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www.publish.csiro.au/journals/app *Australasian Plant Pathology*, 2003, **32**, 403–409

# *Phytophthora cinnamomi* in native vegetation communities of southern Victoria — morphological variation and paragyny among isolates

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*Abstract.* Morphology has often been used as an indicator of variability within species. The present study investigated morphological and physiological characteristics of isolates of *Phytophthora cinnamomi* collected from diseased vegetation communities at Anglesea, Victoria, and isolates collected from other regions in the State. Characteristics studied included growth rate on potato-dextrose agar (PDA), corn-meal agar and V8-juice agar at 24°C, growth rate on V8 agar at 15°C, colony morphology on PDA, sporangial and gametangial morphology, sporangial production and mating type. Phenotypic variation was demonstrated in radial growth rate, colony morphology and sporangial dimensions. Sporangial and oogonial dimensions and sporangial production were not significantly different between isolates from different geographical regions. All isolates were found to be of the $A_2$ mating type suggesting variation was derived asexually. Paragynal associations, in an organism characteristically defined as amphigynal, were observed following crossing with $A_1$ isolates. This is the first such study undertaken in southern Victoria. The findings highlight the importance of appropriate management of an area of such high conservation value as the Anglesea Heath to contain the current infection and to prevent introduction of new isolates into the area.

*Additional keywords:* amphigynous, dieback, morphology, paragynous, phenotype, variability.

## Introduction

*Phytophthora cinnamomi* has attained prominence as the causal organism of one of the most destructive pandemics of native vegetation ever recorded in Australia (Irwin *et al*. 1995). In Victoria, the pathogen causes dieback and decline in susceptible woodland and heathland communities in south-western, eastern and southern areas of the State, resulting in a decline in floral and faunal diversity, species number and changes in species composition.

*P. cinnamomi* is one of the most easily identified *Phytophthora* species with distinguishing features including coralloid hyphae, prominent hyphal swellings and abundant chlamydospores. The pathogen has been the subject of several morphological studies, primarily as a basis for diagnostics and classification (Gerrettson-Cornell 1989; Stamps *et al.* 1990; Waterhouse 1970) but also to describe phenotypic variation within, and among, pathogen populations (Hüberli *et al.* 2001; López-Herrera and Pérez-Jiménez 1995; Shepherd *et al*. 1974). These studies highlight the scope of variation observable within *P. cinnamomi*. Victorian populations of *P. cinnamomi* have not been investigated in depth, and no studies have examined the diversity in the population in the Anglesea Heath, in southern Victoria, Australia, where disease is severely affecting heathland and open forest communities (Wilson *et al*. 2000).

The Anglesea Heath is the most diverse vegetation community recorded in Victoria (Australian Heritage Commission 1991) with over 620 flora species documented in the area. At least eight of these species are rare or threatened at the national level. The area also provides habitats for 29 mammal species, including the endangered *Pseudomys novaehollandiae* (Parks Victoria and Alcoa World Alumina Australia 2002). Further highlighting its conservation value, the Anglesea Heath is listed on the National Estate Register as having significant ecological values.

A management plan for the Anglesea Heath has been developed (Parks Victoria and Alcoa World Alumina Australia 2002) with one of the main priorities being to control *P. cinnamomi*. Limited knowledge of variation among *P. cinnamomi* isolates in the Anglesea population has consequences for the development of disease management

 10.1071/AP03041 0815-3191/03/030403

strategies, as our understanding of the potential of the pathogen population to change over time is minimal. The current study examined the micro- and macro-morphological characteristics of *P. cinnamomi* isolates from the Anglesea Heath in southern Victoria, with the aim of improving our knowledge of the diversity and structure of the pathogen population in these species-rich vegetation communities. Morphological and physiological traits of these isolates are described and compared with the characteristics of *P. cinnamomi* isolates from additional geographic regions in Victoria to determine the extent of the variation within, and between, populations.

## Methods

*Isolate collection and culture maintenance*

Soil samples for isolation of *P. cinnamomi* were collected from Wilson's Promontory National Park (WPNP), Brisbane Ranges National Park (BRNP), Otway National Park (ONP) and from the principal study site, the Alcoa Lease Area near Anglesea, in Victoria. *P. cinnamomi* was isolated from soil by baiting with *Eucalyptus sieberi* cotyledons (Marks and Kassaby 1974) and subculturing onto PARPH (*Phytophthora* selective medium; Erwin and Ribeiro 1996). The identity of collected isolates was confirmed based on morphological characteristics (Erwin and Ribeiro 1996) and the identification key of Stamps *et al*. (1990). Additional isolates were provided by Dr Ian Smith and Paul Clements at the Forest Science Centre, Heidelberg, Victoria. All isolates were passaged through *E. sieberi* seedlings prior to use in experiments as a preventative measure against potential loss of characteristics and virulence resulting from continuous subculturing and storage (Erwin 1983). Working cultures were maintained on 10% V8 agar medium (Erwin and Ribeiro 1996) at 24°C in the dark.

*Morphological phenotypes*

The following morphological characteristics of *P. cinnamomi* were examined: (i) growth rate, (ii) colony morphology, (iii) sporangial dimensions and numbers, (iv) mating type, (v) oogonial dimensions, and (iv) paragynal associations. Dimensions of reproductive structures were determined directly using a compound microscope (Axioskop, Zeiss Pty Ltd, Victoria, Australia) and were measured using an ocular micrometer.

*Growth rate on nutrient medium*

Growth rates were determined by subculturing isolates onto corn-meal agar (CMA; Sigma Chemical Company, MO, USA), 10% clarified V8 agar (10% CV8; Campbells Soups Australia, Victoria, Australia; Erwin and Ribeiro 1996) and potato-dextrose agar (PDA; Sigma Chemical Company). The cultures were incubated at 24°C in the dark for 3 days. Agar plugs (3 mm × 3 mm) were cut from the edge of 3-day-old colonies and transferred to fresh CMA, 10% CV8 and PDA medium, respectively, to minimise the effect of nutrient medium on growth rate. Three nutrient media plates (90 mm diameter) containing 20 mL of medium were used for each isolate. Plates were sealed with Parafilm and incubated at 24°C in the dark. Colony diameters were measured in two directions, perpendicular to one another, through the centre of the inoculum plug daily for 5 days and the average daily radial growth rate (mm/day) of each isolate calculated. The experiment was repeated twice.

*Colony morphology*

To investigate differences in colony morphology, isolates were grown on PDA at 24°C in the dark for 3 days. A mycelial plug was then taken from the edge of the colony and transferred to fresh PDA medium to minimise effects of the growth medium on colony morphology. Cultures were incubated at 24°C in the dark. After 7 days, colony morphology was recorded as rosaceous, petaloid or undefined based on descriptions given by Erwin and Ribeiro (1996). The experiment was repeated three times.

*Sporangial production*

Sporangia were produced using the method of Byrt and Grant (1979) up to the cold shock incubation, which induces release of zoospores. Briefly, a miracloth disc on 10% CV8 was inoculated with five mycelial plugs (2 mm × 2 mm) taken from the actively growing edge of a *P. cinnamomi* colony and incubated at 24°C in the dark for 5 days. The colonised miracloth was transferred to 100 mL of 5% clarified V8 juice broth (CV8B; Erwin and Ribeiro 1996) and the culture incubated on an orbital shaker (90 rpm) at 24°C in the light for 16 h. The 5% CV8B was then decanted and the miracloth washed three times at 15 min intervals with 10 mL of mineral salts solution (MSS; Chen and Zentmyer 1970). The colonised miracloth was incubated in 100 mL MSS for a further 20 h. It was then rinsed with 50 mL MSS, placed in a sterile Petri dish and covered with 20 mL MSS.

Sporangia were examined microscopically and the length and width of 60 randomly selected sporangia per isolate, per flask, were measured and the average length:width ratio of each sporangium calculated. The experiment was conducted in duplicate.

The number of sporangia produced by each isolate was determined by counting the number of sporangia within the field of view under 200× magnification. Sporangia in five fields of view were counted for each flask, and the average calculated for each isolate. The experiment was repeated three times.

*Mating type*

Mating type was determined by growing each *P. cinnamomi* isolate on 10% CV8 agar medium for 3 days at 24°C in the dark. A mycelial plug (2 mm × 2 mm) was then taken from the actively growing edge of the colony and transferred to the centre of one half of a fresh 10% CV8 agar plate. Two known $A_1$ isolates, H1012 and H1003 (provided by Dr Adrienne Hardham, Australian National University, Canberra, Australia) were cultured under conditions similar to those described above. A mycelial plug of an $A_1$ isolate was placed in the centre of the adjacent half of the plate. Each *P. cinnamomi* isolate was paired with each of the two $A_1$ isolates. The plates were then incubated in the dark at 24°C for 7 days, or until the mycelium from both isolates had grown together. Mating type was determined using a light microscope to observe the presence or absence of oogonia. Where oogonia were present, the isolate was characterised as $A_2$. The absence of oogonia suggested an $A_1$ mating type. The experiment was duplicated.

*Gametangia*

Oogonia and antheridia were produced as described above. The length and width of 100 randomly selected oogonia were measured and the average calculated. The experiment was repeated twice.

*Paragyny*

Following incubation as above, oogonia and antheridia were located on the culture plate and three pieces of agar (5 mm × 5 mm) were cut from the culture and placed onto separate microscope slides. The slides were placed on a hot plate at 60°C and a cover slip was applied with gentle pressure to flatten the warm agar. Three hundred oogonia were counted on each slide for each isolate pairing and the number of paragynal associations recorded. The experiment was conducted in duplicate.

**Table 1. Macro-morphological phenotypes of *Phytophthora cinnamomi* isolates from Victoria categorised by geographical region of isolation**

| Phenotype | Anglesea ($n$ = 29) | BRNP ($n$ = 4) | WPNP ($n$ = 4) | Otway Ranges ($n$ = 7) |
|---|---|---|---|---|
| | *Growth rate mm/day (mean ± SEM)*[A] | | | |
| 10% CV8 at 15°C | 12.1 ± 0.24 a | 12.9 ± 0.31 a | 12.4 ± 0.26 a | 12.5 ± 0.12 a |
| 10% CV8 at 24°C | 15.2 ± 0.33 a | 16.1 ± 0.74 a | 15.1 ± 0.98 a | 16.7 ± 0.74 a |
| PDA at 24°C | 7.3 ± 0.16 a | 7.2 ± 0.26 a | 6.5 ± 0.53 a | 7.1 ± 0.32 a |
| CMA at 24°C | 8.6 ± 0.3 a | 9.7 ± 0.5 a | 9.1 ± 0.3 a | 7.4 ± 0.3 a |
| | *Colony morphology on PDA at 24°C (%)*[B] | | | |
| Undefined pattern | 9.7 a | 25.0 b | 25.0 b | 0.0 c |
| Rosaceous | 51.6 a | 75.0 b | 25.0 bc | 28.6 bd |
| Petaloid | 38.7 a | 0.00 b | 50.0 c | 71.4 a |

[A]Row values followed by the same letter are not significantly different ($P > 0.05$).
[B]Colony type was not always consistent for a given isolate i.e. some isolates demonstrated both rosaceous and petaloid colonies, or undefined colony morphology where no pattern was distinguishable, even though the experiment was repeated under similar conditions. In this case, the most commonly observed pattern was recorded.

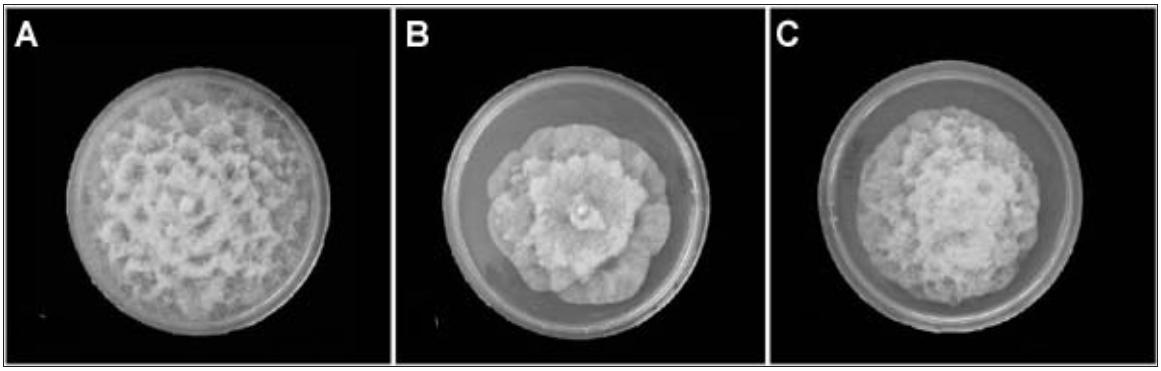


**Fig. 1.** Morphology phenotypes of *Phytophthora cinnamomi* isolates grown on PDA for 7 days at 24°C. (*A*) rosaceous; (*B*) petaloid; (*C*) undefined colony patterns.

*Data analysis*

Data were examined for assumptions of homoscedasticity, normality and outliers. Transformations were performed where required, for use of parametric tests (Dytham 1999; Fowler and Cohen 1990). The arcsine square root transformation was performed on proportion data. One-way ANOVAs and the Least Significant Difference (LSD) test ($P$ = 0.05) were used to test for significant effects. Pearson's Test and the Spearman Rank Test were used to test for correlation between data. Where data could not be transformed to fit a normal distribution, non-parametric tests were used. Data for growth rate on V8 agar at 15°C did not fit the normal distribution and could not be corrected by transformation so they were analysed using the non-parametric Kruskal Wallis test. Analyses were performed using statistics software (SPSS Base 10.0, SPSS Inc., Illinois USA).

## Results

*Colony morphology*

There was no difference in growth rate on any of the defined media between isolates obtained from each of the four regions (Table 1). Colony morphology of the isolates was described as rosaceous, petaloid or undefined (Fig. 1). The 'undefined' classification was given to those isolates that did not display any characteristic pattern as described by Erwin and Ribeiro (1996). For all isolates, the greatest proportion of colony types were rosaceous (56%), followed by petaloid (37.5%) and 16.5% of colony patterns were undefined. There were significant differences between the proportion of isolates that had a particular morphological type and the region from which they were derived; for example, the proportion of isolates with rosaceous colonies was higher in the Anglesea and BRNP group of isolates than in the WPNP and Otway Ranges groups.

*Growth rate on 10% CV8 agar*

Growth rate on 10% CV8 agar was significantly different between isolates ($P$ = 0.003) at 15°C, but not at 24°C ($P$ = 0.973). At 15°C, DU021 from Anglesea was the slowest growing isolate and DU028 from Anglesea the most rapid. At 24°C, the slowest growth rate was displayed by DU042

**Table 2. Micro-morphological phenotypes of *Phytophthora cinnamomi* isolates from Victoria categorised by geographical region of isolation**

| Phenotype | Anglesea ($n$ = 29) | BRNP ($n$ = 4) | WPNP ($n$ = 4) | Otway Ranges ($n$ = 7) |
|---|---|---|---|---|
| | *Sporangia (mean ± SEM)*[A] | | | |
| Sporangial length (μm) | 96.9 ± 0.6 a | 109.0 ± 2.3 b | 100.2 ± 1.7 a | 99.5 ± 1.9 a |
| Sporangial width (μm) | 60.5 ± 0.4 a | 70.5 ± 1.5 b | 61.4 ± 1.1 a | 64.8 ± 1.3 bc |
| Length:width ratio | 1.64 ± 0.02 a | 1.57 ± 0.02 b | 1.65 ± 0.02 a | 1.55 ± 0.02 b |
| Number produced | 15.3 ± 0.8 a | 13.9 ± 2.8 a | 16.1 ± 1.6a | 14.7 ± 1.3 a |
| | *Gametangia (mean ± SEM)* | | | |
| Oogonial diameter (μm) | 39.6 ± 0.1 a | 40.0 ± 0.4 a | 40.1 ± 0.4 a | 38.6 ± 0.5 b |
| Paragynous associations (%) | 0.87 ± 0.06 a | 0.75 ± 0.2 a | 0.72 ± 0.1 a | 1.46 ± 0.24 b |

[A]Row values followed by the same letter are not significantly different ($P > 0.05$).

from Anglesea and the fastest by CFTT89.3 from the Otway Ranges. There was no significant difference in growth rate at either temperature between isolates based on the geographical region from which they were isolated ($P > 0.231$) (Table 1).

*Growth rate on CMA*

Growth rate on CMA at 24°C was significantly different between isolates ($P = 0.012$). Isolate DU042 was the slowest growing isolate on CMA, whereas CFTT91 from the Otway Ranges was the fastest growing isolate. There was no significant difference in growth rate ($P = 0.218$) between isolates based on their geographical region of isolation (Table 1).

*Growth rate on PDA*

Growth rate on PDA at 24°C was significantly different between isolates ($P < 0.001$). The fastest growth rate was displayed by isolate DU028, whereas DU041 from the Otway Ranges had the slowest radial growth rate. Differences in radial growth rate were significant between isolates from Anglesea and WPNP. There was no difference ($P = 0.363$) in growth rates of other isolates based on their geographical region of isolation (Table 1).

*Correlation of growth rates on various media*

Using the Pearson Correlation, growth rate at 24°C on the three media was significantly correlated between PDA and CMA ($P = 0.011$, $r = 0.264$). Correlations between V8 and PDA ($P = 0.392$, $r = –0.096$) and V8 and CMA ($P = 0.976$, $r = –0.003$) were low. Using the Spearman's Rank Test, correlation between growth rates on 10% CV8 at 15°C and 24°C was low ($P = 0.130$, $r = 0.159$).

*Sporangia*

Sporangial dimensions and sporangial numbers were significantly different between all *P. cinnamomi* isolates ($P < 0.001$). Based on geographical region of isolation, sporangial lengths of Anglesea isolates were significantly shorter than those of isolates from the BRNP ($P < 0.001$) (Table 2). No differences ($P > 0.068$) were observed in sporangial lengths between isolates from other populations. Sporangia were significantly wider in isolates from the BRNP and the ONP compared with isolates from other regions ($P < 0.019$) (Table 2). Isolate DU001 from Anglesea produced sporangia with the greatest length:width ratio, whereas DU022 from Anglesea had the smallest sporangial length:width ratio.

The length:width ratio was significantly greater in isolates from Anglesea and WPNP relative to isolates from the BRNP ($P = 0.049$) and the ONP ($P = 0.01$). Based on geographical region of isolation there were no significant differences ($P > 0.151$) in the number of sporangia produced between isolates from the various Victorian locations (Table 2). The greatest number of sporangia were produced by isolate DU007 from the BRNP, whereas isolate DU010 from Anglesea produced the least.

*Mating type*

All *P. cinnamomi* isolates collected from Victoria were of the $A_2$ mating type as gametangia were always formed upon crossing isolates with the $A_1$ isolates.

*Oogonia*

No significant differences were observed in oogonial dimensions between the crossings with each $A_1$ isolate (i.e. H1012 an H1003), so data were grouped and analysed together. Differences between oogonial diameters were significant between isolates ($P < 0.001$) and geographical region of isolation ($P = 0.012$) (Table 2). Isolates from the ONP produced oogonia with significantly smaller oogonial diameters ($P < 0.01$) than isolates from other regions sampled. Oogonia produced by isolate DU017 from Anglesea had the greatest oogonial diameters and the smallest oogonia were produced by isolate DU0039 from the ONP.

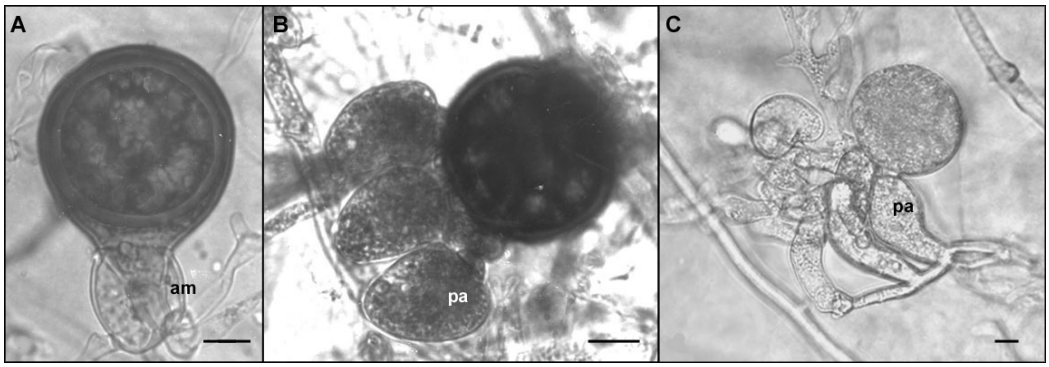


**Fig. 2.** Oogonial associations in *Phytophthora cinnamomi* observed in Anglesea isolates. (*A*) Typical amphigynous association, am = amphigynous antheridium; (*B*) paragynous antheridia, pa = paragynous antheridium; (*C*) paragynous antheridia, pa = paragynous antheridium. Scale bars represent 10 μm.

*Paragyny*

All isolates displayed paragynal associations following crossing with the $A_1$ isolates. Paragyny was observed in less than 3% of gametangial associations. There was no difference ($P > 0.05$) in the proportion of paragynal associations when the alternate $A_1$ isolates were used and data from the two crossings were analysed together. Where present, oogonia were observed to have one or more paragynous antheridia (Fig. 2). Differences in the proportion of paragynal associations were significant ($P = 0.015$) between the ONP isolates and isolates from the other three geographical regions (Table 2). The greatest number of paragynal associations was observed in isolate DU007 from the BRNP and isolate DU029 from Anglesea had the least.

## Discussion

The occurrence of greater variation may enhance the potential for the pathogen to survive adverse conditions, or overcome or resist control measures. Where the level of variation is high, there is the potential for the pathogen to evolve and adapt to its environment as selection pressure creates a situation where the pathogen is forced to change to survive. This study demonstrates the occurrence of morphological variation among Victorian isolates of *P. cinnamomi* in consensus with the morphological plasticity observed in other studies examining variation in *P. cinnamomi* (Hüberli *et al.* 2001; Zentmyer *et al.* 1976). In Australia, the majority of recent research has been conducted on Western Australian isolates. Limited studies, using small numbers of isolates, have also demonstrated phenotypic and physiological variation among *P. cinnamomi* isolates from north-eastern Victoria, New South Wales, South Australia, Queensland and Tasmania (Gerrettson-Cornell 1980; Shepherd and Forrester 1977; Shepherd *et al*. 1974). This is the first study to provide detailed information regarding the phenotypic variation in the pathogen population at Anglesea, Victoria. It should be noted that sample sizes from some populations were small (e.g. the sample sizes from BRNP and WPNP where $n = 4$) and care must be taken when comparing growth rates between populations from different geographical regions.

The majority of isolates displayed rosaceous and petaloid colonies, concurring with previous studies (Hüberli *et al.* 2001; Luo *et al.* 1988; Zentmyer 1980). Instability of colony morphology was reported by Hüberli *et al.* (2001) who observed variation in colony type among isolates following incubation under a range of temperatures. The variability in colony morphology demonstrated by *P. cinnamomi* isolates, even upon replication of the same isolate, highlights the variation among isolates within a population. It also suggests that colony morphology is a relatively unstable characteristic, and care should be taken when using it as a trait for identification of *P. cinnamomi*.

Except on PDA, variation in growth rate was not significant for isolates based on geographical region. This perhaps suggests that isolates originated from a similar source and that growth rate does not provide an evolutionary advantage, and thus has not been selected for over time (Zentmyer *et al.* 1976). The lack of correlation of growth rates between the various media emphasises the influence of medium composition, nutrition and environmental factors on growth rate of *P. cinnamomi*.

In this study, variation in sporangial production was significantly different among Victorian isolates but not between isolates from the various geographical regions. Sporangia arguably form the most important spore-bearing stage in the lifecycle of *P. cinnamomi*, providing the potential for immense inoculum production and infection of hosts following release of zoospores. While Byrt and Grant (1979) suggest that sporangial numbers do not necessarily provide an accurate representation of the number of zoospores produced, Shea *et al*. (1978) found sporangial formation to coincide with high densities of the pathogen in the soil and an increase in infection of susceptible hosts. Thus, the ability of an isolate to produce greater numbers of sporangia may provide it with the potential to release more zoospores.

Sporangial morphology is used as a criterion for identification of *P. cinnamomi*. Sporangial dimensions observed in Victorian isolates of *P. cinnamomi* were within the range (45–75 μm long) described previously (Erwin and Ribeiro 1996; Stamps *et al.* 1990). Although both sporangial lengths and widths were significantly different at the isolate level, dimensions were relatively stable based on isolate geographical region and the only significant differences observed were between the Anglesea and Brisbane Ranges National Park isolates. These results are in accordance with those of Hüberli *et al.* (2001) who concluded that sporangial morphology was relatively stable among *P. cinnamomi* isolates in Western Australia.

Oospores provide the pathogen with a mechanism for long-term survival and a basis for generation of genetic variation (Elliot 1983). The $A_2$ mating type is more common in Australia (Pratt *et al*. 1972), and no $A_1$ mating types were found to occur in the isolates sampled from Victorian populations. This is advantageous for disease management as it indicates that the populations are likely to remain genetically stable over time and there is less potential for the pathogen to evolve and, for example, overcome host resistance or develop tolerance to fungicides. It also accentuates the importance of quarantine measures to prevent future introduction of the pathogen from external sources. Furthermore, the introduction of the second mating type may increase the production of oospores that may remain dormant as resistant structures in the soil or plant material, thus promoting long-term survival of the pathogen in the area (Zentmyer *et al.* 1979). Management strategies need to be implemented in the Anglesea Heath to avoid introduction of external isolates into the area and subsequent generation of genetic variation via sexual reproduction.

Although *P. cinnamomi* is categorised in Group IV of the *Phytophthora* key (Stamps *et al.* 1990) and is typically considered to form amphigynous antheridia during sexual reproduction, paragynal associations have been observed in this study, confirming earlier reports (Hüberli *et al*. 1997). Hüberli *et al.* (2001) suggested that paragyny is widespread in *P. cinnamomi*. Although paragynal associations were observed in all isolates in this study, they occurred in less than 3% of oogonial-antheridial interactions. This is much lower than the proportion of paragyny observed by Hüberli *et al.* (2001), suggesting variability in the incidence of paragyny within the species *P. cinnamomi*, as has been demonstrated in other species (Gao *et al.* 1998; Savage *et al.* 1968). Paragynal associations may be influenced by environmental or physical factors. Gao *et al.* (1998) found antheridial configuration in *P. boehmeriae* to be markedly influenced by the constitution and nutrient concentration of culture media. This is consistent with the different medium used in this study, relative to that used by Hüberli *et al.* (1997) (10% CV8 agar compared with V8 agar). However, Gao *et al.* (1998) suggest that paragynous antheridia are favoured by low nutrient concentrations, conflicting with observations of Hüberli *et al.* (1997, 2001) who observed greater proportions of paragynal associations on V8 agar, relative to those observed here on clarified V8. Clarified V8 has fewer nutrients, including sterols, relative to the standard V8 agar (Englander and Turbitt 1979).

The Anglesea Heath has a broad range of users in addition to the Alcoa mining operations, including recreational four-wheel-driving, motorbike and horse riding, and mountain biking. Such activities have the potential to contribute to the distribution of the pathogen. The listing of the Anglesea Heath on the National Heritage Register emphasised its conservation significance and species diversity. This study provides valuable information for the future management of the Anglesea Heath. An understanding of the extent of variation within species and populations, and how this variation occurs, poses important considerations for disease management. Knowledge of the variation in the pathogen population and the absence of the $A_1$ mating type in the Anglesea Heath emphasises the importance of hygiene and quarantine to prevent the spread of the pathogen into currently uninfested areas, and to avoid the introduction of new genes into the current population.

## Acknowledgements

R. Daniel was the recipient of an Australian Postgraduate Award (Industry) funded by the Australian Research Council in association with Alcoa World Alumina Australia. We thank Dr A. Hardham, Dr I. Smith and P. Clements for contribution of isolates, and E. Thomas for her assistance at WPNP.

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Received 20 November 2002, accepted 17 April 2003