Temporal analysis of charcoal root rot in forest nurseries under different pathogen inoculum densities and soil moisture content

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ABSTRACT

The progress of charcoal root rot (CRR) disease in *Pinus radiata* seedlings caused by *Macrophomina phaseolina* was evaluated in greenhouse and field trials. In one greenhouse trial *P. radiata* was sown in a sandy soil with pathogen inoculum densities (ID) ranging from 25 to 1000 cfu.g⁻¹. After two months, the seedlings were established under three soil moisture content (SMC) levels corresponding to 100%, 75% and 50% of field capacity (FC). In one field trial, *P. radiata* was sown on microplots infested with ID of 50 to 250 cfu.g⁻¹. In the greenhouse trial the disease incidence was related with the pathogen ID and SMC. At 100% FC, the seedlings remained asymptomatic until the experiment ended, however *M. phaseolina* was able to infect the roots. In the field trial, the disease progress curves were similar to those of the greenhouse trial in response to ID and SMC but the plant mortality due to CRR incidence did not exceed 50%. The monomolecular model was the one that best fitted the disease progress curve data for both trials and most treatments. Knowledge of the existing ID of *M. phaseolina* in the soil, combined with information on SMC, may be important for predicting CRR epidemics in *P. radiata* nurseries.

Key words: *Macrophomina phaseolina*, *Pinus radiata*, epidemiology.

INTRODUCTION

Charcoal root rot (CRR) caused by the fungus *Macrophomina phaseolina* (Tassi) Goid. causes significant losses in *Pinus radiata* D. Don bare-root nurseries in Chile. In the 1980's the pathogen was detected in forest nurseries located in the Libertador Bernardo O'Higgins and Bío-Bío Regions, and was also found in *P. radiata* and *Eucalyptus globulus* Labill. plantations up to six years old (Gonzalez, 1993). CRR was considered a serious problem in bare-root conifers in nurseries causing approximately 30% mortality in the southwestern United States (Fraedrich & Smith 1994). Among the most important factors for the development of the disease are high temperatures and low moisture content in the soil (Wyllie, 1989). *Macrophomina phaseolina* infects plants within a wide temperature range (20 to 35°C) and is influenced by water stress conditions (Ali & Ghaffar, 1991; Diourte et al., 1995; Kending et al., 2000). Conditions of low moisture content in the soil leading to drought stress have been recognized as the most important predisposing factor for CRR (Reuveni et al., 1982), increasing the susceptibility of the host and decreasing the

activity of antagonistic microorganisms to *M. phaseolina* (Palti, 1981). Another factor that affects the incidence of CRR is the amount of inoculum in the soil at the beginning of the growing season. *Macrophomina phaseolina* sclerotia are propagules that allow the survival of this fungus in the soil. These are formed in the host during the parasitic phase and released into the soil through the decomposition of the infected host tissue (Cook et al., 1973; Meyer et al., 1974). Unlike other soilborne pathogens, *M. phaseolina* can grow and produce great amount of sclerotia under drought conditions (Olaya & Abawi, 1996).

There is a direct relationship between levels of pathogen inoculum in the soil and disease incidence (Filho and Dhingra 1980, Short et al., 1980). The relationship between inoculum density of *M. phaseolina* and the incidence of CRR varies according to the susceptibility of the host and prevailing environmental conditions, as demonstrated for plant species such as soybean [*Glycine max* L. (Merr)], spurge (*Euphorbia lathyris* L.) and sesame (*Sesamum indicum* L.) (Ganghopadhyay et al., 1982; Pineda et al., 1985).

For conifers, and particularly for *P. radiata*, the epidemiology of CRR is unknown. The objective of this research was to determine the effect of soil moisture content and inoculum density of *M. phaseolina* on the progress of CRR in *P. radiata* seedlings.

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MATERIALS AND METHODS

Pathogen strain and inoculum production

The UDC-019 strain of *M. phaseolina*, isolated from the roots of *P. radiata* seedlings with symptoms of CRR and collected from a commercial nursery ("Carlos Douglas" Mininco Forest, Yumbel, Bio-Bio Region, Chile), was used in all trials. The isolate was stored in Difco potato-dextrose agar medium (PDA) at 4°C (Valiente et al., 2008). A potato broth medium (potato, 200 g.L⁻¹ + glucose, 20 g.L⁻¹) was used for inoculum production. This media was prepared in 50 mL flasks, and seeded with four 0.5 cm discs collected from 4-days old actively growing colonies in PDA. The flasks were kept for one month at 30°C in darkness. At the end of this period, the mycelium with sclerotia formed on the surface of the medium was filtered, air dried, crushed in a mortar and sieved to 0.71 mm to produce a homogeneous inoculum.

Greenhouse trial

Study site

This study was carried out from January to April 2007 in the experimental greenhouse at the University of Concepción (Bío-Bío Region, Chile), under controlled conditions of temperature and soil water moisture.

Soil and infestation with pathogen inoculum

A sandy soil collected from the "Carlos Douglas" nursery was used as a substrate for seedling growth. It originated from the top soil layer (0-20 cm depth) and had a fine sandy texture: 20% of particles equal or greater than 1 mm, 43% between 0.5 and 1.0 mm, 27% between 0.5 and 0.25 mm, and 10% with less than 0.25 mm. Field capacity (FC) was of 12.8%, permanent wilting point of 2.6% and available moisture of 10.2%. Before use the soil was sieved to 16 mm, sterilized with formalin (2%) and then placed in 1650 cm³ pots. The bottom half of the pot contained sterile sand while the top layer contained a homogeneous mixture of soil and inoculum of *M. phaseolina*. Inoculum density (ID) levels in this portion of soil were 0, 25, 50, 100, 250, 500 and 1000 cfu.g⁻¹.

Trial establishment

Seeds of *P. radiata* were provided by the "Proplanta Ltda" forest nursery, with 90% germination capacity and 80% germination vigour. The latter was determined by the Czabator index (Benedetti et al., 2012). Two days after soil infestation with the pathogen, *P. radiata* was sown at a 0.5 cm depth at a rate of 30 seeds per pot, replicated five times. The pots were kept in the greenhouse for 28 days after sowing, and approximately 90% of seedlings emerged. During this period, pots were irrigated daily with 300 mL of sterile distilled water until total emergence of seedlings. After that, the pots were moved to a controlled temperature chamber where the temperature was maintained at either

21±1°C or 30±1°C and a photoperiod of 12/12 hours of light and dark. After 26 days in the chamber constantly irrigated at FC, the seedlings were subjected to three different soil moisture regimes (SMC): 50% FC (0.015 MPa), 75% FC (0.0225 MPa) and 100% FC (0.03 MPa), for two months.

Evaluations

Seedling mortality (%) was evaluated during the course of the trial, and at the end of the experiment the incidence and severity were measured as the degree of root colonization by *M. phaseolina* in asymptomatic plants (%). In the field trial records were taken of the environmental temperature (°C) with a thermo hygrometer Model 303C. Soil temperature (°C) was recorded for both greenhouse and field studies with a mercury glass thermometer Model VLQ-20260 (base placed at 10 cm depth). Soil moisture content was measured by the gravimetric method.

(i) Disease incidence. Seedlings bearing symptoms of wilt, yellowing and foliage necrosis were collected every 3 days from the 63th to the 117th day and evaluated at the Laboratory of Forest Pathology. The seedlings were observed under a stereoscopic magnifying glass (x25) to determine the presence of microsclerotia of *M. phaseolina* on the collar and the roots. Plants with no signs of the pathogen had their roots cut into 5 mm segments, dipped in 50% alcohol for 30 s, transferred to 0.5% sodium hypochloride for 1 min and rinsed in sterile water and then placed on a modified selective medium (Mihail & Alcorn, 1982). The original recipe for this medium contained the following ingredients: Difco potato dextrose agar (39 g.L⁻¹), Difco Bacto-Agar (10g.L⁻¹), streptomycin sulfate (250 µg active ingredient/mL) and chloroneb (Demosan 65WP). However, here, chloroneb was replaced by propamocarb hydrochloride (Previcur, 695 mg active ingredient.mL⁻¹). The emergence of the pathogen from the roots and formation of typical black colonies (due to the presence of abundant microsclerotia of *M. phaseolina*) was evaluated for each sample after four days of incubation at 27°C.

(ii) *Macrophomina phaseolina* in asymptomatic plants.

At the end of the trial disease incidence was evaluated on seedlings and CRR severity was measured as root colonization per plant (%). Twenty-five seedlings were randomly collected for each pathogen ID level and soil water moisture levels. Pathogen isolation was performed by the following procedure: roots were washed and brushed in running water to remove adhering soil, and the roots were subsequently dried with paper towels. From each seedling, four 5 mm-long roots segments were randomly cut, dipped in 50% alcohol for 30s, transferred to 0.5% sodium hypochloride for 1 min and rinsed in sterile water. The root pieces were placed in Petri dishes containing the modified selective medium (Mihail & Alcorn, 1982) and incubated for 6 days at

30°C. After incubation, samples were searched for *M. phaseolina*. Root colonization by the pathogen was estimated as a proportion between the root fragments with *M. phaseolina* and the total number of fragments for each treatment. The experimental unit was one Petri dish containing four root fragments per replication. *Macrophomina phaseolina* incidence was calculated as the number of seedlings infected with the pathogen in relation to the total number of seedlings evaluated from each replication.

Experimental design and statistical analysis

The trial was conducted as a 3x7 factorial experiment (three SMC levels and seven IDs), using a completely randomized design with five replications. The control consisted of sterile sand without inoculation with the pathogen. The experimental unit for disease incidence was one pot (30 seeds of *P. radiata*). The experimental units for incidence and root colonization in asymptomatic seedlings were five seedlings and twenty root pieces, respectively. For all the variables evaluated normality and homogeneity of variances were determined by the Shapiro-Wilk and Bartlett's test, respectively ($P > 0.05$), and the Tukey multiple range test (SAS Institute, 2002) was used to determine significant differences between treatments ($\alpha = 0.05$). In the case of any interaction between the factors an analysis of contrasts was performed to verify significant differences between treatments of interest.

Temporal analysis

The data on seedling mortality was used for the preparation of a disease progress curve. Mortality was calculated as the percentage of dead seedlings in relation to total seedlings evaluated for each treatment. Gompertz, logistic and monomolecular models were used in their non-linearized form in the analysis of disease incidence progress (Campbell & Madden, 1990). The best model was selected by considering the smallest value of root mean square error (RMSE). The progress rate corresponded to the r coefficient for each model. The disease incidence data was transformed by $(\sqrt{\%}/100)$. The adjustment of the mathematic models and the temporal analysis of disease data were made with the Statistical Analysis System (SAS Institute, 2002) and Statistica version 6.0.

Nursery trial

Area

The trial was conducted in a commercial nursery of *P. radiata* (Proplantas Ltda.) located in Quinchamalí, Bio-Bío Region, Chile (36°37'23"S; 72°21'23"W) from January to April 2008. The region has a warm mediterranean climate, with an average annual temperature of 14°C, an average maximum in the warmest month (January) of 28.8°C and a average minimum of the coldest month (July) of 3.5°C. The mean annual rainfall is 1093 mm and July is the rainiest month with 217 mm (Novoa, 1989).

Soil and infestation with pathogen inoculum

The experimental area was located on a soil formed sediments of the Itata river. The soil is laminated, dark brown to black, sandy, with a simple grain structure and little biological activity. The trial was in an area of known occurrence of CRR. Before sowing, the natural *M. phaseolina* ID was estimated according to the procedure described by Mihail & Alcorn (1982). Soil sampling followed a two-diagonal systematic pattern, and five soil samples of 25 g were taken from the top layer (0-12 cm). The natural pathogen ID ranged from 5 to 20 cfu.g⁻¹. The soil was artificially infested with the pathogen up to 10 cm depth with 0, 50, 75, 100, 125, 150 and 250 cfu.g⁻¹.

Trial establishment

The microplots consisted of plastic containers (32 cm diameter x 34 cm height), where manual incorporation of microsclerotia was made for each pathogen ID level assigned. Two months after soil infestation with the pathogen, *P. radiata* was sown at 0.5 cm depth using 40 seeds per microplot. The seeds of *P. radiata* used in the experiment were as described for the greenhouse trial. Every 10 days the soil temperature (°C) and soil moisture (%) at 10 cm depth were taken from a weather station of the Proplanta nursery located 1 km from the study site. The experiment was replicated four times.

Evaluations

The plants presenting wilting symptoms, yellowing and foliage necrosis were collected every 4 d from day 41 to 125th and evaluated for disease intensity. Evaluation was as described for the greenhouse trial.

Experimental design and statistical analysis

The nursery trial was organized in a completely randomized block design with four replications. Each block included seven treatments (pathogen ID levels) and the experimental unit was a microplot with 40 seeds. The control consisted of microplots without pathogen infestation. Statistical analysis and temporal analysis of the disease progress curve were as described for the greenhouse trial.

RESULTS

Greenhouse trial

Death of *P. radiata* began 7 days after seedlings were submitted to the three SMC levels. Some seedlings showed symptoms of wilting followed by necrosis in needles, the main roots were shorter and lacked lateral rootlets. In the SMCs at 50% and 75% FC, the disease incidence levels were related with the pathogen ID levels in the soil (Figure 1A and 1B). *Pinus radiata* seedlings at 100% FC remained free of CRR symptoms until the end of the trial.

FIGURE 1 - Progress curve of charcoal root rot caused by *Macrophomina phaseolina* on *Pinus radiata* seedlings at different pathogen inoculum densities and soil moisture content levels (greenhouse trial). **A**. 50% field capacity (0.015 MPa); **B**. 75% field capacity (0.0225 MPa).

Controls had a seedling mortality which did not exceed 10% and displayed symptoms of attack by *Fusarium* spp. and not of CRR. Similarly, at 100% FC a seedling mortality of 3% was observed to be associated with infection by *Fusarium* spp. In both cases, this pathogen was isolated from the neck and roots of the wilted plants.

In the first 69 days, incidence of the disease increased rapidly and was higher for treatments with pathogen ID's ≥ 100 cfu.g⁻¹ as compared with treatments with ID's ≤ 50 cfu.g⁻¹. At the end of the study (117th day), for ID ≥ 250 cfu.g⁻¹, the disease incidence was significantly higher ($P < 0.001$) than with pathogen ID's ≤ 100 cfu.g⁻¹ (Figure 1A and 1B). Thus, from 250 to 1000 cfu.g⁻¹, the seedlings mortality exceeded 80% for both SMC levels tested, and from 25 to 100 cfu.g⁻¹, the mortality varied from 32 to 68% at 50% FC, and from 42 to 62% at 75% FC (Figures 1A and 1B). The interaction between pathogen ID and SMC was highly significant ($P < 0.0001$), since at 100% FC no seedling mortality by *M. phaseolina* was observed. No significant difference ($P = 0.207515$) in the disease incidence at 50 and 75% FC was observed for all pathogen ID levels tested.

In general, disease progress fitted well within the Gompertz and monomolecular models ($P < 0.001$). At 50% and 75% FC, from 50 to 1000 cfu.g⁻¹ the monomolecular model was the best to describe disease progress, and the Gompertz model was the best for the pathogen ID of 25 cfu.g⁻¹ (Table 1).

The disease progress rates (r) increased with the increase of the pathogen ID levels in the soil. The plants maintained at 50% FC showed slightly higher rates of progress when compared with the pathogen ID of 50 and 250 cfu.g⁻¹ at 75% FC (Table 1). At the end of the trial the symptomless plants were shown to have root infections by *M. phaseolina* for all SMC levels evaluated. As a general trend, when the SMC decreased and the pathogen ID level

increased, the incidence of asymptomatic seedlings infected with *M. phaseolina* increased (Figure 2A, 2B and 2C). At 100% FC the incidence of asymptomatic plants infected with *M. phaseolina* was lower compared with 50% and 75% FC ($P < 0.001$) (Figure 2A). The general trend of infection at 50% and 75% FC was similar and proportional to the initial ID. As the pathogen ID increased, the incidence of asymptomatic plants infected by the pathogen also increased. At 50% and 100% FC, root colonization by *M. phaseolina* did not follow the expected trend as there was greater colonization (about 60%) in the lower ID value (25 cfu.g⁻¹). In contrast, at 75% FC root colonization increased with increasing pathogen ID. A positive correlation ($R^2 = 0.73$) was observed between the incidence of asymptomatic seedlings infected with *M. phaseolina* and root colonization by the pathogen (Figure 3).

Nursery trial

Death of *P. radiata* seedlings began 10 days (day 40) after 100% emergence of the plants, for all *M. phaseolina* ID levels. The seedlings showed the characteristic symptoms of the disease (wilting followed by necrosis of needles and stems). The seedling mortality of *P. radiata* increased following increasing ID levels in the soil. Controls without pathogen infestation had limited seedling mortality caused by *Fusarium* spp. (5%). In the first 90 days, seedling mortality exceeded 20% for all pathogen ID levels evaluated, except for the ID of 50 cfu.g⁻¹ (approximately 12% mortality). At the end of the trial (125th day), for the lowest ID the mortality did not exceed 25%, and for the other ID levels the mortality varied between 27% (50 cfu.g⁻¹) and 38% (250 cfu.g⁻¹) (Figure 4).

During the summer (January-March), maximum soil temperature reached 32°C and then decreased to a minimum

TABLE 1 - Fitness of monomolecular, logistic and Gompertz models to the progress of charcoal root rot disease of *Pinus radiata* caused by *Macrophomina phaseolina* and rates of *P. radiata* mortality at different inoculum densities (ID) and soil moisture content levels in a greenhouse trial

ID (cfu.g ⁻¹)	Model	50% FC (0.015 MPa)			75% FC (0.0225 MPa)		
		RMSE	a	(r)	RMSE	a	(r)
25	Monomolecular	0.1828	-0.0210	0.065	0.179	-0.002	0.002
	Logistic	0.1688	0.0030	0.020	0.211	0.023	0.034
	Gompertz	0.1670	0.0008	0.002	0.200	0.017	0.012
50	Monomolecular	0.2377	0.0006	0.003	0.309	0.008	0.002
	Logistic	0.2435	0.036	0.002	0.323	0.029	0.031
	Gompertz	0.2592	0.028	0.013	0.318	0.024	0.012
100	Monomolecular	0.4717	0.028	0.003	0.132	0.008	0.003
	Logistic	0.5025	0.062	0.026	0.307	0.038	0.033
	Gompertz	0.4932	0.051	0.011	0.279	0.030	0.013
250	Monomolecular	0.5959	0.002	0.004	0.419	-0.003	0.005
	Logistic	0.6219	0.045	0.037	0.454	0.046	0.038
	Gompertz	0.6141	0.033	0.016	0.437	0.032	0.017
500	Monomolecular	0.4566	0.03	0.005	0.342	0.014	0.005
	Logistic	0.4847	0.069	0.028	0.390	0.059	0.033
	Gompertz	0.4711	0.056	0.013	0.370	0.045	0.015
1000	Monomolecular	0.5149	0.069	0.007	0.350	0.047	0.005
	Logistic	0.5485	0.109	0.024	0.379	0.081	0.026
	Gompertz	0.5365	0.089	0.012	0.369	0.067	0.012

*FC = field capacity; RMSE = root mean square error; *r* = disease progress rate.

FIGURE 2 - Incidence of *Macrophomina phaseolina* (%) in asymptomatic seedlings of *Pinus radiata* and pathogen root colonization (severity), growing at different pathogen inoculum densities and soil moisture content levels (greenhouse trial). **A.** 100 % field capacity (0.03 MPa); **B.** 75% field capacity (0.0225 MPa); **C.** 50% field capacity (0.015 MPa). Vertical bars show the standard error.

of 15°C in April (Figure 5). The monomolecular model was the one that best described the disease progress under field conditions, having the lowest RMSE (Table 2).

Disease progress rates (*r*) increased with the initial ID, whereas the lowest ID levels yielded lower progress rates compared with the highest ID evaluated. Plants that remained asymptomatic until the end of the study had latent root infections by *M. phaseolina*. *Trichoderma* spp., *Fusarium* spp. and various bacteria grew from the roots and showed antibiotic activity against the pathogen in culture (unpublished data). The maximum incidence of

asymptomatic seedlings infected with *M. phaseolina* was 35%. With increasing ID, the number of seedlings infected with *M. phaseolina* increased until a level of 100 cfu.g⁻¹ was reached and then decreased gradually until 250 cfu.g⁻¹. In contrast, root colonization by the pathogen increased following the increasing levels of initial pathogen inoculum, except for the treatment with 150 cfu.g⁻¹ (Figure 6A). There was a positive significant correlation ($R^2 = 0.52$, $P < 0.001$) between the incidence of asymptomatic seedlings infected with *M. phaseolina* and disease severity on roots (Figure 6B).

FIGURE 3 - Correlation between the percentage of asymptomatic seedlings infected with *Macrophomina phaseolina* and pathogen root colonization. Pearson correlation coefficients ($N = 180$).

FIGURE 4 - Progress curve of charcoal root rot caused by *Macrophomina phaseolina* on *Pinus radiata* seedlings at different pathogen inoculum densities (nursery trial).

DISCUSSION

Results of the greenhouse trial indicate that pathogen ID levels and SMC levels have an important role in the development of CRR. Therefore, a pathogen ID ≥ 100 cfu.g⁻¹, in an environment conducive to the disease, can potentially lead to high mortality (up to 50%) in the bare-root production of *P. radiata*. These results agree with those obtained by Valiente et al. (2008), who found that the mortality of *P. radiata* increased with increasing ID, reaching up to 60% of CRR incidence with 200 cfu.g⁻¹. Regarding the SMC, mortality of *P. radiata* seedlings maintained at low soil humidity was higher, coinciding

FIGURE 5 - Soil temperature average at 10 cm of depth during the study period from January to April 2008.

with the results obtained by Russin et al. (1995) who found that low soil moisture was a determining factor for CRR occurrence.

The relationship between seedling mortality and ID found in this study was not consistent with that found for some other pathosystems. In sorghum, for example, the increase of disease incidence was not related with the density of *M. phaseolina* inoculum in the soil, even under conditions favorable for disease development (Gonzalez et al., 2007). These discrepancies can be explained by several factors including the degree of susceptibility of the host (Mayek-Pérez et al., 2001) and the aggressiveness of the pathogen strain (Pratt et al., 1998).

Disease progress in the greenhouse and nursery trials were similar. In both situations a direct relationship between the pathogen ID and plant mortality was found. However, under nursery conditions plant mortality was lower as a response to ID. Higher ID levels seem to be necessary in natural soils compared with sterile soil for equivalent disease levels to be reached. This is thought to be a result of *M. phaseolina* having a low saprophytic ability, being unable to compete for substrate with other microorganisms in the soil, or having an inherent difficulty to reach the roots of the host plant (Dhingra & Sinclair, 1978).

M. phaseolina has been recognized as a pathogen which is favored by drought conditions (Abawi & Pastor-Corrales, 1990). The fungus can grow even under low water potential and is also known to attack a large range of different hosts (Diourte et al., 1995; Olaya & Abawi, 1996). Cotton plants (*Gossypium herbaceum* L.) inoculated with *M. phaseolina*, growing in a sandy soil and kept under water stress and high temperature, showed a high CRR incidence (about 75%). However, under adequate irrigation and lower soil temperature, incidence was lower (Ghaffar & Erwin 1969). Similarly, sorghum plants maintained under water stress showed more severe symptoms of CRR compared with plants free from water stress (Diourte et al., 1995).

Disease incidence and the degree of root colonization in asymptomatic plants had a direct relationship with *M.*

TABLE 2 - Fitness of monomolecular, logistic and Gompertz models to the progress of charcoal root rot disease of *Pinus radiata* caused by *Macrophomina phaseolina* and rates of *P. radiata* mortality at different inoculum densities (ID) in a nursery trial

ID (cfu.g ⁻¹)	Model	RMSE	<i>a</i>	(<i>r</i>)
50	Monomolecular	0.202	0.0251	0.0025
	Logistic	0.287	0.0486	0.0217
	Gompertz	0.288	0.0399	0.0091
75	Monomolecular	0.400	0.0905	0.0039
	Logistic	0.480	0.1488	0.0150
	Gompertz	0.453	0.1181	0.0083
100	Monomolecular	0.526	0.1022	0.0046
	Logistic	0.596	0.1699	0.0164
	Gompertz	0.571	0.1322	0.0095
125	Monomolecular	0.566	0.0315	0.0047
	Logistic	0.592	0.0856	0.0232
	Gompertz	0.577	0.0646	0.0120
150	Monomolecular	0.503	0.0324	0.0052
	Logistic	0.553	0.0952	0.0234
	Gompertz	0.527	0.0704	0.0126
250	Monomolecular	0.380	0.0435	0.0058
	Logistic	0.488	0.1165	0.0223
	Gompertz	0.441	0.0857	0.0126

*RMSE = root mean square error; *r* = disease progress rate.

FIGURE 6 - A. Incidence of *Macrophomina phaseolina* (%) in asymptomatic seedlings and pathogen root colonization (severity) in *Pinus radiata* at different pathogen inoculum densities in a nursery trial. Vertical bars show the standard error. B. Correlation between the incidence of asymptomatic seedlings infected with *M. phaseolina* and pathogen root colonization. Pearson correlation coefficients (N = 70).

phaseolina ID in the soil at levels of 50% and 75% FC. Some studies have demonstrated the lack of a consistent correlation between the severity of infection and host mortality. Disease symptoms in the field are most evident under environmental conditions that impair plant vigor, such as poor soil fertility (Sinclair & Backman, 1989), high plant density (Pearson et al., 1984; Sinclair & Backman, 1989), low soil moisture (Meyer et al., 1974; Ali & Ghaffar, 1991; Kendig et al., 2000), high temperature (Mihail, 1989), and root damage (Canaday et al., 1986). In this study, a good correlation was found between seedling mortality and pathogen root colonization in asymptomatic

plants. Plants maintained at field capacity had no mortality by *M. phaseolina* and remained asymptomatic until the end of the experiment for all ID levels evaluated. The absence of symptoms could be attributed to the fact that seedlings having an abundant water supply (constantly at field capacity) are less susceptible to pathogen attack. Moreover, at 100% FC there is less oxygen available for pathogen growth, diminishing its ability to grow through the soil and to infect the roots (Wyllie & Gangopadhyay, 1984). Saturated soils are known to also affect the sclerotia of other fungal pathogens such as *Sclerotinia minor* (Abawi et al., 1985), *Verticillium dahliae* (Ioannou et al., 1977) and

Rhizoctonia solani (Ploetz & Mitchell 1985). The damage to sclerotia in soils under high moisture content is related to the inability of the sclerotia of regulating their water content and the interference on their constitutive state of dormancy (Cook & Al-Hamandi, 1986). The low availability of oxygen or increased carbon dioxide levels, as well as other volatile components found in wet or flooded soil, may also produce a negative effect on the sclerotia (Zaki & Ghaffar, 1988). Moreover, Wyllie & Gangopadhyay (1984) verified that the germination and production of microsclerotia of *M. phaseolina* is affected by the concentration of oxygen and carbon dioxide in the soil. The pathogen infected *P. radiata* seedlings maintained at field capacity without expressing symptoms of the disease. The use of asymptomatic plants infected with *M. phaseolina* in afforestation programs can be a potential risk of spreading the pathogen among commercial nurseries and to new plantations of *P. radiata* in Chile.

Thus, at 100% FC, mortality did not happen even under a certain level of root infection (15%). Although *M. phaseolina* root infection is often associated with high levels of water stress and high temperatures (Ghaffar & Erwin, 1969), in our study SMC levels regarded as incapable of causing plant stress were shown to favor seedling mortality by *M. phaseolina*. The observed mortality was associated with the high level of pathogen inoculum in the soil.

A lower mortality (38%) was recorded in the field trial as compared with the greenhouse trial ($\geq 80\%$). These differences can be attributed to the competitive pressure on *M. phaseolina* under natural conditions as compared with its performance in a sterile soil.

In vitro assays have demonstrated that fungi such as *Aspergillus flavus*, *A. fumigatus*, *Penicillium citrinum*, *Rhizobium japonicum* and *T. viride* can inhibit the growth and production of microsclerotia of *M. phaseolina*, and strains of *T. viride* and *A. niger* were reported parasitizing *M. phaseolina* (Pineda & Gonella 1988). Viability of biocontrol of *M. phaseolina* by components of the microbiota have been demonstrated for various crops (Alagarsamy & Sivaprakasam, 1988; Ehteshamul-Haque et al., 1990; De la Cruz & Hubbel, 1974).

For both experiments, the progress of CRR was found to generally fit better in the monomolecular model and in some cases in the Gompertz model. CRR is a monocyclic-type disease, highly dependent on initial inoculum in the soil, and the disease progress rate increases with increasing amounts of initial inoculum. At higher ID in the soil sclerotia are more numerous, increasing the likelihood of infection by the pathogen in early stages of crop development, leading to plant death. At this stage plants are more vulnerable, having fewer roots and a weaker ability to respond to infection. These results established the importance of pathogen inoculum density and soil moisture content in the progress of CRR on *P. radiata* nurseries. Production of bare-root plants of *P. radiata* in nurseries can be affected when levels of inoculum of the pathogen are >50 cfu.g⁻¹ and plants are

under water stress. This may lead to high mortality and production of infected but asymptomatic plants which may die under field conditions in the first year of planting.

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