2

CABI PLANT PROTECTION SERIES

# *Phytophthora*

## A Global Perspective

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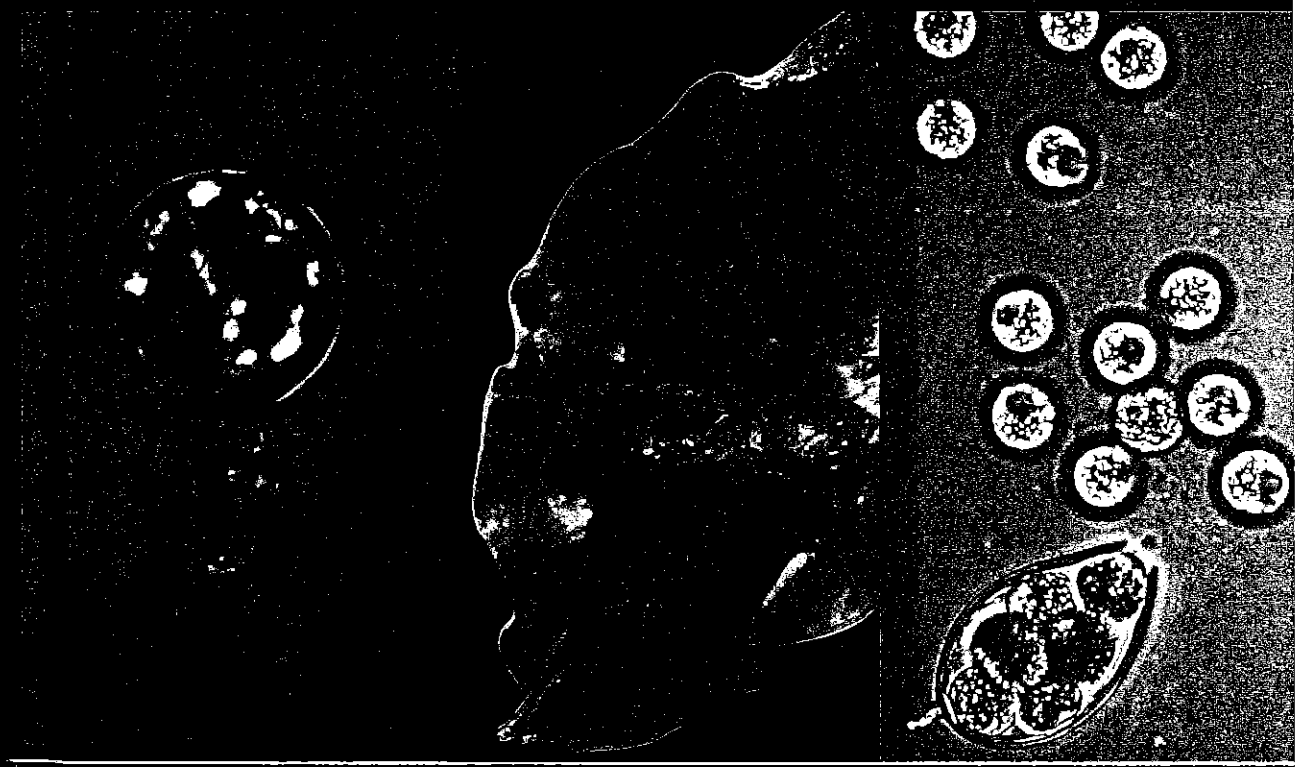

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# 17 *Phytophthora pinifolia*: the Cause of Daño Foliar del Pino on *Pinus radiata* in Chile

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## 17.1 Introduction

*Phytophthora pinifolia* was first observed in *Pinus radiata* plantations in 2004 in the coastal areas of the Arauco province of Chile. *P. pinifolia* causes a disease known locally as 'daño foliar del pino (DFP)', which translates as pine foliar damage. *P. pinifolia* is unusual because it is the only known *Phytophthora* infecting the needles and succulent tissue of any *Pinus* spp. (Durán *et al.*, 2008). *P. pinifolia* was formally described in 2008 and the origin is unknown (Durán *et al.*, 2008; Wingfield, 2008).

Symptoms of DFP were first observed in July of 2004, and by October of 2004 there was mortality of 1- and 2-year-old plantations (Wingfield, 2008). Currently, DFP-infected trees are present in plantations in the coastal Bío Bío and Los Ríos regions and in some young plantations in the Maule region. Annual monitoring indicates affected areas increased from 3300 ha in 2004 to 54,000 ha in 2006; by 2007 they had decreased to about 2000 ha and have remained at this level through to 2011 (R. Ahumada, unpublished). The overall reduction is probably the result of many factors including: (i) non-conducive environmental conditions; (ii) removal of infected trees over large areas; and (iii) implementation of research-based strategies to reduce the impact of DFP. Research-based strategies include: (i) the use of models to define risk levels at different sites; (ii) methods to select tolerant planting stock; and (iii) appropriately timed chemical treatments.

## 17.2 Phylogeny

*P. pinifolia* has unbranched sporangiophores and non-papillate, sub-globose to ovoid sporangia. Sequence analysis of the internal transcribed spacer (ITS) region places it into Group 6 of the phylogeny-based classification of Cooke *et al.* (2000), and its closest relatives are *Phytophthora megasperma*, *Phytophthora gonapodyides*, *Phytophthora thermophile* and *Phytophthora litoralis*. Other Group 6 species include *Phytophthora humicola*, *Phytophthora inundata*, *Phytophthora gibbosa*, *Phytophthora gregata*, *Phytophthora rosacearum* and several undescribed species (Cooke *et al.*, 2000; Jung *et al.*, 2011). Group 6 species are either sterile or inbreeding and occur primarily in forest or riparian ecosystems (Brasier *et al.*, 2003; Kroon *et al.*, 2004). *P. pinifolia* is ecologically and morphologically

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distinct because other Group 6 species are soil-borne and *P. pinifolia* is found above ground. In addition, *P. pinifolia* occasionally produces caducous sporangia that do not proliferate internally or externally; a trait distinct from *P. gonapodyides*, *P. megasperma*, *P. inundata* and *P. humicola*.

## 17.3 Disease Characteristics and Spatial Distribution

*P. pinifolia* attacks the needles and the cambium of succulent tissues. Infected needles die relatively quickly (Fig. 17.1A) and may lead to defoliation (Fig. 17.1B) (Durán *et al.*, 2008). Chile has a Mediterranean climate, and symptoms are generally observed from autumn (May) to late spring (November) during the rainy season. Infected needles often have black bands (Fig. 17.2A), which are resinous, translucent areas (Fig. 17.2B). The tops of infected trees have a greyish appearance and turn brown at the end of spring. Infected trees in 1- and 2-year-old plantations typically die, while trees in 3–6-year-old plantations suffer needle damage and defoliation and survive. In adult plantations (older than 6 years) DFP is limited to the needles and the succulent tissue of young branches and the trees do not die (Durán *et al.*, 2008). *P. pinifolia* does not survive in the felled green timber of infected trees and wood from infected trees is not a risk for subsequent infections. Since 2008 ongoing investigations by Bioforest (a research company of the Arauco Group) indicate that *P. pinifolia* is not transported with timber from Chile (Ahumada *et al.*, 2012).

In Chile *P. pinifolia* is found primarily in coastal areas from Constitución to Valdivia, with the highest incidence in the Arauco province. Areas with high levels of infection have high humidity for most of the year due to their proximity to the Pacific coast. Environmental conditions substantially influence disease severity and disease is most severe in plantations with a southern exposure where the trees remain cooler and moister for longer periods (Fig. 17.1C). The spread of DFP in trees older than 2 years has a clear pattern, where disease starts in the lower canopy, which is cool and wet due to less sunlight, and then progresses upwards. The highest levels of infection are observed from June to September, followed by defoliation from July to November.

## 17.4 Isolation and Identification

*P. pinifolia* can be isolated on CARP media (cornmeal agar specific medium with 0.01 g benomyl, 0.01 g pimaricin, 0.2 g ampicillin, 0.01 g rifampicin), and recovery from freshly infected needles, particularly those with resinous bands, is relatively simple and mycelium is visible in 5–7 days. In addition, sporangia and zoospores can be visualized by placing infected needles on a glass slide in calcofluor fluorescent brightener (0.001%) for 30 s and examining them microscopically under a fluorescent light (Fig. 17.2C). Isolates grown on V8 I (354 ml V8 juice buffered with 5 g of $CaCO_3$, 15 g agar and 1 l distilled water; Erwin and Ribeiro, 1996) and CA (corn meal agar) media are characterized by fluffy mycelium with regular to rosaceous or petallate margins and growth is optimal at 25°C (Erwin and Ribeiro, 1996). The sporangia are occasionally detached from the medium-length pedicels (Durán *et al.*, 2008). Oogonia and antheridia have not been observed, and *P. pinifolia* is thought to be sterile (Durán *et al.*, 2008).

Molecular identification of *P. pinifolia* can be accomplished using species-specific PCR primers or a PCR-restriction fragment length polymorphism (RFLP) assay (Durán *et al.*, 2009) (see Martin, Chapter 3, this volume). There are two *P. pinifolia*-specific primer sets. One is based on the ITS region of the ribosomal RNA (rRNA) gene and the other is based on the ras-related protein gene *Ypt*1 (Durán *et al.*, 2009). The PCR-RFLP assay was developed according to Drenth *et al.* (2006) and results in a unique profile compared with other *Phytophthora* spp. In Chile *P. pinifolia* is routinely identified using genomic DNA from infected plants using either species-specific primers or the PCR-RFLP assay.

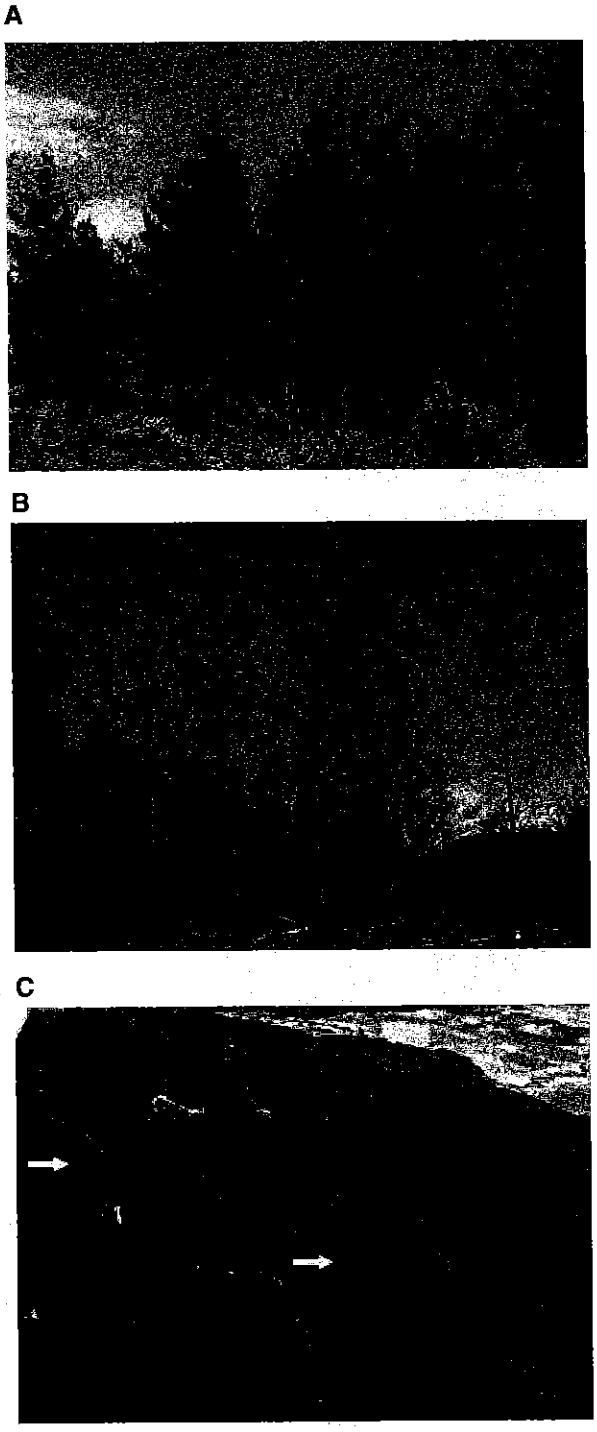


**Fig. 17.1.** Symptoms of *Phytophthora pinifolia* on *Pinus radiata*. **(A)** Infected adult trees, **(B)** defoliated trees, and **(C)** aerial photo of infected trees with a southern exposure (white arrows) and adjacent healthy trees with a northern exposure (black arrows).

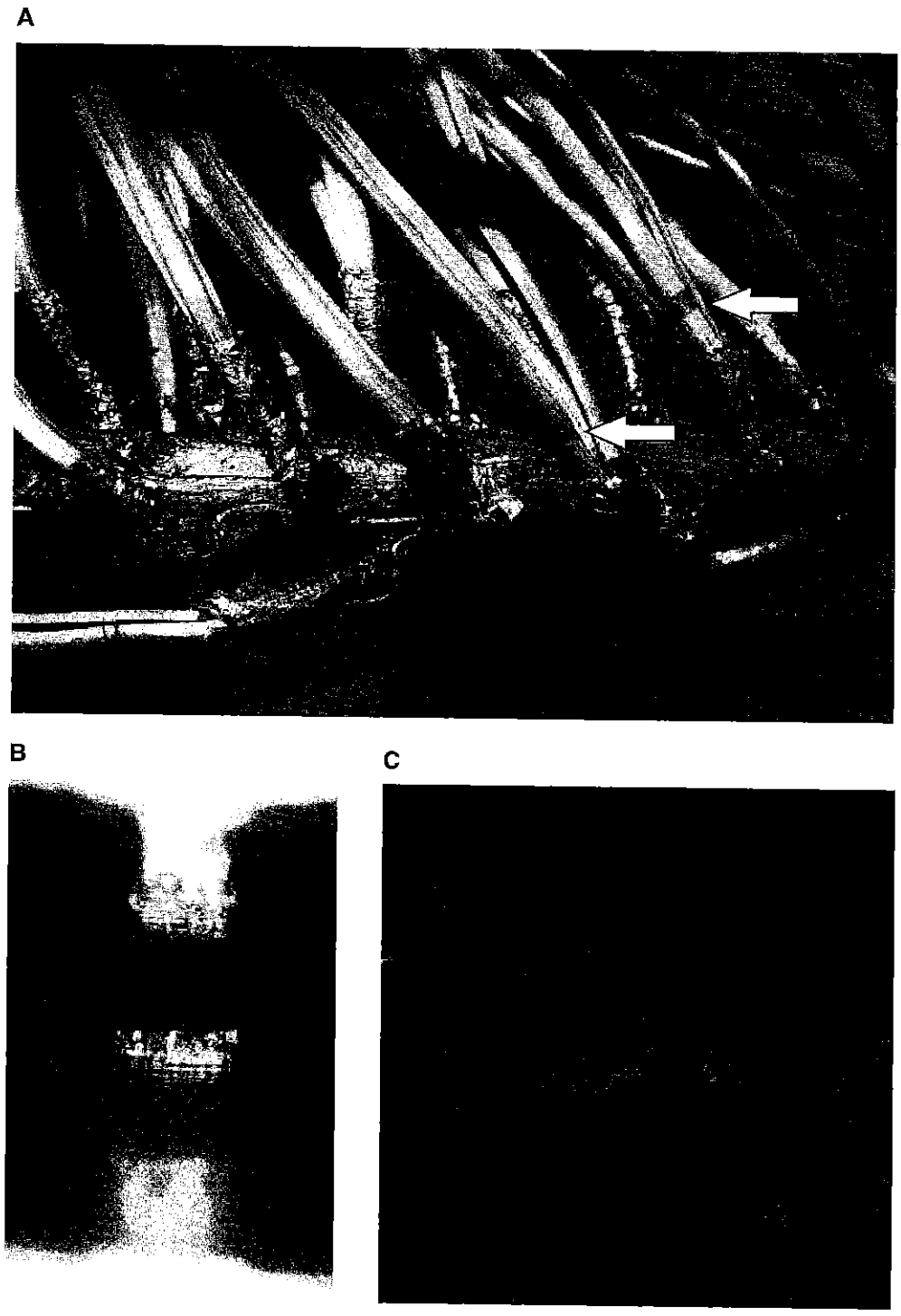


**Fig. 17.2.** *Phytophthora pinifolia* on needles of *Pinus radiata*. **(A)** Infected needle with black bands (arrows), **(B)** close-up of the resinous translucent band and **(C)** sporangia on the needle surface observed by fluorescent light microscopy.

A large number of isolates of *P. pinifolia* have been collected in Chile and analysed using amplified fragment length polymorphism (AFLP) markers. Results indicate a single clonal lineage is dominant and the overall population structure is clonal (Durán *et al.*, 2010). *P. pinifolia* appeared suddenly in Chile in 2004 across a very limited area, and it is probably an introduced pathogen. Interestingly, other *Pinus* species and conifers (e.g. *Pinus pinaster* and *Pseudotsuga menziesii*) found in areas with DFP are not infected, and it appears that *P. pinifolia* is host specific. While the origin of *P. pinifolia* is currently unknown, it may originate from areas where *Pinus radiata* or related species are native. Future studies are needed to determine the origin of *P. pinifolia* as this might prevent its accidental movement to new environments.

## 17.5 Epidemiology

*P. pinifolia* is spread via sporangia. Sporangia are produced on green needles and on dead needles in the trees or that fall to the forest floor. The majority of sporangia are produced during the winter months and needle wetness and temperature are key factors for infection and the development of DFP symptoms. Along the coast of the Arauco province the frequent mist and rain and the temperatures experienced are conducive to the development of DFP symptoms (Del Pozo and Del Canto, 1999).

The incidence of new infections has been monitored using healthy trap plants, and new infections occur throughout the year with the highest incidence during the wet winter and spring months. The area affected by DFP on the Arauco coast province has varied considerably between 2006 and 2011, from a high of 54,000 ha to the current low of 2000 ha. The number of days that are favourable for *Phytophthora* development has been estimated using the Hyre model (Hyre, 1954), and there were 141 favourable days in 2006 and between 51 and 60 favourable days between 2007 and 2011. Clearly, wet weather favours disease development.

## 17.6 Management of the Disease

The management of DFP in *P. radiata* stands includes: (i) selection of clones tolerant to the infection; (ii) selection of sites that do not have the conditions for disease development; and (iii) use of specific fungicides. Tolerant clones are identified by placing young clonal plants under the canopies of trees with high levels of infection. Clones exhibit a range of tolerance and are ranked to select tolerant clones for planting in high risk areas. The selection of tolerant clones is a long-term programme, but it is already providing promising results. In addition, rapid laboratory and greenhouse screening techniques are being developed to improve the efficiency of selecting tolerant clones.

Low risk sites are identified based on knowledge gained from monitoring areas with severe disease incidence (roughly 120,000 ha from 2005 to 2009). The monitoring includes annual aerial photography of the same areas when symptoms are the most severe (October). Monthly climatic data is used to estimate the dew point and relative humidity, and a model has been developed to estimate the amount of time each month where the temperature is above 7°C, the minimum temperature where *P. pinifolia* can grow. Another model estimates the length of time with >90% relative humidity (assumed to be a condition favourable for *P. pinifolia* infection). The models confirm that the coastal area of the Arauco province (also called the Arauco Gulf) is the most favourable for infection by *P. pinifolia*, and aerial surveys in 2006 confirmed a direct relationship between the number of days favourable for infection and the amount of damage observed.

Several fungicides have been tested for DFP control. Systemic products such as metalaxyl and mefenoxam (phenylamides), propamocarb HCL (carbamate), dimetomorph and mandipropamid (carboxylic acid amides) and other fungicides including fosetyl-aluminium (ethyl phosphonate) and salts of phosphorous acid (phosphite) have been considered for their efficacy to reduce infection by *P. pinifolia*. The use of

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chemicals in young plantations consistently reduced the damage of DFP. Several trials carried out between 2008 and 2011 indicate spraying with phenylamides and mefenoxam two to four times/year reduced symptoms of DFP by up to 90% and reduced plant mortality to less than 5%. Other formulations of mefenoxam with chlorotalonil and mancozeb, sprayed four times during the year, reduced DFP by 77% and 96% and mortality to 2% and 14%, respectively.

Phosphites sprayed alone or alternated with phenylamides showed promising results for the management of *P. pinifolia* in the field. Phosphites sprayed four times/year alone gave a control of between 73% and 99% with 700 cc/hL (equivalent to 2.9 g $P_2O_5$/l and 1.9 g $K_2O$/l) and 1400 cc/hL (equivalent to 5.8 g $P_2O_5$/l and 3.8 g $K_2O$/l), respectively. The variation in results could be a consequence of the different chemical formulations. The use of phosphites in rotation with other fungicides could reduce the emergence of resistant strains of *P. pinifolia* to phenylamides. An important advantage of phosphites is the translocation between both phloem and xylem (Guest and Grant, 1991). Fosetyl-Al and phosphite are less effective than metalaxyl-Mz (metalaxyl) and metalaxyl-M (mefenoxam). In addition, phosphites are treated as fertilizers and have a low environmental impact.

To achieve the best control, application of fungicides should occur before the resinous bands on the needles are first observed. On the Arauco coast, applications are made from April to July in plantations older than 1 year. For establishment of plantations, plants are treated in the nursery before they are planted. For areas with high risk of infection, plants are treated every 3 months with phosphites to prevent the development of DFP. Adult plantations are only treated with phosphite if located in the highest risk areas.

## 17.7 Conclusions and Future Research

*P. pinifolia* has been studied intensely since it was first recognized. Damage was severe in commercial plantations and much of the work has occurred in the private sector. A better understanding of the climatic conditions favouring disease has made it possible to develop strategies for management, which, thus far, appear to be working. The clonal nature of the pathogen in Chile and its apparent specificity to *Pinus* spp. suggests that it was accidentally introduced into the country. Studies to determine its origin may be helpful to identify pathways of introduction and reduce the risk of *P. pinifolia* being introduced into other areas of the world.

*P. pinifolia* is one of the most important pathogens to affect plantation-grown pines in the last decade and it may be a threat to *Pinus radiata* in other parts of the world. This includes areas where *P. radiata* is planted as a non-native and where the species is native. Very little is known regarding the susceptibility of other *Pinus* spp., but preliminary studies suggest that relatives of *P. radiata* are susceptible to infection (R. Ahumada, unpublished data). Clearly, continued research is needed to prevent the spread of *P. pinifolia* to new areas.

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