laboratory conditions and in the field. The laboratory trials were conducted at Bioforest research facilities in Concepción. The screening facility was set to provide a photoperiod of 12 h of artificial light, at 75% relative humidity, a temperature of between 18–22ºC and the containers were irrigated daily for 1 h.

The field experiment was installed on the Llico farm (37°22'45"S; 73°58'W; Arauco province). A shade house (70% shade netting) was erected for this inoculation study and a fogging system was installed and applied for 15 minutes, three times per day (10, 14 and 18 hours) to maintain a high level of humidity over the foliage of the inoculated trees. The shade house was surrounded by a plantation of *Eucalyptus globulus* trees so as to reduce any chance of natural infection by *P. pinifolia*.

Inoculation of Pinus *spp. with* P. pinifolia *mycelium plugs*

Twenty plants (15-month-old, an average of 22 cm tall and 0.8 cm average diam. at the substrate level) of each of thirteen *Pinus* spp. including varieties [*P. arizonica* (PAR), *P. durangensis* (PDU), *P. greggii* mix of families (PGR1), *P. greggii* var *australis* (PGR2), *P. greggii* var *greggii* (PGR3), *P. maximinoi* (PMA), *P. muricata* (PMU), *P. patula* mix of families (PPA1), *P. patula* var *longipedunculata* (PPA2), *P. patula* var *patula* (PPA3), *P. pinaster* (PPI), *P. radiata* (PRA) and *P. taeda* (PTA)] were established in 140 cc containers. The plants were acclimatized for two weeks prior to inoculation in the screening facilities using the conditions described above. Small bark discs (4 mm diam.)

were removed from succulent tissue of the stems, between 10 to 12 cm from the growing tips and a plug of mycelium (3-week-old culture of *P. pinifolia* grown on CA) of similar size was placed into the wounds. Discs of clean CA were used as negative controls. Fifteen plants of each species and the varieties were inoculated with the isolate CMW 33986 of *P. pinifolia* and five plants per species were inoculated as controls. Inoculation wounds were covered with Parafilm to reduce desiccation and contamination. Four weeks after inoculation, the Parafilm and the bark around the inoculation points was removed with a sterile scalpel and the lesion lengths were recorded.

Re–isolation was attempted from the leading edges (top and bottom) of the lesions on all inoculated plants. Small pieces (< 5 mm^2) of succulent infected tissue from the leading edges of the lesions were plated onto CARP medium to re–isolate the inoculated organism and ensure that it was associated with the lesions. The identity of 10% of the resulting cultures that were characteristic of *P. pinifolia* was confirmed using the PCR specific primers (Durán et al. 2009).

Analysis of data was conducted separately for all *Pinus* species, using the linear model of analysis of variance (ANOVA) and means were separated based on LSD (Least Significant Difference) using the software Statistica V9 for Windows (StatSoft Inc., 2004).

Inoculation of P. radiata *with* P. pinifolia *zoospores*

This experiment was conducted with a suspension of zoospores and sporangia using a mixture of the three isolates as described above. An equivalent volume of zoospore suspension (adjusted to approximately 5 x 10^4 zoospores ml^{-1}) was transferred to forty 2 L

plastic bags, in which individual *P. radiata* plants were immersed (75% of their foliage) overnight (~12 h). After immersion, half of the plants (20) were placed in the screening facility in the laboratory and the other half (20) transported in a cooler box, to the shade house in the field on the Llico farm. Forty plants, with the same characteristics as those inoculated, were used as controls and were dipped into sterile distilled water overnight. Half of the plants were placed in the laboratory in an area separate from the plants inoculated with *P. pinifolia,* to avoid cross contamination and the other half were transported to the field and placed in the shade house together with the inoculated plants. All plants were inspected weekly for the appearance of symptoms.

Four weeks after the inoculation, symptoms were recorded on all inoculated plants. Evaluations included the presence (1) or absence (0) of resinous bands on the needles of both inoculated and control plants. A needle was considered to be infected if it had at least one resinous band. A sample (~10%) of the bands produced on the needles was used for re–isolation of the inoculated pathogen using the same methodology described above for plants inoculated with mycelial plugs.

A Bayesian approach was used for data analyses. The variable response was assumed to be a binomial random variable and the percent of infection was set to a beta prior distribution. The analyses were separated between the plants in the laboratory and the plants maintained in the field, in order to avoid possible confounding effects in the responses measured. Bayesian credible intervals for each proportion (π) were computed using Win BUGS (Bayesian Inference Using Gibbs Sampling) software (Lunn et al. 2000). The Gibbs sampling chain was run for 11,000 iterations with the first 1,000 iterations used as burn-in.

The convergence of the Gibbs chains was checked using the Heidelberger and Welch's test (Heidelberger and Welch 1983).

Fluorescence microscopy was used to provide an additional confirmation of the presence of *P. pinifolia* on the foliage. A sub-sample of 10% of the needles with resinous bands were immersed in a solution of Calcofluor Fluorescent Brightener 28 (Sigma-Aldrich, St. Louis) at 0.001% in 0.05 M Tris-HCl (pH 8.0) for 30 sec. These needles were mounted on glass slides and examined under a fluorescence microscope (Olympus CX31, Olympus America Inc.) for the presence of sporangia or mycelium on the needle surfaces.

Monitoring the presence of sporangia in plantations

Six field sites were selected for monitoring the presence of *P. pinifolia* inoculum in a natural environment. This monitoring was carried out between May 2009 and December 2011. The selected sites were located in three 7-year-old *P. radiata* plantations that have been historically infected with *P. pinifolia*. Each monitoring site consisted of a block of approximately 100 m^2 (20 m x 5 m) from which 10 trees with typical symptoms of *P. pinifolia* damage were selected. From each tree, 20 needles were sampled twice each month and transported to the laboratory for analysis. In the laboratory, a minimum of 50 needles from the total collection (~200 per site) having clear resinous bands were selected for detailed examination. For each needle, a section of approximately 4 cm was cut around the resinous band. These needle pieces were immersed in a solution of Calcofluor Fluorescent Brightener 28 for 30 sec and then mounted on glass slides for microscopic observations. All observations were made using an Olympus fluorescence microscope as above, to quantify the presence of inoculum (sporangia, zoospores and mycelium) on the

needle surface. The sections of every needle examined corresponded to approximately 30 stomata and thus, approximately 18 000 stomata (30 stomata x 50 needles x 6 areas x 2 sampling periods) were examined each month.

The data recorded using fluorescence microscopy were tabulated and then plotted as monthly average of sporangia per needle (or per resinous band abbreviated as RB). The monthly rainfall was also recorded at the sampling sites during the same period of observation. The average of inoculum (sporangia) per month was plotted together with the rainfall for the entire evaluation period.

Results

Inoculation experiments

Inoculation of Pinus *spp. with* P. pinifolia *mycelium plugs*

Eleven of the 13 inoculated *Pinus* spp. or varieties developed wilting shoots and lesions around the inoculation points within 30 days (Fig. 1). The percentage of plants with symptoms ranged from 13% in the *P. patula* mix of families to 100% in both *P. radiata* and *P. muricata* while *P. pinaster* and *P. taeda* seedlings were free of any symptoms when this experiment was terminated. Mean inner lesion lengths ranged from 17.2 mm in the *P. patula* mix of families to 54.6 mm in *P. durangensis*. Other species with large average lesion lengths were *P. radiata* (50 mm), *P. greggii* var *greggii* (49.3 mm) and *P. arizonica* (48.9 mm), while the species with the lowest average lesion lengths were *P. greggii* var *australis* (24.3 mm), *P. greggii* mix of families (28.9 mm) and *P. patula* var

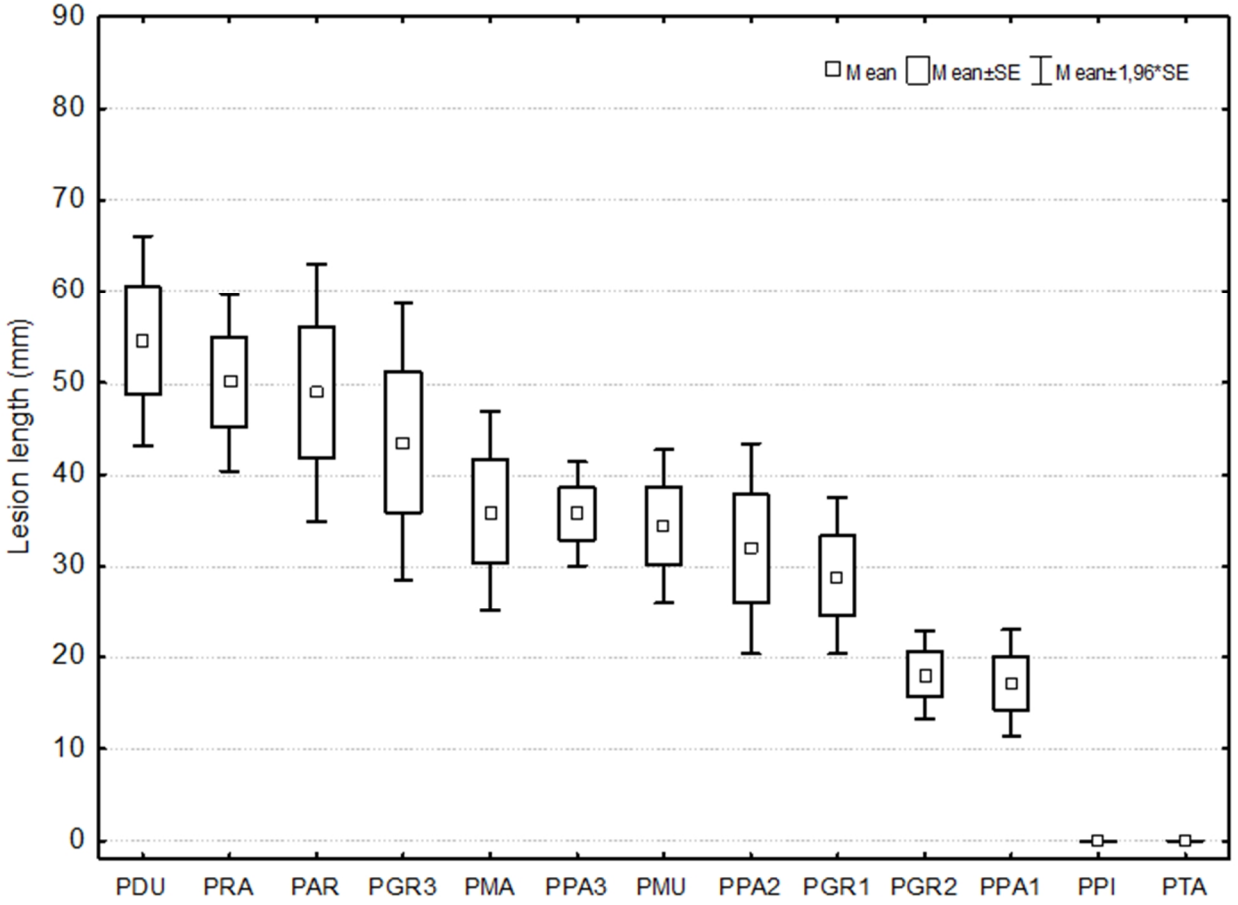

Fig. 1 Average lesion lengths on 13 *Pinus* spp. (or varieties) inoculated with *P. pinifolia* isolate (CMW33986) into the succulent stem tissue with a plug of agar covered with mycelium or sterile agar in the case of the controls. PDU = *Pinus durangensis*; PRA = *P. radiata*; PAR = *P. arizonica*; PGR3 = *P. greggii* var *greggii*; PMA = *P. maximinoi*; PPA3 = *P. patula* var *patula*; PMU = *P. muricata*; PPA2 = *P. patula* var *longipedunculata*; PGR1 = *P. greggii* mix of families; PGR2 = *P. greggi* var *australis*; PPA1 = *P. patula* mix of families; PPI = *P. pinaster* and PTA = *P. taeda*

longipedunculata (31.9 mm). *Pinus patula*, *P. greggii* and its varieties tested showed a wide range of symptoms and lesion lengths.

Phytophthora pinifolia was consistently re-isolated from the lesions and the identity of the cultures selected was confirmed in 100% of the cases using PCR-specific primers. None of the control plants in the all species treated, showed any evidence of symptoms and *P. pinifolia* could not be isolated from them.

Inoculation of P. radiata *with* P. pinifolia *zoospores*

After 4 weeks, plants inoculated with the *P. pinifolia* zoospore suspensions showed the characteristic symptoms on the needles but a non-uniform distribution of the resinous bands (Fig. 2A). No stem necrosis was observed on any of the inoculated *P. radiata* plants. Seventy seven percent of the inoculated plants in either the laboratory or the field trials showed the presence of these resinous bands characteristic of the DFP (Fig. 2B). There was no evidence of symptoms on untreated plants either in the laboratory or the field trial (Fig. 2C).

Eighty-three percent of the inoculated plants maintained in the laboratory developed symptoms, while those maintained in the field showed a lower incidence of symptomatic seedlings (71%). The Bayesian credible interval, however, showed that these differences were insignificant and the only statistically significant differences were those between the inoculated plants and the controls (Table 1).

More than 95% of the isolations made from the resinous bands yielded isolates having characteristic *P. pinifolia* morphology and those analysed with PCR specific-primers were

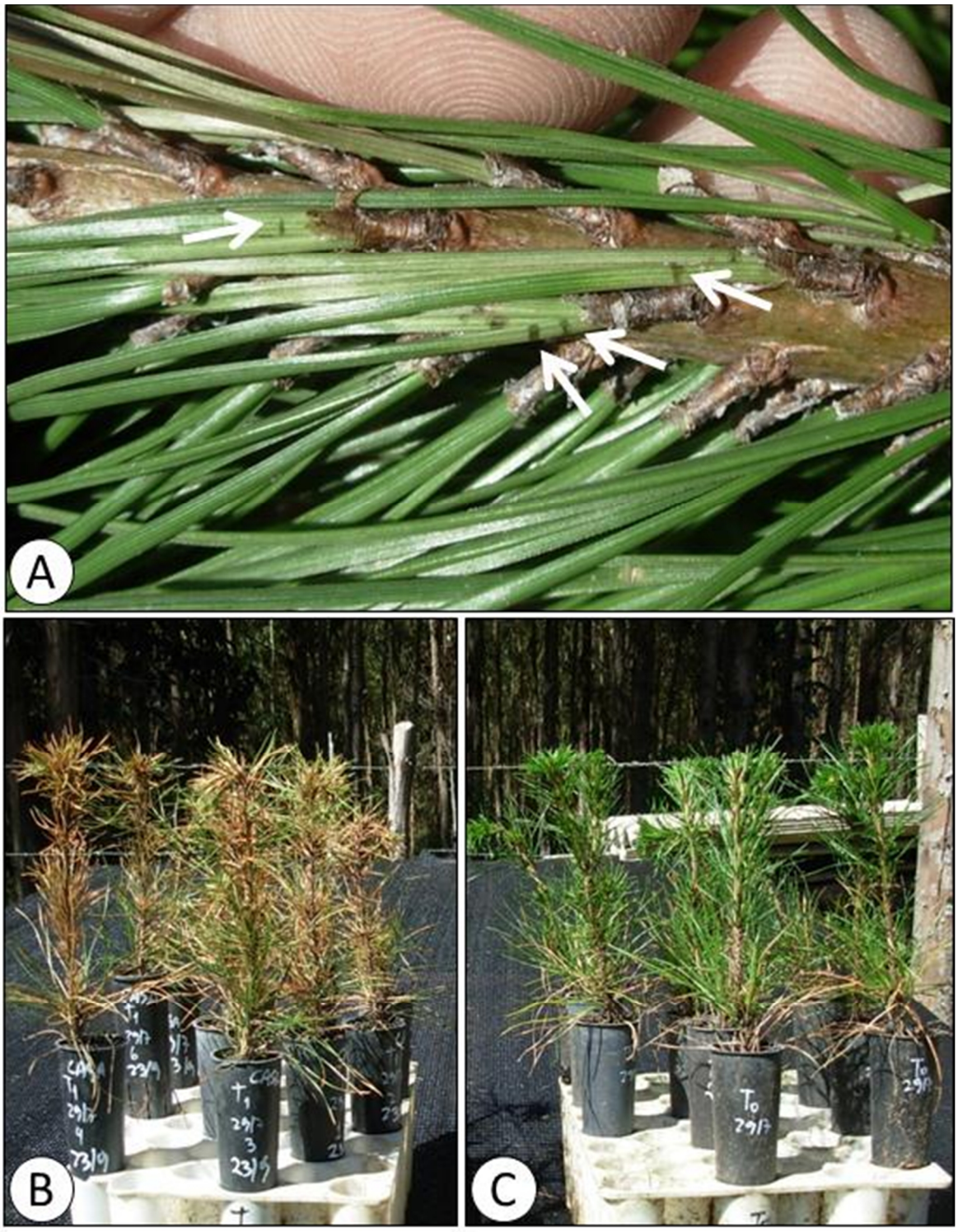

Fig. 2 Seedlings used in the inoculation trials. (A) Seedlings with resinous band (black bands) highlighted with the white arrows, after the inoculation (B) Seedlings inoculated with *P. pinifolia* zoospore suspensions with dying or brown foliage, and (C) seedlings used as controls and inoculated with sterile water with green non-infected foliage

Table 1

Incidence of *P. pinifolia* symptoms on *P. radiata* plants inoculated with zoospores and maintained in the laboratory or under field conditions.

Treatments	mean		median	sd	MC error [1]	Credible Interval [1]	
						2.5%	97.5%
Control	0.0238	**a**	0.0166	0.02335	2.21E-01	0.0064	0.0879
Laboratory inoculation	0.8333	**b**	0.8380	0.05683	6.27E-01	0.7092	0.9278
Field inoculation	0.7136	**b**	0.7168	0.06964	7.43E-01	0.5669	0.8386

[1]. MC error refers to mean square error and Credible intervals proportion (π) were computed using Gibbs sampling. Values in column of means followed by the same letter are not significantly different according to the Bayesian credible interval.

all confirmed to be of this organism. It was possible to consistently produce the necessary amount of inoculum for the all experiments and zoospores were shown to have an average of 86% viability.

Monitoring the presence of sporangia in plantations

Monitoring the presence of *P. pinifolia* sporangia and zoospores on infected needles in the field showed that the characteristic resinous bands were present (Fig. 3) during the entire assessment period between May 2009 and December 2011. The variation in the incidence of resinous bands on the needles was closely correlated with the number of sporangia present on the needles. The highest monthly value for inoculum was observed in September 2009 with 16.7 sporangia per resinous band (RB), September 2010 with 14.6 sporangia per resinous band and then in July 2011 with 6.3 sporangia per resinous band. There was a clear decreasing trend in the average of inoculum from 2009 to 2011, with an annual average of 11.7, 8.3 and 1.8 sporangia per resinous band, respectively (Fig. 3). The average rainfall between May to November (rainy season) showed a similar trend of that observed for the sporangia. The highest annual rainfall between May to December was of 1,489 mm in 2009, decreasing to 1,368 mm in 2010 and then to 1,154 mm in 2011 (Fig. 3).

Fluorescence microscopy made it possible to confirm the presence of sporangia that typically emerged from the stomata on the resinous bands and sporangia were found in about 62% of the needles sampled (Fig. 4A-D). Despite the fact that 84% of the sporangia were open (Fig. 4B, 4C), zoospores (Fig. 4E-F) were seen only infrequently.

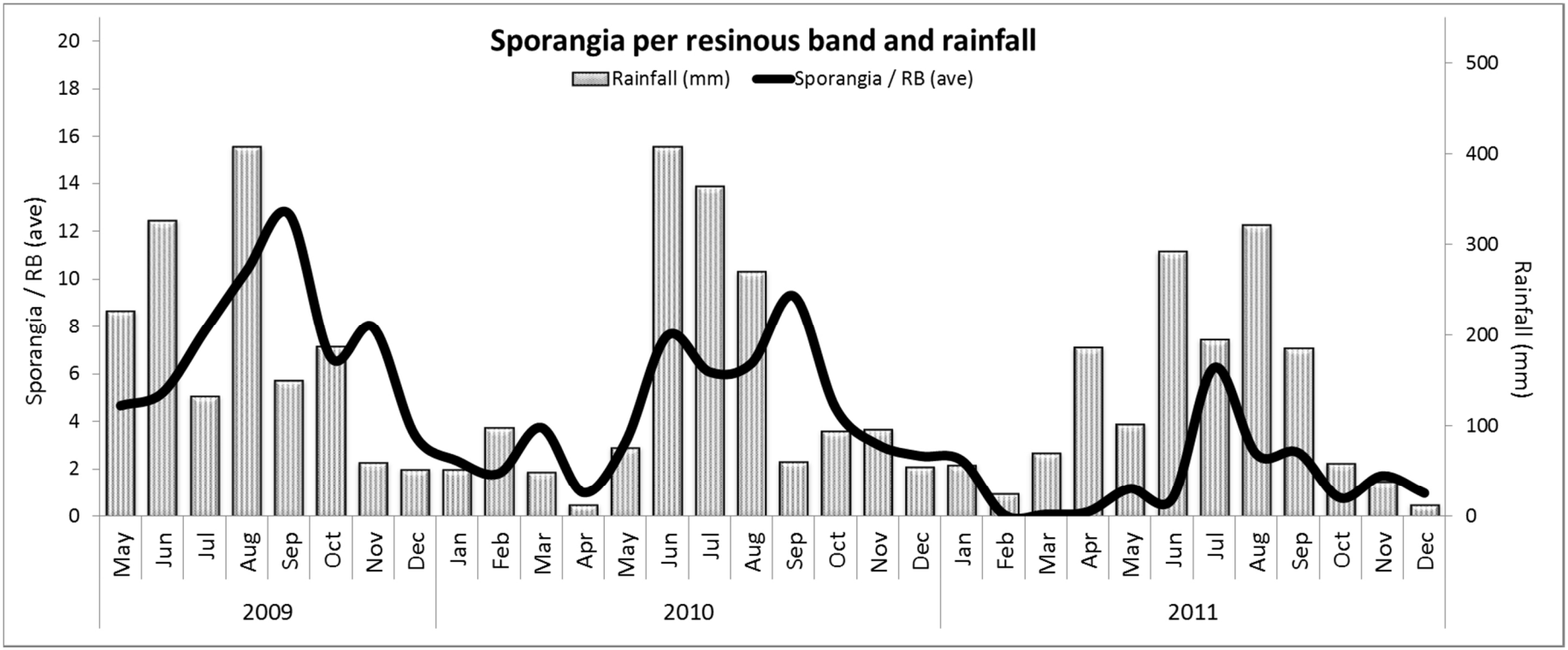

Fig. 3 Mean monthly rainfall in 2009, 2010 and 2011 (bars) and the average number of sporangia on resinous bands (RB), (solid line)

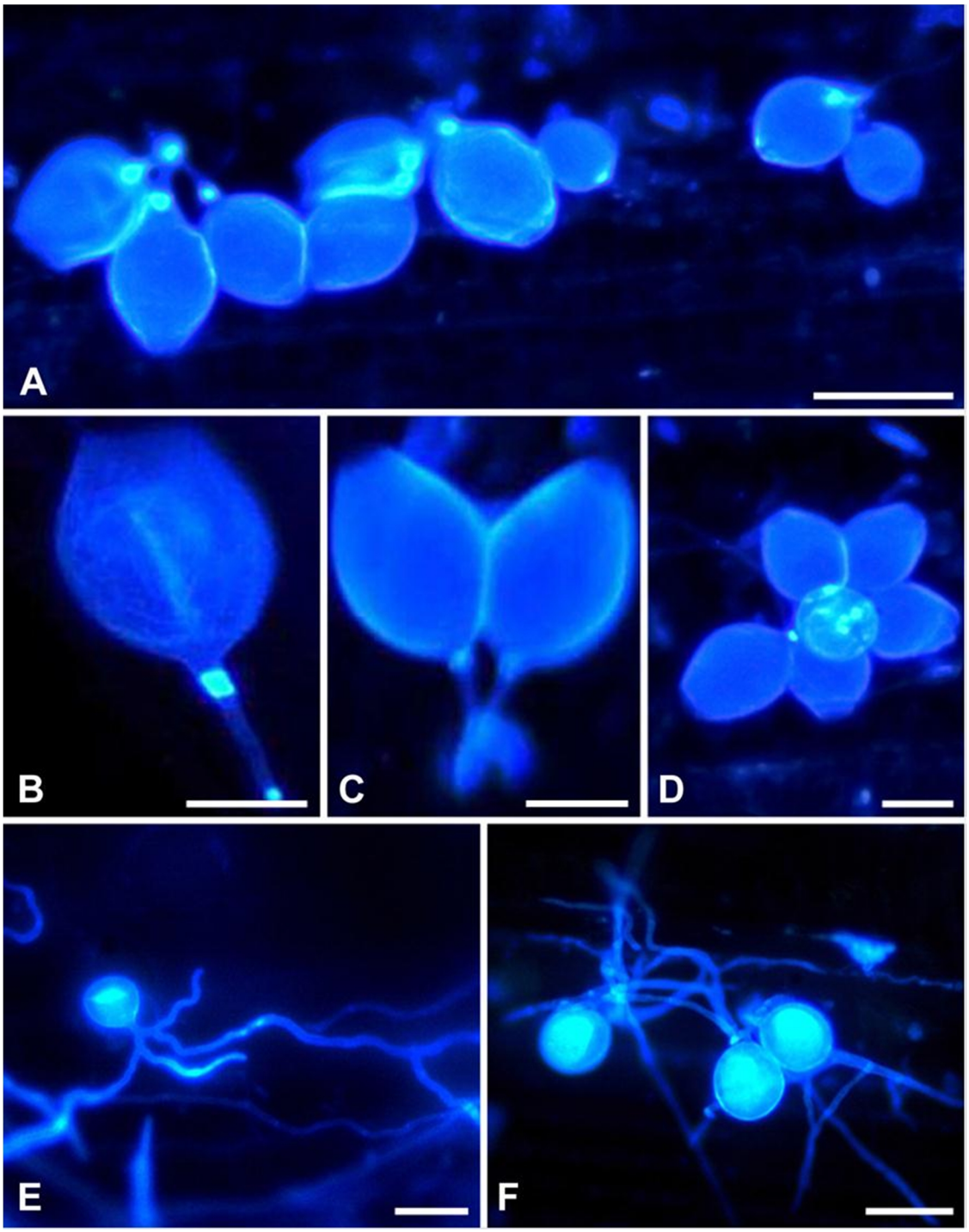

Fig. 4 Sporangia (A-D) and germinating zoospore (E-F) seen on the surface of *P. radiata* needles having symptoms of infection by *P. pinifolia* (resinous bands) viewed with fluorescence microscopy). Scale bars are: A=50 µm, B-D=25 µm and E-F=10 µm

Discussion

While it has been relatively certain that *P. pinifolia* causes the needle disease DFP on *P. radiata* in Chile, it has never been shown experimentally that this organism can cause symptoms identical to those associated with natural infections. Results of this study have resolved this problem and have completed Koch's postulates, showing unequivocally that *P. pinifolia* causes DFP on *P. radiata.*

In this study, where *P. radiata* tissue was immersed in zoospore/sporangial suspensions, infection occurred only on needles and never on the succulent stem tissue. This is an important observation because green stem tissue is commonly seen to be infected under field conditions (Durán et al. 2008). Results of this study also confirm that natural infection occurs mainly via the needles and that *P. pinifolia* moves down the needles to infect the stem tissue. Despite these observations, it is not clear whether the succulent tissue of young stems can be infected directly although this seems not to be the case from the results of the present study.

Pathogenicity tests made by inoculating stems of 13 different *Pinus* spp. and varieties with mycelial plugs, showed a significant variation in the susceptibility of the species tested, from apparently resistant species such as *P. pinaster* and *P. taeda* to highly susceptible species including *P. durangensis*, *P. radiata* and *P. arizonica*. Despite the fact that *P. pinifolia* caused infections on various *Pinus* spp., operational field monitoring carried out since 2006 has never found evidence of infection in other plants such as *Nothofagus* spp., *Eucryphia cordifolia*, *Luma apiculata*, *Chusquea quila*, *Aristotelia chilensis,* which are very common in the area (unpublished data). This is in contrast to many other

Phytophthora spp., such as *P. ramorum* that has a wide range of hosts (Davidson et al. 2003; Rizzo et al. 2005; Tooley and Browning 2009). The fact that *P. pinifolia* has been found only in Chile and its low genetic diversity (Durán et al. 2010) suggests strongly that it is a pathogen specific to *Pinus* spp.

Stem inoculation with mycelial plugs gave similar results to those observed in a previous study (Durán *et al*., 2008). The inoculated seedlings showed a general chlorosis of the needles, but did not produce the characteristic symptoms on the needles. The stem inoculations were, however, substantially more efficient in terms of the time needed to prepare inoculum and the evaluation of symptoms. Lesion lengths were easily measured and showed a clear tendency in terms of susceptibility. This inoculation method has been used many times as a test of the susceptibility of different hosts to *Phytophthora* spp. (Rizzo et al. 2002; Hüberli et al. 2002; Oh et al. 2006; Durán et al. 2008; Greslebin and Hansen 2010) and appears to offer a reliable means to estimate broad differences in susceptibility of *Pinus* spp.

Phytophthora pinifolia is unlike other aerial *Phytophthora* spp. where sporangia are caducous and serve as the major dispersal propagules. This is for example the case of *P. ramorum, P. kernoviae, P. cactorum, P. infestans* and *P. capsici*, which typically produce caducous sporangia, as the main propagules for dissemination (Erwin and Ribeiro 1996; Hansen et al. 2003; Brasier et al. 2005; Rizzo et al. 2005). Results also showed that sporangia are produced prolifically from the stomata of infected needles and specifically from the areas where the typical resinous bands are present. Empty sporangia can be seen suggesting that zoospores are released from the intact sporangia on the needles. The sporangiophores appear to twist and break at some distance from the sporangia, although

this was observed only occasionally. The strong coastal winds and the rain-splash during the rainy season on the coast of the Biobío region of Chile would facilitate this form of dispersal. This suggests that these structures can also represent a means of spread for *P. pinifolia* but our observations suggest that this happens infrequently and it is a question that deserves further study.

The zoospore/sporangia inoculation technique used in this study was technically challenging and is unlikely to be suitable in its current form for large scale experimentation and resistance screening. This is in contrast with other systems, such as those of Hansen et al. (2005) who found that leaf-dip inoculation represented a rapid and reliable method to predict susceptibility to *P. ramorum* infection. In the present study, inoculation with zoospores reproduced the typical symptoms of Daño Foliar del Pino, but it was variable and inconsistent in the level of infection observed. For example, the number of resinous bands produced per plant in the laboratory was different to that seen under field conditions, although these differences were not statistically significant. In contrast with *P. ramorum,* which is very easy to inoculate by spraying of zoospores (P. Resser, pers. comm.), this has not been achieved with *P. pinifolia* thus far. It will thus be important to develop a more complete understanding of the factors influencing infection by *P. pinifolia*, in order to standardize the protocols for resistance screening.

Detailed monitoring of sporangial development highlighted key aspects of the biology and epidemiology of *P. pinifolia*. It was evident that sporangial development was closely linked to rainfall patterns. Not only did sporangial development correlate with the onset and seasonal variation of rainfall, but it was also remarkably closely linked to the amount of rainfall in a particular period of time. The same patterns have also been observed with *P.*

ramorum in California and Oregon, which has a Mediterranean climate with a distinct wet and dry season, and sporulation of the pathogen with subsequent plant infection appear to be primarily restricted to the rainy season (Davidson et al. 2005; Rizzo et al. 2005).

This study allowed a deeper understanding of *P. pinifolia* as the cause of the Daño Foliar del Pino on *P. radiata* in Chile, because it was possible to reproduce symptoms identical to those observed in natural infections. The pathogenicity tests showed that *P. pinifolia* is not specific to *P. radiata* and was able to infect many of the other *Pinus* spp. and hybrids that were artificially inoculated. It was also shown that two of the 13 tested species (*P. pinaster* and *P. taeda*) appeared to be most resistant. In the case of *P. pinaster,* this confirmed field observations where the species shows no symptoms when grown close to heavily infected *P. radiata* trees. The sporangial monitoring demonstrated that propagules of *P. pinifolia* remain on the foliage throughout the year, but their presence is very closely correlated with climatic conditions, of which rainfall is most important.

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