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Variation in pathogenicity among South African isolates of *Phytophthora cinnamomi*

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Abstract

Phytophthora cinnamomi isolates from South Africa were evaluated for differences in growth rate *in vitro* and levels of pathogenicity towards *Eucalyptus smithii* in the field. Inoculations were conducted in the field in summer and winter in two subsequent years at two locations in South Africa using 59 *P. cinnamomi* isolates. The isolates differed significantly in growth rate *in vitro*, as well as in levels of pathogenicity to *E. smithii* in the field. Growth rate *in vitro* was significantly influenced by interactions with culture age, geographic origin and genetic background as determined using isozymes. Levels of pathogenicity in the field were influenced by season of inoculation and average minimum temperatures at trial sites. The host from which *P. cinnamomi* isolates were originally obtained did not significantly affect levels of pathogenicity in the field. Culture age had a significant negative effect on growth rate *in vitro* and pathogenicity in the field. Significant differences in levels of pathogenicity could be found for different multilocus isozyme genotypes. Geographic origin and mating type of *P. cinnamomi* isolates had no significant effect on levels of pathogenicity in the field. A positive correlation was found between growth rate *in vitro* and levels of pathogenicity in the field. Levels of variation for pathogenicity within A1 mating type isolates were significantly lower than for A2 mating type isolates. Results of this study provide valuable information on selection of *P. cinnamomi* isolates for future resistance/tolerance screening assays of *Eucalyptus* germplasm in South Africa.

Abbreviations: PRS 94, 95 – Piet Retief summer inoculations in 1994 and 1995; PRW 94, 95 – Piet Retief winter inoculations in 1994 and 1995; RMS 94, 95 – Richmond summer inoculations in 1994 and 1995; RMW 94, 95 – Richmond winter inoculations in 1994 and 1995.

Introduction

Phytophthora cinnamomi Rands is an oomycetous, soilborne fungal pathogen of many ornamental and woody plants (Zentmyer, 1980). It is heterothallic with two mating types, A1 and A2. Its geographic center of origin is unknown, although isozyme studies have indicated that *P. cinnamomi* was introduced into South Africa (Linde et al., 1997) and Australia (Old et al., 1988, 1984) based on low levels of gene and genotypic diversity in these locations. As a pathogen of forest trees, it is probably most significant in die-back of jarrah (*Eucalyptus marginata*) in western Australia, where it was first described in 1965 (Podger et al., 1965). It infects mainly roots and feeder roots and has a host range of close to a 1000 different plant species (Zentmyer, 1980). *P. cinnamomi* is also able to induce cankers in various tree species, such as stripe canker in

cinnamon in the tropics (Rands, 1922) and root-collar cankers in *E. marginata* in Australia (Shearer et al., 1981). In South Africa, mortality of *Eucalyptus* spp. planted in commercial forestry areas is predominantly associated with root-collar cankers (Linde et al., 1994).

Commercial forestry is one of the largest sectors of the South African economy. More than 1.5 million ha of land is utilised for plantations of mainly *Pinus*, *Eucalyptus*, and *Acacia* spp. (Van der Zel, 1994). Root diseases of *Eucalyptus* and *Pinus* spp. associated with *Pythium* and *Phytophthora* spp. were most prevalent in nurseries in the late 1970s (Donald and von Broembsen, 1977). Alteration of cultivation procedures and improvement of sanitary control, resulted in a dramatic decrease in root diseases caused by Oomycetes. However, established *Eucalyptus* trees, especially cold tolerant *Eucalyptus* spp. planted in South Africa, are particularly susceptible to *P. cinnamomi*. High levels of susceptibility in *E. fastigata* and *E. fraxinoides*, have made them unsuitable for use in commercial forestry (Wingfield and Knox-Davies, 1980). Another cold tolerant species, *E. smithii*, is also susceptible to *P. cinnamomi* root disease (Wingfield and Kemp, 1994). Mortalities of these *Eucalyptus* spp. are usually restricted to one to two-year-old trees, whereas basal cankers may persist in more mature trees until harvest.

Breeding and selection programmes for *P. cinnamomi* disease-resistant planting stock in South Africa focused mainly on avocados (Botha et al., 1990). The importance of cold tolerant *Eucalyptus* spp. as high quality pulp producers, and a limited number of suitable *Eucalyptus* spp. to plant at high elevation areas, has prompted the South African forestry industry to breed and select for resistance/tolerance to *P. cinnamomi*. Breeding and selection programmes in *E. marginata* (Stukely and Crane, 1994) and *Pinus radiata* (Butcher et al., 1984) carried out in Australia, have shown that resistance is under strong genetic control. However, the long term success of breeding and selection programmes will be partly dependent on variation in pathogenicity of the pathogen population. The aims of the current investigation were to assess variation in levels of pathogenicity for the South African *P. cinnamomi* population, investigate the influence of climate and growth rate *in vitro*, culture age, geographic origin, mating type, and multilocus isozyme genotype background of *P. cinnamomi* isolates on levels of pathogenicity. This information will be important to implement efficient and reliable screening assays towards *P. cinnamomi* in germplasm of *Eucalyptus* in South Africa.

Materials and methods

Isolates

Fifty-nine (39 A2 and 20 A1) South African *P. cinnamomi* isolates were tested for growth rate *in vitro* and pathogenicity to *E. smithii* in the field. Isolates were collected between 1977 and 1993 from the south and southwestern Cape region (collectively referred to as Cape region) and from the northeastern parts of South Africa (collectively referred to as Mpumalanga). Isolates were divided into two subpopulations based on isolation dates. Isolates collected from 1977 to 1986 are referred to as 'old' isolates (32 Cape and eight Mpumalanga isolates) and those collected from 1991 to 1993 as 'new' isolates (10 Cape and nine Mpumalanga isolates). The *P. cinnamomi* isolates from Mpumalanga were collected from various hosts including avocado, *Eucalyptus*, and *Pinus* spp., whereas isolates from the Cape were collected from commercial *Eucalyptus* and *Pinus* spp., native fynbos (unique flora of the southwestern Cape), and native forest tree species. The A1 mating type isolates were predominantly obtained from native flora while A2 mating type isolates were predominantly obtained from commercial crops. Details on isolation, maintenance of cultures, determination of mating type, isozyme analysis, and definition of multilocus isozyme genotypes are provided elsewhere (Linde et al., 1997).

Growth rate in vitro

Blocks (2 mm^3) were cut from margins of 3-day-old *P. cinnamomi* cultures grown on potato dextrose agar (PDA – Difco). Four blocks of each isolate were transferred to a Petri dish containing 20 ml PDA. Three Petri dishes were used for each isolate. Cultures were incubated at 25 °C in the dark for 72 h before taking measurements. Petri dishes were arranged in a complete randomised block design. Growth studies were duplicated once. Colony diameter data were analysed by ANOVA (SAS Institute Inc., 1989) for possible interactions with culture age, mating type, geographic origin, and multilocus isozyme genotype background of isolates. Test statistics were approximately normal distributed. Cape *P. cinnamomi* isolates were also analysed separately by ANOVA to account for the absence of A1 isolates from Mpumalanga.

Assessment of pathogenicity in the field

The level of pathogenicity of each *P. cinnamomi* isolate was assessed through inoculation of established 3-year-old *E. smithii* trees at two localities, namely Piet Retief (Mpumalanga) and Richmond (KwaZulu Natal). These two trial sites were selected because temperatures at Richmond are usually lower than those at Piet Retief. Inoculations were conducted in summer (December–February) and winter (April–June) to determine the existence of seasonal influences on levels of pathogenicity. Apart from obvious temperature differences between winter and summer, temperature differences between trial sites would possibly allow further distinction on levels of pathogenicity. Trials were conducted during 1994 and repeated in 1995. Temperature and rainfall data for the two study sites were obtained from nearby weather stations for the duration of each trial.

P. cinnamomi isolates were grown on PDA for 7 days at 25 °C. Mycelial discs were used to inoculate wounds on the stem of each tree, 1.3 m above the ground, after the bark had been removed with a 10-mm diameter cork borer. In each trial, each isolate was used to inoculate 20 trees in a randomised block design. For control inoculations, twenty trees in each trial were inoculated with a sterile disc of PDA. Wounds were sealed with masking tape to prevent desiccation. Thus 160 trees for each isolate with a total of 9600 trees were inoculated. Lesion lengths in the secondary phloem (Shearer et al., 1987; Tippett et al., 1983) were measured 8 weeks after inoculation. Re-isolations onto a selective medium (Tsao and Guy, 1977) were made from control and inoculated trees.

Data analysis

Stem lesion data was analysed using ANOVA (SAS Institute Inc., 1989). ANOVA for lesion length data in the field was similar to that for growth rate *in vitro* as measured by colony diameter, except that additional interactions with locality and season of inoculation were included as block factors. Least significant differences (LSD) were calculated using the harmonic means of numbers on which each means was based. Test statistics were approximately normally distributed. Controls were excluded from statistical analysis as lesions did not develop in these trees. Because A1 mating type isolates from Mpumalanga were not included in this study, lesion length data for Cape *P. cinnamomi* isolates were also analysed separately to distinguish between levels of pathogenicity of mating type isolates.

Relationships between lesion length and other variables (average maximum and minimum temperatures, and rainfall) were investigated by calculating Pearson's coefficient of correlation (Snedecor and Cochran, 1980). To determine the relationship between the growth rate *in vitro* and level of pathogenicity in the field among *P. cinnamomi* isolates, a natural growth function based on a least square analysis (Patron and Innis, 1972) was used in which: Growth = $A(1 - e^{B\text{Lesionlength(cm)}})$. This is a non-linear relationship where A = the maximum value of the function and B = the parameter that controls the rate at which the function approaches '*A*'. A correlation index (Schumann et al., 1980) was calculated for this non-linear relationship to test the significance of the influence of growth rate *in vitro* and pathogenicity. Bartlett's test for homogeneity of variances (Snedecor and Cochran, 1980) was conducted to test for group variances in heterogeneity of colony diameter and stem lesion data within 'old' and 'new', as well as within A1 and A2 mating type subpopulations of *P. cinnamomi*.

Results

Growth rate in vitro

Variation in growth rate

Significant differences ($P < 0.05$) were observed for growth rate *in vitro* among *P. cinnamomi* isolates with colony diameters for individual isolates ranging from 19.9 to 45.5 mm after 72 h. All main effects [culture age × origin × multilocus isozyme genotype (mating type)] significantly ($P = 0.0001$) affected growth rate (Data not shown).

Pathogenicity in the field

Variation in levels of pathogenicity

Significant differences ($P < 0.05$) in lesion length on *E. smithii* caused by individual *P. cinnamomi* isolates were found with lesion lengths ranging from 5.2 to 27.4 cm. Lesion length data represent lesion lengths on 160 *E. smithii* trees inoculated with each isolate and represent an average of all trials (season × locality). Successful re-isolations of *P. cinnamomi* could be made from inoculated trees.

Influence of locality and climate

Lesion lengths associated with different isolates of *P. cinnamomi* differed significantly ($P = 0.001$) for each locality and season (Figure 1A). Lesion lengths for PRS 94 and 95 were the longest, followed by RMW 94 and 95, PRW 94 and 95, and then RMS 94 and 95. Lesion lengths for the Piet Retief summer inoculations in 1994 were significantly ($P < 0.05$) longer than those at the same site in 1995. Except for the summer inoculations at Piet Retief, lesion lengths from 1994 inoculations did not differ significantly from those of 1995.

Average maximum temperatures (Figure 1B) and average minimum temperatures (Figure 1C) varied considerably for the winter and summer inoculations. Average maximum temperatures in the winter were consistently lower at Richmond trials than at Piet Retief. In summer trials, average maximum temperatures for 1994 Richmond and Piet Retief trials were approximately the same, whereas temperatures were lower at Richmond for the 1995 trial (Figure 2B). Average lesions length and average maximum temperatures at trial sites did not correlate ($r = 0.30$) significantly. Average lesion lengths and average minimum temperature did correlate significantly ($P < 0.05$; $r = 0.42$). Rainfall was higher during summer than winter inoculation trials (Figure 1D), but no significant correlation ($r = 0.23$) between lesion length and rainfall was found.

Influence of culture age

Average lesion lengths of 'old' Cape and Mpumalanga *P. cinnamomi* isolates were significantly smaller ($P < 0.05$) than those for 'new' isolates (Figure 2). Culture age × multilocus isozyme genotype significantly ($P = 0.0002$) affected levels of pathogenicity (Figure 2). Average lesion lengths of 'old' isolates belonging to multilocus isozyme genotypes 1, 6, and 7 were significantly ($P < 0.05$) smaller than those

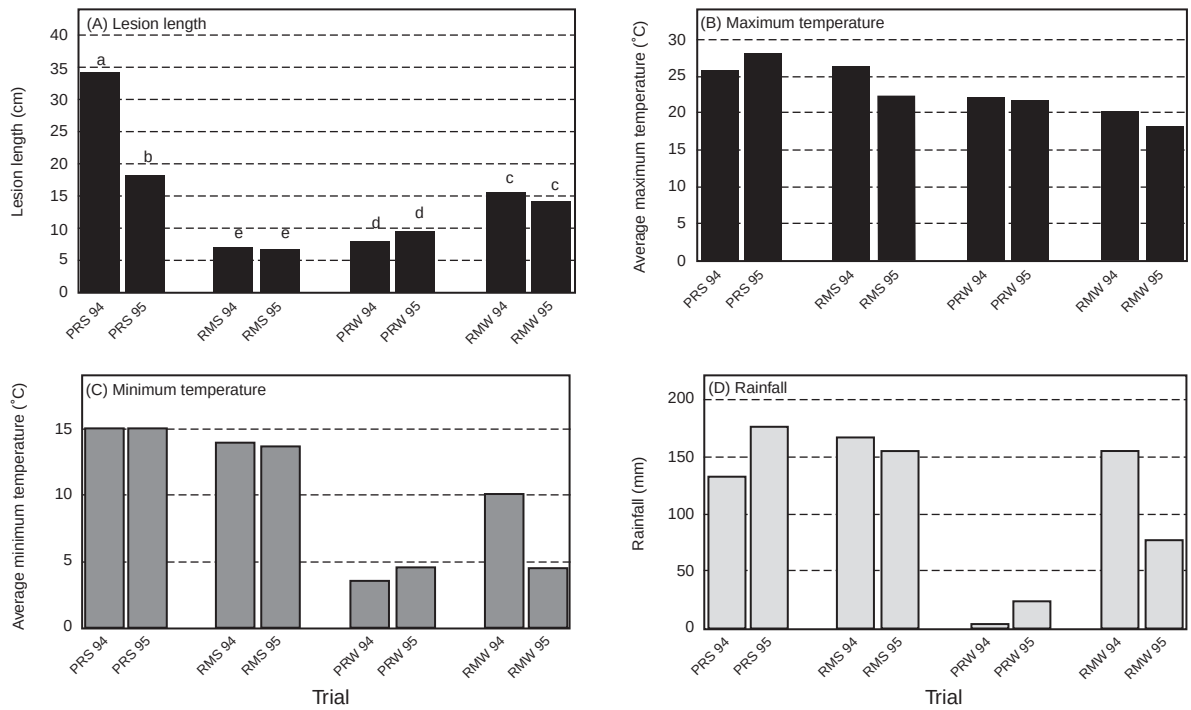

Figure 1. Data for 3-year-old *Eucalyptus smithii* trees inoculated with *Phytophthora cinnamomi* in winter or summer at two localities, Piet Retief and Richmond. (A) Average lesion lengths on *Eucalyptus smithii*, 8 weeks after inoculation with 59 *Phytophthora cinnamomi* isolates. Each bar represents the average lesion length of 1180 trees. Bars topped by the same letter at each trial are not significantly different ($P < 0.05$) as analysed by ANOVA, (B) average maximum temperature recorded for each locality for duration of the trial, (C) average minimum temperature recorded for each locality for duration of the trial, (D) average rainfall recorded at locality for duration of the trial. PRS = Piet Retief summer inoculation, RMS = Richmond summer inoculation, PRW = Piet Retief winter inoculation, RMW = Richmond winter inoculation, 94 and 95 indicate to inoculations conducted during 1994 and 1995 respectively.

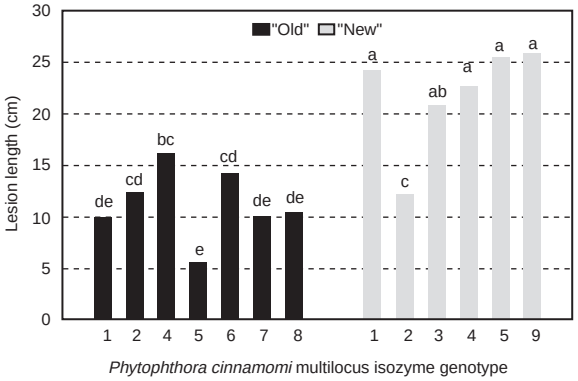

Figure 2. Average lesion lengths on 3-year-old *Eucalyptus smithii* trees inoculated with 59 *Phytophthora cinnamomi* isolates representing different multilocus isozyme genotypes, of 'old' and 'new' isolates. Data represented are averages of lesion length data obtained eight weeks after inoculations at two trial localities during winter and summer. Bars topped by the same letter at each multilocus isozyme genotype are not significantly different ($P < 0.05$) as analysed by ANOVA.

from similar multilocus isozyme genotype's of 'new' isolates. Average lesion lengths associated with multilocus isozyme genotypes 2 and 4 did not differ significantly between 'old' and 'new' isolates (Figure 2).

Influence of mating type

Average lesion length of 'old' A1 mating type isolates (multilocus isozyme genotype 4) was significantly ($P < 0.05$) greater than that of 'old' A2 mating type isolates belonging to multilocus isozyme genotypes 1, 7, and 8, but did not differ significantly from 'old' A2 mating type isolates belonging to multilocus isozyme genotypes 2 and 6. Average lesion length for 'new' A1 mating type isolates (multilocus isozyme genotype 4) was significantly ($P < 0.05$) greater than that of A2 mating type isolates belonging to multilocus isozyme genotype 2, but did not differ significantly from A2 mating type isolates belonging to multilocus isozyme genotypes 1, 6, 7, and 9 (Figure 2).

Influence of geographic origin of isolates

No significant differences in average lesion length could be found when comparing the sets of isolates from Cape and Mpumalanga (Table 1).

Host adaptation

Average lesion lengths on *E. smithii* associated with four isolates from *Eucalyptus* (24.9 cm) and eight isolates from *Ocotea bullata* (23.7 cm) did not differ significantly ($P < 0.05$) from each other.

Table 1. Average lesion lengths on *Eucalyptus smithii* in the field eight weeks after inoculation with 'old' and 'new' *Phytophthora cinnamomi* isolates from the Cape and Mpumalanga

Culture age	Origin	No. of isolates	Lesion length (cm)[a]
'Old'	Cape	32	13.6 b
	Mpumalanga	8	12.6 b
'New'	Cape	10	23.1 a
	Mpumalanga	9	22.3 a

[a]Lesion length is an average of lesion lengths on 160 *Eucalyptus smithii* trees inoculated in summer and winter with *Phytophthora cinnamomi* isolates at two localities, Piet Retief and Richmond. Means followed by different letters were significantly different at $P < 0.05$.

Correlation between growth rate in vitro *on level of pathogenicity in the field*

Significant ($P < 0.001$) correlation ($r_1 = 0.51$) was found between growth rate *in vitro* of isolates and their level of pathogenicity in the field. The relationship between growth rate and level of pathogenicity was non-linear as calculated by a natural growth function and expressed as predicted growth.

Level of variation within 'old' and 'new' isolates of A1 and A2 mating type

Growth rate in vitro

Variances within 'old' and 'new' as well as within A1 and A2 mating type isolates were not significantly different according to Bartlett' s test for homogeneity of variances (Table 2).

Level of pathogenicity in the field

Level of variation within 'old' ($S_i^2 = 19.291$) and 'new' ($S_i^2 = 17.757$) *P. cinnamomi* isolates were not significantly different at 5% level according to Bartlett's test for homogeneity of variances ($\chi^2 = 0.288$; $P = 0.592$). Variances within A1 mating type isolates ($S_i^2 = 17.017$) differed significantly ($P < 0.05$) from that of A2 mating type isolates ($S_i^2 = 25.678$) ($\chi^2 = 4.327$; $P = 0.038$) (Table 2).

Discussion

In this study, significant ($P < 0.05$) differences in *in vitro* growth rate and level of pathogenicity in the

Table 2. Bartlett's test for homogeneity of variances for growth rate *in vitro* and level of pathogenicity in the field within 'old' and 'new', and mating type *Phytophthora cinnamomi* isolates

	P. cinnamomi population	Degrees of freedom	Variance (S_i^2)	χ^2	*P*
Colony diameter *in vitro*	'Old'	80	1.91	2.977	0.315
	'New'	38	1.16		
	A1	40	1.29	1.662	0.197
	A2	78	1.87		
Lesion length in the field	'Old'	273	19.29	0.288	0.592
	'New'	126	17.76		
	A1	133	17.02	4.327[a]	0.038
	A2	266	25.68		

[a]Variances differ significantly at $P < 0.05$.

field to *E. smithii* among 59 South African *P. cinnamomi* isolates have been identified. Growth rate *in vitro* of *P. cinnamomi* isolates was significantly affected by an interaction with culture age, geographic origin, multilocus isozyme genotype, and mating type characteristics of isolates. Level of pathogenicity was negatively affected by culture age which interacted with multilocus isozyme genotype, especially in the 'old' *P. cinnamomi* population. Geographic origin of isolates did not affect levels of pathogenicity in the field. Variation in lesion length within A1 mating type isolates was significantly ($P = 0.038$) lower than in A2 mating type isolates, which is consistent with the low levels of genetic (isozyme) diversity that were observed previously among A1 mating type isolates (Linde et al., 1997).

The results of this study indicate that significant variation in pathogenicity is present among South African *P. cinnamomi* isolates. Dudzinski et al. (1993) also showed significant differences in pathogenicity towards a susceptible *E. marginata* clone among 42 *P. cinnamomi* isolates. These results are in contrast to findings of Podger (1989) who concluded that 14 *P. cinnamomi* isolates tested on five highly susceptible host species, did not differ significantly in pathogenicity. Those isolates were obtained from 10 different species of host plants and from 14 localities across Australia, and represented three of the four multilocus isozyme genotypes of the fungus that are known to occur in Australia (Podger, 1989). Therefore, uniformity in levels of pathogenicity cannot be attributed to isolates having the same genetic background, but may be because levels of pathogenicity were determined on highly susceptible host species. The high susceptibility of test species made it impossible to distinguish between isolates with different levels of pathogenicity. The larger sample sizes used by Dudzinski et al. (1993), and the more extensive experimental approach in the current study provide a finer level of resolution regarding pathogenic variation among *P. cinnamomi* isolates. Significant variation in levels of pathogenicity contrasts with the low levels of genetic diversity in the South African (Linde et al., 1997) and Australian (Old et al., 1988, 1984) *P. cinnamomi* populations as identified using isozymes.

The influence of climate on levels of pathogenicity was inconsistent in this study. Levels of pathogenicity were not always the highest in summer as was found in Australia and France. In those studies, trunk and stem inoculations on *Eucalyptus* spp., *Banksia grandis* (Shearer et al., 1988; Tippett et al., 1989) and red oaks (*Quercus rubra*) (Robin et al., 1994) with *P. cinnamomi*, have shown that pathogenicity as assessed by fungal growth rate in trees, increases in summer. Furthermore, average lesion lengths did not correlate with average maximum temperatures in this study, but rather correlated ($P < 0.05$; $r = 0.42$) with average minimum temperatures at trial sites. Correlation between minimum temperature data and lesion development in red oak trunks, suggested that temperature is most likely the climatological factor that limits disease in red oak forest stands (Robin et al., 1994). However, the effect of minimum temperatures on pathogen growth cannot entirely account for low levels of pathogenicity during winter. This is confirmed by low levels of pathogenicity found in the summer

Richmond trial, compared to winter inoculations at Piet Retief and Richmond. Furthermore, although correlation between average minimum temperature and average lesion length was significant, it was very low and did not entirely explain low levels of pathogenicity in winter.

Results of this study showed no relationship between rainfall and levels of pathogenicity. These results might have been different if the relative water content of phloem was used as a criterion, because phloem water content varies with timing of rainfall (Tippett and Hill, 1983) and site (Tippett and Shea, 1985). It has been shown that relative water content of red oak (Robin et al., 1994) and *E. marginata* (Tippett et al., 1987) bark is related to average length of lesions in excised bark. However, no correlation was observed between the average relative water content and average linear lesion extension in red oak trunks (Robin et al., 1994). It could also not be conclusively shown that relative water content in non-excised *E. marginata* stems is correlated with lesion development (Tippett et al., 1987). This suggests that relative water content also does not explain different levels of pathogenicity found during winter in this study. Various other possible explanations exist such as the physiological and chemical changes in tree cortical tissue during the year (Kramer and Kozlowski, 1960; Srivastava, 1964).

Growth rate of *P. cinnamomi* isolates *in vitro* was positively correlated ($P < 0.001$; $r = 0.51$) with level of pathogenicity in the field. This suggests that fast growing isolates could be selected in the laboratory for utilisation in resistance assays. This might eliminate the need to inoculate trees to determine relative levels of pathogenicity. However, since this was not an absolute relationship, it is suggested that fast growing isolates should be tested in the field to verify their levels of pathogenicity. Those isolates with the highest levels of pathogenicity could then be used in further screening trials. Growth rate *in vitro* was negatively affected by the length of time the isolates were maintained in pure culture. This was observed in some isolates with interactions between culture age, origin, multilocus isozyme genotype, and mating type of isolates, making it difficult to predict which isolates would be fast growing. It is common that fast growing fungal isolates are more pathogenic than slow growing isolates as in *Ophiostoma ulmi* (Brasier and Webber, 1987).

In this study, for most of the multilocus isozyme genotypes, no differences in pathogenicity were detected between the A1 and A2 mating types. In the 'new' *P. cinnamomi* population, lesion length of A1 mating type isolates (multilocus isozyme genotype 4) differed significantly ($P < 0.05$) only from A2 mating type isolates belonging to multilocus isozyme genotype 2. Levels of pathogenicity for A1 and A2 mating type isolates from Australia also did not differ significantly from each other (Dudzinski et al., 1993). Previous reports demonstrating variation in pathogenicity between mating types of *P. cinnamomi* to *Nothofagus* (Weste, 1975), as well as to camellia and avocado (Zentmyer and Guillemet, 1981), included only a limited number of isolates (1 or 2 isolates of each mating type), preventing meaningful statistical analysis. Thus, differences in pathogenicity observed in those studies may represent pathogenic variation among *P. cinnamomi* isolates, as was found in the present and Australian study (Dudzinski et al., 1993) and not a correlation with mating type.

Cape and Mpumalanga regional *P. cinnamomi* isolates did not show significant differences in levels of pathogenicity. This result was not surprising as low levels of genetic differentiation have been identified between these two populations using isozyme analysis (Linde et al., 1997). Isolates may thus be selected without considering geographic origin in South Africa, for future disease resistance/tolerance screening assays.

The relationship between pathogenicity and different multilocus isozyme genotypes in the South African *P. cinnamomi* population is difficult to simplify and shows interaction with culture age. Sample sizes of most of the multilocus isozyme genotypes tested were extremely small because only limited numbers of individuals of some of the multilocus isozyme genotypes were identified in a previous study (Linde et al., 1997). Small sample sizes make statistical analysis unreliable, although significant ($P < 0.05$) differences between multilocus isozyme genotype's were identified in this study. Significant differences for growth rate *in vitro* and pathogenicity in the field was observed among different multilocus isozyme genotypes. Population genetic studies using isozymes have recently shown that sexual reproduction is rare, if not absent, in the south African *P. cinnamomi* population (Linde et al., 1997). The lack of sexual reproduction, giving rise to more or less stable clonal lines over time, provides the simplest explanation for this apparent linkage between multilocus isozyme genotype and pathogenicity.

The level of pathogenicity of South African *P. cinnamomi* isolates that were obtained from *Eucalyptus* spp. (from commercial forest plantations) was comparable with that of isolates from *Ocotea bullata* (an indigenous forest tree species). This suggests that

host adaptation may not have occurred in South Africa. Ideally, these isolates also should have been inoculated onto *O. bullata* to test reciprocal pathogenicity on that host. Host of origin also did not affect levels of pathogenicity towards different *Quercus* hosts (Robin and Desprez-Loustau, 1998). Host specialisation to avocado and camellia, and the presence of strains or 'races' in *P. cinnamomi*, were suggested previously (Zentmyer and Guillemet, 1981). However, only one isolate of each mating type was used preventing any meaningful conclusions to be drawn from that study.

Valuable conclusions can be drawn from this study regarding selection of highly pathogenic *P. cinnamomi* isolates to be used and the implementation of resistance/tolerance screening assays of *Eucalyptus* germplasm in South Africa. For future disease resistance/tolerance screening assays, highly pathogenic *P. cinnamomi* isolates should be used, and these can be partly selected using *in vitro* assays. Geographic origin and mating type of *P. cinnamomi* isolates do not significantly influence pathogenicity, as long as isolates have not been in storage for an extended period. Disease resistance screening assays should be conducted during summer, rather than winter months in order to identify resistance/tolerance more easily.

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