RISKS AND THREATS DUE TO GENETIC VARIATION IN *PHYTOPHTHORA CINNAMOMI* FOR DISEASE MANAGEMENT IN NATURAL VEGETATION ECOSYSTEMS

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SUMMARY

Phytophthora cinnamomi is often genetically diverse in disease fronts and diseased areas. *P. cinnamomi* has considerable ability to produce a wide range of pathogenic phenotypes measured from 'plant clone by isolate by environmental factor interactions' in each of the three pathogen clonal lineages of *P. cinnamomi* isolated in Australia. *P. cinnamomi* lineages are defined by microsatellite types. In a few disease areas, despite both mating types occurring in the same square metre of soil, or 50 g soil sample or bait plant, no evidence of sexual reproduction (genomic recombination) has been found in the field. Isolates are sexually competent in the laboratory and all lineage combinations are strongly outcrossing. Hence the substantial variation in lineages in a range of traits associated with disease development must be arising asexually. Preventing movement of any *P. cinnamomi* contaminated material is critical to maximising disease control and minimising risks of introducing new strains which may threaten plant communities, their diversity and the integrity of these ecosystems. Phosphite or other intended phytophthoracide usage, which does not eliminate *P. cinnamomi* in plant nurseries can, by camouflaging disease, increase potential risks and threats by spreading different strains in diseased materials.

INTRODUCTION

Phytophthora diseases are major problems in wildlands where the pathogen is introduced, or as in the case of the alder *Phytophthora*, it is a hybrid between introduced species (Brasier *et al.* 1999; Hansen 2000). Some other major forest diseases, such as white pine blister rust and *Dothiostroma* disease of *Pinus radiata,* are also the result of recent host jumps. None of these devastating diseases have simple control measures.

Disease caused by *P. cinnamomi* has often been called 'dieback', but it is more aptly termed '*Phytophthora* root and collar rot' (Colquhoun and Hardy 2000). *P. cinnamomi* is a pan global pathogen which has most probably been dispersed by trade and with human migration. Most of its host plant species are non-coevolved associations and the number and diversity of hosts are very large (see e.g. Gerrettson-Cornell 1973; Brown 1976; Zentmeyer 1980; Broembsen 1984; Broembsen and Kruger 1985; Weste and Marks 1987; Podger and Brown 1989; Wills and Keighery 1994). The host range of *P. cinnamomi* for Australian plant species is especially large including many woody perennial species, and some rare and endangered species for which *P. cinnamomi* is a major threat to their continuing existence in their 'native vegetation' (Peters and Weste 1997; Weste 1994; Shearer and Tippett 1989; Wills 1993; Shearer and Dillon 1995). The term 'wildlands' equates to 'natural vegetation' in European usage or 'native vegetation' in Australian usage and as this paper is substantially about impacts of *P. cinnamomi* on Australian vegetation, 'native vegetation' will be used frequently. In south western Australia, it is estimated that approximately 2000 of the 9000 plant species are susceptible (Wills 1993). In plant communities in this region, disease is a major disaster because there are up to 40 susceptible species per ha (Shearer 1990). Rare and endangered animal species are also put at increased risk by *P. cinnamomi* when their habitats have a predominance of susceptible host plants.

Fig. 1. Death of *Banksia* and many understorey shrubs due to a *Phytophthora cinnamomi* diseasefront on either side of a track in a Bassendean sand community

Fig. 2. Spasmodic seedling regeneration of *Eucalyptus marginata* (jarrah), *Banksia* species. and other susceptible understorey species sometimes occurs after *Phytophthora cinnamomi* disease.

Disease impacts are large on susceptible plant communities and some landscapes due to losses of genetic resources, plant community structure and key species which are animal food (Figs 1, 2). As well as high species diversity, genetic diversity within species is high and exists as a mosaic within landscapes of south western Australian *P. cinnamomi* susceptible flora (Hopper 1992; Keighery *et al.* 1994). This may also apply to communities in Tasmania. The consequences of *P. cinnamoni* disease in native ecosystems for biodiversity are far greater than just the losses of plants and larger animals (Wilson *et al.* 1994). For the myriads of small organisms, (fungi, bacteria or insects) associated with shoots, roots and litter, which are the major components of biodiversity, the repercussions are largely unknown. Indications of the possible extent of decline in small organism biodiversity can be gauged by analogy. These include insects associated with individual *Eucalyptus* tree canopies (Majer *et al.* 1999) and the diversity of

ectomycorrhizal associates in the vegetation of 3 ha of *E. marginata* forest, *Eucalyptus* woodlands or heathlands (Bougher and Tommerup 1996; Tommerup and Bougher 1999; Glen *et al.* 2000a, b). *P. cinnamomi* disease centers may have low impact on ground dwelling insects in south eastern Australia, however effects are largely unknown in south western Australia where the disease impacts are often greater than in the south east (Wills 1993; Shearer 1994; Shearer and Dillon 1995, 1996; Newell 1997).

In wildlands, considerable management challenges are raised when *P. cinnamomi* has large impacts on highly diverse communities and threatens ecosystem integrity. It is a soil borne disease and the early development stages in roots and butts are often camouflaged. This increases the challenges for broadscale management over large tracts of land. Effective means of reducing the spread of the pathogen are quarantine, restricting access, including for logging operations, to only dry seasons, and adherence to hygiene with a high level of public cooperation (Brandis and Batini 1985; Shearer and Tippett 1989; Colquhoun, 2000). More recently the strategic application of phosphite has been used to control *P. cinnamomi* disease in some rare and endangered plant communities (Hardy *et al*. 2000). The possibility of introducing naturally resistant lines of some susceptible plant species is being investigated (McComb *et al*. 1994; Colquhoun, 2000). Plant breeding programs selecting for resistance to *P. cinnamomi* should take account of the considerable genetic and pathogenic phenotypes in the pathogen (Tommerup 1995; Tommerup *et al*. 1997; Dobrowolski *et al.* 1998c; Hüberli *et al.* 2000b). Fundamental factors to disease management and control are (i) how much genetic variability is in a pathogen population, (ii) how and when that variability flows through the population and (iii) how it affects the pathogen's capacity to cause disease, survive and reproduce.

We hypothesise that the basis of the adaptive variability of *P. cinnamomi* is that it has a large amount of genetic variation in pathogenesis characters relative to other characters (Tommerup 1995; Tommerup *et al.* 1997). In order to identify genetic diversity and relationships the development of markers is a prerequisite to discriminate amongst strains and make comparisons among local, regional and national isolates of *P. cinnamomi*. Unequivocal markers are also a prerequisite for examining mechanisms of genetic exchange, determining the extent of genetic variation due to asexual and sexual reproduction and providing distinct characters linked to quantitative traits.

GENETIC MARKERS IN *P. CINNAMOMI*

Old *et al.* (1984, 1988) showed that the A1 mating type of *P. cinnamomi* had nine different isozyme types and the A2 had two. Oudemans and Coffey (1991) examined more world-wide isolates and probably found additional variation in the A1 isozyme type and still only two isozyme types for A2. Whether there is additional variation in the South African *P. cinnamomi* isolates can not be discerned as reference isolates were not used (Linde *et al.* 1997).

As an initial class of markers for analysing variation in populations of *P. cinnamomi* in native vegetation we used RAPD–PCR (random amplified polymorphic DNA-polymerase chain reaction). We used 700 arbitrary primers including primers with and without microsatellite sequence anchoring at the 3' or 5' end to analyse variation in 100 isolates from all known Australian and Papua New Guinea and a few Asian and USA isozyme types (Fig. 3; Tommerup 1995). The Papua New Guinea A1 isolates, which are distinctly isozymically different to the Australian A1, (Old *et al.* 1988) had distinctly different RAPD-PCR patterns and they also differed from isolates from other regions. The RAPD–PCR investigations have (i) revealed considerable homology amongst all isozyme types; (ii) shown unique bands consistently associated with each isozyme type and other unique bands associated with mating type; (iii) raised the possibility that some genome regions of A1 isozyme type 1 and A2 isozyme type 2 may be more closely related to each other than they are to A2 isozyme type 1; (iv) revealed bands associated with some pathogenesis factors and supported the hypothesis that pathogenicity factors and isozyme types are not closely linked; (v) demonstrated a substantial genetic variability within each mating and isozyme type in some regions of the genome and indicated that variation due to asexual and/or possibly infrequent sexual mechanisms have occurred; and (vii) provided evidence from 2000 markers suggesting clonal relationships among some isolates within isozyme types in Australia (Tommerup 1995; Fig. 3). However, this study showed RAPD-PCR would most probably not provide sufficient genetic information for fine scale population genetic analysis.

Taiwanese isolates of *P. cinnamomi* examined for genetic diversity with RAPD–PCR DNA appear to have similar patterns of variation and to be genetically limited, although distinction at the isozyme type level was not analyzed. Taiwanese isolates did not cluster into groups corresponding to their mating types, however, bands associated with A1 and A2 mating types were found (Chang *et al.* 1996). Genetic differentiation among isolates from the same location was significantly high between the mating types, indicating that no hybridization occurred between A1 and A2 mating types in that situation. Although genetic differentiation between isolates from avocado and those from other sources was significantly strong indicating that host specified races might occur in *P. cinnamomi* associated with some crop species Chang *et al.* (1996). An unexplored possibility is that particular isozyme types have developed in avocado plantations, probably due to anthropocentric induced founder events, as in Papua New Guinea (Arentz and Simpson 1986; Old *et al.* 1988).

Due to the possibility that isozyme types of *P. cinnamomi* in native vegetation represented clonal lineages (Tommerup 1995), we chose to develop microsatellite markers. Unlike RAPD–PCR markers, they are codominant (i.e. can distinguish heterozygotes from homozygotes), which is important for a diploid organism. Once developed, they are easily used and require minimal material unlike RFLP markers. They are more polymorphic than isozymes and RFLPs and being PCR markers they are efficient to use in large populations (Dobrowolski *et al.* 1997, 1998a).

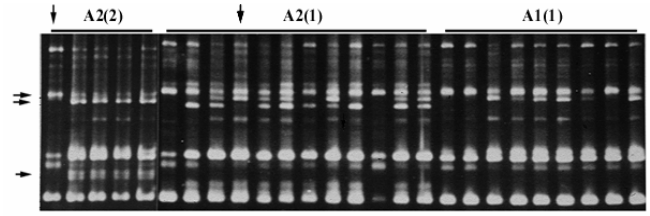

Fig. 3. RAPD–PCR variability within isozyme types of Australian isolates of *P. cinnamomi*. Arrows indicate variable bands (→) within isozyme types and isolates representing distinct patterns (↓) (after Tommerup 1995).

SEXUAL RECOMBINATION IN OOSPORES.

Old *et al.* (1984, 1988) found both mating types of *P. cinnamomi* in southern and eastern Australia and they co-occurred in the same patches of native vegetation. Indeed they were found in the same 50 g soil sample. Their isozyme analysis showed no evidence of sexual recombination in isolates from agricultural or native ecosystems. However, isolates of these isozyme types are sexually competent (Dobrowolski *et al.* 1997, 1998b). Dobrowolski *et al.* (1997, 1998b) studied the inheritance of microsatellite markers and demonstrated unequivocally for the first time that genetic exchange

and recombination occurred in single oospore progeny. They used progeny (Fig. 4) from four controlled crosses of the A1 isozyme type 1 with two isolates of the A2 type 1 and two of A2 type 2 produced and germinated axenically (Tommerup and Catchpole 1997).

Analysis of sexual progeny also allowed them to test if the markers were inherited in a Mendelian fashion, prior to analysing field populations. The majority of progeny inherited alleles at the microsatellite loci in a non-Mendelian manner, that is, showed aberrant sexual recombination sometimes at all loci (Fig. 5). In *Phytophthora infestans* genetic markers linked to mating type were prone to duplication, transposition, deletion and other aberrant sexual recombination (Judelson 1996). In their study, mating type was not linked to any microsatellite locus so it is unlikely to explain the aberrant sexual recombination (Dobrowolski *et al.* 1998b).

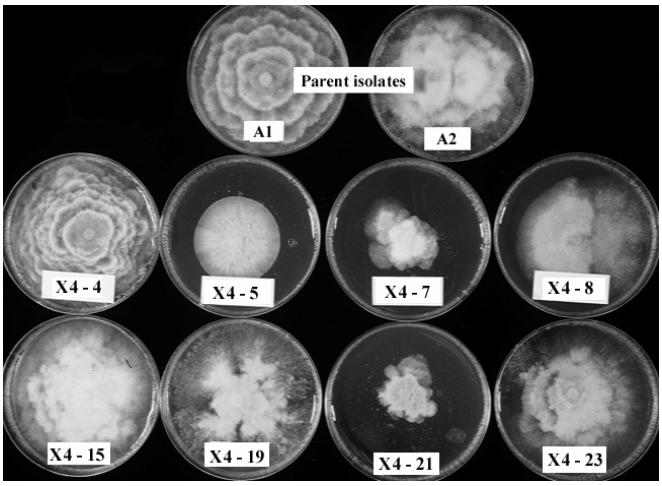

Fig. 4. Phenotype variation in growth of eight single oospore progeny of a pair of A1 and A2 parent isolates of *Phytophthora cinnamomi* (after Tommerup and Catchpole 1997).

All 200 progeny analyzed in detail were outcrossed as determined by the inheritance of alleles at the microsatellite loci tested (Dobrowolski *et al.* 1998b). High levels of heterozygosity at these loci in the parents and progeny show that *P. cinnamomi* is basically diploid and this supports the cytological evidence of Brasier and Sansome (1975) and Sansome (1980). Dobrowolski *et al.* (1998b) concluded that the high level of aberrant sexual recombination in this diploid pathogen could be explained by a high frequency of imperfect meiosis (e.g. nondisjunction, unequal crossing over) leading to additions and deletions in the chromosome complement of the sexually derived progeny.

Dobrowolski *et al.* (1998a) also developed microsatellite mitochondrial markers for *P. cinnamomi* to determine if and how they vary in Australian and non-Australian isolates. For the loci examined, the Australian isolates were uniform for all mating and isozyme types. Interestingly, all the A2 isozyme type 1 and type 2 isolates from throughout the world had no variation in the microsatellite loci. The only variation found was in A1 isolates from Japan and Papua New Guinea. Because the loci are uniform in the Australian populations they are not valuable for analysis of mitochondrial inheritance in sexual progeny of the three isozyme types. They obviously may be valuable for analyzing recombination amongst pairs of isozyme types differing at these loci.

The potential exists for oospore production in the wild. This could be due to interaction between A1 and an A2 mating types, or selfing including for the *Trichoderma* effect, or by intraspecific interactions (Brasier 1971; Shepherd 1978; Zentmeyer 1980; Old *et al.* 1984; Chambers *et al.* 1995). Lack of evidence for sexual reproduction in the field (Old *et al.*1984, 1988; Dobrowolski *et al.* 1998c) may not necessarily indicate its failure to ever occur. The progeny may not persist because they are may be less ecologically fit than their parents or unable to compete with the vast numbers of propagules of the parent types and not persist (Dobrowolski *et al.* 1998c, 2000).

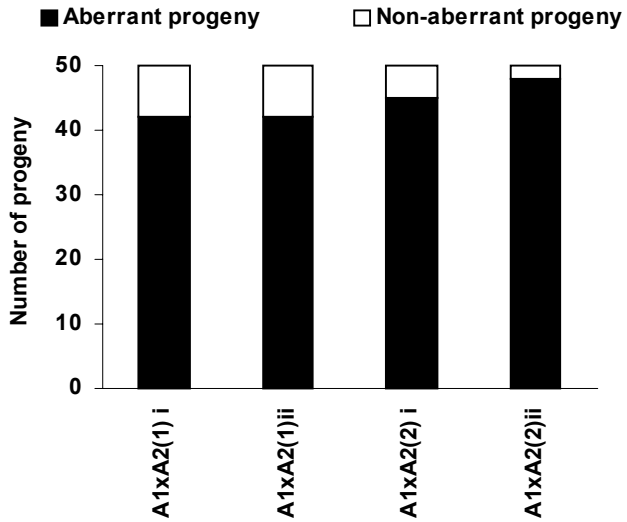

Fig. 5. Histogram of numbers of progeny of *Phytophthora cinnamomi* from each cross having aberrant and non-aberrant inheritance of alleles at the microsatellite loci tested (after Dobrowolski *et al*. 1998b).

ASEXUAL VARIATION: A PREDOMINANT MECHANISM OF CHANGE IN *P. CINNAMOMI* IN WILD POPULATIONS?

Two factors contributed to our hypothesis that considerable variation in Australian populations of *P. cinnamomi* may be asexually derived: (i) the pattern of variation in RAPD–PCR seen amongst isozyme types; and (ii) the lack of sexual reproduction in wild populations (Dudzinski *et al.* 1993; Old *et al.* 1984, 1988; Tommerup 1995; Dobrowolski *et al.* 1999). Morphological variation in isozyme lineages is continuous e.g. colony morphology under defined conditions, asexual and sexual reproductive structures (Hüberli *et al.* 1997a, b, 2000b). We have not found any of these phenotypic characters to be sufficiently distinct for genetic analysis based on studies of either field populations in Australia or isolates from most of the isozyme types identified world-wide, or of 200 single spore progeny of crosses from all Australian isozyme types. Clearly genetic analysis of field populations was needed to test our hypothesis that variation is derived asexually. The microsatellite markers developed on sexual progeny could be used to distinguish clonal lineages (Dobrowolski *et al.* 1999, 2000)

Genetic diversity of *P. cinnamomi* in disease fronts of native vegetation

To survey the genetic structure of *P. cinnamomi* in disease fronts of south western Australia and assess the potential for sexual reproduction Dobrowolski *et al.* (1998c, 1999) hierarchically sampled three disease fronts. Tissue (bark or lesion in wood) and adjacent soil samples were taken from diseased plants; up to 100 per disease front. *P. cinnamomi* was isolated by direct plating of tissue on selective agar and baiting of flooded soil samples with *Eucalyptus sieberi* cotyledons. Mating type was determined by

pairing with known A1 and A2 tester isolates. DNA extractions of 640 isolates were analyzed with four microsatellite loci.

The isolates grouped into three clonal types as indicated by their multilocus microsatellite genotypes, two of A2 and one of A1 mating type. One clonal type was common to all three disease fronts, and the other two were found only at site three. Because no recombinants were found, Dobrowolski *et al.* (1998c) concluded that any variation was asexual in origin, despite recovering an A1 isolate from tissue and an A2 isolate from the adjacent soil sample on one occasion, and A1 and A2 isolates were obtained from soil samples within 1m proximity. A size change in one allele (but no recombination) distinguished the site one isolates from site two and some site three isolates. With few exceptions, our hierarchical sampling showed that multiple isolates from a single tissue or soil sample were identical. No association was evident between host species and *P. cinnamomi* clonal type at any disease front.

Dobrowolski *et al.* (1998c) concluded that founder effects gave rise to the genetic diversity of *P. cinnamomi* in the three disease fronts. Sites one and two were the result of single introductions of *P. cinnamomi* which are clonaly related but asexually divergent. The disease front at site three was caused by the introduction of three clonal types. Although the potential for sexual reproduction exists in disease fronts, we found no evidence for it. However, we have found genetic variation within clonal types (Dobrowolski *et al.* 2000). The significance of this to management in wildlands is that every new introduction of the pathogen has the potential to be genetically different and with new capacities to cause disease.

PATHOGENESIS: A QUANTATIVE TRAIT

Dudzinski *et al.* (1993) unequivocally showed pathogenesis varied among isolates and that the range in phenotypic diversity in pathogenesis was unrelated to mating or to both A2 isozyme types and an A1 isozyme type. They found a greater variation among the A2 isozyme types than the A1 type. This confirmed earlier research indicating variation in pathogenesis among isolates (Zentmeyer 1980; Shearer *et al.* 1988) and it has been reconfirmed in Australia, France and South Africa (Hüberli *et al.* 1998, 2000b; Robin and Desprez-Loustau 1998; Linde *et al.* 1999). Variation in pathogenesis is broad and continuous within mating types and within isozyme types indicating that the trait is quantitative (Fig. 6) (Dudzinski *et al.* 1993; Tommerup *et al.* 1997; Tommerup 1998; Hüberli *et al.* 2000a,b).

A series of disease associated phenotypes have been described involving variation in isolate pathogenesis, physiology and inoculum production. Presently, no trait has been shown to be unequivocally a genetic one and no genetic marker has been unequivocally associated with any phenotype variation. *P. cinnamomi* isolate phenotypes vary from a biotroph to an aggressive necrotroph (Hüberli *et al.* 2000a, b). This has now been shown for 120 isolates in 1-5 different clones of *E. marginata* in field experiments with 5-7 year old trees, controlled environment and glasshouse experiments (Dudzinski *et al.* 1993; Hüberli *et al.* 2000a,b). Whether the variation is arising by episodic or progressive evolution is as yet unknown. Host variation impacts on disease expression so that some isolates in host clonal lines or seedlings produce a 'biotrophic' response with large areas of colonized tissue with no macroscopically visible lesions (O'Gara *et al.* 1997; Hüberli *et al.* 2000a). This behavior, if it occurrs in a wide range of species, is significant to recognising disease in wildlands and to quarantine procedures where accurate disease assessment and disease free certification are critically important.

Environment-by-pathogen isolate combinations produce variable interactions which are part of the total complexity. Large shifts in the behavior of isolates due to interactions changing with environmental factors can pose difficulties for diagnosing disease levels because the host response may vary from nil to a large disease expression. That individual isolates from a population have relatively large variation in disease expression in *E. marginata* clones under standardized conditions means that there are likely to be concomitantly large interactions between environment and disease expression in the forests. Which environmental factors are most influential is more difficult to define in native ecosystems than in controlled environment interactions.

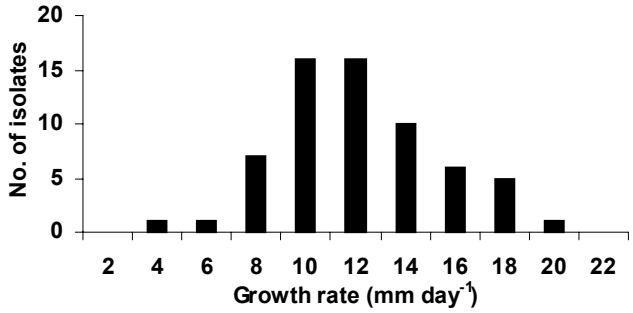

Fig. 6. Pathogenesis phenotypes of *Phytophthora cinnamomi* A2 mating types isolates from the northern *Eucalyptus marginata* forest grow at up to five-fold different rates in *E. marginata* (after Hüberli *et al.* 2000b).

Inheritance of resistance to *P. cinnamomi* is also probably quantitative (Tommerup *et al.* 1997). It has long been recognized that species of *Eucalyptus* vary in their resistance to *P. cinnamomi* (Marks *et al.* 1972). Variation in resistance of half sib families of *Eucalyptus regnans* and *E. marginata* is continuous (Harris *et al.* 1983; Harris *et al.* 1985; McComb *et al.*1990; Stukely and Crane 1994). Major differences among species in susceptibility to *P. cinnamomi* occur in many genera including *Eucalyptus*, *Banksia* and *Acaia* and between codominant woody species in plant communities (Marks *et al.* 1981; McCredie *et al.* 1985; Tippett *et al.* 1985; Wardlaw and Palzer 1988; Noble 1989).

Defining resistance-susceptibility responses in clonal *E. marginata* lines is strongly dependent on environmental factors and pathogenesis-by-environment interactions. Host water availability and temperature are two factors for which interaction effects have been defined to some extent. Temperature changed resistance of clonal *E. marginata,* seedling *E. marginata* and seedling *E. calophylla*, and the pathogenesis phenotypes of several *P. cinnamomi* isolates (Grant and Byrt 1984; Hüberli *et al.* 1998). One-year old *E. marginata* clones selected as resistant or susceptible at 20º C using one isolate were (i) equally susceptible at 25-30º and at 15º C and, (ii) for clone 2 susceptible not resistant at 20º C when testd against another isolate (Fig. 7) (Hüberli *et al.* 1998). Lesion size in roots of *E. marginata* in the forest is associated with temperature (Shearer *et al.* 1987a,b; Tippett *et al.* 1983). Cold soils were unfavorable to inoculum build up and to disease development by *P. cinnamomi* in susceptible tree species, and disease was markedly reduced below 15º C (Fagg *et al.* 1973).

Many different forest and laboratory studies involving inoculation of moderately resistant *Eucalyptus* species stems and roots have shown that high bark and phloem moisture favors lesion development by *P. cinnamomi* (Tippett and Hill 1983; Smith and Marks 1985, 1986; Tippett *et al.* 1987; Bunny *et al.* 1995) Also viability of *P. cinnamomi* in 10-week-old and 1-year-old lesions was decreased as tree water deficits increased (Bunny *et al.* 1995). Impeded drainage and any form of water-logging can exacerbate predisposition of susceptible tree and shrub species to *P. cinnamomi* and increase *Phytophthora* root and collar rot disease hazard rating for sites (Christensen 1975; Marks *et al.* 1975; Shea *et al.* 1983; Colquhoun 2000; Old and Dudzinski 2000). Management of soil moisture and soil temperature have implications for land hygiene measures during logging and clearing

operations (Kassaby *et al.* 1977). Under a closed canopy, low soil temperatures resulted in poor establishment of *P. cinnamomi* and low disease expression.

Increased disease in resistant hosts at higher temperatures and high phloem and bark moisture has large implications for disease control with global warming, especially if summer rainfall increases. Disease management strategies in wildlands may need to be revised based on long term predictions.

Undefined soil factors have an effect on disease development in native vegetation (Marks and Smith 1983). Susceptible host species in some soil types very rarely have disease, as in the Quindulup or Spearwood dune systems of the Swan coastal plain (west coast of S. W. Western Australia) where no disease centres were found. *Banksia attenuata* was apparently disease free yet on the adjacent Bassendean dune system 60% of *B. attenuata* was devastated by disease (Hill *et al.* 1994; Shearer and Dillon 1996). The reasons for the low disease impact are currently unknown and they are clearly important to disease management.

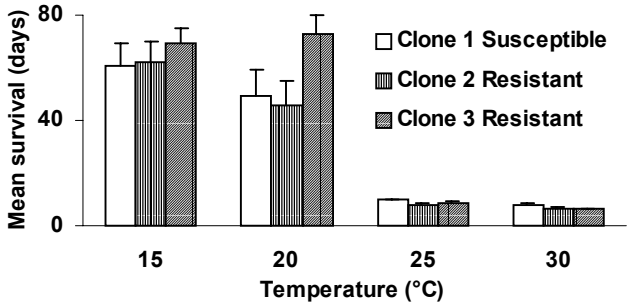

Fig. 7. Temperature changes resistance of susceptible and resistant *E. marginata* clones to *P. cinnmamomi* (after Hüberli *et al.* 1998).

CONTROL OF *P. CINNAMOMI* IN LONG LIVED WOODY PLANT SPECIES

Disease control and rehabilitation pose considerable challenges in wildlands where disease impact is greatest such as in Bassendean dune systems or Tasmanian or Victorian heath communities. Disease alters ecosystem functions and the pathogen persists for several years after plants die (Hill *et al.* 1994, 1995). *P. cinnamomi* will probably not be eliminated from diseased wildlands. Resistant hosts and strategic use of chemicals may reduce its impacts.

Breeding and selecting for resistance

Resistance selection has been successful in several species including *E. marginata*, *E. regnans*, *Castanea sativa* and *Pinus echinata* (Ruehle *et al.* 1984; Harris *et al.* 1985; Salesses *et al.* 1993; Colquhoun 2000). Breeding and selecting for resistance may be more durable if screening stages take account of *P. cinnamomi* pathogenesis variation (Tommerup *et al.* 1997; Tommerup 1998). The cryptic nature of lesion development by some isolates, even in pathogen clonal lineages, and the host-pathogen interaction variation induced by some environmental conditions are a particular challenge to experimental design (Dudzinski *et al.* 1993; Dobrowolski *et al.* 1998c, 1999; Hüberli *et al.* 2000a,b). If resistance is sufficient to enable reseeding and regeneration then not only will rehabilitation be improved but the possibility of natural selection for enhanced resistance by geneflow through the community be enhanced. Geneflow in disjunct and widely separated populations of *Eucalyptus* species may have occurred for up to a maximum of 50 km, however, in forests it may occurr over greater distances (Sampson *et al*. 1995 Adams 1997). Future tree generations may evolve sufficient resistance to not only complete a life cycle but to perpetuate. Only then will it be known if resistance is durable. Development of mature plants of dominant species is important to ecosystem rehabilitation.

Natural regeneration of some susceptible plant species, following a major decline in the population due to *P. cinnamomi*, has occurred in open forest, woodland and heathland of the *E. marginata* forest in Western Australia and the Grampians, Victoria, Australia. Whether the host resistance in reseeding species has increased or whether it is disease escape is not known (Weste and Kennedy 1997; McDougall 1998; Weste *et al*.1999). As for outplanting of deliberately selected resistant individuals, the potential for naturally evolving resistance offers hope of increasing the diversity of indigenous species in disease sites. Rehabilitation of plant community structure has benefits which flow on to other biodiversity that depend on the plants for survival. Self reproducing and sustaining communities offer the greatest long-term protection of genetic diversity within species.

Chemical control

Phosphite (phosphonate) has been successfully used to control *P. cinnamomi* in *E. marginata* and *Banksia* species in forests and woodlands (Komorek *et al*. 1997; Shearer and Fairman 1997a,b). Recent research with other native vegetation species in different plant commuities has indicated two emerging complexities. Firstly the chemical may need to be applied every 1-3 years, as a spray treatment, to control the pathogen in some species on some sites rather than only every four or more years as found for injection of *E. marginata* and *Banksia* species (Komorek *et al*. 1997; Shearer and Fairman 1997a,b;Wilkinson *et al*. 1999a, 2000; Hardy 2000). Secondly, evidence of *P. cinnamomi* resistance to phosphite treated plants is increasing among isolates from native vegetation which has not been exposed previously to phosphite (Wilkinson *et al*. 1999b; Hüberli *et al.* 2000b) and among isolates from phosphite treated orchards (Duvenhage 1994). Phosphite does not kill *P. cinnamomi* in soil and does not always kill the pathogen in plants (Ali and Guest 1998). Phosphite or intended phytophthoracide usage which does not eliminate *P. cinnamomi* in plant nurseries can, by camouflaging disease, increase potential risks and threats to native plant communities. These increased risks and threats are caused by spreading *P. cinnamomi*, having different pathogenic phenotypes or strains with other ecological adaptations, in diseased materials. Should phosphite and any phytophthoricide which does not kill the *P. cinnamomi* be banned from use in nurseries unless there is adequate testing to demonstrate that the pathogen has been eliminated? Or should those chemicals be banned from plant nurseries if they allow *P. cinnamomi* to evolve strains which are more phosphite resistant?

Disease prevention and minimising its spread

Rapid dispersal of *P. cinnamomi* occurs when infested soil is carried from infested to uninfested sites. Hygiene management can reduce spread of *P. cinnamomi* (Batini and Cameron 1971; Kassaby *et al.* 1977; Colquhoun 2000). Any new introductions of the pathogen have the potential to introduce new genetic variation (Dobrowolski *et al.* 2000) including new pathogenicity types and increase disease impact on remaining or regenerating vegetation.

Quarantine

In wildlands at the local, regional, national and international scale quarantine deals with similar issues, namely minimizing risks of introducing new *P. cinnamomi* strains. That leads to reduced threats to plant communities, their diversity and the integrity of their ecosystems.

Lack of evident lesions due to biotrophy or phosphite treatment are a threat to quarantine and disease free certification. These threats could be minimised in the plant nursery trade but may be

less tractable to management in wildlands (Huberli *et al.* 2000a). Visual inspection as the main criterium for assessment as 'disease-free' is inadequate for resistant and susceptible plant species. Testing for disease-free certification needs to be augmented by molecular probe methods with or without baiting and plating (Dobrowolski and O'Brien 1994; Brasier *et al.* 1999; Cooke *et al.*2000; Hüberli *et al.* 2000a). Diagnostics are virtually available for routine use to detect importation of these pathogens. Diagnostics to detect current pathogens and new pathogens introduced by global trade are available in research laboratories. They have enabled e.g. detection of hybridization between *Phytophthora* species and the resulting disease in trees which previously had no known *Phytophthora* disease (Brasier *et al.* 1999).

Scale of the management problem

The cost of disease caused by *P. cinnamomi* in native vegetation or wildlands is probably in the billions. It has colonized a vast range of woody plants; in a wide range of ecosystems world-wide (Zentmyer 1980; Hansen 2000; Old and Dudzinski 2000). It threatens the survival of biodiversity in large areas of land and its control is difficult. There are economic costs adding to others due to its impact on ecosystems such as degradation and erosion. Forest, nature conservation, tourism and wild honey harvesting are other examples of industries negatively impacted upon by the pathogen.

Opportunities for *P. cinnamomi* to vary in Australian native vegetation, and probably other wildlands, are abundant. The pathogen has a mosaic distribution in forests, woodlands and woody heaths, all of which are very genetically diverse. These host communities inhabit a range of soils and climates. Host resistance in some *Eucalyptus* species, interacts with climate, such as in drought induced resistance, and possibly soil nutrition and may lead to evolution in *P. cinnamomi*. We have shown the pathogen produces vast numbers of zoospores in disease fronts under conducive conditions e.g. at least one per 40-200 µm sand grain (Tommerup and Dobrowolski pers. comm.). This creates a huge potential for fostering rare pathogen variation in host-environment circumstances new to the pathogen. Has *P. cinnamomi* evolved with new hosts, and in new environments? If it has what are the implications for disease management? We have developed a suite of microsatellite tools to test these hypotheses and are in the process of developing additional suites of them to begin mapping the *P. cinnamomi* genome and linking pathogenicity phenotypes and chemical resistance traits to markers.

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