STUDIES ON MYRTLE WILT

by

Jillian Mary Packham, B. Sc. Hons., (U. C. N. W., Bangor)

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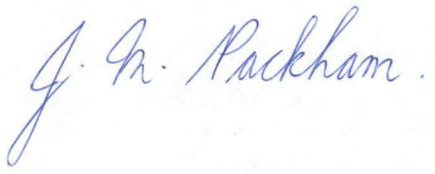
This thesis is dedicated to the memory of my father,
the late Brian James Packham B. Sc.,
who instilled in me a great love of natural history.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text of the thesis.



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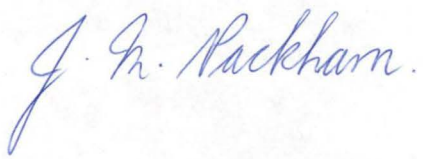

J. M. Packham

ABSTRACT

Nothofagus cunninghamii, or myrtle, is the dominant tree species in many Tasmanian and Victorian cool temperate rainforest communities. The main cause of myrtle death in undisturbed stands is the disease myrtle wilt, which is caused by the pathogenic hyphomycete *Chalara australis.* Early literature, aerial surveys and aerial photography indicated that myrtle wilt was endemic in at least part (and possibly most) of the range of myrtle, but that in some areas, disease levels may have increased in the recent past.

In Tasmania, measured mortality rates were found to be variable but not escalating, with no apparent overall trend. A new estimate of annual mortality due to myrtle wilt was calculated to be 0.61% p.a. Logging, thinning and roading of myrtle-dominated rainforest led to increased myrtle wilt incidence. For some disturbed areas, there was evidence that after an average of nine years, elevated myrtle wilt mortality levels declined to background levels. The spread of myrtle wilt into areas adjacent to disturbances was clearly detectable up to 180 m from the disturbance, although not all sites were affected.

Small, experimental stem wounds on myrtle saplings provided suitable infection courts for *C. australis* spores, with most infections occurring within 14 days. Functional root grafts commonly occurred in young myrtles, and experimental inoculation, root excavation and sectioning strongly indicated underground, tree to tree transfer of *C. australis.* Re-isolations of *C. australis* were made from these trees, and characterisation of these isolates verified spread via root grafts. Root grafting probably predisposes stands to epidemics, plays a major role in the spread of myrtle wilt, and causes the clumped pattern of infected trees. Clumping occurred on a scale of 2.5-14 m and gave rise to patches of dead and diseased myrtles, often resulting in large gaps in the forest canopy.

Floristic studies showed that there was no distinct set of species which characterised myrtle wilt gaps, but that there were generally more myrtle seedlings than in control forest. Data from one site suggested that the vegetation composition in large, old gaps was reverting to that of the surrounding forest, and that myrtle was self replacing in such gaps. The probable long-term effects of myrtle wilt on Tasmanian myrtles were investigated using a simple population model.

In summary, if current levels of myrtle wilt continue, it is unlikely that the disease will lead to any permanent change in forest structure. In undisturbed forest, myrtle wilt acts primarily as a mechanism facilitating stand rejuvenation.

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CONTENTS

1. INTRODUCTION

AN INTRODUCTION TO MYRTLE WILT

Nothofagus cunninghamii (Hook.) Oerst. or myrtle, is the dominant tree species in many of the Tasmanian and Victorian cool temperate rainforest communities. The main cause of myrtle death in undisturbed Tasmanian stands is the disease myrtle wilt (Elliott *et al.* 1987).

The phenomenon of myrtle wilt, or myrtle dieback as it was initially called, was first noted by Howard (1973a) in both disturbed and undisturbed forests in north-west Tasmania. The deaths of large groups of mature myrtles were associated with accelerated attack by an ambrosia beetle, thought to be a vector of a fungal pathogen. The beetle was identified as *Platypus subgranosus* Schedl, the mountain pinhole borer (E. C. Zimmerman, unpublished data).

The pathogen was found to be a previously unrecorded hyphomycete, *Chalara australis* Walker and Kile. A vascular stain disease rather than a true vascular wilt, *C. australis* produces dark brown radial streaks in the wood of infected myrtles. Symptoms are wilting, followed by leaf death; the dead leaves being retained on the trees, giving them an orange/brown appearance (Kile and Walker 1987; Kile *et al.* 1989).

Kile and Hall (1988) showed that *C. australis* was not dependent on *P. subgranosus* for its spread or entry into trees, and that infection actually occurred prior to attack by these beetles. Wounding of trees was shown to produce a direct infection site for *C. australis* (Kile and Walker 1987). The tunnels and frass of *P. subgranosus* were found to be a good early indicator of the disease, but not its cause (Kile and Hall 1988).

In Tasmania, extensive areas of myrtle-dominated rainforest are in conservation reserves (Kirkpatrick 1991), and myrtle is used in the furniture, craftwood and paper industries. In Victoria, myrtle is largely confined to drainage lines which are particularly vulnerable to disturbance (Read 1991). Myrtle wilt is therefore a potentially serious threat, both to conservation of the species and to management of stands for timber production.

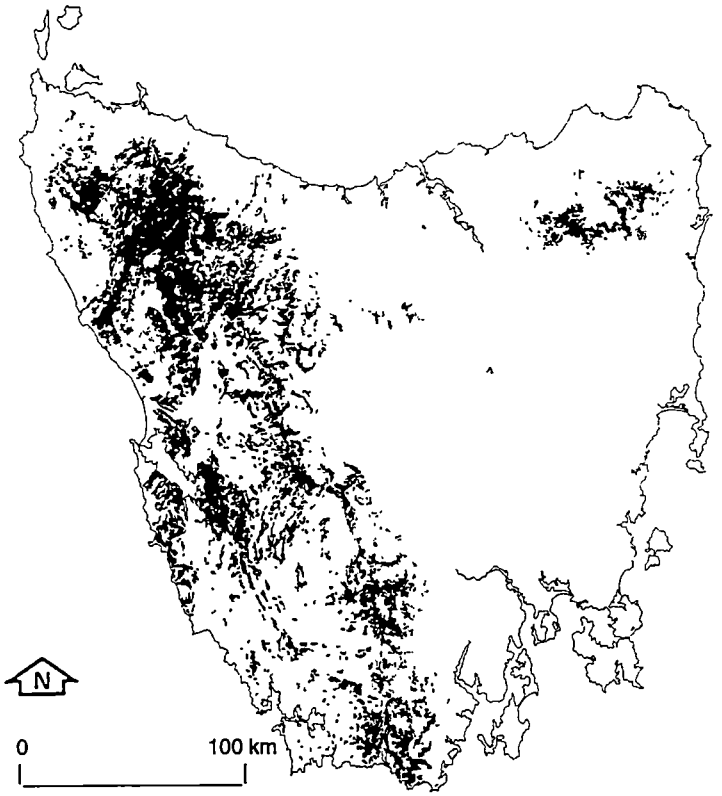

Figure 1.1 The distribution of cool temperate rainforest in Tasmania.

(From Hickey *et al.* 1993.)

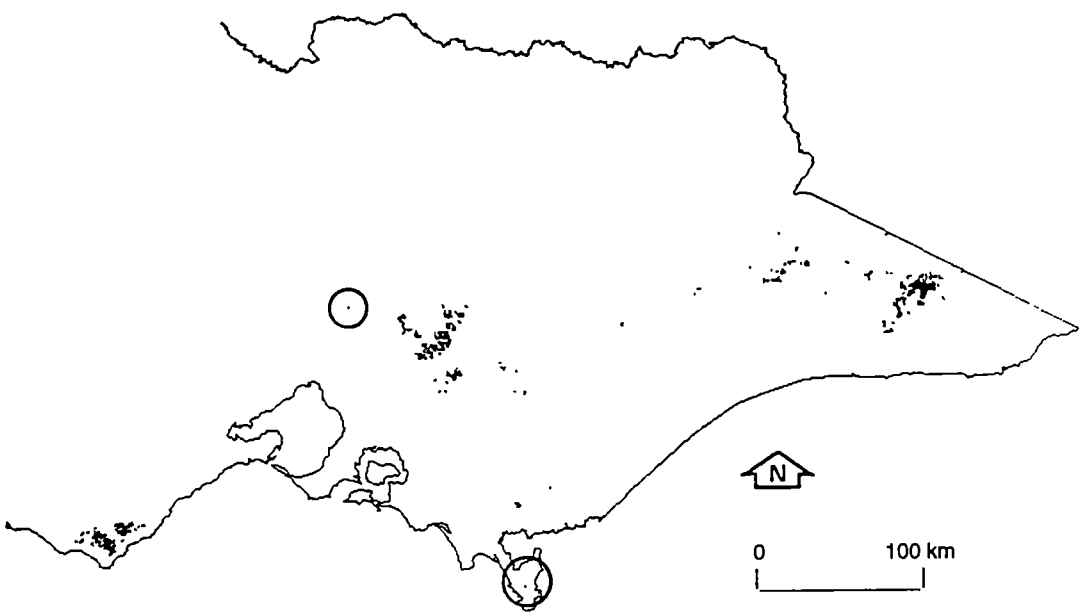

Figure 1.2 The distribution of cool temperate rainforest in Victoria.

(From Cameron 1992.)

SPECIES NOMENCLATURE

Species nomenclature follows: Buchanan *et al.* (1989) for the Tasmanian flora; Hnatiuk (1990) for the mainland Australian flora; Hill and Read (1991) for *Nothofagus* species; Schedl (1972) and Roberts (1979, 1986) for *Platypus* species; Nag Raj and Kendrick (1975) and Kile and Walker (1987) for *Chalara* species; and Seifert *et al.* (1993a) for *Ceratocystis* and *Ophiostoma* species. Other references are cited in the text.

NOTHOFAGUS

The genus *Nothofagus* (Fagaceae) is considered to be an important Gondwanic relict, with extant species being distributed in the southern cool temperate zone; in Argentina and Chile (nine species), south-eastern Australia (three species) and New Zealand (five species); also occurring in the cooler tropical highlands of New Guinea (13 species) and New Caledonia (five species)(Wardle 1984; Hill 1990; Hill and Read 1991). Its distribution does not overlap those of the other genera in the Fagaceae (Hutchinson 1973). The genus is divided into four subgenera: *Nothofagus; Lophozonia; Brassospora and Fuscospora* (Hill and Jordan 1993).

Of the three Australian species, *N. cunninghamii* (myrtle) is the most widely distributed, being the dominant tree in many of the Tasmanian and Victorian cool temperate rainforest communities (Figures 1.1 and 1.2). *N. moorei* (antarctic beech) is found in the cool temperate rainforests of northern New South Wales and southern Queensland, while *N. gunnii* (fagus), is a deciduous Tasmanian native[1], restricted to high altitude areas but co-occurring with myrtle in parts of its range (Jarman *et al.* 1984, 1991; Robertson and Duncan 1991). *N. cunninghamii* and *N. moorei* belong to the subgenus *Lophozonia,* while *N. gunnii* belongs to the subgenus *Fuscospora* (Hill and Jordan 1993).

Myrtle occurs in pure rainforest, as an understorey to *Eucalyptus* species in mixed forest, and in many swamp, riparian and scrub forest communities (Jarman *et al.* 1984, 1991; Kirkpatrick *et al.* 1988; Cameron 1992; Pannell 1992). Tasmanian rainforest vegetation has been divided into broad groups on the basis of community structure and floristics. Three of these groups contain myrtle as a dominant species:

- callidendrous rainforest has tall, well formed trees and an open understorey;
- thamnic rainforest has well formed trees of moderate height, and a distinct shrub layer;

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[1] The term "native" has been used in this context in preference to "endemic", which is used in its epidemiological sense elsewhere in the text.

- implicate rainforest has shorter trees, a tangled understorey and an uneven broken canopy (Jarman *et al.* 1984, 1991).

Typically callidendrous forest occupies more fertile sites than thamnic or implicate forest. In Tasmania, myrtle-dominated rainforest is very extensive and occurs in large and often continuous areas in the west/south-west, while in the east it is usually confined to small, remnant patches (Jarman *et al.* 1984; Neyland 1991; Neyland and Brown 1994). The distribution of mixed forest (with a mature myrtle understorey) is similar but extends further to the north and east, into drier and more fire-prone sites (Hickey and Savva 1992). Swamp forests containing myrtle are largely restricted to north-west Tasmania (Pannell 1992).

In Victoria the definition of cool temperate rainforest is broader and includes some secondary rainforest communities. Myrtle is generally restricted to small patches (often gully communities), and its distribution is limited to sites in the Central Highlands, the Otway Ranges and the Strzelecki Ranges, with some isolated occurrences on Wilsons Promontory (Cameron 1992). Victorian primary rainforest which contains myrtle, broadly resembles Tasmanian callidendrous rainforest.

THE *CERATOCYSTIS* COMPLEX AND *CHALARA*

The form genus *Chalara* belongs to the Fungi Imperfecti (Deuteromycotina: Hyphomycetidae). Where teleomorphs (perfect forms) of *Chalara* are known, they are *Ceratocystis* species (Ascomycotina).

The genera *Ceratocystis, Ophiostoma, Ceratocystiopsis* and *Sphaeronaemella* together form what has been termed 'the *Ceratocystis* complex' (De Hoog and Scheffer 1984). The taxonomy of this group has been revised by De Hoog (1974), De Hoog and Scheffer (1984), and Harrington (1987). A synopsis of the taxonomic revisions of *Ceratocystis* is given by Perry (1991). Wolfaardt *et al.* (1992) produced a synoptic key and computer database for identification of species of *Ceratocystis sensu lato.* Subsequent revisions have been made by Hausner *et al.* (1993a, 1993b).

The *Ceratocystis* complex

Species within the *Ceratocystis* complex may have several anamorphs belonging to different form genera (of which *Chalara* is only one). Upadhyay and Kendrick (1975) listed 16 possible conidial anamorphs: *Hyalodendron; Sporothrix; Hyalorhinocladiella; Verticicladiella; Pesotum; Hyalpesotum; Pachnodium; Leptographium; Graphium; Graphilbum; Acremonium; Chalara; Chalaropsis; Thielaviopsis; Phialocephala* and *Phialographium.* However, Nag Raj

and Kendrick (1975) considered *Chalaropsis* and *Thielaviopsis* to be synonyms of *Chalara,* while Upadhyay (1981) included *Gabardnaudia, Graphiocladiella* and *Phialographium.* Wingfield *et al.* (1991) considered *Phialographium* and *Pesotum* to be synonyms of *Graphium,* although this has been disputed by Upadhyay (1993a).

While *Ophiostoma* was at one stage regarded as a synonym for *Ceratocystis* (Upadhyay and Kendrick 1975), more recently, differences between the genera have become apparent. De Hoog and Scheffer (1984) proposed the separation of *Ceratocystis* Ellis and Halst. *sensu lato* into *Ceratocystis sensu sticto,* which was restricted to species with *Chalara* anamorphs; and *Ophiostoma,* which was composed of species which lacked *Chalara* anamorphs, had rhamnose in their cell walls, and were resistant to cycloheximide. Upadhyay (1993b) considered that the absence of information on the cell walls of some species, rendered the taxonomic application of such information premature. However, Nag Raj and Kendrick (1993) suggested that *Chalara* anamorphs of *Ceratocystis* species were likely to be of monophyletic origin. Samuels (1993) considered that *Ceratocystis, Ophiostoma* and *Ceratocystiopsis* were distinct genera, whose anamorphs indicated different lines of derivation. The Ophiostomataceae were retained as a distinct family of the Xylariales.

Phylogenetic analysis of partial ribosomal DNA sequences supported the separation of *Ceratocystis* and *Ophiostoma,* and it was suggested that the teleomorphic similarities of the two genera were due to convergence, possibly due to the independent evolution of insect dependant ascospore dispersal mechanisms. It was suggested that *Ophiostoma* should remain the sole genus of the Ophiostomataceae, which should be the sole family within the Ophiostomatales, whereas *Ceratocystis* would be best disposed within the Microascales (Hausner *et al.* 1992, 1993a, 1993c, 1993d). *Ceratocystiopsis* was reduced to synonymy with *Ophiostoma* (Hausner *et al.* 1993b).

Using similar methods, the results of Spatafora and Blackwell (1994) supported the separation of *Ceratocystis* and *Ophiostoma. Ceratocystis* and *Sphaeronaemella* were more closely related, and were proposed as sharing a recent common ancestor with members of the Microascales.

Some species in the *Ceratocystis* complex e.g. *Ophiostoma ulmi* (Buism.) Nannf., are heterothallic, requiring two mating types to produce the teleomorph. Others, e.g. *Ceratocystis piceae,* are homothallic, but effectively self-fertile (Webster 1970). The limited number of *Chalara australis* isolates examined to date have been of one mating type, but are self-sterile (T. C. Harrington, personal communication). The teleomorph of *C. australis* has

not been found, implying that the second mating type is either absent or at a very low frequency in the population (C. M. Brasier, personal communication).

The *Ceratocystis* complex, with its imperfect forms, has a worldwide distribution (Upadhyay 1981) and includes a number of economically and ecologically important species. Diseases caused by these organisms occur on diverse hosts (mainly angiosperms), and include vascular wilts, vascular stain diseases, canker diseases, root and stem rots and rots of fruits, tubers, seed pods, leaves and buds. Major methods of dispersal of pathogenic species are via soil, wind and water, root-grafts, insect or animal vectors, insect frass or via pruning and other tools (Kile 1993).

Ceratocystis species are some of the primary fungal colonisers of timber, and are able to invade both moribund and living tissue (Dowding 1984). Many are wood-staining fungi, both saprophytes and pathogens (Seifert 1993). The majority of the saprophytic wood-staining fungi are found in bark beetle galleries (Hutchison and Reid 1988).

In general, *Ceratocystis* species are most frequently reported from habitats other than beetle galleries (Malloch and Blackwell 1993). However, some *Ceratocystis* and *Ophiostoma* species exhibit complex symbiotic relationships with insects. While *Ophiostoma* species are generally identified with bark beetles, *Ceratocystis* species may be associated with ambrosia beetles, but tend to form less specialised arthropod host relationships (Spatafora and Blackwell 1994). Notable pathogens include *O. ulmi* and *O. novo-ulmi* Brasier, causal agents of Dutch elm disease, and *Ceratocystis fagacearum* (anamorph *Chalara quercina*), causal agent of oak wilt.

Dutch elm disease

Ophiostoma ulmi and *O. novo-ulmi* are vectored by bark beetles (Webber 1990). Both conidiospores (asexually produced) and ascospores (sexually produced) are carried by the beetles, although conidiospores are probably the most important. Trees become infected via both the feeding groves and the breeding tunnels made by the insects (Webber and Brasier 1984). Root grafting is known to be an important mechanism for local disease spread (Neely and Himelick 1963).

A non-aggressive strain of *O. ulmi* is believed to have been endemic to Great Britain and adjacent parts of Europe since the 1920s. An origin has been postulated near Antwerp (Belgium) in 1900-1905 (Holmes 1990). However, in the 1970s an aggressive, highly pathogenic strain of the disease was discovered (Brasier 1979). This strain has now been

recognised as a separate species, *O. novo-ulmi* (Brasier 1991), which has two distinct races. The NAN race is thought to have originated in North America and is now spreading eastwards through Europe. The EAN race probably originated in Central Europe and is spreading westward. The NAN and EAN races of *O. novo-ulmi* are considered jointly responsible for the recent outbreaks of Dutch elm disease in Western Europe, and are progressively replacing the less aggressive *O. ulmi* (Brasier 1979).

The current Dutch elm disease pandemic has encompassed most of Europe, eastern North America and south-west Asia. During the 1970s the disease killed 20 million elms (*Ulmus* Linn. species) in Great Britain alone. There is evidence that the disease will not only attack mature elms, but also the seedlings and suckers that arise to replace them (Webber and Brasier 1984). Thus over the next 40 years the disease is predicted to reduce European field elms to an understorey or scrub population, with occasional escapes in mountain valleys (Brasier 1983). Dutch elm disease has also been proposed as a possible cause of the Neolithic elm decline in north-west Europe (Perry and Moore 1987; Turner and Hodgson 1991). In Italy, a recent survey identified only about 100 large (bole diameter greater than 1 m) elms in the country (Mittempergher 1989).

Chalara

The form genus *Chalara* comprises over 80 known species, of which about ten are known plant pathogens, a number of these having considerable economic importance (Kile and Walker 1987): *C. thielavioides* is associated with root and graft rot in walnut, carrot and lupin; *C. neocaledoniae* causes a vascular stain disease in coffee and guava and *C. populi* causes a canker disease in poplar and willow (Kile 1993).

It is difficult to assess the distribution of *Chalara* in Australasia and South America. Nag Raj and Kendrick (1975) listed 70 species, of which 28 were recorded from New Zealand and one from Australia. Later workers have added to this list (e.g. Kile and Walker 1987; Old *et al.* 1991) but the distribution of known species still probably reflects that of mycologists, rather than that of *Chalara!*

Kile and Walker (1987) listed *Chalara* species recorded on plants in the family Fagaceae. Most were saprophytic, with some causing timber spoilage. There were only three parasitic species: the *Chalara* anamorph of *Ceratocystis fimbriata* on *Fagus* and *Quercus* species; *Chalara quercina* causing oak wilt of fagaceous species in North America; and *C. australis* causing myrtle wilt of myrtle in south-east Australia. They concluded that the Fagaceae, with

one third of the known species of *Chalara* recorded on them, may have some significance as hosts for the genus.

Oak wilt

Chalara quercina has affinities with *C. australis*, both in terms of its morphology (Nag Raj and Kendrick 1975) and in causing a vascular infection of a Fagaceous host (Kile and Walker 1987). Oak wilt was described from *Quercus* species by Henry *et al.* (1944), and was at that stage widespread in much of Wisconsin, and also present in Minnesota, Iowa and Illinois (USA). The anamorph was described by Henry (1944) and the teleomorph (*Ceratocystis fagacearum*), discovered in culture, was described by Bretz (1952).

The disease is vectored by nitidulid beetles. These beetles carry both conidiospores and ascospores to wounds caused by woodpeckers, which they use for sap feeding (Dowding 1984; Webber and Brasier 1984). Local spread of the disease also occurs via root grafts (Kuntz and Riker 1950, Beckman and Kuntz 1951).

Anderson and Anderson (1963) investigated the rate of spread of oak wilt in southern Wisconsin and south-eastern Minnesota between 1955 and 1959. They monitored both the new infection centres, which became established through relatively long distance spread of the fungal spores, and the increase in size of existing centres, as the fungus spread through root grafts and by local spread of spores. They concluded that there was a slow, steady build up of the disease likely to result in substantial long-term losses. By 1981 the disease had been reported over a wide area from 18 states, and was found to be attacking all native species of oak, regardless of size, age or vigour (Upadhyay 1981). However, Tryon *et al.* (1983) have reported abundant oak regeneration in oak wilt infection centres in West Virginia.

***Chalara australis* and myrtle wilt**

In the field, myrtle wilt has only been observed to kill myrtles, although when artificially inoculated with *C. australis* the Tasmanian native *Trochocarpa gunnii* (Epacridaceae) died, with *Nothofagus gunnii* also being susceptible. The disease is considered a potential hazard to *Nothofagus* species found outside Tasmania, particularly the deciduous members of the genus. Additionally *C. australis* may grow saprophytically and sporulate on dead xylem tissues of native Tasmanian shrub and tree species, and this has the potential to increase inoculum availability (Kile 1989).

In myrtles, *C. australis* causes brown, radial streaks across multiple growth rings, suggesting that it is not a true vascular wilt. Unlike *Ceratocystis fagacearum* and *O. ulmi*, it does not become systemic in the vascular system, but is generally restricted to the roots and lower to mid stem (Kile and Walker 1987). Clumping of diseased trees and relationships between disease incidence and stand density are considered indicative of below ground spread (Elliott *et al.* 1987; Kile *et al.* 1989).

Conidiospores are produced by sporulating black felts which sometimes form on the bark of infected myrtles or other wood surfaces, mainly in the autumn and winter (Kile *et al.* 1989). Two endoconidial forms are produced (Kile and Walker 1987; Kile 1993). Inoculum appears to be air or water dispersed, and this has been confirmed in rainforest areas by inoculum-trapping experiments, using fresh myrtle billets and rainwater collection. Infection of wounds to the trunk, crown or roots, is thought to be the origin of most new disease foci (Kile *et al.* 1989).

These results contrast strongly with those of Dowding (1984), who found that the sticky conidiospores of *Ceratocystis* species were very short lived and susceptible to sunlight and UV light, although ascospores were longer lived. Since spores were produced under the bark, he deduced that adaptations for air dispersal were unnecessary, and that spore stickiness could not be an adaptation to dispersal by rain splash since the site of spore production and the infection court were usually protected from heavy rain. He concluded that the spores were adapted for transfer by arthropods.

Kile and Walker (1987) reported myrtle wilt in the Otway Ranges of Victoria, while Elliott *et al.* (1987) showed the disease to be widespread in undisturbed Tasmanian myrtle forest. A number of workers have reported the presence of myrtle wilt in remote Tasmanian sites (Jarman *et al.* 1984; Working Group for Nature Conservation 1987, Kile *et al.* 1987).

The closest known relative of *Chalara australis* is a new species of *Chalara*, which has been isolated from wounds in *Eucalyptus sieberi* and *E. obliqua* in Victoria (Old *et al.* 1991). The distributions of both species are known to adjoin or overlap that of myrtle in Victoria (Hogan 1944; D. G. Cameron, personal communication), and *E. obliqua* commonly occurs with myrtle in Tasmanian mixed forest (Kirkpatrick *et al.* 1988).

BARK AND AMBROSIA BEETLES AND *PLATYPUS*

The Coleopteran subfamilies Platypodinae and Scolytinae (Curculionidae) contain a number of genera which attack trees and timber (Lawrence and Britton 1991), and are arguably responsible for killing more trees than any other natural cause (Wood 1982). The Scolytinae have a world-wide distribution whereas the Platypodinae are predominantly tropical. The group can be divided on the basis of feeding habits; the phloeophagus scolytids are known as bark beetles, and the xylomycetophagus platypodids and scolytids as ambrosia beetles (Batra 1963; Francke-Grosmann 1967; Cooke 1977). *P. subgranosus* is thus an ambrosia beetle.

Bark and ambrosia beetles are usually the primary colonists of recently injured, standing trees and newly felled logs (Wood 1982). Bark beetles are the most important invertebrate colonists and their activities facilitate the entry of other organisms. These beetles may become vectors of sticky-spored *Ceratocystis* species, when emerging adults come into contact with fungal inoculum (Dowding 1984; Carpenter *et al.* 1988).

The host specificity of bark beetles has been shown to be lower in tropical than in temperate regions. This was thought to be due to the greater species diversity and heterogeneity of tropical forests; in such an environment polyphagy can reduce the problems of host-finding. In contrast ambrosia beetles (which are relatively more important in the tropics), show low host specificity in both regions. However, these beetles feed on the same ectosymbiotic ambrosia fungi, regardless of the host tree species, and it is really the fungi that are polyphagous, although it is the beetle that selects the host (Beaver 1979).

Whilst ambrosia beetles generally have an extremely wide host range, they usually occur on diseased or dead trees (Francke-Grosmann 1967). Thus, in general, with ambrosia beetles, hosts tend to be selected on the basis of their health status rather than their species.

Bark beetles

Bark beetles inhabit bark and sapwood which is rich in easily digestible nutrients. However, many of them are associated with specific fungi in ectosymbiotic relationships. Some fungi are transported by the beetles, either in specialised organs, on the integument or in the gut; others are wind-dispersed (Francke-Grosmann 1967; Graham 1967). The *Ceratocystis* complex (and associated hyphomycetes) are often involved (Upadhyay 1981). These may be saprophytic, blue-staining species, e.g. *Leptographium procerum* (Kendrick) Wingfield and *Ceratocystis ips*, associated with attack by *Dendroctonus terebrans* (Olivier) on *Pinus*

species (Rane and Tatar 1987), or they may be pathogenic, e.g. *O. ulmi* and *O. novo-ulmi,* vectored by *Scolytus scolytus* (F.) and *S. multistriatus* (Marsham), on *Ulmus* species (Webber 1990). Beetles may also be instrumental in bringing together the two mating strains in heterothallic species e.g. *C. fagacearum* (Upadhyay 1981).

Ambrosia beetles

Ambrosia beetles are not primarily responsible for the death of trees, and inhabit dead or dying wood, often heartwood. They are wood borers but not wood feeders, and have a symbiotic relationship with the ambrosial fungi which are cultured in their tunnels and used as a food source. They are attracted to unhealthy trees, or to those suffering from a temporary reduction in vitality as a result of environmental conditions, and require wood with a relatively high moisture content (Fisher *et al.* 1953; Cooke 1977).

Ambrosia fungi are transported by adult scolytids in specialised depressions or invaginations of the body surface, known as mycangia (Batra 1963) or mycetangia (Francke-Grosmann 1967). In platypodids the mycetangia, if present, have a much simpler form, being only pits or notches in the integument. The taxonomy of ambrosial fungi has been reviewed by several authors, and most are hyphomycetes (Batra 1963; Francke-Grosmann 1967). It is uncertain whether ambrosia fungi are specific to beetle species, groups of beetles or to the host plant (Fisher *et al.* 1953).

Ambrosia beetles may also be important disseminators of saprophytic wood staining fungi (Fisher *et al.* 1953). Blue-stain fungi, including *Ceratocystis* species, are often associated with beetle tunnels, although in relation to the ambrosia fungi these are 'weed' fungi (Cooke 1977; Francke-Grosmann 1967).

While bark beetles are commonly vectors of pathogens, ambrosia beetles have not been shown, conclusively, to transmit pathogenic fungi, and attack by native ambrosia beetles on healthy, living trees in natural forest is unusual. Specifically, *P. subgranosus* is a secondary factor in myrtle wilt, the beetles attacking trees already infected by *Chalara australis* (Kile and Hall 1988).

Platypodid ambrosia beetles

Platypodid ambrosia beetles are secondary borers of newly dead and moribund trees. Optimal conditions for attack and breeding occur where there has been little moisture loss, but where the wound response of the tree has been inhibited. Natural forests provide breeding sites in old trees; trees primarily attacked by fungi or insects; trees which have

suffered from drought, fire, floods, gales, lightning, sudden exposure and sunburn, volcanic eruptions and similar calamities (Kalshoven 1960).

Host selectivity in ambrosia beetles generally (Beaver 1979), and in platypodids specifically (Kalshoven 1960), is thought to be low. However, work by Browne (1958) in the humid tropics of south-east Asia indicates that this may be an oversimplification. He found that 30 percent of the known ambrosia beetle species were highly host selective, with selectivity occurring throughout particular genera, and with closely related species tending to select the same hosts. Hosts were normally selected at the level of botanical family, with very few families being affected. Highly evolved groups were more selective than primitive groups. It is possible that the specificity of the beetles for certain trees is the result of the specific requirements of their particular fungus (Fisher *et al.* 1953).

Browne (1958) found that the Fagaceae were attractive to ambrosia beetles in general, but also provided the hosts of many selective species. Notably, *Platypus* species in the section *Platypi spinulosi* were typically associated with Fagaceae. He also found that *Platypus* were borers of large timber, and did not record any species attacking timber less than 7.5 cm in diameter.

The predominantly tropical distribution of the platypodids is reflected in the number of species recorded by Schedl (1972) from Australasia and South America: New Guinea (123 species); New Caledonia (one species); Australia (30 species); New Zealand (three species) and Argentina (14 species).

***Platypus subgranosus* and myrtle wilt**

Platypus subgranosus was first described by Schedl (1936), and is in the section *Platypi semiopaci* of the genus (Schedl 1972). It has been identified from Tasmania, Victoria and Queensland.

The blue-stain fungus *Leptographium lundbergii* Lagerberg and Malin has been constantly isolated from the tunnels *of P. subgranosus* and was thought to be the ambrosia fungus (Webb 1945). However, Batra (1963) noted that in relation to the beetles, this was a non-specific fungus, which neither formed the characteristic ambrosia phase, nor was actually observed to serve as their food source.

In Tasmania *P. subgranosus* was apparently first collected in 1922, from the St Patricks River and Hobart areas (Schedl 1942), and later from Waratah (Schedl 1936). The first host

records were of dead and dying myrtles (Howard 1973a), but it has subsequently been found in the dead logs of other rainforest species: *Atherosperma moschatum; Eucryphia lucida; Pittosporum bicolor; Phyllocladus aspleniifolius; Anodopetalum biglandulosum* (Elliott 1978) and *Lagarostrobos franklinii* (H. J. Elliott, unpublished data). It has also been recorded from unhealthy specimens of the introduced *Pinus radiata* D. Don (Elliott and De Little 1984). It is widely distributed throughout the state, in rainforest and mixed forest (Elliott *et al.* 1982; 1987).

In Victoria it was recorded from fire-killed timber in the Central Highlands, following the devastating bush fires of 1939. *Eucalyptus regnans, E. delegatensis, E. goniocalyx, E. obliqua* and myrtle were all attacked. *P. subgranosus* attacked only unhealthy or damaged green trees, or dead trees which had not become too dry. Standing, fire-killed trees and those in dry log dumps were not badly attacked; this was in contrast to those in water-sprayed dumps where sprays provided ineffective water coverage. Although a number of other *Eucalyptus* species were involved, *E. goniocalyx* and myrtle appeared to be most susceptible (Hogan 1944; 1948).

In Queensland it has been recorded from the Dividing Range (Hogan 1948), Palen Creek, Mt Glorious and Brisbane, from *Scolopia brownii* (fallen log), *Brachychiton populneum* (sawn timber), *B. acerifolium, Eucalyptus maculata* and from *Pterocymbium beccarii* K. Schum., introduced from New Guinea (Schedl 1979).

In Tasmania *P. subgranosus* attack on standing myrtles is highly indicative of myrtle wilt. The comprehensive survey of Elliott *et al.* (1987) found very few live myrtles with evidence of having survived *P. subgranosus* attack (which would indicate they were never infected with *C. australis,* but were attractive to the beetle). Although *P. subgranosus* will attack wounded or burnt myrtles, Kile *et al.* (1992) showed that only myrtles infected with *C. australis* suffer sustained attack. Hogan (1944) indicated that standing, fire-killed trees were unlikely to be attacked, probably due to the relatively low moisture content found in dead standing trees. Ethanol (produced from fermenting wood) is known to act as a primary attractant and boring stimulant for *P. subgranosus* and it is thought that volatiles produced from diseased trees attract the beetles (Elliott *et al.* 1982, 1983; Kile *et al.* 1992).

Elliott *et al.* (1987) found that there was a very low proportion (1.2%) of standing myrtles which were dead or dying, but not attacked by *P. subgranosus.* This would indicate either that trees dying from causes other than myrtle wilt are not attractive to the beetle, or, that not all *C. australis* infected trees are necessarily attacked by it. Even if the latter were true, it

would only lead to a slight underestimate of *C. australis* infected trees. Thus *P. subgranosus* attack on standing myrtles is a relatively accurate indicator of myrtle wilt.

Kile and Hall (1988) undertook a series of studies to assess *P. subgranosus* as a vector of *C. australis* and this work has been extended by Candy (1990). *P. subgranosus* was found to lack mycetangia and is therefore considered to be fairly primitive. *C. australis* could not be isolated from any of the beetles emerging from infected trees or billets, or from those trapped in flight. This was attributed to the fact that most of a *P. subgranosus* brood emerges from an attacked tree the second summer after infection. These beetles are unlikely to become contaminated (and therefore unlikely to be vectors) because after this time the survival of *C. australis* in the above-ground wood is minimal. However, *C. australis* was successfully isolated from *P. subgranosus* within infected trees (particularly those trees currently dying or recently dead), so it is possible that the beetles and their larvae promote within-tree spread of the fungus.

For recently wounded trees there was no apparent relationship between *P. subgranosus* attack and *C. australis* infection: some trees which were not attacked by *P. subgranosus* became infected with *C. australis,* while some attacked trees remained uninfected for nine weeks. Whilst the theory of a direct vectoring role for *P. subgranosus* was disproved, it was conceded that a small percentage of the beetles may carry and transmit *C. australis*. Also, Kile *et al.* (1989) indicated that wind-borne beetle frass (contaminated with *C. australis* conidia and phialides) could be an inoculum source, particularly during the summer months. If proven, this would imply a degree of mutualism between *P. subgranosus* and *C. australis*.

GONDWANIC PARALLELS?

In brief, the distributions of *Nothofagus*, *Chalara/Ceratocystis* and *Platypus* are, respectively, Gondwanic, worldwide and largely tropical, with both *Chalara/Ceratocystis* and *Platypus* occurring on a wide range of host species. This being the case, numerous, phylogenetic parallels with myrtle wilt appear unlikely, although both *Chalara* (Kile and Walker 1987) and *Platypus* (Browne 1958) have been shown to exhibit a degree of specificity for the Fagaceae.

However, since the distribution of *Nothofagus* does not overlap those of other members of the family (Hutchinson 1973), an attempt has been made to collate the accessible information relating to *Chalara,* the *Ceratocystis* complex and *Platypus* species on *Nothofagus* hosts, and this is shown in Figure 1.3. In addition to the summaries by Kile and

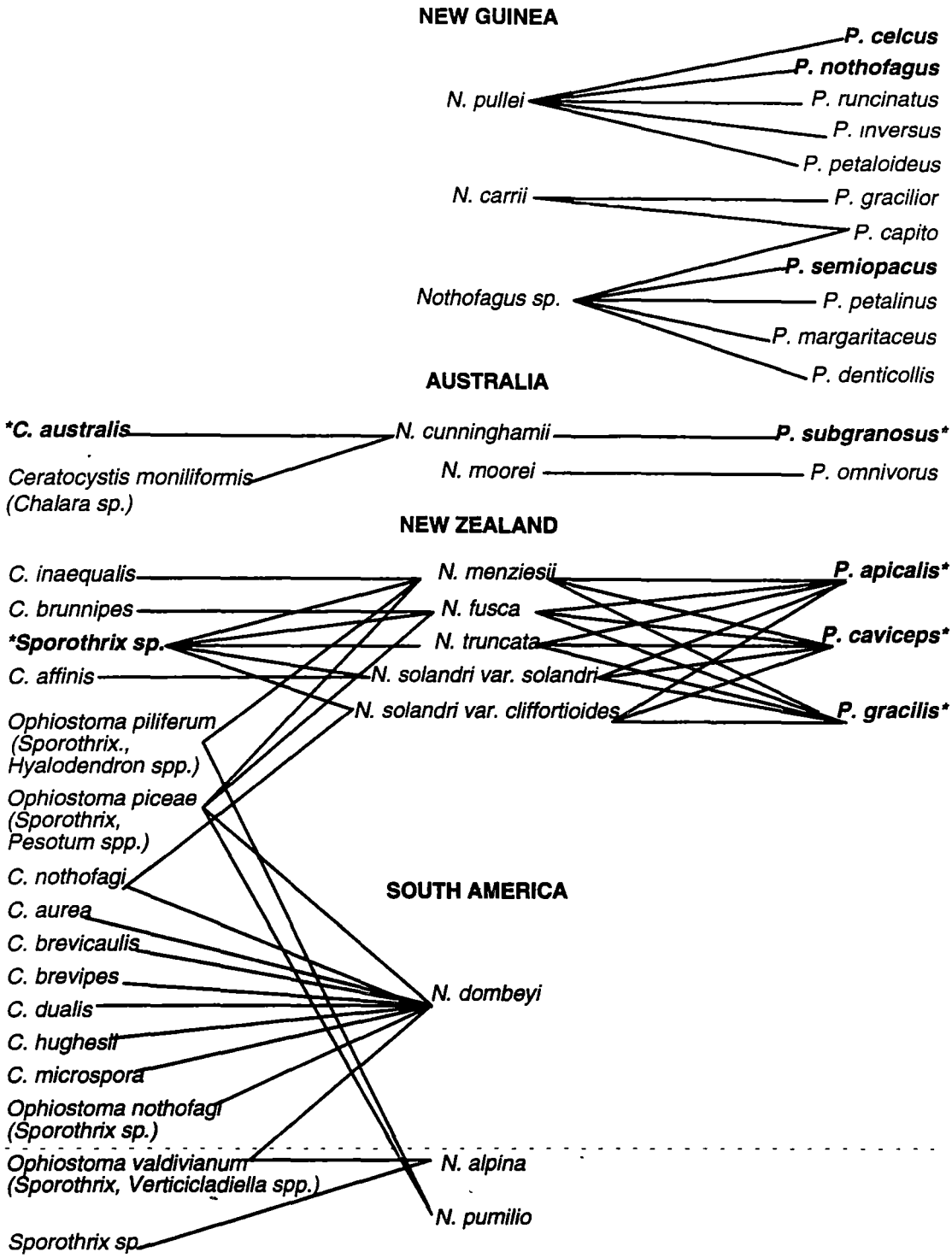


Figure 1.3 Associations of *Nothofagus*, *Chalara*, the *Ceratocystis* complex and *Platypus*.

- *Nothofagus* species above the dotted line are evergreen, those below it are deciduous
- Anamorphs of *Ceratocystis* and *Ophiostoma* species shown in parentheses
- * Denotes a known fungal/ambrosia beetle association
- Species shown in **bold** are those known to attack living *Nothofagus* hosts

Walker (1987) and Wardle (1984), the following sources were used: Faulds (1973); Milligan (1974); Roberts (1979, 1986, 1987); Browne (1983); Butin and Aquilar (1984); P. Hadlington (unpublished data); H. Peredo (unpublished data, cited in USDA (1993)).

Although there are no relevant records from *Nothofagus* in New Caledonia, *Chalara neocaledoniae* is known to occur on other hosts in this region (Kile and Walker 1987). Of the *Chalara* species, only *C. nothofagi* is known from *Nothofagus* hosts in both New Zealand and in South America. However, it is notable that two *Ophiostoma* species, *O. picea* and *O. pilifera* have been recorded from both New Zealand and South American *Nothofagus* species. *O. pilifera, O. picea* and *O. valdiviana* have been found on both evergreen and deciduous *Nothofagus* species.

The apparent absence of *Platypus* species on *Nothofagus* in South America is thought to be due to two main reasons. Firstly, there are no host records at all for the great majority of South American Platypodinae; secondly there are no records of any Platypodinae in Chile. Particularly in the south of the country (within the range of *Nothofagus*), enough work has been done to make it likely that the family is actually absent there, although reasons for this absence are not clear (R. A. Beaver, personal communication).

The only known pathogens are *C. australis*, from Tasmania and Victoria, and an unknown species of *Sporothrix* from New Zealand (Faulds 1973). Except for an unidentified *Ceratocystis* species isolated from *Platypus* tunnels in *N .fusca* (Faulds 1973), these are also the only species from the *Chalara/Ceratocystis* complex known to have an association with *Platypus* species.

Sub-lethal attack by *Sporothrix* is associated with the formation of pathological wood (Litchwark 1978) in *N. fusca* and *N. menziesii,* and Wardle (1984) concluded that there was a relationship between *Platypus* attack and the formation of core rots in New Zealand *Nothofagus* species. It is of interest that in New Guinea, *P. celcus* and *P. nothofagus* were recorded from live *N. pullei,* but only from trees which had heart rot. *P. semiopacus* was also recorded from live *Nothofagus* (Roberts 1979). Arentz (1988) assumed that this was because the *Nothofagus* species were under stress, possibly contributing to the observed *Nothofagus* dieback. Unfortunately, nothing is known about the cause of the heart rot in this instance.

Tasmania and Victoria have a relatively simple system with *C. australis* being confined to myrtle (Kile 1989) and associated with only one species of *Platypus; P. subgranosus*

(Howard 1973a) which is not specific to *Nothofagus* (Hogan 1948). In New Zealand the system involves five species of *Nothofagus*: *N. fusca* (red beech); *N. menziesii* (silver beech); *N. truncata* (hard beech); *N. solandri* var. *solandri* (black beech) and *N. solandri* var. *cliffortioides* (mountain beech). The pathogenic *Sporothrix* species kills all the New Zealand *Nothofagus* species (Milligan 1974). The three species of *Platypus* involved; *P. apicalis*, *P. caviceps* and *P. gracilis* each attack the five *Nothofagus* species, although only *P. caviceps* is specific to *Nothofagus* (Milligan 1979). In both Tasmania and New Zealand *Platypus* related dieback is generally the most common (proximate) cause of death of mature *Nothofagus* (Milligan 1974; Elliott *et al.* 1987).

In Tasmania the role of *P. subgranosus* in vectoring *C. australis* has been specifically investigated and three of the four criteria necessary to prove a vector relationship could not be shown. These systematic studies have yet to be completed in New Zealand (Kile and Hall 1988).

In New Zealand there were early indications that *Platypus* was not absolutely necessary for the introduction of *Sporothrix*, since it was isolated from some trees which had been drilled but not inoculated with the fungus (Faulds 1973, 1977). However, Milligan (1972), having observed fungal staining in trees earlier attacked by *Platypus*, assumed that this pathogenic fungus (or complex) depended on the beetles for transmission. He stated 'in the writer's *opinion, Platypus appears* to be the vector of a fungus which invades the sapwood ...' (my emphasis) In a later publication he stated 'Evidently a pathogenic sapstain fungus is transmitted by the beetles ...'(Milligan 1974). These conclusions were drawn from wide observations, but were not (and were never claimed to be) experimentally proven facts. In fact Faulds (1977) states 'Whether under natural conditions *Platypus* is a vector of the pathogen or whether the pathogen incidentally invades *Platypus* wounds is not known. However, as the fungus was recovered from trees into which it had not been inoculated it can obviously invade *Platypus*-like wounds in the absence of beetles ....'

In view of this, it is unfortunate that the speculated role of *Platypus* as a vector of *Sporothrix* has been accepted as fact by later authors. This aspect needs clarification, as does the identity of the *Sporothrix* species. Despite this, the New Zealand system clearly represents the closest known parallel to myrtle wilt, at least in terms of species' associations.

On the New South Wales/Queensland border, *Platypus* attack in dead *N. moorei* was noted by the author in 1989. Howard (1973a), had previously noted its absence in this area. The trees were standing dead, along a walking track and some of them were damaged. There

was evidence of dark staining around the *Platypus* holes, and in felled trees black stains were observed in the sapwood only. Samples could not be taken on this occasion, but *Platypus* attack in *N. moorei* logs has been previously recorded (K. Fairey, unpublished data), the only species known being *P. omnivorus* (P. Hadlington, unpublished data). *Ceratocystis/Chalara* species have not been recorded from *N. moorei.*

DISEASE, DISTURBANCE AND DIEBACK IN *NOTHOFAGUS* FORESTS

Mueller-Dombois (1988a) stated 'From a pathological viewpoint, dieback can always be considered as symptomatic of a disease, even when biotic agents play only a minor role in the causal chain. From an ecological viewpoint, however, it should be possible to distinguish between dieback as the result of disease and dieback as a natural phenomenon.'

Insects and diseases have frequently been implicated in death and dieback in *Nothofagus* forests (Milligan 1972, 1974; Faulds 1977; Litchwark 1978; Arentz 1983, 1988; Skipworth 1983; Hosking and Kershaw 1985; Hosking 1986; Hosking and Hutcheson 1986; Elliott *et al.* 1987; Ash 1988), but their true role is not always obvious.

Models of disease, disturbance and dieback

A number of models of disease, disturbance and dieback have been developed, and a consideration of these, with reference to *Nothofagus* forests, helps to define the questions which need to be answered if we are to understand the ecological significance of myrtle wilt. They may also suggest strategies for disease management (Hosking 1989).

Figure 1.4 combines and adapts a number of these ideas and models in a flow chart, designed to elucidate the role of a known pathogen in a natural forest system. The numbers on the flow chart correspond to those of the relevant paragraphs below.

1. Mueller-Dombois (1988b) defined stand-level dieback (canopy dieback) as being the unseasonable loss of crown foliage of a large number of trees in a forest stand. This was in contrast to shoot-tip dieback or crown dieback of isolated single trees or small groups of trees in a forest, which is a normal occurrence in any uneven or all-aged forest. Stand-level dieback takes two main forms: tree-to-tree dieback, in which many adjacent trees are affected; or salt and pepper dieback, where dying trees occur repeatedly in a matrix of healthy trees. It is manifest as a spatially recurring pattern (Mueller-Dombois 1983b).

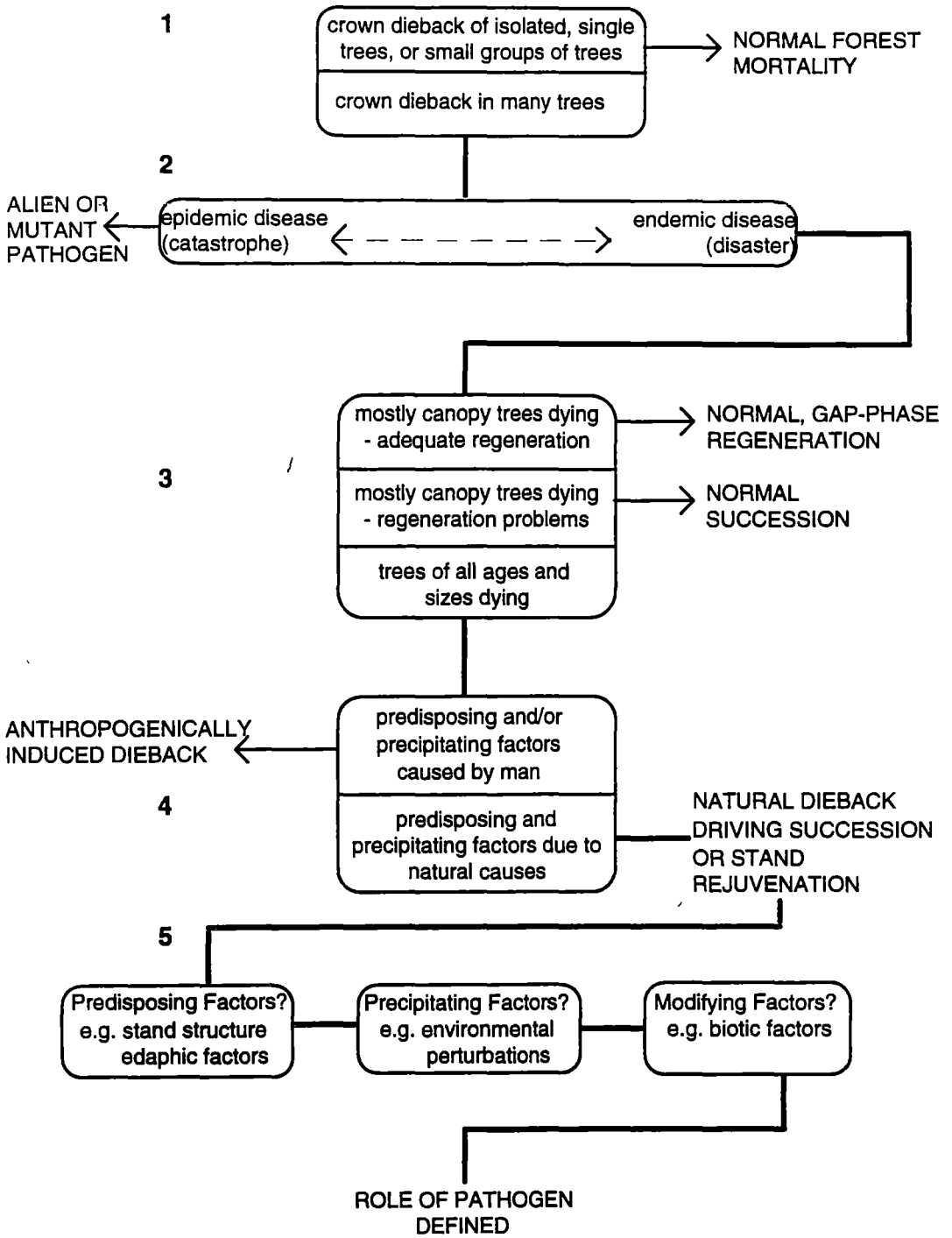


Figure 1.4 Flow chart to assess the role of a known pathogen in a natural forest system.

2. Diseases have traditionally been classified as epidemic or as endemic (in the sense of being continually present in one place). Van der Plank (1975) viewed epidemic and endemic disease as a continuum, with local or micro-epidemics being inevitable even in a mainly endemic situation, due to variation in local conditions. Harper (1977), discussing the effect on plant populations, made a distinction between types of large-scale disturbance: a disturbance too infrequent or irregular to exert a sustained selection pressure was termed a catastrophe; one which occurred with sufficient frequency and regularity to exert a selection pressure on the plant population was termed a disaster. Clearly epidemic disease can be catastrophic, while endemic disease is generally merely disastrous in evolutionary terms! In the case of an alien, epidemic disease, stand-level dieback is unlikely to require further explanation (Mueller-Dombois 1988a); the role of endemic disease, however, is not usually so clearly defined.

3. Mueller-Dombois (1988a) emphasised the need to study not only the dying tree population, but the whole community, and cited the three structural classes recognised by Hosking (1986), for the types of stand-level dieback found in New Zealand's *Nothofagus* forests. The first two classes were considered to be natural successional processes; the third class could represent a true decline disease:
   - Stands in which mostly old canopy trees are dying but which show adequate regeneration of the canopy species;
   - Stands in which mostly old canopy trees are dying but which also have re-establishment problems;
   - Stands in which all ages and sizes are affected by dieback.

   Read *et al.* (1990) used similar concepts in a predictive model of *Nothofagus* dynamics in New Guinea.

4. A three factor theory of forest dieback and disease-induced decline has been developed by a number of workers over the last 30 years and this work has been reviewed by Stewart (1989). Mueller-Dombois (1988a) summarised the decline disease theory and related it to natural dieback. The theory recognises a combination of dieback causes which operate in a chain reaction as: (1) predisposing; (2) precipitating; and (3) modifying factors.
   - Predisposing factors are those which contribute to the loss of vigour with the aging of a stand. They include stand structure, composition and age, extreme edaphic conditions (e.g. nutrient imbalances) and the periodically recurring perturbations that affect a forest area e.g. climatic extremes.

- Precipitating factors are those which synchronise or trigger forest dieback. They are usually fluctuating or recurring environmental perturbations e.g. seismic vibrations, climatic extremes (causing flooding, drought, frost damage, salt spray, storms) or pests and pathogens. Precipitating factors are not always distinct from predisposing factors.
- Modifying factors are those which accelerate or stall dieback and are often biotic factors such as pests and diseases, which attack the already dying stand.

Dieback can be seen as an anthropogenically caused disease if either predisposing or precipitating factors are produced by man. If both have natural origins, stand-level dieback can be viewed as a mechanism driving ecological succession and/or stand rejuvenation (Mueller-Dombois 1988a).

5. When the predisposing, precipitating and modifying factors are known, the role of a pathogen in stand-level dieback can be defined.

Stand-level dieback in *Nothofagus* forests

There is a good deal of knowledge about the operation of the three factors in New Zealand *Nothofagus* forests and this has been reviewed by Stewart (1989) and, less specifically, by Ogden (1985). Species specific regeneration patterns, stand structure and soil stability appear to be important factors predisposing some areas to severe dieback. Ogden (1988) suggested that the synchronised physiological responses to temperature which lead to mast seeding in *Nothofagus*, may also predispose trees to succumb to subsequent environmental stress.

Nothofagus solandri var. *cliffortioides* in New Zealand has a relatively high light requirement for regeneration and occurs in almost pure stands in areas which are subject to periodic destruction by landslips (Mark *et al.* 1989), and to large scale disturbances such as snow storms and windthrow, which may act as precipitating factors (Allen and Wardle 1985). Regeneration and stands therefore tend towards being even aged or two aged (Wardle 1974), and the potential for wide scale dieback at a certain stage in the stand development is high, i.e. cohort senescence is a factor predisposing stands to dieback (Mueller-Dombois 1983b). Skipworth (1983) suggested that dieback in *N. solandri* var. *cliffortioides* in the Tongariro National Park was due to an unusually high percentage of old trees (predisposing factor), lowered water tables (precipitating factor), and to *Platypus* and their associated fungi (modifying factors).

In contrast *N. menziesii*, with a lower light requirement for regeneration, tends towards continuous regeneration and uneven-aged stands on optimal sites, and is less likely to suffer from widespread synchronous dieback (Wardle and Allen 1983), although it can form even-aged stands following landslips (Stewart 1986). Also, in high altitude 'cloud forests' in the Kaimai Ranges, almost continuous soil waterlogging leads to shallow root systems, predisposing stands to dieback precipitated by drought (Jane and Green 1983). *N. menziesii* and *N. fusca* in mixed (and pure) stands usually have gap phase regeneration processes (Stewart and Rose 1990). However, spatial analysis of age classes in one *N. fusca/N. menziesii* stand indicated that tree deaths had been clumped in patches up to 40 m across (Duncan and Stewart 1991).

Dieback of *N. fusca* and *N. menziesii* in the Northern Kaimanawas (New Zealand) appears to have been precipitated (triggered) by storms, with subsequent build up of *Platypus* numbers, presumably with the increase in dead and dying material available for breeding sites (Milligan 1972; Hosking 1977). *Platypus* beetles and *Sporothrix* infection would in this case have been modifying (accelerating) factors. Drought has also been shown to be a precipitating factor in a number of areas (Skipworth 1983; Hosking 1986; Hosking and Kershaw 1985; Hosking and Hutcheson 1986, 1988) with pests and diseases again acting only as modifying factors.

In New Guinea nutrient deficiencies or drought and frost are suspected of being precipitating factors in *Nothofagus* stands predisposed to dieback by tree senescence in an even-aged stand structure. In such cases the presence of *Platypus* beetles in live *Nothofagus* is merely taken as an indication of the stands being under stress (Arentz 1983, 1988). Ash (1988) implicated a soil pathogen in dieback of mature *Nothofagus* on Mt Giluwe, with *Platypus* species again being seen as a secondary factor. In the absence of any other widespread disturbance, such dieback is seen as necessary for *Nothofagus* regeneration. However, Read *et al.* (1990) suggested that past volcanic events may be responsible for the discontinuous size structures found in many *Nothofagus* populations; in other areas the size structures of the population implied continuous regeneration of *Nothofagus*.

A number of South American studies are also relevant: Veblen and Ashton (1978) studied the effects of so-called catastrophic influences (mass movement, vulcanism and fire) on the *Nothofagus*-dominated vegetation of the Valdivian Andes in Chile. It was apparent that the fast growing *N. dombeyi* and *N. alpina* forests were favoured by such disturbances, and were dependent on them for stand regeneration (Veblen *et al.* 1980, 1981), as was *N. pumilio* (Veblen *et al.* 1977). Armesto *et al.* (1992) reported that windstorms may be an

important force influencing regeneration of *N. pumilio*. *N. obliqua* also depends on partial or complete destruction of old-growth stands for regeneration (Veblen *et al.* 1979a). Such disturbances would be merely disasters in evolutionary terms (Harper 1977).

In the absence of volcanic disturbance, the continuously regenerating *N. botuloides* is replacing *N. pumilio* in high altitude stands in south central Chile (Veblen *et al.* 1977). At higher elevations, mixtures of *N. dombeyi* and *N. alpina* (montane zone), and *N. dombeyi* and *N. pumilio* (subalpine zone) are known to regenerate in gaps (Veblen 1985a). In northern Patagonia large-scale disturbances in the form of fire are important for the regeneration of *N. dombeyi.* However, in the absence of species which could replace it, *N. dombeyi* persists by gap-phase regeneration (Veblen and Lorenz 1987, Veblen 1989a). *N. nitida* appears to be continuously regenerating in gaps in *N. nitida/ Podocarpus* forests in southern Chile, and, in contrast to other mixed species *Nothofagus* forests in Chile, the forest canopy composition may be relatively stable (Innes 1992). Successional stage can also be important; during postglacial succession in Patagonia, moraines were first colonised by *N. betuloides,* followed by *N. antarctica* (Armesto *et al.* 1992).

Competition from understorey species strongly influences the regeneration pattern of *Nothofagus.* In both mid-elevation and subalpine *Nothofagus* forests in Chile, *Chusquea* bamboos inhibit seedling development of the dominant tree species. At mid-elevations *N. dombeyi* and *N. alpina* regeneration is dependant on stand-devastating disturbances; in subalpine forests *N. dombeyi, N. betuloides* and *N. pumilio* will also regenerate under canopy gaps where the less favourable microclimate reduces the relative dominance of bamboo (Veblen *et al.* 1979b; Veblen 1982).

In Australia, Porada (1980) reported a progressive crown dieback in *N. moorei* forests around Wauchope in New South Wales. This occurred in undisturbed stands as well as in selectively logged areas. Incidence of dieback was more apparent in the larger size classes, and there was a relationship between the percentage canopy removal, butt damage and dieback. The general forest structure was one of larger, even-aged *N. moorei* over a sub canopy of other species. This accords with the findings of Read and Hill (1985a, 1985b), that *N. moorei* does not regenerate continuously (except at high altitudes and latitudes) due to competition from other shade tolerant species. Once again, cohort senescence (Mueller-Dombois 1983b) probably predisposes these stands to dieback. Neither Howard (1973a) nor Porada (1980) found any evidence of *Platypus* in the area. However, in the latter case, the absence of *Platypus* appears to have been (wrongly) deduced from the presence of healthy coppice; a deduction which only holds when *Chalara australis* is present.

A BRIEF SUMMARY OF THE KNOWN CHARACTERISTICS OF MYRTLE WILT

There are similarities between myrtle wilt and other diseases caused by primary pathogens, such as Dutch elm disease and oak wilt (Howard 1973a, Elliott *et al.* 1987; Kile and Walker 1987). However, in *Nothofagus* forests myrtle wilt appears to be unique, being an often severe and sustained stand-level disease caused by a primary pathogen, and apparently unrelated to environmental stress (Kile *et al.* 1989).

Myrtle wilt - the disease

In summary, both Dutch elm disease and oak wilt are introduced through wounds by insect vectors, and are also spread through root grafts. The causal organisms belong to the *Ceratocystis* group and both asexual and sexual stages are involved. Both diseases kill regeneration as well as mature trees, and have increased their distribution in the recent past.

Myrtle wilt, in common with both Dutch elm disease and oak wilt, is caused by a member of the *Ceratocystis* group, and trees are infected through wound sites. Mortality rates were comparable with those of Dutch elm disease, and like oak wilt, myrtle wilt attacks a fagaceous host (Elliott *et al.* 1987; Kile and Walker 1987).

However, myrtle wilt appears to be unique in that disease spread occurs without an insect vector by means of air/water borne inoculum, and that only the asexual stage of the fungus is involved (Kile and Hall 1988; Kile and Walker 1987; Kile *et al.* 1989).

Key aspects which require clarification in this regard are the existence (or otherwise) of root grafts in myrtles, and their importance for disease spread; the importance of wounds as infection sites and the effect of myrtle wilt on myrtle regeneration. Information on the past and present distribution of the disease is also required.

Myrtle wilt - a dieback factor

Much of the available information on myrtle wilt can be used to begin an assessment of the role of the disease in the rainforest ecosystem (refer to Figure 1.4).

1. Myrtle wilt is an often severe and sustained stand-level disease caused by a primary pathogen (Kile *et al.* 1989). In Tasmania a recent survey of 20 undisturbed sites showed that on average 24.6% of standing myrtle were dead or dying from the disease, with 1.6% of the live trees currently dying due to wilt. All sites showed evidence of the disease (Elliott *et al.* 1987).

2. The average mortality rate that was estimated (1.6% pa), is comparable with the early stages of the last Dutch elm disease epidemic in Great Britain (Elliott *et al.* 1987). Myrtle wilt is known to be present in more remote areas (Jarman *et al.* 1984) but comparative data are lacking, as is any collated information on the origins and history of the disease.

3. The effect of myrtle wilt on the floristics of rainforest is largely unknown. Myrtle regeneration (less than 15 cm diameter) has been found beneath canopy gaps caused by wilt (Howard 1973a, 1981; Hickey 1982a), and larger trees are known to have a higher probability of the disease (Elliott *et al.* 1987). However, canopy and sub canopy trees have a similar disease incidence (Elliott *et al.* 1987), and myrtle wilt has occasionally been observed to kill myrtle seedlings (Kile *et al.* 1989). Gap phase regeneration is known from myrtle forests (Read and Hill 1985a) and it has been postulated that myrtle wilt gaps provide a regeneration niche for myrtle, the disease being in equilibrium with other ecosystem processes (Howard 1981; Jarman *et al.* 1984, Ellis 1985).

4. Disturbance has been observed to exacerbate the effects of myrtle wilt (Howard 1973a), although there are few replicated experiments. However, damaged trees are known to have a higher probability of the disease (Elliott *et al.* 1987).

5. The survey of Elliott *et al.* (1987) indicated relationships between myrtle wilt and a number of stand and environmental variables. Disease incidence:
   - was higher in callidendrous than in thamnic-implicate forests;
   - increased in mixed forests with both relative and absolute measures of myrtle density;
   - decreased with increasing altitude;
   - was higher for trees of larger diameter;
   - was higher for trees with stem and crown damage.

Clearly, further investigations are necessary in order to assess the significance and ecological role of myrtle wilt, and in order to recommend strategies for forest conservation and management. Mueller-Dombois (1983a) stated that a knowledge gap existed in the area of canopy dieback, because it fell between the realms of three disciplines; forestry, pathology and ecology. This project represents one attempt to bridge that gap.

PROJECT AIMS

The primary objectives of this project are to:

- collate the existing unpublished information on the rate of spread and impact of myrtle wilt;
- establish permanent plots to monitor rate of spread of disease in different rainforest types;
- record the extent of the distribution of myrtle wilt in Victoria;
- undertake disease assessments in Tasmanian areas remote from human disturbance;
- test the effects of wound age and chemical treatments on infection rates;
- look for root grafting in myrtles, and if present to assess its effect on disease spread;
- investigate the rate of spread of myrtle wilt in relation to the extent and type of disturbance in rainforest;
- study the impact of myrtle wilt on the floristics of rainforest;
- investigate the role of myrtle wilt in the regeneration process of myrtle.

FIELD SITES

Figure 1.5 gives the locations of the Tasmanian study sites mentioned in the text.

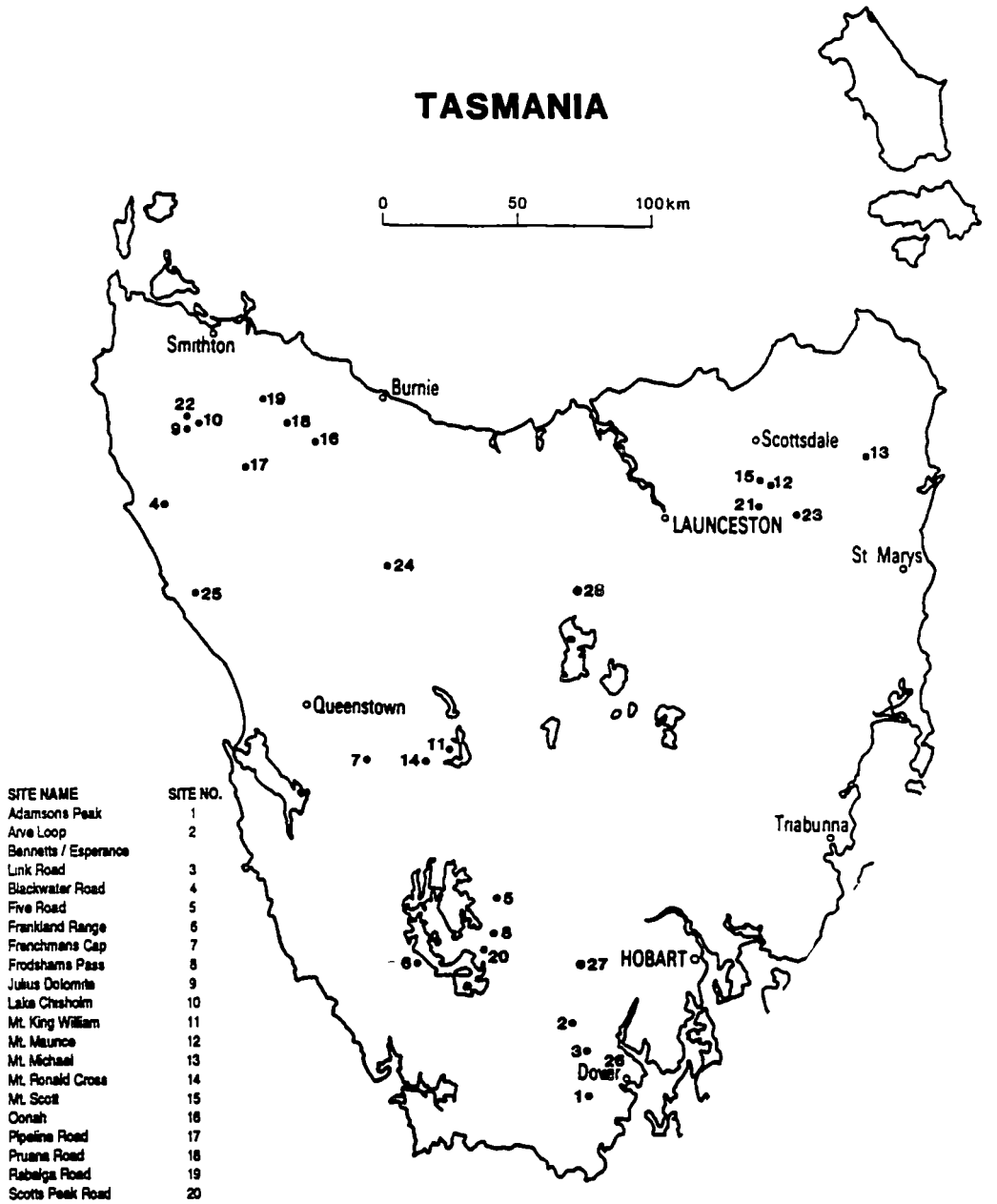


Figure 1.5 Location of study sites in Tasmania.

(Map produced by Forestry Tasmania.)

(Sites 10, 11, 21, 23 & 25 are those of Elliott *et al.* 1987.)

2. MYRTLE WILT IN SPACE AND TIME

2.1 INTRODUCTION

A major problem of dealing with a disease which was reported only 20 years ago is the lack of accurate historical data on its occurrence, distribution and abundance, and the resultant lack of perspective with which the disease is viewed. In short we do not know how long it has been present or whether its severity is increasing (Elliott *et al.* 1982), although both *Platypus subgranosus* and *Chalara australis* are believed to be indigenous to Australia (Hogan 1944; Kile and Walker 1987).

Early Tasmanian references to myrtle

The early Tasmanian literature abounds with references to myrtle and myrtle forests, and these bear investigation. Burns and Skemp (1961) in their record of the Van Diemens Land correspondence of J. D. Hooker , cite letters of R. C. Gunn and A. Cunningham, written 1838-49, which note its presence at Black River near Circular Head, along the Hobart Rivulet, at Macquarie Harbour, and in the vicinity of the Franklin River; also a description, by Gunn, of a myrtle forest from north-east Tasmania. Hooker (1860) later summarised that myrtle was 'common in mountainous and western humid districts, forming a large proportion of the forest'.

Burn (1855) recorded the overland journey of Sir John and Lady Franklin from Hobart to Macquarie Harbour in 1842, following the track cut previously by Calder, and his account contains numerous descriptions of myrtle and myrtle forests: south of Mt Cheyne; at Surprise River, King River, Painters Plains, and at Loddon River; west, south-west of Fatigue Hill; at Bagota Fall and in the Acheron Valley; at Black Forest and in the Franklin Valley.

The reports of G. S. Perrin, the first Tasmanian Conservator of Forests are of particular relevance. He mentioned the use of myrtle on the Mt Bischoff tram line (Perrin 1886b), and after a trip from Waratah to Macquarie Harbour and the West Coast in 1886, described the quality of the myrtle forests on the summit of the Magnet Range and along the Corinna track south of Corinna. East of Strahan he noted that the two-year-old, gravelled Government road passed through a myrtle forest, and myrtle was also recorded from the Queen River area and the Linda Valley (Perrin 1886a).

Later in 1886 Perrin made an expedition to the La Perouse Ranges, the Lune Valley and towards the Picton River, and mapped the type and condition of the forests in the area

(Perrin 1887). Myrtle was evidently widespread and was specifically mentioned in the Picton valley. One note is of interest: 'Considerable numbers of dead King William pine are met with of both species, *Athrotaxis selaginoides* and *A. cupressoides;* the former showing their larger trunks - bare, bleached skeletons glistening white in the sun, and shining at intervals with a silvery lustre among the dark green myrtles and scrub; a few live trees are met with, together with stunted specimens of the latter species, but the majority are dead, probably killed by the great frosts of 1837.' In the same year he recorded the export of myrtle to London, from Burnie and Hastings (Perrin 1887).

In 1887 Perrin accompanied the Deputy Surveyor General and Deputy Commissioner of Lands, C. P. Sprent on a trip from Hobart, via Ouse, Lake St. Clair, Mt Arrowsmith, the Collingwood Valley, Mt Lyell and the Queen River, to Strahan and Macquarie Harbour. The party included J. B. Walker and W. V. Legge. From Lake St Clair to the Linda goldfield they followed the new Linda Track, which had been cut by T. B. Moore four years earlier (Perrin 1887; Walker 1993).

Perrin (1887) recorded that at Mt Arrowsmith (and along the mountain chain), the eucalypt forests gave way to the myrtle forests of the West Coast, this being coincident with a change in geological characteristics. He also noted: 'The descent from Mt Arrowsmith is tolerably steep, but a good roadway has been cut into the gravel round the sides of the mount.' The party later crossed the newly completed bridge over the Franklin and stayed at the road party's camp near the Upper Collingwood. Myrtle forests were reported from the area of the Surprise, Franklin, Collingwood and Cardigan Rivers. He concluded; 'Myrtle is the prevailing feature of the timbered lands, and exists in immense quantities.'

Walker (1887) noted myrtle scrub below Mt Gell, and myrtle forests on the lower slopes running down into the Franklin River gorge. Legge (1887) specifically mentioned myrtle groves on the western shore of Lake St Clair, near Mt Rufus and at the foot of Mt King William and noted the prominence of myrtle along the rest of the route to the West Coast, and compared this with the solitary specimens seen around Mt Wellington. Of particular note is his description of the Mt King William forest: 'a splendid beech grove, in which I measured a monarch of the forest which was 27 feet in girth.'

On subsequent trips Perrin reported myrtle from the area of Russells Falls River and Marriotts Look-out (Perrin 1889), and from Schnells track on the north side of the River Huon (Perrin 1890). In a later summary report he mentioned the large myrtles of the Pieman

Valley being extensively used in the mining fields; also patches of myrtle about Mt Lyell, Strahan and Zeehan (Perrin 1898).

W. T. H. Brown, Perrin's successor, in trips to the north-east, recorded a dense covering of myrtle at Mt Maurice, also noting it at Diddleum and along the track in the vicinity of Mt Barrow, with large, good quality myrtle timber found east, south-east of Mt Albert (Brown 1890). He also recorded a collection of ornamental woods (including myrtle) to be sent to London as an experiment (Brown 1891).

By the beginning of this century, the properties of myrtle timber were well known in Tasmania, although relatively little had been exported. The railway from Emu Bay to Zeehan, which passed through much myrtle forest, was thought likely to improve its prospect of utilisation (Green 1903), although the export situation appeared little changed by 1920 (Counsel 1920).

The new Conservator of Forests noted of myrtle: 'It also possesses the advantage of being gregarious, and large forests are available for exploitation' (Irby 1920). He reported: 'Next to the eucalypts this is the most abundant tree in Tasmania. It predominates in the forests of the West Coast, and is plentiful in the north-west and north-east.' He also noted that it did not appear to regenerate after fire (Irby 1921).

Rodger (1928) also recorded it as being 'very susceptible to fire, and the most successful regeneration is consequently found where the canopy has been broken and the ground exposed without the agency of fire, as in old road cuttings.' However he concluded: 'It is, however, very faulty in the log... To grow into sound timber, it apparently requires fair soil at a fair elevation, say over 800 feet. Consequently, there are very few areas which are economical milling propositions.'

However, even by 1923, Irby was arguing the case for a pulp and paper industry for the State, based on the suitability for pulping of a number of eucalypt species and myrtle. The first pulpwood concession area, granted in 1924, was crown land in the vicinity of the Burnie to Zeehan railway, now held by Associated Pulp and Paper Mills Ltd (APPM). Other concessions followed during the 1920s and 30s and the Tasmanian industry was established. Ground surveys made at this time of the remote South Arthur forests (north-west Tasmania), gave brief stand descriptions but apparently no references to myrtle dieback (Mesibov 1978).

During the 1960s and 70s, woodchip exports to Japan led to an escalation in logging activity. Of particular note, APPM was given permission to export chips from its pulpwood concession area and this commenced in 1972-3 (Carron 1985). Until 1973 references to the orange/brown myrtles or groups of dead myrtles typical of myrtle wilt, are, in retrospect, conspicuous by their absence.

The recent history of myrtle wilt in Tasmania

The first record of myrtle wilt was made by Howard (1973a) in the Surrey Hills area, in the APPM concession. She reported: 'In undisturbed forest, the death of a single tree is often followed year by year, with the death of surrounding trees, of all ages and sizes (excluding poles and saplings with a diameter of less than six inches). Following disturbances, typically by cull felling, or access road construction, which create large gaps in the canopy, rapid and widespread deaths occur of a large number of trees.' At that time she noted *Platypus* infestation (and by inference myrtle wilt) as having been observed in north-west and western Tasmania.

Mesibov (1978) made a pilot study of rainforest decadence, comparing black and white aerial photographs of the South Arthur rainforest taken in 1946, 1952, 1971-2 and 1976. Whilst it was not possible to estimate the rate of loss of myrtle crowns, dead and dying myrtles were clearly identifiable in the area at each date. Colour aerial photography of forestry areas in Tasmania was not used until 1972 (D. Boyer, personal communication).

Observations made in the late 1940s of the McPartlans Pass area and the route to Federation Peak (via the old Port Davey track), and those made in the early 1950s of the Florentine Valley, indicated occasional myrtle deaths. Dying myrtles did not become noticeable in the Florentine until the mid 1950s, several years after roads were constructed. However, when the Burma (Styx) Road was constructed c.1960, dieback was immediately apparent (W. D. Jackson, personal communication). Observations of the Mt Maurice area in the 1960s indicated the absence of myrtles with orange/brown foliage (characteristic of wilt) until the 1970s, after the extensive opening up of the surrounding area for logging activities (R. C. Ellis, personal communication). Isolated myrtles dying of wilt have been observed from the air in NW Tasmania since 1976-7 (M. Brouder, personal communication).

Howard (1981), reported the absence of *Platypus* (and, by inference, myrtle wilt) in some areas of Tasmania which had marginal rainfall for myrtle, the implication being that the disease was present in the other myrtle forests of Tasmania. Elliott *et al.* (1982) reported myrtle wilt as occurring throughout the range of myrtle in Tasmania, although the only

specific site mentioned was the Arve Valley. However, in the mid 1980s, Elliott *et al.* (1987) investigated 20 undisturbed sites throughout Tasmania, and in each case found evidence of myrtle wilt. Also, Neyland (1991) and Neyland and Brown (1994) reported myrtle wilt in some remnant rainforest patches in eastern Tasmania (areas of marginal rainfall for myrtle).

Myrtle wilt in remote areas of Tasmania

The questions of the origins of myrtle wilt and of its present distribution in remote areas are related, but while the former can only be answered partially using circumstantial evidence the latter can be investigated.

Although myrtle wilt has traditionally been viewed as a problem of disturbed areas (Howard 1973a; Elliott *et al.* 1982; Hickey and Felton 1991), Elliott *et al.* (1987) recorded its presence in undisturbed sites, and it has also been noted in more remote areas (Jarman *et al.* 1984; Working Group for Rainforest Conservation 1987, Kile and Walker (1987). Since its history and details of long distance inoculum spread are still unclear, it is obviously of interest to ascertain the presence or absence and levels of myrtle wilt in a number of sites remote from any human disturbance. The present study explores some possible methods.

The Victorian situation

In Victoria the distribution of myrtle is discontinuous, and limited to sites in the Central Highlands, the Otway Ranges and the Strzelecki Ranges, with some isolated occurrences on Wilsons Promontory (Cameron 1992).

In the late 1960s and early 70s a comprehensive study of myrtle was undertaken in these areas (Howard and Hope 1968; Howard 1973b, 1973c, 1973d; Howard and Ashton 1973). In contrast to the Tasmanian situation, in Victoria no *Platypus* infestation was found during this four-year study (Howard 1973a), and it was still reported as absent in 1981 (Howard 1981). However, Weste (1975) reported myrtle dieback from the slopes of Mt Donna Buang in the Central Highlands. This was investigated and found to be unrelated to *Phytophthora cinnamomi* Rands. It was assumed to be due to exposure and *injury* as a result of major *road works* (my emphasis).

Photographs dating from 1980 (or earlier), indicate the presence of myrtle wilt at the Binns Road landslip site in the Otways (I. Roberts, personal communication), and in 1980 *C. australis* was successfully isolated from a dying myrtle along Youngs Creek Road in the Otways (G. A. Kile, unpublished data). In 1980/81 myrtle wilt was recorded from a number of Otways' sites, using aerial photographic interpretation surveys (P. McHugh, unpublished

data). Elliott *et al.* (1982) noted it as occurring throughout the range of myrtle in Victoria, although the only location mentioned was a trial site in the Otway Ranges. Kile and Walker (1987) also reported the disease from the Otway Ranges. Brinkman and Farrell (1990) noted it as widespread within the myrtle forests of the Otways, although the full extent and rate of spread of the disease were not known (DCE 1991).

Cool temperate rainforest in the Otways forms an open forest, and this has been attributed to past disturbance, and to 'beech dieback' (Brinkman and Farrell 1990). Cameron (1992) wrote 'with the demise of mature *Nothofagus* throughout the Otways as a result of the current epidemic of myrtle wilt, an increasing proportion of the Otway rainforests remain dominated only by blackwood (*Acacia melanoxylon*).' No evidence of the disease was found in some of the more accessible myrtle stands at Wilsons Promontory (Seales Cove Track and Ferny Glade), when visited in 1992 (S. Gumley, personal communication). In Paradise Valley on the eastern side of Wilsons Promontory, there were no trees obviously dying of myrtle wilt in 1993 (T. W. May, personal communication).

At the start of this project the presence and extent of the disease in the remainder of Victoria's myrtle forests were unknown. The survey of myrtle wilt in the Central Highlands and Strzelecki Ranges (Packham and Kile 1992) was conducted as part of the following study.

Hypotheses

The history and distribution of myrtle wilt in south-east Australia are unclear. However, the apparently numerous possibilities derive from three basic hypotheses:

- The disease has long been present, but levels have recently changed (increased);
- The disease has long been present, but there has been a recent change (increase) in its distribution;
- There has been an introduction/evolution of a new disease/strain in recent times, with a spread from its point of origin.

These possibilities will be discussed in the light of all the available evidence.

2.2 MATERIALS AND METHODS

Tasmanian remote sites

Surveys were carried out in four areas remote from human disturbance: Mt Ronald Cross, the Frankland Range, Adamsons Peak and Frenchmans Cap. Since access was by helicopter, time, both on the ground and in the air, was limited. Thus for every survey a

consistent minimum amount of information was recorded, i.e. the number of observed myrtles which were healthy, dying of myrtle wilt, or dead. With the later surveys, it was possible to collect additional information as expertise was developed.

Disease and damage assessments were based on the system of Elliott *et al.* (1987), given in Tables 2.1 and 2.2. These health status classes were modified to suit the different survey techniques, as is outlined below. However, in all cases the percentage of damaged myrtles, and the percentage of myrtles currently dying of myrtle wilt, were each calculated as a proportion of the number of *live* myrtles; the percentage of diseased myrtles (myrtle wilt), and the percentage of dead and dying myrtles (all causes), were each calculated as a proportion of the number of *standing* myrtles (includes dead, standing trees). Details of survey locations are given in Appendix 1.

Table 2.1 Health status classes (HSC)

1. Healthy
2. Healthy, with old *Platypus subgranosus* attack on lower stem
3. Healthy, but with current *P. subgranosus* attack (frass accumulation on lower stem)
4. Dying (orange/brown foliage retained on the tree) with current *P. subgranosus* attack
5. Dead <3 years (fine twigs remaining on the tree); *P. subgranosus* attack (old or current) present
6. Dead >3 years (main branches only remaining on the tree); *P subgranosus* pin holes present on lower stem
7. Dead, but with no evidence of *P. subgranosus* attack
8. Tree alive but with major crown dieback, cause unknown

Table 2.2 Damage classes (DC)

1. No damage
2. Broken limbs in crown
3. Stem damage
4. Broken limbs in crown and stem damage

Tasmanian remote sites - ground surveys

Mt Ronald Cross

The undisturbed rainforest/scrub myrtle forest on the western slopes of Mt Ronald Cross was visited and the health status of myrtles of more than 15 cm (approximate) diameter at breast height (DBH) along an estimated 10 m x 225 m transect recorded. Health status classes are given in Table 2.3.

Table 2.3 Modified health status classes used in the Mt Ronald Cross ground survey and the Adamsons Peak aerial transects.

1.	Healthy
4.	Dying with orange/brown foliage (characteristic of myrtle wilt)
5.	Dead <3 years (fine twigs remaining on the tree)
6.	Dead >3 years (main branches only remaining on the tree)

Frankland Range

The implicate rainforest (I1.1 after Jarman *et al.* 1991) to the north-east of Orb Lake in the Frankland Range was visited. The health and damage status (classes defined in Tables 2.1 and 2.2 respectively) and DBH of myrtles (of more than 15 cm DBH) within two parallel 10 m x 150 m transects (centre-lines 50 m apart) were recorded, the transects being subdivided into 10 m contiguous sections.

Tasmanian remote sites - aerial transects

Frankland Range

Two aerial transects were flown over the Frankland Range using the method below. A horizontal line marked on a transparent sheet was taped to the helicopter window. This had been calibrated previously to define a 20 m wide transect at a height of 50 m. The pilot flew at an estimated 50 m above canopy level, at a slow speed (20-30 knots where possible), and the position of the transect was marked on the map. The health status of all mature myrtle trees falling within the transect was recorded both on cassette and by an assistant, using the simplified system outlined in Table 2.4.

Table 2.4 Modified health status classes used in the Frankland Range aerial transects.

1.	Healthy
4.	Dying with orange/brown foliage (characteristic of myrtle wilt)
6.	Dead

A ±20 m x 600 m transect was flown over rainforest to the north-east of Orb Lake in the Frankland Range. The lower part of this transect covered approximately the same area as the ground survey.

A ±20 m x 700 m transect was flown over rainforest to the north, north-east of Sanctuary Lake in the Frankland Range.

Adamsons Peak

Following work in the Frankland Range the following modifications were incorporated into the method which was then used for two aerial transects in the Adamsons Peak area.

- Slow helicopter speeds (20-30 knots) were maintained by flying up valleys and by contour flying;
- A piece of card, with a slit cut out of it to define the transect, was taped to the helicopter window, replacing the transparent sheet. The slit had been calibrated previously to define a 20 m wide transect at a height of 50 m. The width of the slit was adjustable and the card served to black out trees not on the transect and aid counting;
- Trees dead less than three years (fine twigs present) were easily distinguishable from those dead more than three years and were recorded separately. The modified health status classes used were thus identical to those given in Table 2.3.

A ±20 m x 4.7 km transect was flown over rainforest to the south of Adamsons Falls on the slopes of Adamsons Peak.

A ±20 m x 2.1 km transect was flown over rainforest in the Creekton Rivulet gully on the slopes of Adamsons Peak.

Frenchmans Cap

Since it was by this time deemed possible to distinguish trees which had major crown dieback (cause unknown) from healthy trees and those dying of myrtle wilt, the health status

classes used for the four sets of aerial transects in the Frenchmans Cap area, were again modified and are given in Table 2.5.

Table 2.5 Modified health status classes used in the FrenchmansCap aerial transects

1.	Healthy
4.	Dying with orange/brown foliage (characteristic of myrtle wilt)
5.	Dead <3 years (fine twigs remaining on the tree)
6.	Dead >3 years (main branches only remaining on the tree)
8.	Tree alive but with major crown dieback, cause unknown

Two ±20 m wide transects were flown over rainforest east of Lake Gertrude in the Frenchmans Cap area. The first was 600 m long, and the second 500 m long. The results of the two were combined.

A ±20 m x 1.4 km transect was flown over rainforest to the south-west of Pine Knob in the Frenchmans Cap area.

A ±20 m x 800 m transect was flown over rainforest in a gully south-east of Lake Millicent in the Frenchmans Cap area.

Two ±20 m wide transects were flown; the first over rainforest east of Lake Cecily; the second to the north-west of the lake, up the side of Frenchmans Cap. The first transect was 450 m long, and the second 1.1 km long. The results of the two were combined.

Remeasurement of a 26-year-old transect at Mt Maurice, north east Tasmania

Grid Ref (1:100 000): Forester 508252 506250

Altitude: 930 m

On 18 May 1964 a transect was established on the slopes of Mt Maurice. It was 20.1 m (one chain) wide and 241.4 m long and ran from (old growth) callidendrous myrtle forest (C2.1 after Jarman *et al.* 1991) through areas of regrowth myrtle and open grown myrtle (myrtle woodland) into an area dominated by *Eucalyptus delegatensis*. The DBH of trees and shrubs was measured and the transect was mapped in detail, including the position of dead standing trees, stumps and logs (R. C. Ellis, unpublished data).

At the time of establishment the surrounding district had not been opened up to extensive logging activities although there had been selective logging of rainforest along the nearby Ben Ridge Road, Bennett Road and Ding Dong Spur from the late 1950s, and there was some selective logging of eucalypts at the end of the transect in 1962. In 1964 there was no evidence of extensive myrtle dieback in the Mt Maurice area, and there were no currently dying myrtles, with the characteristic orange/brown foliage (i.e. HSC 4), on the transect at the recording date. Myrtle wilt apparently only became an obvious problem after the extension of Ben Ridge Road and the extensive logging of eucalypts for sawlogs and pulpwood which occurred after 1972 (R. C. Ellis, personal communication).

On 13-14 March 1990 the first 140.1 m of the transect were remeasured omitting the area of eucalypt forest. All myrtles dead or alive and greater than 15 cm DBH in 1964 were relocated where possible and classified as:

- live;
- dead standing/stumps (DS);
- not found/windthrown (NF/WT).

Platypus subgranosus attack was noted as an indicator of myrtle wilt. The disease incidence (cumulative dead and dying trees) was calculated for the 1964 and 1990 measurements, and the mean mortality rate per annum attributed to myrtle wilt was calculated.

Tasmanian aerial photography

Effectiveness of black and white photographs

The work of Mesibov (1978) showed that aerial photographs had the potential to indicate the presence of myrtle wilt in a given area. To investigate this, recent colour aerial photographs were used; the positions of orange/brown myrtles dying of myrtle wilt (HSC 4), and dead myrtles with fine twigs (HSC 5), which appeared white, were noted. Black and white reproductions of the photographs were then examined to see if any of these trees were still clearly identifiable.

Early surveys of main rainforest areas

The earliest black and white aerial photographic coverage of most of the rainforest areas in Tasmania dates from the 1940s and 50s. With the aid of a rainforest distribution map (Hickey *et al.* 1993), map sheets representative of the main rainforest areas were selected. For each mapsheet, several flight runs were chosen and photographs showing areas of possible myrtle forest were investigated using a stereoscope. Myrtles with crowns which appeared light grey (HSC 4) or white (HSC 5) were noted. Details of the photographs are given in Appendix 2.

Recent surveys of remnant rainforest patches in eastern Tasmania

An investigation was made of the larger remnant rainforest patches in the east and north-east of Tasmania. In this lower rainfall region the distribution of myrtle is generally restricted to the highlands, riparian communities, and coastal areas where 'cloud tapping' occurs. Recent colour aerial photographs were used with a stereoscope. Dying (HSC 4) and recently dead (HSC 5) myrtles were noted. Details of the photographs are given in Appendix 2.

Victorian ground reconnaissance

The objectives of these surveys were:

- Reconnaissance (ground inspection) of *Nothofagus cunninghamii* stands in central Victoria to determine if myrtle wilt was present and if so its approximate incidence and distribution;
- Reconnaissance (ground inspection) of a number of stands in the Otways containing *N. cunninghamii,* to assess the incidence levels of myrtle wilt in various situations.

A number of sites were visited in the Central Highlands and the Strzelecki Ranges with the aim of sampling the edges of the *N. cunninghamii* distribution (Figure 2.1). When characteristic 'myrtle wilt' (HSC 4) trees were found, surrounding trees were carefully observed for early symptoms of the disease. Sites of different forest types and ages were visited in the Otways (Figure 2.2). Forest type, disturbance history, myrtle wilt incidence and myrtle regeneration status were noted.

Sampling sites were all accessible by car, and site details are given in Appendix 3. The health status classes used in this study are given in Table 2.1. It should be noted that the lack of trees in HSC 4 does not definitely mean the total absence of wilt, since trees in HSC 3 are infected but appear healthy from a distance. Dead trees with old *P. subgranosus* attack (HSC 5 & 6) are indicative but not sure proof of old wilt infections.

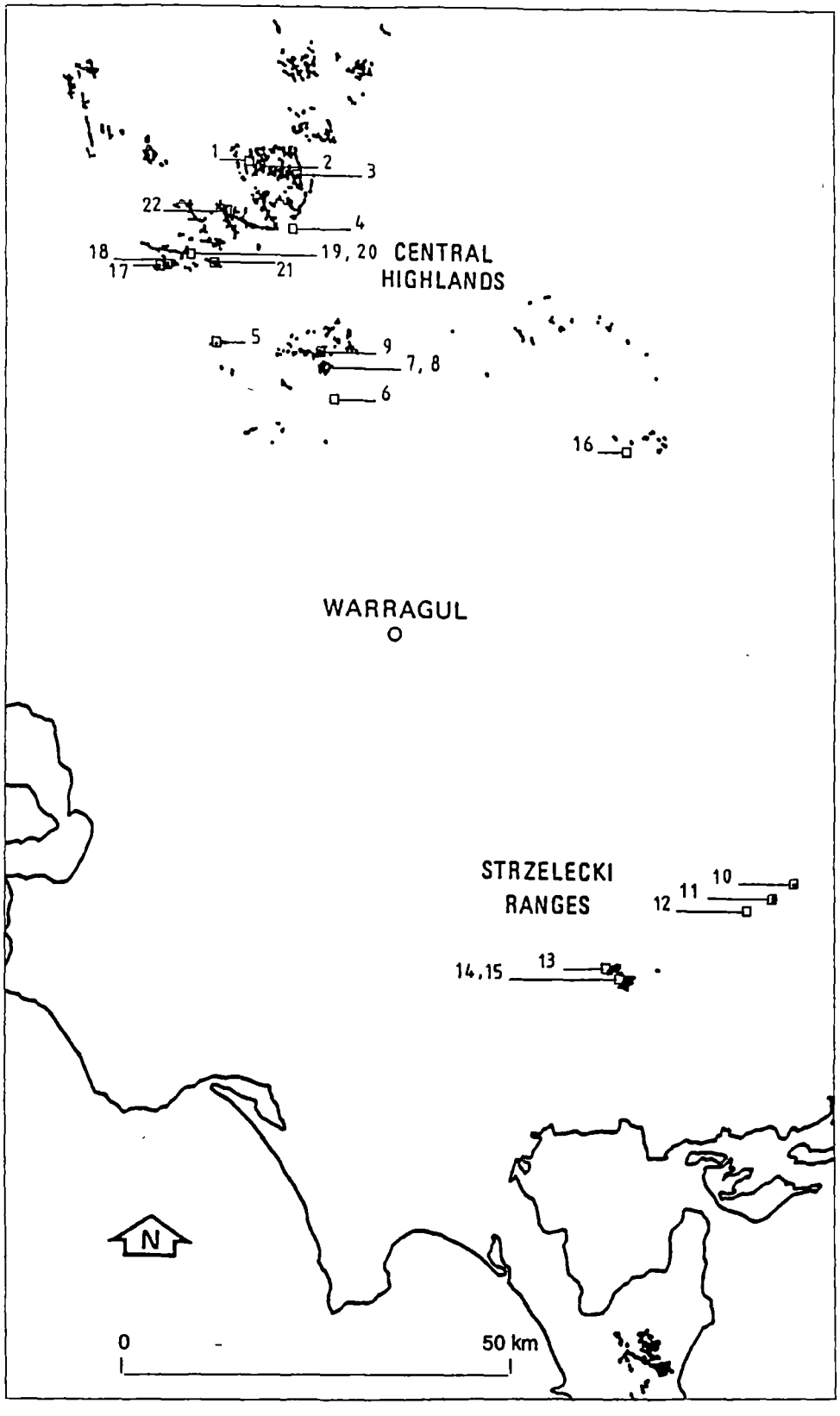


Figure 2.1 Sampling sites in the Central Highlands and Strzelecki Ranges of Victoria.

(Map produced by the Department of Conservation and Natural Resources, Victoria.)

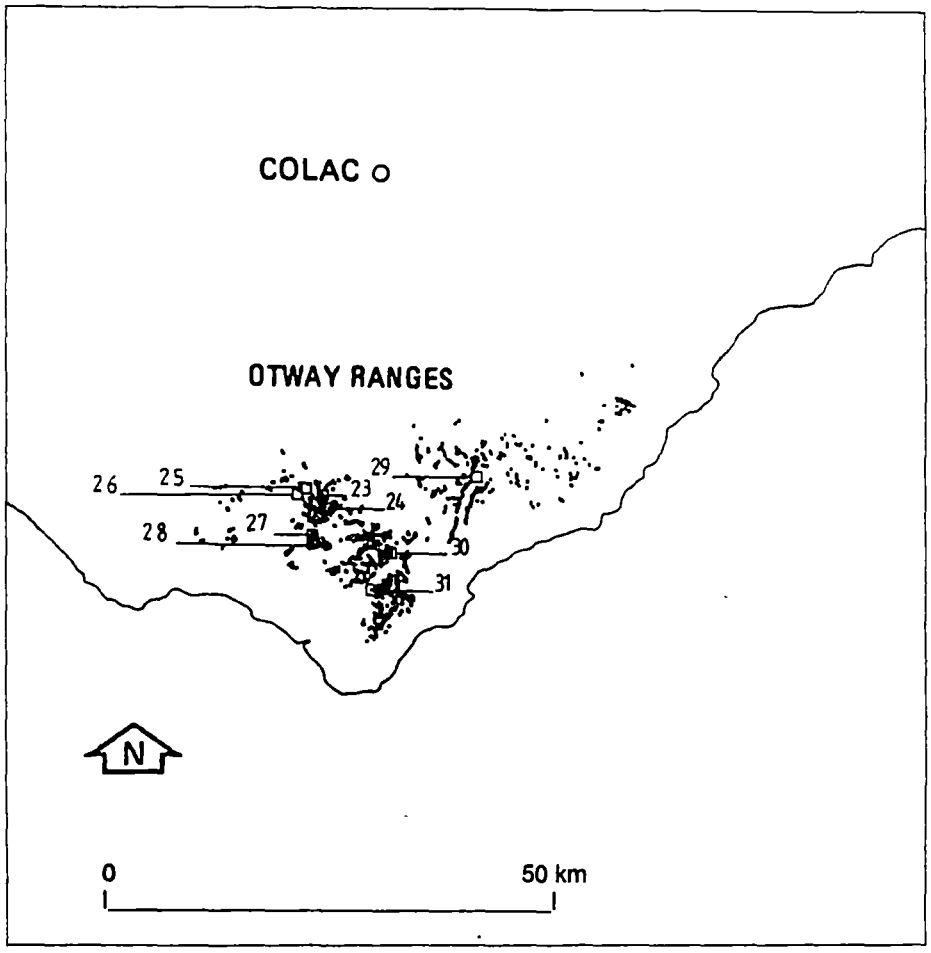


Figure 2.2 Sampling sites in the Otway Ranges of Victoria.

(Map produced by the Department of Conservation and Natural Resources, Victoria.)

2.3 RESULTS

Tasmanian remote sites - ground surveys

Mt Ronald Cross

Myrtles in the forest on the slopes of Mt Ronald Cross were commonly ±20-30 cm diameter. *P. subgranosus* attack was evident when some of the dead trees were closely examined. Several dying trees with the red/brown foliage characteristic of wilt were observed from a distance.

Forty eight percent of the myrtles on the transect were dead and all but one of these had been dead more than three years. No dying trees were encountered along the transect (Table 2.6). One small myrtle on the top of Mt. Ronald Cross was dying back. It had stem damage, but no evidence of *P. subgranosus* attack and the cause seemed unlikely to be myrtle wilt.

Table 2.6 Health status of myrtles in the Mt Ronald Cross ground survey

No. of myrtles in HSC				Totals	%Dying of wilt	%Dead & dying
1	4	5	6		(HSC 4/1-4)	(HSC 4-6/1,4,5&6)
12	0	1	10	23	0%	47.8%

Frankland Range

Results of disease and damage assessments of myrtles on the Orb Lake transect are given in Tables 2.7 and 2.8 respectively. No trees currently dying of myrtle wilt (HSC 4) were encountered on the transect although one was observed further up the valley.

Table 2.7 Disease incidence in the Frankland Range (Orb Lake) ground survey

No. of myrtles in HSC								%Dying of wilt	%Diseased	%Dead & dying
1	2	3	4	5	6	7	8	(HSC 4/1-4)	(HSC 3-6/ 1-8)	(HSC 3-7/1-8)
71	1	0	0	0	3	15	2	0%	3.2%	19.5%

Table 2.8 Recent damage in the Frankland Range ground survey

No. of myrtles in damage class (DC)				% Damaged
1	2	3	4	(DC 2-4/1-4)
71	2	1	0	4.1%

Tasmanian remote sites - aerial transects

Frankland Range

The health status of trees on the Frankland Range transects are given in Table 2.9. Trees dying of myrtle wilt (orange/brown foliage) were observed in both areas, although there were none recorded on the transect at Sanctuary Lake.

Table 2.9 Health status of myrtles on the Frankland Range aerial transects

Site	No. of myrtles in HSC			Totals	%Dying of wilt	%Dead & dying
	1	4	6		(HSC 4/1&4)	(HSC 4&6/1,4&6)
Orb Lake	73	1	7	81	1.4%	9.9%
Sanctuary Lake	37	0	4	41	0%	9.8%
Mean for Frankland Range					0.9%	9.8%

Adamsons Peak

The health status of trees on the Adamsons Peak transects are given in Table 2.10.

Table 2.10 Health status of myrtles in the Adamsons Peak aerial transects

Site	No. of myrtles in HSC				Totals	%Dying of wilt	%Dead & dying
	1	4	5	6		(HSC 4/1&4)	(HSC 4,5&6/1,4,5&6)
Adamsons Falls	134	13	22	47	216	8.8%	38.0%
Creekton Rivulet	128	7	15	29	179	5.2%	28.5%
Mean for Adamsons Peak						7.1%	33.7%

Frenchmans Cap

The health status of trees on the Frenchmans Cap transects are given in Table 2.11.

Table 2.11 Health status of myrtles on the Frenchmans Cap aerial transects

Site	No. of myrtles in HSC					Totals	%Dying of wilt	%Dead & dying
	1	4	5	6	8		(HSC 4/1,4,&8)	(HSC 4,5&6/1,4,5,6&8)
Lake Gertrude	59	4	3	3	0	69	6.4%	14.5%
Pine Knob	42	0	5	11	0	58	0%	27.6%
Lake Millicent	82	1	4	7	0	94	1.2%	12.8%
Lake Cecily	90	0	4	29	2	125	0%	26.4%
Mean for Frenchmans Cap							1.8%	20.5%

Remeasurement of a 26-year-old transect at Mt Maurice, north-east Tasmania

The disease incidence in 1964 and 1990 and the average annual mortality rate due to myrtle wilt from 1964 to 1990 is given in Table 2.12. A distinction has been made between the total of dead and dying trees and those diseased with myrtle wilt, *Platypus* attack being used as an indicator of wilt. The data set is given in Appendix 4.

Table 2.12 Disease incidence and mortality rates due to wilt along a 26-year-old transect at Mt Maurice

Site	% Dead & Dying		% Diseased		Mortality % p.a.	
	1964	1990	1964	1990	Total	Wilt
Old growth myrtle	6.7%	13.8%	≥3.4%	13.8%	0.41%	0.41%
Regrowth myrtle	1.6%	14.3%	1.6%	7.1%	0.77%	0.19%
Myrtle woodland	8.7%	13.0%	8.7%	13.0%	0.18%	0.18%
Transect totals	4.4%	13.9%	≥3.5%	10 2%	0.56%	0.25%

Tasmanian aerial photography

Effectiveness of black and white photographs

A proportion of the myrtle wilt affected trees noted from the colour photographs were also clearly identifiable on the black and white reproductions. Myrtles dying of myrtle wilt (HSC 4) appeared as pale grey, and dead myrtles with fine twigs (HSC 5) appeared white. They were distinguishable from trees with other, more gradual crown diebacks (HSC 7 and HSC 8), since the crowns were of a uniform tone.

Early surveys of main rainforest areas

Dead and dying myrtles indicative of myrtle wilt were found on 1940s and 50s aerial photographs of all the main rainforest areas surveyed; i.e. in the north-west, west and south of the State. The disease was also apparent in the north-east; both in the North East Highlands, and in some remnant stands near the Great Musselroe River. However, no myrtle wilt was observed in the Douglas-Apsley area of eastern Tasmania.

Recent surveys of remnant rainforest patches in eastern Tasmania

Aerial photographs showed up dead and dying myrtles, indicative of myrtle wilt, in all the main areas of remnant rainforest surveyed in the north-east, in the 1980s and 90s (including the Douglas-Apsley area). However, no myrtle wilt was observed from the Tasman/Forestier Peninsula area of south-east Tasmania.

Victorian ground reconnaissance

Central Highlands

Wilt levels in the O'Shannasy Catchment (restricted public access) were low with most dying trees, (HSC 3/4) observed being on the roadside. Some were damaged, probably by the road slasher. One site had no active wilt (HSC 4) but there was evidence of earlier infections (HSC 5/6) at all sites.

In the Brittania Creek and Ada Valley areas there was no evidence of active wilt, but there was evidence of earlier infections. In the area of Charlie Creek a number of roadside trees were dying (HSC 4), several being damaged.

In the Mt. Erica area, the site visited had recently been roaded and there were several damaged roadside trees dying of wilt. However, other apparently undamaged trees >100 m from the road were also infected. There was no obvious evidence of earlier infections.

The Mount Donna Buang, Cement Creek and Acheron Way sites mostly showed evidence of current wilt (HSC 4). These trees were largely restricted to the roadside and often were damaged. There was no obvious evidence of earlier infections.

Strzelecki Ranges

No evidence of current wilt was found in the Tarra or the Bulga National Parks. There were a few distant, old dead stags but these were not examined for evidence of earlier infections.

More surprisingly, no evidence of past or present wilt was found in the disturbed Middle Creek area. There was, however, some crown dieback in the myrtles which was probably due to *Biscogniauxia nothofagii* , a fungus which is believed to invade stressed trees (Whalley *et al.* 1990).

When no evidence of wilt was seen along the Grand Ridge Road, it seemed possible that the whole of the region was free from the disease. However, two neighbouring infection sites were found on the Toora-Gunyah road and it seems that at least one was related to illegal cutting activities: one myrtle had been felled and neighbouring trees were infected. Both the Agnes and Franklin catchments were affected.

Otway Ranges

Most areas surveyed were long, narrow gully communities which often had a disturbance history and/or were vulnerable to damage from neighbouring logging operations or branch shedding by ridge-top eucalypts.

Myrtle wilt incidence was generally high in areas which supported mature myrtles, except where they composed a remnant in a largely regrowth forest. In such cases, the numbers and density of myrtles were low.

Myrtle regeneration was variable, particularly with regard to the presence of older regeneration (poles/saplings). However, only at one site was there a complete absence of myrtle regeneration; in this case a few, remnant, multi-stemmed myrtles stood at the edge of the forest which bordered cleared land.

2.4 DISCUSSION

Tasmanian remote sites

The work on Mt. Ronald Cross indicated that myrtle wilt was present in the forest, as was *P. subgranosus*. With the time available it was only possible to sample a limited number of trees and not possible to closely investigate the likely cause of death of the dead trees (and this was also the case for all the aerial transects). However, the recent survey of Elliott *et al.* (1987) in less remote sites found that only 2.6% of standing, dead myrtles (0.6% of all myrtles), had no evidence of *P. subgranosus* attack. If the same were to be true of this forest then the disease incidence in terms of cumulative dead and dying trees of 48% would be high in comparison with the 24.6% mean found by Elliott *et al.* (1987).

Possible causes could be the effects of fire in nearby forest increasing disease and inoculum levels, and high wind damage due to the exposed position at the forest edge. However the small sample size prevents a direct comparison, particularly as the diseased trees appeared to be clumped.

Validation of aerial transect methods

Comparing the results of the Orb Lake ground survey (Table 2.7) with those of the aerial transect (Table 2.9) a number of discrepancies become obvious. Firstly the ground survey records 92 trees/3 000 m^2 (307 trees/ha) whereas the aerial transect shows 81 trees/12 000 m^2 (67.5 trees/ha). There are a number of possible explanations for this:

a) Change in forest type (the ground survey covers only part of the aerial transect area).

b) Helicopter flying lower than 50 m, decreasing effective transect width.
c) Some trees not counted due to difficulties in the system, e.g. flying speed too high.
d) Sub-dominant trees not visible and therefore omitted (NB ground survey included myrtles down to 15 cm DBH).
e) Many dead stumps recorded in the ground survey are not visible from the air (see below).

Of these, neither a), b) nor c) should affect the recorded ratio of currently dying: healthy trees. a) and b) are in practice unavoidable. c) was probably minimised in later work by flying up valleys and contour flying to keep a low helicopter speed, by using a device to black out trees not on the transect. However d) and e) are probably the most important.

With regard to d), Elliott *et al.* (1987) found that for currently attacked and dying trees (HSC 3 & 4) there was no significant difference in attack between canopy classes. This means that even if sub-dominant trees are not visible from the air, the measured ratio of dying to live trees should not be affected.

e) can be investigated by comparing the proportion of dead trees (HSC 6 & 7) recorded in the ground survey (19.5%) with that from the aerial transect (8.7%). As expected, the aerial transect gave an underestimate but this would not explain much of the recorded difference in myrtle density. Neither would this affect the recorded ratio of dying to live trees. In summary, aerial transects may omit many sub-dominant trees and stumps which will affect the estimates of disease incidence (cumulative dead and dying trees). However, this should not affect the recorded ratios of currently dying to live trees which can thus be directly compared with those of Elliott *et al.* (1987).

Comparison of remote sites with those which are undisturbed, but less remote

For undisturbed but not remote sites, Elliott *et al.* (1987) found an average of 1.6% (range 0%-4.5%) of live trees currently dying of wilt. From Tables 2.6, 2.9, 2.10 and 2.11, the comparable figures for the four remote sites are as follows:

- Mt Ronald Cross ground survey 0%;
- Frankland Range aerial transects 0.8%;
- Adamsons Peak aerial transects 7.6%;
- Frenchmans Cap aerial transects 2.6%

with an average of 2.8% (range 0%-7.6%)

Thus although some remote sites currently have a low mortality due to wilt, all sites investigated to date have had isolated dying trees in the locality, and evidence of past or current *P. subgranosus* attack has been found in all ground surveys.

Adamsons Peak had one of the highest levels of dying trees found in any undisturbed site. The rainforest on this area of limestone geology is likely to be more susceptible to drought than is usual (Duncan and Kiernan 1989). It is possible that *P. subgranosus* may selectively attack drought affected trees, *C. australis* then entering the trees via the wounds caused by their tunnels (Kile *et al.* 1992). Howard (1981) noted the link between dry summers and extensive, *P. subgranosus* related myrtle deaths in disturbed areas and drought is known to precipitate insect and fungi related dieback in New Zealand beech forests (Hosking 1986). The relationship between myrtle wilt and drought on soils of low water-holding capacity, is one which may warrant further investigation.

In the Orb Lake ground survey dead myrtles with both old and current *P. subgranosus* attack (HSC 6) were encountered on the transect but at very low levels, with a disease incidence (cumulative dead and dying trees) of only 3.2% (Table 2.7). This is lower than on any of the sites recorded by Elliott *et al.* (1987) but it should be noted that in many cases stumps had degenerated to such an extent and were so moss-covered that it was impossible to identify *P. subgranosus* pinholes. These trees were classified as dead of other causes (HSC 7), whereas it may well be that many of them had experienced *P. subgranosus* attack in the distant past.

Increase in myrtle wilt at Mt Maurice

At Mt Maurice the incidence of myrtle wilt was measured by the proportion of *P. subgranosus* attacked standing trees and stumps. Kile *et al.* (1992) showed that live myrtles did not sustain *P. subgranosus* attack unless they were infected with *C. australis.* Although the beetle is known to attack fresh myrtle billets (Elliott *et al.* 1982), and also fire-killed timber which has been felled, standing dead trees appear to suffer little damage (Hogan 1944). The only known exception to this is the very dense attack on standing dead myrtles, which had been submerged by a Hydro Electric Commission scheme at Lake Gordon, and were then exposed as the water level fell 30 m due to low rainfall (H. J. Elliott, personal communication). Thus standing, dead myrtles with *P. subgranosus* attack were regarded as having been killed by myrtle wilt. Of these, trees which were dead at the time of the original survey have been recorded as 'diseased 1964'; trees which have died subsequently as 'diseased 1990'.

Myrtle wilt incidence was relatively low, but had increased between 1964 and 1990 (Table 2.12). The mortality due to wilt over the 26 years was relatively low in comparison with the adjusted mean (see Chapter 3) of 0.8% per annum found by Elliott *et al.* (1987) in their survey of 20 undisturbed sites. Mortality due to wilt was highest in the old growth myrtle forest where trees were of larger DBH, and this is in agreement with the findings of Elliott *et al.* (1987). In the area of myrtle regrowth mortality was high, but mostly this was not due to wilt and could have resulted from self thinning.

Tasmanian aerial photography

Myrtle wilt is the only known factor (excluding fire) which rapidly kills whole myrtles and leaves them standing. Thus myrtles with entire crowns consisting of dead leaves (HSC 4) or fine twigs (HSC 5) can be considered indicative of myrtle wilt. Black and white photography does not show up HSC 4 trees as effectively as colour, and even colour photography does not record reliably all the HSC 4 trees (H. J. Elliott, unpublished data). Therefore the apparent spread of the disease into the Douglas-Apsley area since the advent of colour photography could be merely an artefact, particularly since there are very few canopy myrtles visible from the air. In summary, although the method is useful for indicating the presence of myrtle wilt in an area, it is unreliable as an indicator of its absence.

However, the history of myrtle wilt in Tasmania can be traced retrospectively as far back as 1946, when it was apparent in the un-logged and un-roaded South Arthur (Sumac) rainforest. The late 1940s and 1950s photography shows up the disease in all the main rainforest areas in Tasmania. Perhaps the most interesting point is that it was not until 27 years after 1946, that myrtle wilt was actually noted (at apparently high levels), in a severely disturbed area. Until this time it appears to have gone unnoticed, or have been attributed to other causes. This indicates that myrtle wilt could have always been present in Tasmanian (and possibly Victorian) rainforests, but have gone unrecorded until recent times.

The Victorian situation

Myrtle wilt was observed in the Central Highlands and Strzelecki Ranges i.e. near the northern and eastern limits of the host species. The presence of *C. australis* in these areas has subsequently been confirmed by other workers; isolates from the Central Highlands (sites 2, 3, 4, 7 and 8) and the Strzelecki Ranges (sites 15 and 16) were morphologically identical to strains from Tasmania, and had comparable growth rates and the same temperature range for optimal growth (G. A. Kile and M. F. Hall, unpublished data). Disease levels appeared generally low (individual trees or small patches of diseased trees). It is likely that, particularly in the Strzeleckis, many gully communities may be entirely free of

infection although it seems infection is sufficiently widespread that no one patch can be expected to remain free from infection indefinitely.

In the Otway Ranges, rainforest is largely restricted to gully communities which trace various river systems. Few of these areas have a history free from selective logging (Brinkman and Farrell 1990), and being often very narrow they remain extremely vulnerable to disturbance caused when adjacent coupes are harvested etc. Possibly due to the above factors, wilt levels appeared high with many trees currently dying from the disease. The exceptions tended to be in the regrowth forests, where myrtle regeneration and remnant myrtles appeared healthy, and in areas where the numbers and density of mature myrtles were probably too low for the disease to remain endemic in the forest patch (see below).

The cool temperate rainforest of the Otway Ranges is comparatively depauperate in rainforest trees ; myrtle being the only primary rainforest species, and *Acacia melanoxylon* the only secondary rainforest species. With the death of many mature myrtles from myrtle wilt, *A. melanoxylon* is dominating an increasing proportion of rainforest stands (Cameron 1992). *Atherosperma moschatum,* which is usually co-dominant with myrtle in Victoria, is entirely absent from the Otways. Thus in areas of pure rainforest in the Otways, large canopy gaps result, making the effects of wilt highly visible. Both Read (1985) and Read and Hill (1985a) showed that due to its slower growth responses, *A. moschatum* was unable to 'capture' gaps in competition with myrtle on fertile sites. Thus the absence of *A. moschatum* may not influence the final canopy composition. It will, however, influence the sub canopy structure and probably the amount of light reaching the ground in gaps formed by wilt. A separate study of myrtle wilt, regeneration status of myrtles and floristic changes has now commenced in the Otways (Cameron and Turner 1994).

Recent survey and mapping of myrtle wilt incidence (Cameron and Turner 1994) found the disease to be endemic throughout the Otways and Central Highlands. Remote and undisturbed stands experienced at least low, background levels of the disease, which was only absent from small or isolated stands.

Evidence for a change (increase) in levels of myrtle wilt

The absence of references to any form of myrtle dieback up to 1973 could be attributed to the fact that it is much easier to observe an already described phenomenon; e.g. the significance of dead and dying myrtles on the 1946 aerial photographs was not appreciated until the disease had been described (Mesibov 1978). Additionally, in earlier times proponents of the Arcadian view were more likely to expound the wonders of the natural

world, than to describe imperfections such as plant diseases (Podger 1993). Even up to the 1970s, there had been few observations or studies of disease effects in Tasmanian rainforest (H. J. Elliott, personal communication).

However, it could indicate the absence (or very low levels) of myrtle wilt in earlier times. The disease is correlated to tree damage (Elliott *et al.* 1987), and many of the early Tasmanian references do record myrtle in areas of disturbance (mines, tracks, roads, logging activities and fires). Forest condition was obviously considered to be of some importance, and dead trees were noted on at least one occasion, i.e. *Athrotaxis* species in the Picton, the deaths being attributed to severe frosts (Perrin 1887). If myrtle wilt in Tasmania occurred at similar levels to those found recently by Elliott *et al.* (1987) in undisturbed forest (24.6% of standing myrtles dead or dying, with 1.6% live myrtles currently dying), it is perhaps surprising that it was never observed. This is particularly the case in view of the numerous recent observations of aggravated disease in some areas of disturbance. Additionally, the limited evidence from the investigation at Mt Maurice may indicate that disease levels have increased in the recent past.

Endemic disease is defined as being constantly present, whereas epidemic disease is sporadic and occurring within limited time. Endemicity implies both host resistance and that the pathogen does not die out over all its range. This resistance is also related to the environment, less resistance being needed to bring about balance when the environment is less favourable to the disease. Because conditions cannot stay constant (at least on a microscale), it is inevitable that even in an area where disease is mainly endemic there will be local epidemics (Van der Plank 1975).

Thus any localised increase in myrtle wilt incidence, as at Mt Maurice, may be due equally well to the small scale epidemics of an endemic disease as to the progressive colonisation of a new and/or largely epidemic one. In either case, there must have been an increase in pathogenicity or in host susceptibility. An increase in pathogenicity could be due to a new species/strain of the pathogen, whereas an increase in host susceptibility could be due to an environmental change which has led to an increase in disease levels. Susceptibility of individual myrtles to infection is influenced by various factors, some of which may alter with time:

- *Degree of genetic resistance;*

From observation and in keeping with most host/pathogen interactions it seems likely that there is a degree of wilt resistance in the myrtle population.

- Size/Age;

Elliott *et al.* (1987) showed that larger myrtles had a higher probability of infection by myrtle wilt.

- *Damage;*

Damage is known to be correlated with myrtle wilt incidence (Elliott *et al.* 1987). Van der Plank (1975) indicated that the susceptibility of a host to a wound pathogen was related both to the number of wounds, and to the 'infectivity' of the wounds. In the case of myrtle wilt 'infectivity' decreases with wound age (Chapter 5).

- *Inoculum levels.*

The susceptibility of a host to a wound pathogen is also related to the amount of inoculum present (Van der Plank 1975). The amount of inoculum will increase with the number of infected trees. Kile *et al.* (1989) confirmed the presence of air/water borne inoculum of *C. australis* in a rainforest area, although the effective dispersal distance by this mechanism is not yet known.

Thus disease levels would be expected to increase in areas immediately adjacent to a disturbance. Given the increase in logging and roading activity in the last 50 years, and since these areas tend to be the most accessible and visible, it is not surprising that many observations emphasise this point, and this is one explanation for the increase in disease levels at Mt Maurice.

In summary, there is a rather surprising lack of early myrtle wilt observations, and records from one site do show an increase in disease incidence over the last three decades. However, there is insufficient evidence to draw conclusions about any long term or overall changes in myrtle wilt levels in Tasmania.

Evidence for a change (increase) in the distribution of myrtle wilt

Records of myrtle wilt in Tasmania do appear to pre-date those in Victoria, and in Victoria records in the west pre-date those in the east. In both States the only areas now thought to be free of the disease are some stands on the eastern limits of myrtle distribution.

In Victoria myrtle wilt was reported as absent in the 1960s and 70s (Howard 1973a, 1981). Since Howard had systematically investigated myrtle in Victoria, and was the person who later described the disease, this is good evidence that myrtle wilt was either entirely absent from Victoria or at extremely low levels, prior to 1980, when it was recorded from the Otways. Despite the disease having been well described, it was not reported from central or eastern Victoria until the 1990s, which may be another indication of recent spread.

Alternatively it could represent the increased activities of research workers moving outwards from the main centres of population. If early Victorian aerial photographs were available, they would have the potential to clarify this issue.

However, although the first isolation of the pathogen from Victoria was not made until 1980 (G. A. Kile, unpublished data), the record of myrtle dieback from the Central Highlands where trees had been damaged (Weste 1975), is highly indicative of myrtle wilt. Additionally, general field observations on the occurrence and field habits of *P. subgranosus* had previously been made by forest officers (Hogan 1948), and the beetle was known to attack only *unhealthy* or damaged green trees (my emphasis), or dead trees which had not become too dry. Myrtle was given as one of the most susceptible species, although it was never specifically mentioned in relation to disease (Hogan 1944). Thus it is possible that low levels of myrtle wilt were present in the Central Highlands even at this stage.

When a disease occurs over only part of its potential geographical area, not only does the level of disease rise and fall, but the boundary of the diseased area advances and recedes. An advancing disease boundary indicates an area where the disease is epidemic (Van der Plank 1975). Given that myrtle wilt is apparently absent from some Tasmanian and Strzelecki sites and Wilsons Promontory, some change in disease distribution with time should be expected. Disease levels in Tasmanian remote sites are similar to elsewhere, indicating either that myrtle wilt has long been present in these areas, or that there has been very efficient disease spread. Aerial photographs indicated the presence of myrtle wilt in remote areas as early as 1946.

At present there is probably not enough evidence to claim that there has been an extensive increase in myrtle wilt distribution in the recent past. An alternative explanation to the spatial (and temporal) variation in disease records, is the influence of various interacting factors on the susceptibility of myrtle stands to infection. These factors include:

- *Forest structure and composition;*

Elliott *et al.* (1987) showed that incidence of myrtle wilt was related to the density and relative density of myrtles in the stands considered. In the light of subsequent work (Chapter 5) it seems likely that this was due, at least in part, to the facilitation of disease spread through root grafts. They also showed that myrtle wilt levels were lower in rainforest with an implicate structure than in those with a thamnic or callidendrous structure (Jarman *et al.* 1984). In Victoria all myrtle forests have a callidendrous structure.

- *Rainforest patch size and isolation;*

According to the theory of island biogeography, patch size is generally inversely related to the rate of extinction of species, and patch isolation is inversely related to the rate of colonisation/reinvasion (McArthur and Wilson 1967). While this can be applied both to myrtle and to *C. australis,* the processes are likely to be more rapid with the latter because of its shorter life cycle.

- *Stand age structure;*

It has been suggested that there is currently a high proportion of 'mature' or 'over-mature' rainforest which is susceptible to myrtle wilt epidemics (G. A. Kile, personal communication). However, although larger myrtles had a higher probability of myrtle wilt, size class distributions of myrtle stands across 20 Tasmanian sites were found to be relatively uneven-aged, and not significantly related to disease incidence (Elliott *et al.* 1987). If, as with some other *Nothofagus* diebacks (Chapter 1), stand age structure is a predisposing factor for myrtle wilt, this has yet to be demonstrated. Because of the infrequency of some of the critical events involved (see below), this would require systematic investigation of a much larger number of sites.

- *Environmental factors.*

Elliott *et al.* (1987) found a lower disease incidence at higher altitudes. Since *C. australis* has a low temperature optimum for growth (17.5-20^{o}C), increases in average temperatures with decreasing latitude and general elevation could operate to suppress disease development on the margins of the distribution of *N. cunninghamii* (G. A. Kile, personal communication). Howard (1981), reported the absence of *Platypus* (and, by inference, myrtle wilt) from some Tasmanian areas of marginal rainfall for myrtle. She attributed this to the loss of host material after extensive fires.

Thus any apparent spread of myrtle wilt could also be explained by site dependent factors which influence disease levels and visibility.

Evidence for a new disease/strain

At the present time there is no direct evidence for the recent introduction or evolution of a new species/race, and *C. australis* is thought to be native to Australia (Kile and Walker 1987). Under field conditions it is only known to be pathogenic to myrtle, although it can saprophytically utilise other hosts (Kile 1989).

The discovery of a closely related *Chalara* species, a wound pathogen in *Eucalyptus globoidea* and *E. sieberi,* may be relevant (Old *et al.* 1991). Although to date this species has only been found in East Gippsland (Victoria), *E. sieberi* also occurs in Tasmania. A

search for the centre of diversity for *C. australis* could usefully start with the areas where myrtle distribution overlaps that of *E. sieberi* (e.g. site 6, Appendix 3).

Since a new disease/strain will inevitably spread from its point of origin, this hypothesis also implies an increase in disease distribution. As discussed above, evidence for such a spread is inconclusive. The increased disturbance in myrtle forests in recent times, together with a raised awareness of 'dieback' problems are probably sufficient to account for the relatively recent 'discovery' of myrtle wilt.

Comparison of the Tasmanian and Victorian situations

A comparison of the situation in Victoria with that in Tasmania raises some interesting issues. In Tasmania, myrtle-dominated rainforest occurs in large and often continuous areas in the west/south-west of the State, where myrtle wilt appears ubiquitous. In the east myrtle is usually confined to small remnant patches, in which it has not always been possible to detect active myrtle wilt by aerial surveys or by ground-based studies (M. Neyland, personal communication). Thus the absence of *current* infections in many sites could be due to small patch size. The historic absence of the disease from such a patch could only be proved by the absence of *P. subgranosus* tunnels in standing dead myrtles, and this aspect was not investigated.

In Victoria the situation is very different from that of most of Tasmania, with myrtle forest being confined to small patches which are often gully communities (Read 1992). Additionally the regions in which rainforest is found are often widely separated e.g. Central Highlands and Strzelecki Ranges (Figure 1.2). Thus the situation in the Strzeleckis (with the lack of evidence of old infections and only one area with new infections) could be explained by its geographic isolation.

In a large or contiguous patch of myrtle forest the continual presence of low levels of disease could be expected. Disease would spread slowly from a number of foci (usually damaged trees) killing all susceptible trees. New foci would develop as other trees were damaged and infected by airborne inoculum. Myrtle regeneration in the gaps formed by these deaths would help to maintain an uneven age structure and a mosaic of patches of different ages. Since smaller trees are less likely to be damaged and infected, such a composition may well serve to moderate the effects of subsequent outbreaks. In regard to such myrtle populations, outbreaks of the endemic wilt can be seen as a disaster rather than a catastrophe since the return interval is of the same order of magnitude as the myrtle longevity (Harper 1977; Chapter 7), and the evolution of genetic resistance is thus highly

probable. This would appear to be the current situation in western Tasmania, and possibly in the Central Highlands of Victoria.

In the extreme opposite case of a relatively small, isolated myrtle patch remaining uninfected for generations, genetic resistance would be likely to be lower. Since many of these outlier patches may arise from coppice regeneration after fire (Howard 1973a), they may tend to be even aged. Also, a lack of gap phase regeneration could result in a stand becoming older and more even aged, making disease spread more likely, i.e. cohort senescence (Mueller-Dombois 1983b). Thus a single disease focus could lead to an epidemic with the majority of the trees dying. This would be even more likely when the epidemic was precipitated by a disturbance in which numerous trees were damaged and there were multiple disease foci. In such a case the epidemic of wilt could be termed a catastrophe. It is also questionable whether in such cases, sufficient seed trees and cover would remain for satisfactory myrtle regeneration. This extreme situation is thought to be unlikely because of the ability of airborne inoculum to infect wounds, although it could possibly represent the situation in some of the Strzelecki Ranges, Wilsons Promontory and eastern Tasmanian sites.

In the more likely case of a small but less isolated patch of myrtle forest being frequently recolonised by myrtle wilt, the 'island effect' on the fungus being slight, the situation would be more akin to that of a large, continuous patch. Various, intermediate situations could occur depending on the isolation of the patch. This is probably the situation in most of the sites in Victoria, with the susceptibility of trees (and thus of myrtle rainforest patches) being largely determined by the degree of disturbance (damage).

2.5 SUMMARY

Myrtle wilt appears to be ubiquitous in Tasmania's extensive myrtle forests, with disease levels in remote areas being similar to those in undisturbed but less remote sites. In Victoria, the distribution of myrtle is confined to small patches in widely separated regions. Myrtle wilt has been found in all the main regions, but is apparently absent from some Strzelecki sites and from Wilsons Promontory.

Although aerial photographs indicate that myrtle wilt was present in all the main rainforest areas in Tasmania in the late 1940s and 1950s, in the early literature there is a rather surprising lack of observations which could be attributed to the disease. Also, records from one site do show an increase in disease incidence over the last three decades. However, there is not sufficient evidence to draw firm conclusions about any long term or widespread

changes in myrtle wilt levels. Neither is there enough evidence to claim that there has been an extensive increase in myrtle wilt distribution in the recent past.

An alternative explanation to the spatial (and temporal) variation in disease records is the influence of various interacting factors on the susceptibility of myrtle stands to infection. Any apparent spread of myrtle wilt can also be explained by site dependent factors which influence disease levels and visibility.

There is no direct evidence that a new species/race has recently evolved or been introduced. The increased disturbance in myrtle forests in recent times, together with a raised awareness of 'dieback' problems, are probably sufficient to account for the relatively recent 'discovery' of myrtle wilt.

In summary, the available information indicates that myrtle wilt is endemic in at least part (and possibly most) of the range of myrtle in Tasmania and Victoria, although disease levels may have increased (at least in some areas) in the recent past. However, other interpretations are possible, and the need for permanent monitoring sites and reliable data cannot be overemphasised.

3. INCIDENCE LEVELS AND MORTALITY RATES

3.1 INTRODUCTION

Although myrtle wilt was first described in 1973 (Howard 1973a), even by 1982 little was known about the dynamics of the disease; in particular data on mortality rates, rate of spread and pattern of attack were lacking. In order to provide this type of information, a permanent plot was established in 1982 in the Arve Loop near Geeveston and remeasured every two years (H. J. Elliott, unpublished data). Pattern analysis of the 1982 data has been attempted by a number of workers and a summary is given below.

Candy (1982), after dividing the plot into three subregions and analysing nearest neighbour pairs, showed that for one of these regions, there was a positive association of attacked trees with other attacked trees and similarly for un-attacked trees. He also found increasing intensity of attack from south to north. Clayton (1982), re-analysed the data, accounting for the fact that nearest neighbour distances were reflexive (i.e. A is the nearest neighbour of B and B is the nearest neighbour of A). His findings indicated that for the third subregion, the first subregion (for distances greater than 6 feet) and for the area as a whole, the diseased trees were more regularly dispersed than if the disease occurred randomly. Diggle (1983a), used a Dirichlet tessellation to compute an index to show clumping of diseased trees. This was significant, especially when the definition of nearest neighbours involved a distance threshold (maximum distance between nearest neighbours), with a value of 10 m or more. He concluded that there was fairly strong evidence that diseased trees were clumped.

More recently the survey by Elliott *et al.* (1987) of 20 undisturbed Tasmanian sites indicated that a mean of 24.6% (range 9.4-53.4%) of myrtle trees were dead or dying due to myrtle wilt. It also predicted that, across the sites, myrtle wilt was killing an average of 1.6% (range 0-4.5%) of live myrtles per year, the assumption being that trees were taking only one year to die. The estimated annual mortality rate of 1.6% was then found to be comparable to that of the early stages (later stages having escalating mortality rates), of the most recent epidemic of Dutch elm disease in Great Britain, a finding which has been viewed, not surprisingly, with some alarm.

The same survey showed that disease incidence was higher in callidendrous than in thamnic-implicate forests, and increased in mixed forests with both relative and absolute measures of myrtle density. Additionally, diseased trees were shown to be clumped, with the degree of clumping being dependent on the nearest neighbour distances within sites. In

1988 and 1989 five further plots were established in different locations and rainforest types, to facilitate monitoring of mortality rates due to myrtle wilt on a long-term basis. For each site the aims were:

- to determine the change in levels (rate of spread) of myrtle wilt using the incidence of new attack;
- to obtain information on the development of the disease by analysis of the pattern of attacked trees.

3.2 MATERIALS AND METHODS

Arve Loop plot

A 3.5 ha long-term plot was set up in 1982, located near the Arve Loop in the Arve Valley in south-west Tasmania (H. J. Elliott, unpublished data). The vegetation type was mixed forest (Gilbert 1959), having a eucalypt overstorey with rainforest understorey. Botanically the rainforest component was classified as thamnic (Jarman *et al.* 1984). The area was selectively logged early this century but has remained undisturbed for many years (Elliott *et al.* 1982). The plot was sited away from the road edge, to avoid any effects of roading disturbance.

In January-March 1982, the location, diameter at breast height (DBH) and health status classes (HSC) of 327 myrtle trees were recorded. Trees of DBH less than 5 cm were excluded. The health status classes are given in Table 3.1 and are similar to those of Elliott *et al.* (1987), but include trees which became downers (i.e. trees which had fallen down). The health status of the trees was reassessed in 1984, 1986 (H. J. Elliott, unpublished data), and in 1988, 1990 and 1992 during the present study. In 1990, the DBH was remeasured, and trees which had grown into the 10 cm DBH class were included in the survey (although it was decided not to include trees which had grown into the 5-10 cm DBH class). Damage assessments were made in 1990 and 1992, using the damage classes previously defined in Table 2.2. Recording methods and assumptions made are given in Appendix 5. Details of the plot are given in Appendix 6.

Table 3.1 Health status classes (HSC) used in the Arve Loop and rate of spread (ROS) plots

1. Healthy, with normal green crown
2. Healthy, with old *Platypus subgranosus* attack on lower stem
3. Healthy, but with current *P. subgranosus* attack (frass accumulation on lower stem)
4. Dying (orange/brown foliage retained on the tree) with current *P. subgranosus* attack
5. Dead <3 years (fine twigs remaining on the tree); *P. subgranosus* attack (old or current) present
6. Dead >3 years (main branches only remaining on the tree); *P. subgranosus* pin holes present on lower stem
7. Dead, but with no evidence of *P. subgranosus* attack
8. Tree alive but with major crown dieback, cause unknown
9. Dead and down. Previously attacked by *P. subgranosus*
10. Dead and down. Not previously attacked by *P. subgranosus*

Platypus subgranosus attack was used as an indicator of *Chalara australis* infection and trees dead or dying, with evidence of *P. subgranosus* attack, were assumed to have been killed or to be dying of myrtle wilt. Alternative methods of presenting the 1982 data have been used by other workers and these are given in Appendix 7.

The number of trees in HSC 3-6/1-8 expressed as a percentage, indicated the proportion of standing myrtles which were diseased, i.e. had been/were being attacked by *P. subgranosus/C. australis.* HSC 1-4 were combined to give the total available number of live trees at each sampling date (HSC 4 trees were considered to be dying rather than dead and HSC 8 trees were not considered). The number of trees in HSC 4/1-4 were expressed as a percentage, to indicate the proportion of live myrtles currently dying due to myrtle wilt. The number of trees which died of myrtle wilt between measurements was expressed as a percentage mortality per annum, calculated on a monthly basis.

Rate of spread (ROS) plots

Sufficient circular plots of 50 m radius to give 200 myrtle/site were established in each of the three main rainforest types:

- Callidendrous (C) — Simons Rd, Five Rd
- Callidendrous/Thamnic (C/T) — Lake Chisholm
- Thamnic (T) — Arve Loop#, Pipeline 18 Mile Peg
- Implicate/Thamnic (I/T) — Frodshams Pass

refers to the Arve Loop plot described above

Additional details of plots are given in Appendix 6.

Once a homogeneous area of the desired forest type was selected, the first plot was located when the first recently attacked (HSC 3, 4 or if necessary 5) tree more than 100 m from the road edge was found. The centre of the plot was generally located 50 m on from this tree on the same bearing, the attacked tree being on the edge of the plot. The second and subsequent plots were similarly located when a currently attacked tree was found, ideally more than 150 m from the edge of the preceding plot. Orientation from the first plot depended on the size and shape of the rainforest area.

The centre of each plot was marked with a metal picket and all myrtles within a 50 m radius with a DBH of more than 15 cm were individually numbered and surveyed into the centre peg. Their DBH was measured and recognisable myrtle stumps of more than 2 m in height were included. As trees were surveyed they were assessed for disease (Table 3.1), and for recent damage (Table 2.2), using the slightly modified system of Elliott *et al.* (1987). Damage assessments were only made on trees which were still alive or which had been dead less than 3 years, i.e. HSC 6, 9, 10 and some long dead HSC 7 trees were not assessed.

The effect of rainforest type on the initial (1988/1989) incidence of myrtle wilt and of recent damage, was tested by analysis of variance (ANOVA), adjusted for altitude by using it as a covariate. The data were first transformed using the arcsin of the square root of the percentage of trees diseased/damaged. The Arve Loop plot was omitted in the test on recent damage since this variable had not been measured throughout, and for the ANOVA the Lake Chisholm plot which was callidendrous/ thamnic was taken to be thamnic. To assist in the interpretation of results the more comprehensive data set of Elliott *et al.* (1987) was partially re-analysed. Student's t tests were used to explore the effects of rainforest type (callidendrous *cf.* thamnic/implicate) and forest type (mixed forest *cf.* pure rainforest) on disease incidence, and on absolute and relative myrtle densities.

Since establishment, all plots have been assessed for disease and damage every two years. The percentage of trees diseased and dying through time, and the annual mortality due to myrtle wilt were calculated as for the Arve Loop plot. It is intended that at every fourth re-measurement (i.e. every eight years) all numbered trees will have their DBH remeasured, and any myrtles which have grown into the 15 cm DBH class will be included in the survey.

Pattern analysis

Distribution of diseased trees

The distribution of myrtles on the Arve Loop plot was displayed by plotting the position of trees and coding according to their health status classes (excluding HSC 7, 8 and 10).

Analyses of both the 1992 Arve Loop data and the most recent ROS plot data used cumulative diseased trees (HSC 3, 4, 5, 6, and 9). For each plot the distribution of diseased trees was mapped. The spatial arrangement of these trees was then examined using the methods of Diggle (1983b) and Ripley (1977), as employed by West (1984). These methods allow the simultaneous testing of pattern at different scales, the lower distance class limit being determined by the accuracy of mapping, and the upper by plot size. In this case accuracy was 0.5 m and ten distance class limits were selected; at 0.5, 2, 3.5, 5, 6.5, 8, 9.5, 11, 12.5 and 14 m.

Test for complete spatial randomness

The method of Diggle (1983b) was used to determine whether the distribution of diseased trees on each plot differed from complete spatial randomness, i.e. whether clumping or regularity (over-dispersion) was present. This was achieved by a comparison of the frequency distribution of nearest neighbour distances between diseased trees with the frequency distributions of a spatially random distribution, using the empirical distribution function (EDF). Values for a random distribution were computed from 19 simulated, random arrangements of diseased trees on each plot. The EDF values for observed and simulated data were then plotted against each other, over the range of selected distance classes.

An observed EDF falling entirely within the simulation envelope indicates a random distribution throughout the range of distance classes investigated. Observed EDFs which fall above or below the simulation envelope indicate, respectively, clumping or regularity. The significance value of any departure from randomness depends on the number of simulations; in this instance, with 19 simulations and a prior expectation of clumping, the test can be considered one-tailed, and the significance level 0.05.

Scale of pattern

The method of Ripley (1977) was used to define the scale of any departure from randomness found when using the procedure described above.

The function $\hat{K}(t)$ depends on the number of diseased trees within a distance t (m), of a given diseased tree, and is given by:

$$\left(\frac{\hat{K}}{pi}\right) \text{ x } (0.5 - t).$$

For each data set this function was plotted against the distance t, using the range of distance classes given above. This was compared to the results obtained from 19 simulations using randomly distributed data.

Once again, departure from randomness is indicated by the observed value falling outside the simulation envelope at any distance class value; values falling above the envelope indicate clumping at that distance class, whereas values falling below the envelope indicate regularity.

Transect remeasurements

To study the change in disease incidence, a number of the transects through rainforest used by Elliott *et al.* (1987) were relocated and reassessed (or partially reassessed) approximately five years after initial delineation. The sites remeasured were Mt King William (thamnic/callidendrous), Simons Road (callidendrous) and Tombstone Creek (mixed forest, callidendrous understorey). Recording methods followed those of Elliott *et al.* (1987) and health status classes are those given in Table 2.1 (downers not being recorded). Mortality rates were calculated as for the Arve Loop plot.

Estimated and actual mortality rates

Re-measurement of the Arve Loop plot and the Simons Road and Tombstone Creek transects provided the opportunity to test the assumption of Elliott *et al.* (1987), that currently dying trees (HSC 4) remain in this state for only one year, thereby giving a good estimate of the actual mortality rate due to wilt.

Whole sites

The observed (actual) number of trees which had died between measurements was compared with the expected (estimated) number of dead trees using the chi squared statistic. The first null hypothesis was that trees remain in HSC 4 for one year, thereby giving a good estimate of the annual mortality rate. The expected number of dead trees was calculated by multiplying the number of trees currently dying (HSC 4) at the first measurement by the number of years between measurements. Other null hypotheses were that trees remain in HSC 4 for two years, three years or four years, the expected number of

dead trees being divided by two, three or four respectively. (The Mt King William transects were excluded since there were no HSC 4 trees at the first measurement.)

An actual value for the number of years trees spend in HSC 4 was obtained by the following procedure. The observed number of trees which had died (cumulative over all sites) was calculated, and for each null hypothesis (i.e. one, two, three or four years spent in HSC 4), the expected number of dead trees (cumulative over all sites) was also calculated. A simple regression model was then fitted (expected number of dead trees against time spent in HSC 4). The model was used to predict the actual number of years spent in HSC 4 (the point at which the expected value was equal to the observed value).

Arve Loop trees

Mortality rates were further investigated by looking in more detail at the records of the Arve Loop plot from 1982 to 1988, the health status of individual trees having been assessed every two years. During this time a number of healthy trees became infected, passed through HSC 4 (dying, orange/brown leaves) and died of myrtle wilt. The number of recording dates at which each of these trees were observed to be in HSC 4 was noted.

Since (for the purposes of this investigation) the respective times at which they entered or left HSC 4 (i.e. the times of symptom development or tree death and foliage loss) were unknown, trees recorded in HSC 4 only in 1982 or in 1988 were omitted from this study. Therefore it can be deduced that:

- trees not observed to be in HSC 4 at any of the recording dates must have spent from zero to two years (average one year) in HSC 4;
- trees observed to be in HSC 4 at only one recording date must have spent from zero to four years (average two years) in HSC 4;
- trees observed to be in HSC 4 at two recording dates must have spent more than two years in HSC 4;
- trees observed to be in HSC 4 at three recording dates must have spent more than four years in HSC 4;
- trees observed to be in HSC 4 at four recording dates must have spent more than six years in HSC 4.

Individual trees

There is other circumstantial evidence which can be used to investigate mortality. Kile and Walker (1987) experimentally wounded a number of myrtles in the Arve Loop area (but not in the Arve Loop plot) and these subsequently became infected with *C. australis*.

Observations were made on the timing of symptom development (entry into HSC 4), tree death and foliage loss (trees moving from HSC 4 to HSC 5 when all foliage is lost) until the date at which all trees were felled.

They stated 'Foliage retention after death was variable. On trees which had lost 20-40% of their foliage prior to death, the residual foliage was lost within 6-10 months, while on trees which died with a high proportion of foliage present, most was lost within 18 months'. When the records of individual trees were investigated (G. A. Kile, unpublished data) the mean time from symptom development (entry into HSC 4) until the trees were felled could be ascertained together with the amount of foliage remaining on each tree at this time.

3.3 RESULTS

Arve Loop plot

The percentages of myrtles diseased and dying through time are given in Figure 3.1. These percentages are comparable with those presented by Elliott *et al.* (1987). The number of trees in each health status class through time, and estimates of mortality rates are given in Appendix 8.

Rate of spread plots

The percentages of trees diseased and dying through time, and the number of trees in each health status class through time with estimates of mortality rates, are shown in Figure 3.1 and Appendix 8 respectively. There was a significant effect of rainforest type on myrtle wilt incidence at the first measurement (Table 3.2). The callidendrous sites had a higher disease incidence than the thamnic sites and the implicate site. The effect of altitude on disease incidence was also significant, with myrtle wilt levels being lower at higher altitudes. Re-analysis of the data set of Elliott *et al.* (1987) indicated that the absolute and relative myrtle densities were greater in callidendrous than thamnic/implicate rainforest, and higher in pure rainforest than in mixed forest (Appendices 9 and 10).

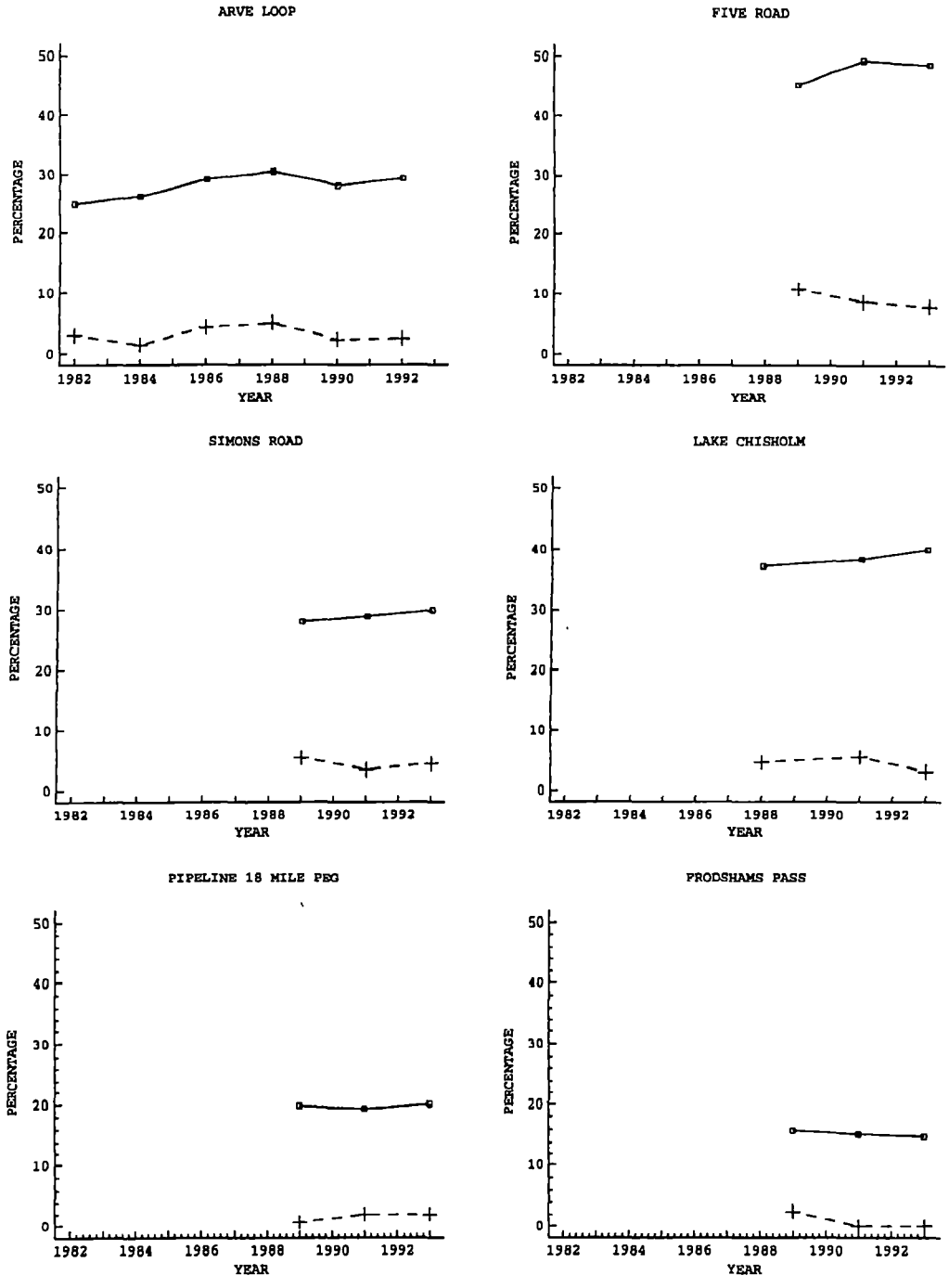


Figure 3.1 Myrtle wilt incidence over time, on the Arve Loop and ROS plots.

Percentage of trees currently dying of myrtle wilt shown by broken line.

Percentage of diseased trees (cumulative dead and dying of myrtle wilt) shown by unbroken line.

Table 3.2 ANOVA of percentage of diseased trees by rainforest type, adjusting for altitude (data transformed to arcsin square root of percentage diseased)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Altitude	32.716	1	32.716	29.290	0.0325*
MAIN EFFECT					
Rainforest type	214.623	2	107.311	96.076	0.0103*
RESIDUAL	2.234	2	1.117		
TOTAL	249.572	5			

NB In this and following tables, 5%, 1% and 0.1% significance levels are shown by *, ** and *** respectively. Non-significant values are denoted by NS.

The incidence of recent damage in the rate of spread plots at the first measurement, is given in Table 3.3.

Table 3.3 Recent damage in rate of spread plots

Site	Rainforest type	No. of myrtles in damage class				% Damaged
		1	2	3	4	(DC 2-4/1-4)
Simons Rd	Callidendrous	209	25	12	3	16.06%
Five Rd	Callidendrous	204	56	6	2	23.88%
Lake Chisholm	Callidendrous/Thamnic	208	11	10	4	10.73%
Pipeline 18 Mile Peg	Thamnic	192	6	8	0	6.80%
Frodshams Pass	Implicate/Thamnic	152	11	2	2	8.98%

The significant effect of rainforest type on the incidence of recent damage is shown in Table 3.4. The callidendrous sites had a higher incidence of recently damaged trees than the thamnic sites and the implicate site. The effect of altitude on damage incidence was unclear.

Table 3.4 ANOVA of percentage of damaged trees by rainforest type adjusting for altitude

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Altitude	3.271	1	3.271	13.635	0.1661 NS
MAIN EFFECT					
Rainforest type	124.778	2	62.389	260.059	0.0432*
RESIDUAL	0.240	1	0.240		
TOTAL	128.289	4			

Pattern analysis

Distribution of diseased trees

The distributions of diseased and healthy myrtles on the Arve Loop plot in 1982 and 1992 are displayed in Figure 3.2. Since downers are included in the later figures, they represent the cumulative effects of myrtle wilt over the 10 year period. The fate of the various disease foci can thus be followed.

The cumulative distributions of myrtle wilt infected trees for the Arve Loop and the ROS plots (based on the last measurements to the end of 1993), are given in the top rows of Figures 3.3-3.8. They indicate the positions of all the trees recorded as dead or dying of myrtle wilt, since the plots were established. Other trees have been omitted.

Test for complete spatial randomness

Plots of EDF values for observed and simulated data are given in the middle rows of Figures 3.3-3.8. With the exception of two of the four Lake Chisholm plots and one of the two Pipeline 18 Mile Peg plots, the analyses indicated that diseased myrtles were clumped.

Scale of pattern

Plots of the $\hat{K}(t)$ function against distance t (m), are given in the bottom rows of Figures 3.3-3.8. Within the range investigated, the analyses indicated that the scale of clumping varied from 2 m (Five Road, plot 3) to 14 m (Arve Loop and Simons Road plots).

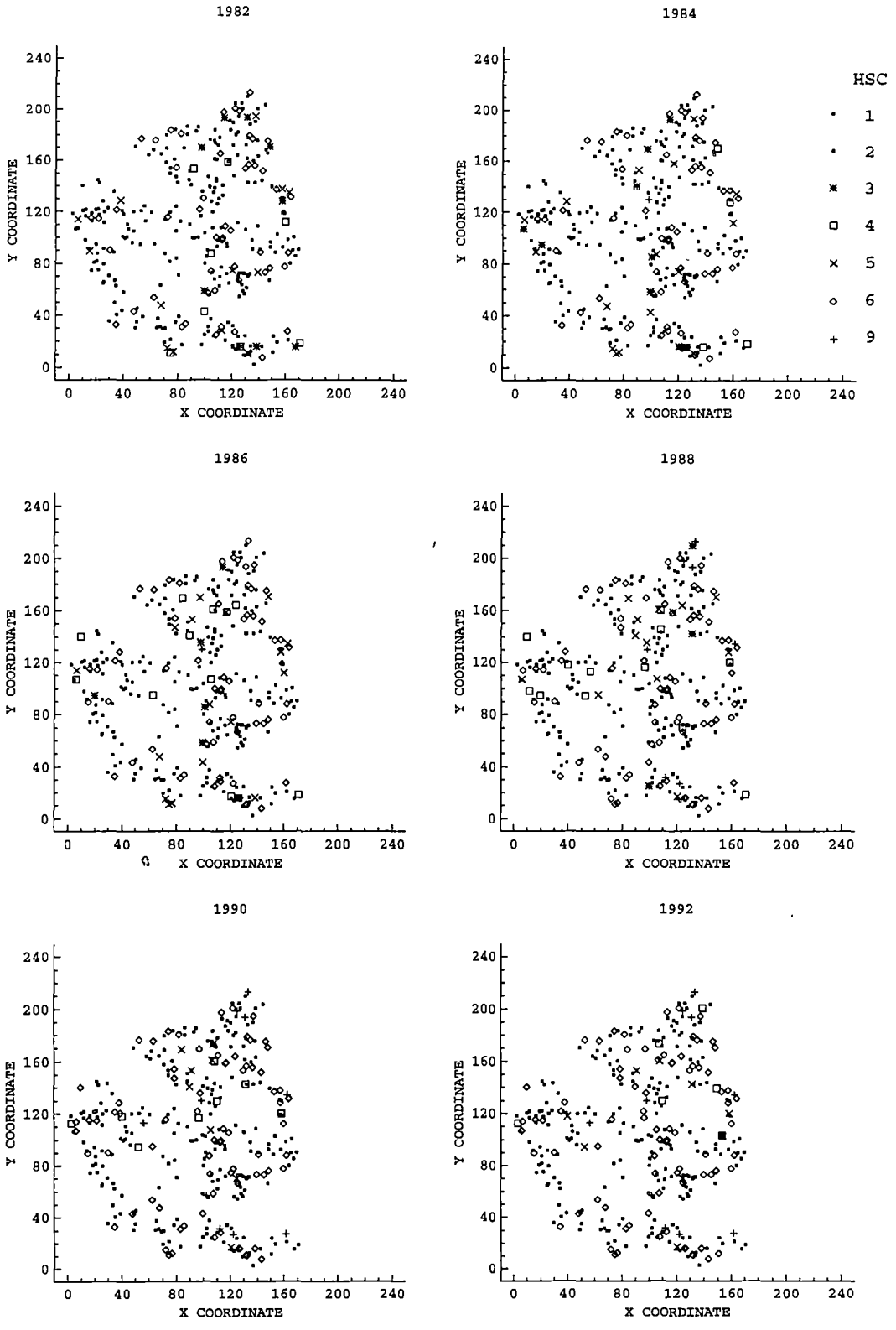
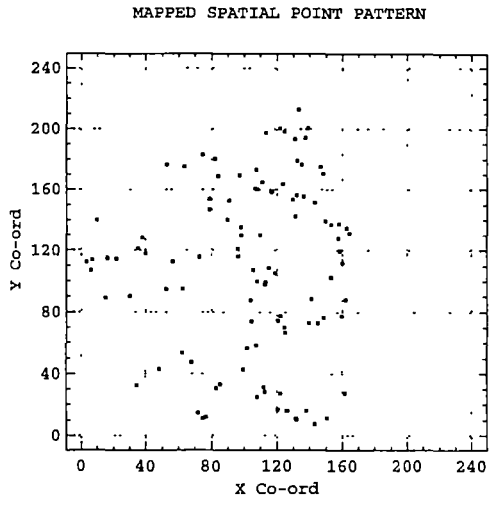
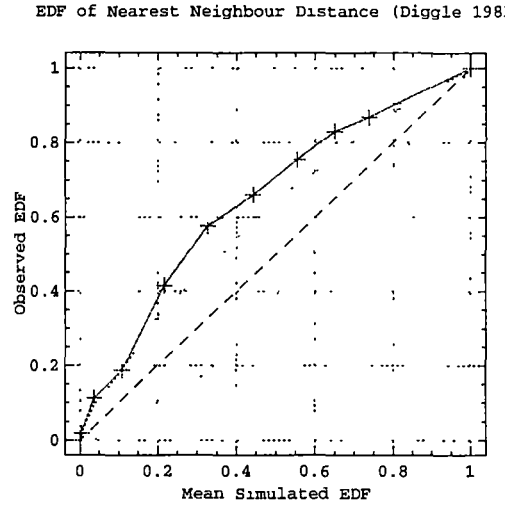


Figure 3.2 The distribution of healthy and myrtle wilt infected trees on the Arve Loop plot from 1982 to 1992. (X and Y coordinates are given in m; Y axes run from south to north.)

Cumulative distribution of myrtle wilt infected trees. This indicates the positions of all the trees recorded as dead or dying of myrtle wilt, since plot establishment. Other trees have been omitted. (X and Y coordinates are given in m, and Y axes run from south to north.)



Plots of EDF values for observed and simulated data. Observed EDF values are shown by unbroken lines; simulation boundaries by dotted lines. Observed values falling above or below the simulation envelope indicate clumping or regularity respectively (Diggle 1983b).



Plots of the $\hat{K}(t)$ function against distance t (m). Observed $\hat{K}(t)$ values are shown by unbroken lines; simulation boundaries by dotted lines. Observed values falling above or below the simulation envelope indicate clumping or regularity respectively. The $\hat{K}(t)$ plot also indicates the scale at which any such departure from randomness occurs (Diggle 1983b).

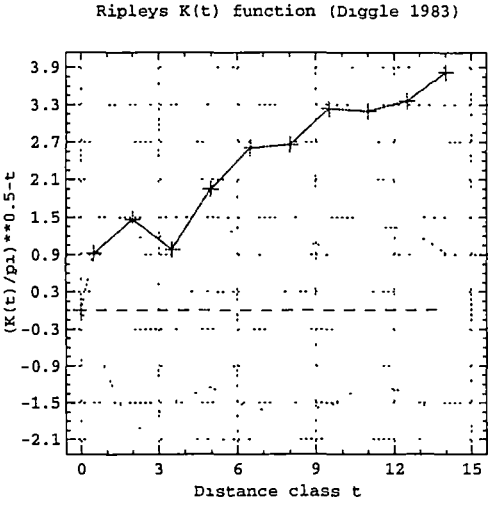


Figure 3.3 Pattern analysis of myrtle wilt infection for the Arve Loop plot.

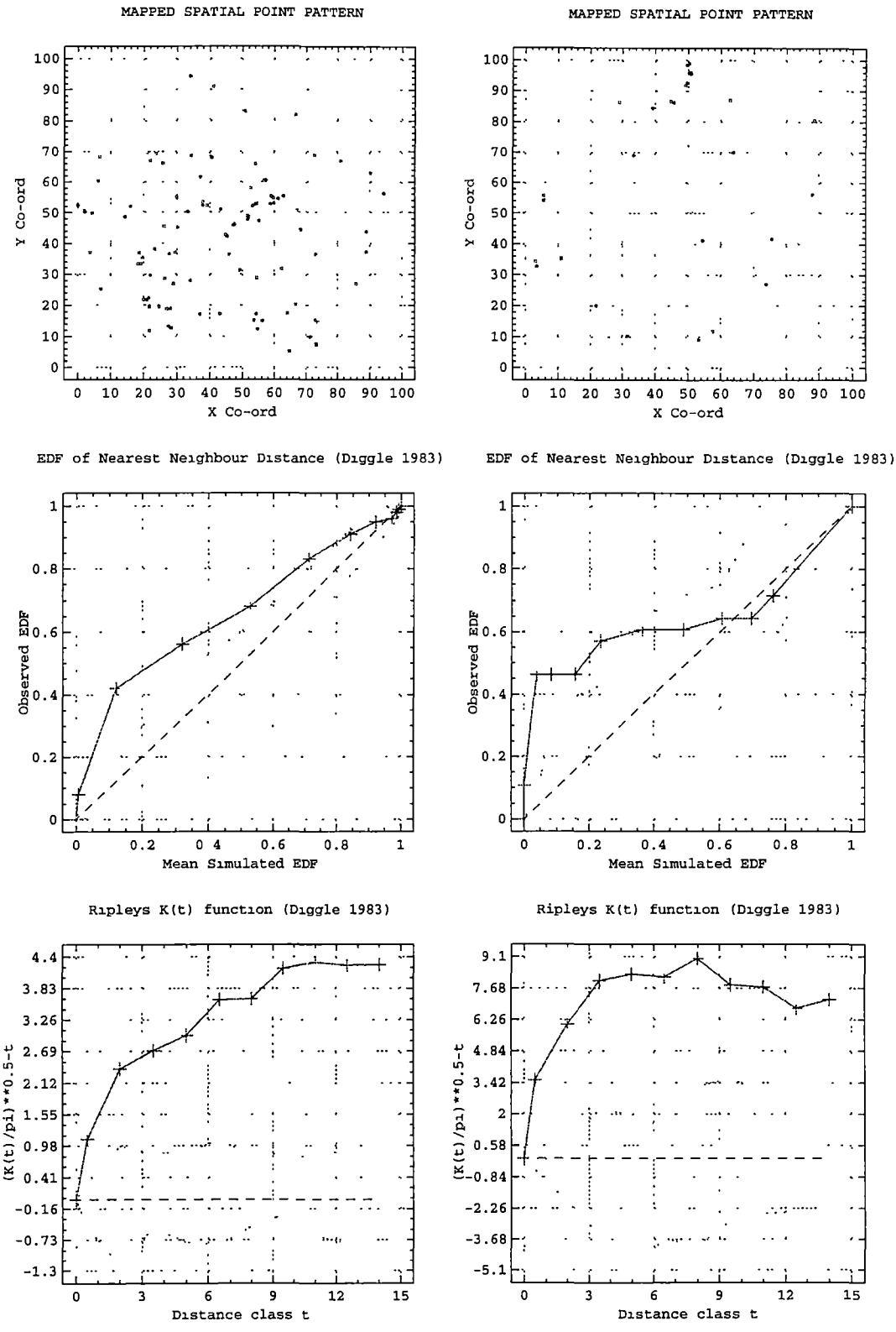


Figure 3.4 Pattern analyses of myrtle wilt infection for the Simons Road (left) **and Frodshams Pass** (right) **plots.** (See Figure 3.3 for interpretation.)

Cumulative distribution of myrtle wilt infected trees. (top)

Plots of EDF values for observed and simulated data. (middle)

Plots of the $\hat{K}(t)$ function against distance t (m). (bottom)

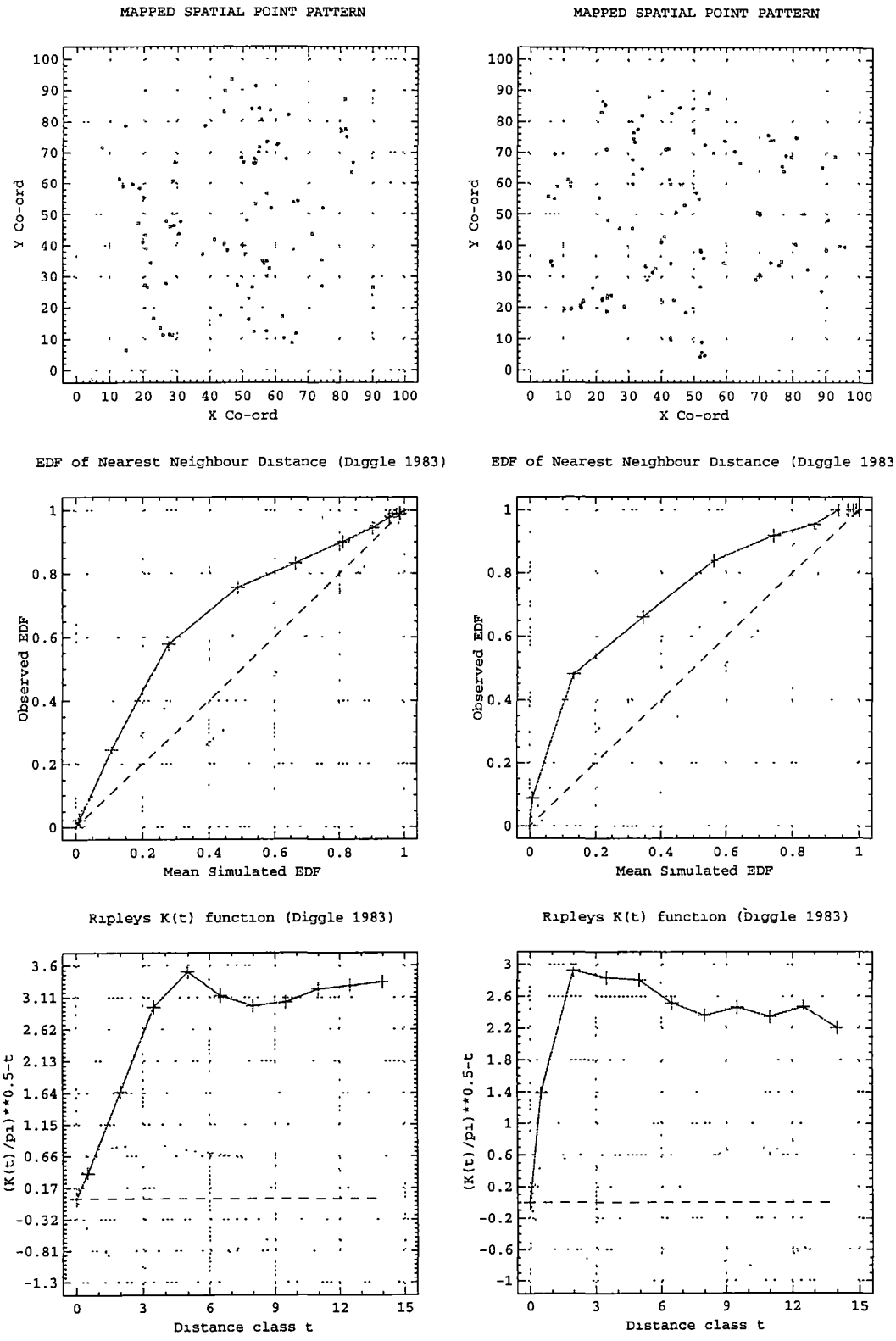


Figure 3.5 Pattern analyses of myrtle wilt infection for Five Road, plots 2 (left) **and 3** (right).

(See Figure 3.3 for interpretation.)

Cumulative distribution of myrtle wilt infected trees. (top)

Plots of EDF values for observed and simulated data. (middle)

Plots of the $\hat{K}(t)$ function against distance t (m). (bottom)

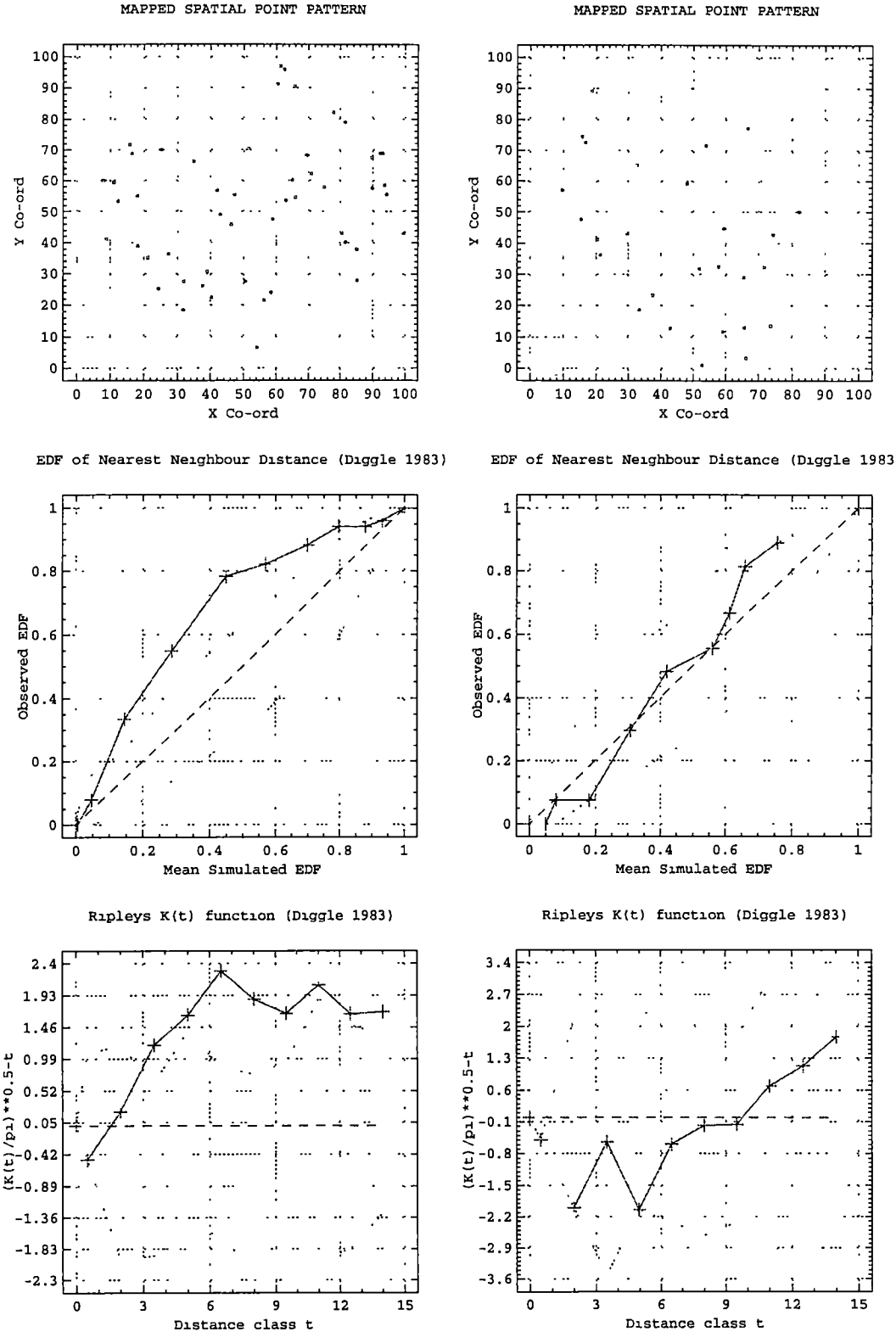


Figure 3.6 Pattern analyses of myrtle wilt infection for Lake Chisholm, plots 1 (left) **and 2** (right).

(See Figure 3.3 for interpretation.)

Cumulative distribution of myrtle wilt infected trees. (top)

Plots of EDF values for observed and simulated data. (middle)

Plots of the $\hat{K}(t)$ function against distance t (m). (bottom)

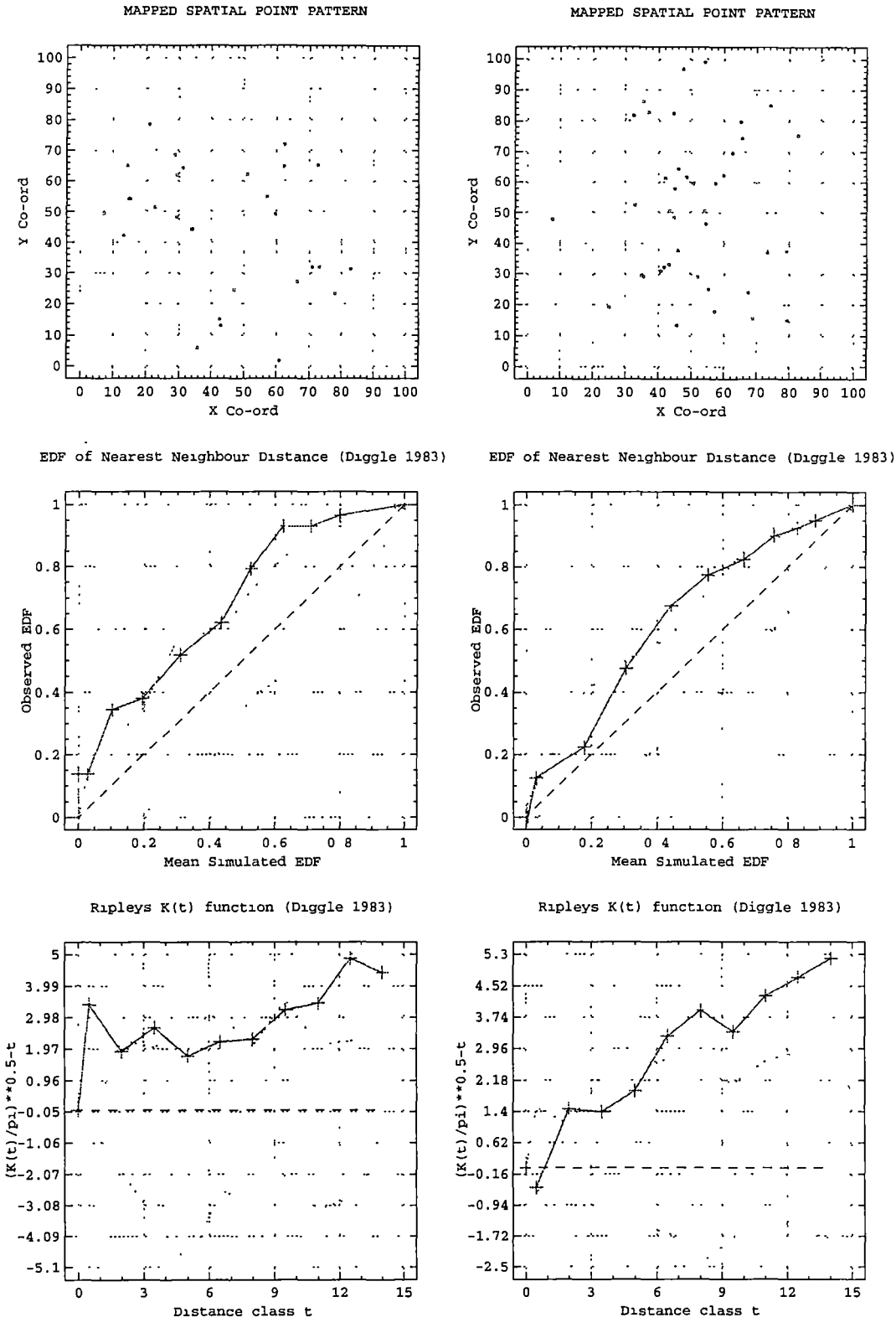


Figure 3.7 Pattern analyses of myrtle wilt infection for Lake Chisholm, plots 3 (left) **and 4** (right).

(See Figure 3.3 for interpretation.)

Cumulative distribution of myrtle wilt infected trees. (top)

Plots of EDF values for observed and simulated data. (middle)

Plots of the $\hat{K}(t)$ function against distance t (m). (bottom)

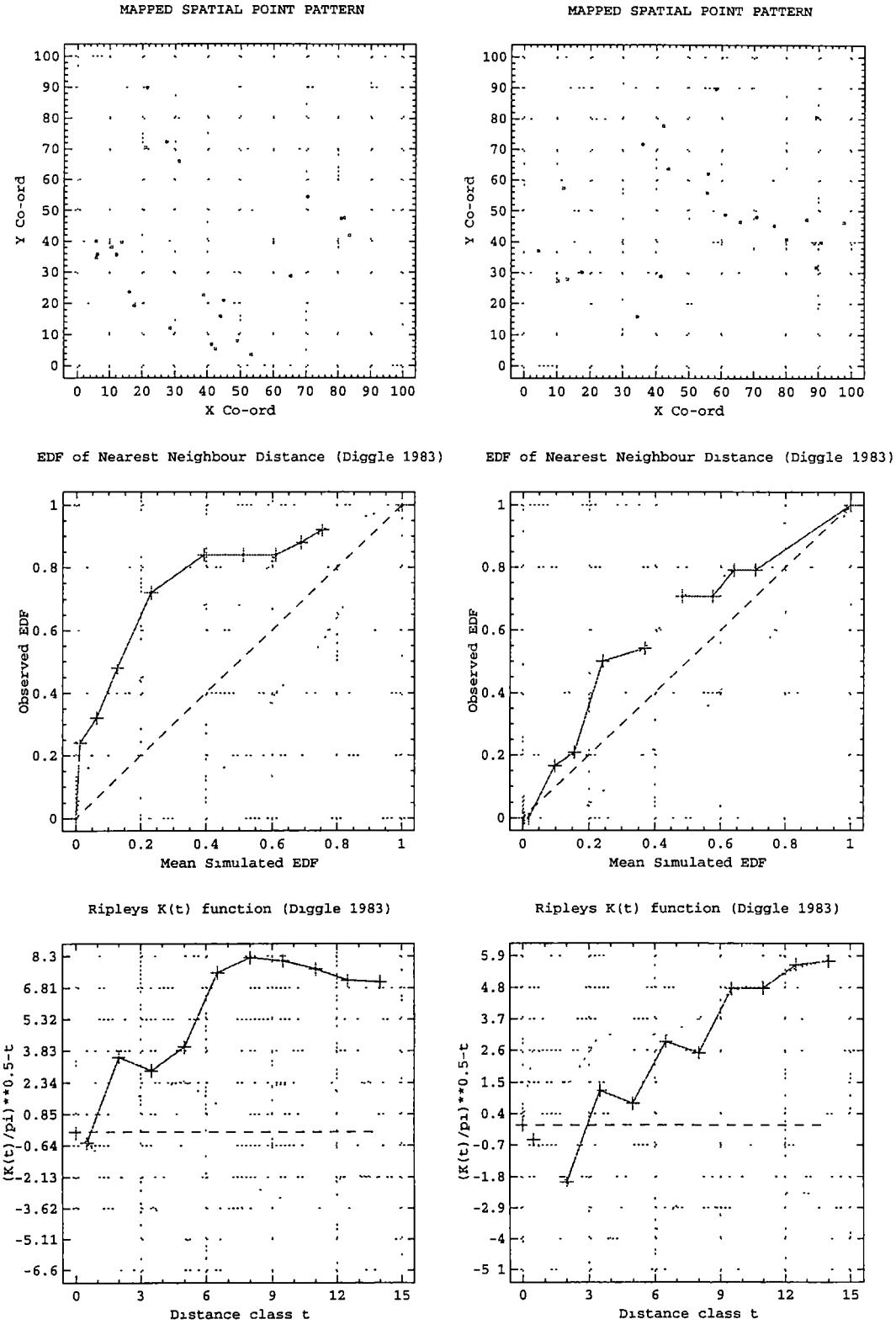


Figure 3.8 Pattern analyses of myrtle wilt infection for Pipeline 18 Mile Peg, plots one (left) **and three** (right). (See Figure 3.3 for interpretation.)

Cumulative distribution of myrtle wilt infected trees. (top)

Plots of EDF values for observed and simulated data. (middle)

Plots of the $\hat{K}(t)$ function against distance t (m). (bottom)

Transect remeasurements

The changes in the number of trees in each health status class on the remeasured transects, the percentage of trees diseased (cumulative dead and dying) due to myrtle wilt and the estimated annual mortality rates are shown in Table 3.5.

Table 3.5 Change in disease incidence and mortality rates on transects, over a five year period

Site and Rainforest type	Date of assessment	No. of myrtles in health status class 1	2	3	4	5	6	7	8	% Diseased (HSC 3-6/1-8)	Mortality (%p. a.)
Mt King William (Thamnic/Callidendrous)	Apr 1984	217	3	1	0	1	35	6	2	13.96%	0%
	Jun 1989	213	4	2	0	0	36	7	3	14.33%	
Simons Road (Callidendrous)	Dec 1983	343	3	2	9	36	53	8	0	22.03%	0.78%
	Nov 1988	312	3	6	10	23	80	14	5	26.27%	
Tombstone Creek (Callidendrous)	May 1984	68	0	4	2	18	29	0	2	43.09%	0.59%
	Jan 1989	61	7	2	2	3	46	2	0	43.09%	

Estimated and actual mortality rates

Whole sites

Table 3.6 shows the results of the chi squared tests of the estimated and actual mortality rates of trees in the Arve Loop plot and the transects at Simons Road and Tombstone Creek (the Arve Loop plot having three measurements). The null hypotheses were that trees remained in HSC 4 for one, two, three or four years (chi squared tables are given in Appendix 11). The first and last of these can be rejected and it can be concluded that trees at these sites remained in HSC 4 for two or three years.

Table 3.6 Results from chi squared tests of estimated and actual mortality at three sites, to test the null hypotheses that trees remain in HSC 4 for one, two, three or four years

No. of years in HSC 4	Chi squared	Degrees of freedom	Significance level
1	39.425	5	0.001
2	5.185	4	NS
3	4.413	3	NS
4	12.199	3	0.01

The observed number of trees which had died (cumulative over all sites) was calculated as 39 (Appendix 11). A multiplicative model of the form $y = ax^b$ gave the best fit for (cumulative) expected number of dead trees plotted against time spent in HSC 4. When the expected value equalled the observed value (39), the model predicted that trees spent an average of 2.6 years in HSC 4. The regression analysis and ANOVA of the model are given in Tables 3.7 and 3.8 respectively.

Table 3.7 Regression analysis for the expected number of dead trees (cumulative over sites) against the number of years spent in HSC 4

Multiplicative model: $y = ax^b$				
Dependent variable (expected number of dead trees)	101	50.5	33.6	25.3
Independent variable (years spent in HSC 4)	1	2	3	4

PARAMETER	ESTIMATE	STANDARD ERROR	t VALUE	SIGNIFICANCE LEVEL
Intercept	4.615	1.772E-3	2604.42	0.00000***
Slope	-0.999	1.865E-3	-535.817	0.00000***

N.B. The intercept is equal to Log a

Table 3.8 ANOVA of the regression model

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MODEL	1.08	1	1.08	287099.7	0.00000***
ERROR	0.0000075	2	0.0000038		
TOTAL (CORR.)	1.0830795	3			

Correlation coefficient = -0.999997

Standard error of estimate = 1.94228E-3

R^2 = 100.00%

Arve Loop trees

The approximate lengths of time for which trees in the Arve Loop remained in HSC 4 are shown in Table 3.9. While most trees remained in HSC 4 for approximately two years there was obviously much variation. However, most trees which differed from the mode spent less than two years in HSC 4. Additionally it is thought unlikely that the tree which spent at least six years in HSC 4 was actually infected with *C. australis* (G. A. Kile, personal communication).

Table 3.9 The number of biennial recording dates at which individual trees in the Arve Loop plot were observed to be in HSC 4

No. of biennial recording dates	Years spent in HSC 4 (range & midpoint)	Number of trees
0	0-2 (1)	4
1	0-4 (2)	12
2	>2	1
3	>4	0
4	>6	1

Individual trees

The amount of time trees spent in HSC 4 (time since symptom onset) before they were felled, and the amount of foliage retained at this time, are shown in Table 3.10. Trees spent

13.4-15.6 months (mean min-mean max) in HSC 4 before they were felled. At this stage all the trees were still in HSC 4 (i.e. had some foliage remaining), and ten of the 14 still had more than 10% of their foliage remaining. Again, the indication is that trees remain in HSC 4 for longer than one year.

Table 3.10 An assessment of foliage retention and the time elapsed since the onset of myrtle wilt symptoms

Tree number	Months from symptom onset to death	Months after death at which foliage was assessed	% Foliage retained at assessment	Months since symptom onset
8	9-13	2	50-60	11-15
12	4-7	2	10 windthrown	6- 9
13	9-11	6	50-60	15-17
15	0.5-2	18	5	18.5-20
23	0.5-2.5	8	40-50	8.5-10.5
25	3-6	8	5-10	11-14
28	2-3.5	13	60	15-16.5
31	7.5-10	8	50-60	15.5-18
33	0-1	18	30-40	18-19
34	2-4.5	12	50	14-16.5
37	0-2	15	30-50	15-17
38	1-2.5	15	40-50	16-17.5
41	1-3.5	7	5	8-10.5
45	8-10	8	50-60	16-18
				mean min 13.4 months
				mean max 15.6 months

3.4 DISCUSSION

Myrtle wilt levels

Arve loop and ROS plots

In the 11 years since its establishment, the Arve Loop plot has generated valuable information on the behaviour of myrtle wilt with time, in an undisturbed situation. There has been a slight increase in disease incidence with time. One reason for this increase is that

after the first measurement, trees growing into the 5-10 cm DBH class were not included, thereby omitting a number of small, healthy trees from the calculations. The measured annual mortality rates are showing no sign of the rapid escalation that was feared, had myrtle wilt displayed similar properties to Dutch elm disease (Elliott *et al.* 1987). Although the ROS plots have not been measured over such a long period, at this stage results do not suggest any short-term escalation in myrtle wilt levels.

The Tasmanian survey of standing myrtles (Elliott *et al.* 1987), indicated an average value of 24.6% diseased (cumulative dead and dying due to myrtle wilt), with a range 9.4-53.4%, and with 1.6% (range 0-4.5%) currently dying due to the disease. Subsequently, a value of 7.1% dying has been recorded in a remote, undisturbed area (Table 2.10). The Arve Loop, with 24.0% diseased in 1982, was very close to the average for all sites, and slightly higher than the 19.9% recorded for a nearby area of the Arve Loop by Elliott *et al.* (1987). The inclusion of recruits to the smallest size class in the 1990 survey is probably responsible for the apparent decrease in disease incidence between 1988 and 1990.

Disease incidence in the ROS plots was within the range expected, with the callidendrous sites having a higher incidence than the thamnic and implicate sites. Since plots are intended to be used for long-term measurement, and are representative of their respective rainforest types, it is significant that these results agreed with those of the more comprehensive survey of Elliott *et al.* (1987), which are further examined in Appendix 10. The effect of rainforest type on disease incidence is not fully understood, but it would seem logical for the optimum conditions of a species specific pathogenic agent to be close to the optimum growing conditions of its host. The dominance of myrtle is greatest in callidendrous (tall, open understorey) rainforest, decreases in thamnic (shrubby) communities, and reaches its minimum in implicate (tangled) rainforest (Jarman *et al.* 1984).

The incidence of recent damage followed the same trend as disease incidence, although whether damage is the cause or the effect of disease in this case was not clear. It is known however, that wounding provides an infection site for *C. australis* (Kile and Walker 1987) and it is reasonable to assume that diseased trees will sometimes cause damage to surrounding trees as they deteriorate. It is most likely damage is both a cause and an effect of high disease incidence and certainly the sites with high disease incidence also had a high incidence of recent damage.

The proportion of currently dying myrtles has been very high at Five Road (10.88% in 1989), although it is now declining. This mid altitude site is totally undisturbed but in places has an

overstorey of old eucalypts which are dying. This is presumably causing considerable damage to the mature, callidendrous rainforest below, and is facilitating entry of the pathogen. Canopy gaps formed by wilt are very large (up to 1750 m^2), and this was the only site studied where myrtle regeneration was not significantly increased in such gaps (see Chapter 6).

Transect re-measurements

There are several factors which may have artificially elevated the disease incidence levels of the remeasured transects. Firstly, trees growing into the 15 cm DBH class have not been assessed so the stand being recorded is an ever-aging one. It is already known that trees of larger diameter are more likely to be infected (Elliott *et al.* 1987) so an apparent increase in disease incidence could be expected for this reason alone. This would also lead to the calculated annual mortality rates being artificially high since a number of small, healthy trees are omitted from the calculation.

Secondly, trees which were originally dead standing but which have been subsequently uprooted, were included in the re-measurement, whereas the original measurement only included stumps more than 2 m in height. The very limited data available (see Chapter 6), indicate a (non-significant) trend for trees which have died of wilt to snap (leaving stumps), rather than to uproot. However, uprooting is still likely to lead to an overestimate of disease incidence.

Despite these inaccuracies, the transect re-measurements provide some useful information. The disease incidence at Tombstone Creek, which had the highest level of all the sites recorded by Elliott *et al.* (1987) had not increased in the intervening five years. Mortality rates show there were some tree deaths but no new infections. It is possible that at this stage all trees susceptible to *C. australis* had already been infected.

Tombstone Creek is a mixed forest site with a eucalypt overstorey and a callidendrous rainforest understorey, and it is interesting to speculate on why such high disease levels were ever reached. One obvious factor is the possible damage caused by the falling branches of eucalypts and the consequent increased probability of wilt. Also, Elliott *et al.* (1987) tabled data which indicated that the mixed forest sites had lower absolute and relative densities of myrtle than the pure rainforest sites (Appendix 10), and their analysis showed that disease incidence increased with the relative density of myrtle in mixed forests. This trend was not evident in the other forest types.

Referring to the tree density data given by Elliott *et al.* (1987), it seems likely that when the myrtle density in absolute terms is between 131 stems/ha (mean for their mixed sites) and 246 stems/ha (mean for their pure rainforest sites) and/or in relative terms between 40% (mean for their mixed forest sites) and 60% (mean for their pure rainforest sites), it reaches an optimum for disease spread, such that any further increase in myrtle density (e.g. that found between different pure rainforest types), results in no further increase in disease (Appendix 9).

In many cases the effects of these factors may nullify each other; a mixed forest may suffer more damage but have a lower relative density of myrtle than an area of pure rainforest. However, Tombstone Creek had the highest relative density of myrtle of all the mixed forest sites studied by Elliott *et al.* (1987), and in addition a eucalypt overstorey and a callidendrous (higher disease risk) understorey. Possibly precipitated by a little roadside disturbance and raised inoculum levels, this proved a disastrous combination!

In contrast to Tombstone Creek, mortality rates had increased at Simons Road between measurements. At this site the cumulative disease incidence was lower and, presumably, there were still some susceptible trees available for infection. However, the Mt King William site, which had a very low disease incidence, had experienced little change and no mortality due to wilt in the five year period. One reason for this could be that the vegetation at Mt King William had a high thamnic component and thamnic forests on average have lower levels of myrtle wilt than callidendrous forests (Elliott *et al.* 1987).

Pattern of disease

Clumping of diseased trees was evident in all forest types considered, (although not on all plots), with the scale of clumping varying from 2.5 to 14 m. Taken with the results of Diggle (1983a), who found significant clumping when the 'grid' size used was greater than 10 m, and of Elliott *et al.* (1987) who found a greater probability of two neighbouring trees both being attacked as the distance between them decreased from 8m to 1m, this leads to the interesting question of the significance of this range of scales.

It could represent the limit of inoculum spread from tree to tree, but given the ubiquity of the disease even in remote sites (Chapter 2), such a limit seems unlikely. The local spread of the disease via root grafts may be a better explanation, with 1-14 m representing the range of between-tree-distances at which such spread can readily occur. The possibility of *C. australis* spread by this means was discussed by Elliott *et al.* (1987), and is investigated further in Chapter 5.

Annual mortality rates

New Tasmanian estimate

On average, trees remain in the 'currently dying' state (HSC 4) for 2.6 years (at least in the three sites investigated), and a more reliable estimate of annual mortality rates may be obtained by dividing the proportion of trees in HSC 4 by a factor of 2.6. Given this recent evidence, it is necessary to modify the original Tasmanian estimate of average mortality due to myrtle wilt (Elliott *et al.* 1987). This is now estimated as 0.61% per annum (range 0-1.73%).

Elliott *et al.* (1987) also found that on average 0.84% of live myrtles had major crown dieback of cause unknown (HSC 8). Although it is not known how long these trees take to die, it can be assumed they take considerably longer than those infected with wilt, which would give a relatively low annual mortality rate due to unknown dieback. Unfortunately annual mortality rates due to windthrow were not considered, but in later studies at Simons Road the number of myrtle deaths due to windthrow and due to unknown causes (HSC 7) were found to be similar (Chapter 6). Thus it should be remembered that the total mortality rates of myrtle will be somewhat greater than the 0.61% per annum caused by wilt.

Short-term effects

Considering myrtle wilt only, and assuming an even distribution of mortality across Tasmanian myrtle forests (which manifestly is not the case), this could lead to the death of a high proportion of mature myrtles. However, given the variation in incidence levels and annual mortality rates it is probable that some stands will remain more or less intact, and the evidence from Tombstone Creek indicates that even in the most severely affected stands some mature myrtles will survive.

This is supported by Runkle (1985), who cited the average disturbance rate of forests as one percent per annum (range 0.5-2%) with a natural return interval of 50-200 years. He maintained that these figures could be reconciled with average tree longevities of 300-500 years because:

- certain trees live longer due to their protected locations or chance deviations from normal (weather) conditions;
- many important forest dominants often persist for many years under a closed canopy, growing very slowly.

Both of these factors are likely to be relevant with regard to myrtle wilt.

Vectored and non-vectored diseases

In answer to the concerns expressed by Elliott *et al.* (1987), myrtle wilt does not appear to be progressing in a way comparable to the recent European epidemic of Dutch elm disease. One probable reason is that the causal organism, *C. australis,* is less pathogenic than *Ophiostoma novo-ulmi.* Another reason may be the difference in dispersal mechanisms: Dutch elm disease is a vectored disease(Webber 1990) whereas myrtle wilt is not (Kile and Hall 1988). Therefore the spread of Dutch elm disease is influenced by the food requirements and host-finding abilities of the vector population.

Table 3.11 Theoretical comparison of disease progression in vectored and non-vectored disease

TIME (years)	VECTORED DISEASE (constant food requirement - 25 trees p.a.)		NON-VECTORED DISEASE (constant mortality rate - 25% p.a.)	
	Tree Nos	Mortality Rate	Tree Nos	Mortality Rate
1	100		100	
		25%		25%
2	75		75	
		33%		25%
3	50		56	
		50%		25%
4	25		42	
		100%		25%
5	0		32	

Considering a vectored disease in its simplest form (Table 3.11), a stable vector population is likely to have a relatively constant food requirement. This will eventually necessitate some form of host-finding and selection by the vectors, and the host tree population may then be virtually eliminated (since tree numbers drop arithmetically). In contrast, a non-vectored disease with a constant mortality rate is unlikely to ever completely eliminate the host population (since tree numbers drop geometrically).

In reality, host and vector populations will be co-dependent, and the host population is unlikely to actually become extinct, as evidenced by biological weed control programmes (Sagar 1974). Additionally, mortality rates even of non-vectored diseases will fluctuate in

space and time (Van der Plank 1975). However, the food requirements and host-finding abilities of the vector population may result in progressively smaller trees being infected, and the drastic reduction of the host population, as has been predicted for European field elms (Brasier 1983). This is not so likely to happen in the case of a non-vectored disease which relies on passive dispersal mechanisms.

Thus the imminent loss of all mature myrtles is very unlikely in undisturbed situations. Whether the species composition and structure of forests can be maintained at present wilt mortality levels is another question, which is considered in Chapter 6. The long-term effects of myrtle wilt on the myrtle population are considered in Chapter 7.

3.5 SUMMARY

Long-term monitoring plots have been established in different rainforest types around Tasmania, with changes in disease incidence and distribution, health status and mortality rates being recorded every two years. Transect re-measurement indicated that there have been no new infections by myrtle wilt in the site which originally had the highest disease incidence. In general, mortality rates are variable but not escalating, with no overall Tasmanian trend apparent after four to ten years of measurement.

Disease and damage incidence are significantly related to rainforest type, with callidendrous sites having the highest levels of disease and damage. There is some indication that damage caused to myrtles by a eucalypt overstorey may increase the probability of myrtle wilt. On most plots diseased trees had a clumped distribution, and this was the case in all the main forest types investigated. On the permanent plots the scale of clumping ranged from 2.5-14 m, which supports the theory that clumping is, at least partially, caused by the transfer of the disease via root grafts between neighbouring trees.

Comparison of estimated and actual mortality rates due to myrtle wilt have indicated that the original Tasmanian estimate of annual mortality was overestimated by a factor of two to three, and this is now calculated to be 0.61% p.a. Due to site variations and to myrtle's ability to persist and grow very slowly under a closed canopy, this mortality rate may be compatible with the currently observed longevity of myrtle.

4. DISTURBANCE AND MYRTLE WILT

4.1 INTRODUCTION

Disturbance can be defined as a disruption of forest structure, and may be classified according to its *effect* on plant populations, and also by its *cause* (see Chapter 1). Forest disturbance may be caused by natural events or processes, either endogenous or exogenous (e.g. browsing, wind, floods, landslips, volcanic activity and fire), or by the activities of man (e.g. logging and thinning operations, road and track construction). This chapter is an attempt to summarise the effects of *anthropogenic* disturbance on myrtle wilt levels.

The connection between high levels of myrtle wilt and logging and roading activities has long been recognised (Howard 1973a, 1981; Elliott *et al.* 1982; Jackson 1983; Read and Hill 1985a; Hickey and Felton 1991). Howard (1973a) noted the link between cull felling and access road construction and the widespread deaths of a large number of myrtles. These deaths were associated with accelerated *Platypus subgranosus* attack and it was inferred that the beetles were vectors of a fungal pathogen. Coppice regeneration from stumps, and from burls on fire-damaged trees, was also killed (Howard 1981).

Since *Platypus* spp. are known to attack logs, green timber, stressed or damaged trees, Elliott *et al.* (1982; 1983) investigated the role of volatiles in the attraction of *P. subgranosus.* Ethanol was found to be an attractant and a boring stimulant, and wounded trees (particularly those with stem wounds) were found to have a higher likelihood of attack by *P. subgranosus/Chalara australis* than undamaged trees (Elliott *et al.* 1987).

Wounding of trees allows direct infection of stems by *C. australis* (Kile and Walker 1987). *C. australis* was not dependent on *P. subgranosus* for its spread or entry into trees since infection actually occurred prior to beetle attack (Kile and Hall 1988). Further investigation indicated that although *P. subgranosus* did attack wounded or burnt trees, only trees infected with *C. australis* suffered sustained attack (Kile *et al.* 1992). However, after selection and attack of stressed trees by *P. subgranosus*, it is possible that *C. australis* may occasionally gain entry via its tunnels (Kile *et al* 1989, 1992). It is also possible that contaminated *P. subgranosus* frass acts as a windborne inoculum (Kile and Hall 1988; Kile *et al.* 1989; Kile, G. A., personal communication).

Howard (1973a, 1981) noted that myrtle seedlings, saplings and pole trees were not normally attacked by *P. subgranosus*, and this was confirmed by Elliott *et al.* (1987) who found no attack in trees less than 12 cm diameter at breast height (DBH). Kile and Walker (1987) reported occasional natural infection by *C. australis* of saplings of 9 cm DBH, but no natural infection of seedlings was observed.

Despite the relevance of these findings to the management of myrtle forests, at the start of this project there were no published data showing the effects of anthropogenic disturbance on myrtle wilt. Hickey and Felton (1991) noted the myrtle wilt related problems caused by attempting to thin myrtle stands older than 40 years, and expressed concern as to the effective survival of seed trees left after logging operations. This was based on findings from a series of rainforest logging, regeneration and thinning trials carried out by the Forestry Commission, Tasmania. The data from these trials which pertain to myrtle wilt have been collated and are presented in this section. Kile *et al.* (1989) included limited data relating to roadside disturbance, which were collected as part of the following study.

4.2 MATERIALS AND METHODS

Rainforest logging and regeneration trials

During the 1970s and 1980s a number of rainforest silvicultural trials were established by the Forestry Commission in the Smithton area of north west Tasmania. These investigated various logging methods and the resultant regeneration. Some of the treatments had special 'dieback' or 'canopy' transects in which the levels of myrtle wilt were monitored. Others generated data which could be manipulated for the same purpose. This summary attempts to draw together the information on myrtle wilt incidence and mortality from the trials and to compare the results with those from undisturbed rainforest.

The Sumac Road logging and regeneration trials involved a number of treatments and a control in the same forest area (callidendrous/thamnic rainforest), with similar soil types. The other individual logging and regeneration trials encompassed a number of treatments, over a range of callidendrous and thamnic rainforest types, on various soils. The trials are summarised in Table 4.1.

Table 4.1 Treatments used in the Smithton logging and regeneration trials

Trial name	Treatment and date carried out
Sumac Area 1	Selectively logged for sawlogs, Nov. 1976
Sumac Area 2	Strip logged, Nov. 1976; some ringbarking, July 1981
Sumac Area 3	Logged for sawlogs and pulpwood leaving shelterwood, also soil scarification to improve regeneration, Feb. 1978; some ringbarking, July 1981
Sumac Area 9	Control; undisturbed rainforest
Pipeline Scarification	Pre-logging soil scarification to give advanced regeneration, Feb. 1984
Pipeline 20 Mile Peg	Logged for sawlogs and pulpwood leaving shelterwood, March 1982
Julius Dolomite	Logged for sawlogs and pulpwood leaving shelterwood, also soil scarification to improve regeneration, May/June 1981
Rabalga Road	Post-logging soil scarification to give advanced regeneration, Dec. 1980 (area previously selectively logged in 1960s)
Pruana Road	Clearfelled with soil scarification to improve regeneration, leaving myrtle seed trees, March 1981

Where 'canopy' or 'dieback' transects were established in the logged areas, myrtles greater than 5 cm DBH were measured within 3 m of the centre line, those greater than 20 cm DBH to within 10 m. In some cases only shelterwood or seed trees remained and the fate of these trees was followed. In all cases only trees greater than 20 cm DBH have been included in the following analyses. Where possible, results were derived from original Forestry Commission field books, with numerous internal reports also being utilised (Blakesley 1980; Hickey 1980, 1981a, 1981b, 1981c, 1982b, 1983a, 1983b, 1983c, 1983d, 1984a, 1984b; Cowell 1985; Jennings 1985, 1987a, 1987b, 1987c; Mesibov 1985a, 1985b, 1985c).

Results were interpreted using the health status class (HSC) classification of Elliott *et al.* (1987), given in Table 2.1. It was assumed that 'healthy' equated to HSC 1, 2 and 3 (HSC 3 is not healthy, but superficially appears so and would have been recorded as such); 'sick' equated to HSC 4 and 8 (regarding HSC 4, this is not strictly accurate because if all leaves were brown, trees were recorded as dead) and that 'dead' equated to HSC 5 and 6. Where death was obviously due to other causes e.g. windthrow or felling (HSC 7), this was noted. Occasionally trees on the transect were not found, and these were assumed to have died of myrtle wilt.

Disease incidence (cumulative dead and dying of wilt) at each measurement, per annum mortality rate due to wilt between successive measurements and total mortality due to wilt over the whole period of measurement were calculated. Disease incidence was then plotted against time. Brief notes on treatment, soil type, vegetation type and tree density, with overall per annum mortality rates due to wilt are given in Appendix 12.

Myrtle thinning trials

Other trials at Smithton tested the effect of thinning on myrtle growth. The trials used even-aged myrtle pole stands of various ages, located at different sites. Each thinned site had at least one unthinned control. Treatments are summarised in Table 4.2.

Table 4.2 Treatments used in the myrtle thinning trials

Trial name	Treatment and date carried out
Sumac Spur 1 Thinned	15 yr old stand, thinned April 1981, pruned to 2m - 2.5m, May 1986
Sumac Spur 1 Control	15 yr old stand, undisturbed rainforest
Pipeline 22 Mile Peg Thinned	15 yr old stand, thinned Oct. 1980 (two plots); thinned and pruned to 2m Feb. 1981 (one plot)
Pipeline 22 Mile Peg Control	15 yr old stand, undisturbed rainforest
Pipeline 26 Mile Peg Thinned	18 yr old stand, thinned Sept. 1983
Pipeline 26 Mile Peg Control	18 yr old stand, undisturbed rainforest
Oonah Thinned	40 yr old stand, thinned April 1984 (four plots)
Oonah Control	40 yr old stand, undisturbed rainforest (four plots)
Rabalga Spur 4Thinned	40 yr old stand, thinned June 1981 (nearby area previously selectively logged in 1960s)
Rabalga Spur 4 Control	40 yr old stand, undisturbed rainforest but with nearby area previously selectively logged in 1960s (two plots)
Blackwater Spur 6 Thinned	65 yr old stand, thinned April 1982
Blackwater Spur 6 Control	65 yr old stand, undisturbed rainforest

All trees were included regardless of their DBH, and results were derived from the original Forestry Commission field books where possible, with a number of internal reports also being utilised (Jennings 1988a, 1988b, 1988c, 1989a, 1989b, 1989c). For the thinning trials, trees were recorded only as 'alive' (equating to HSC 1, 2, 3, 4 & 8) or 'dead' (equating to

HSC 5, 6 & 7). In the absence of other information all deaths (except where noted as windthrown) were attributed to myrtle wilt.

Results were tabulated as for the logging and regeneration trials and the proportion of trees dead due to wilt was plotted against time. Brief notes on stand age, soil type, vegetation type and overall per annum mortality rates due to wilt are included in Appendix 12.

For the only replicated thinning trial (Oonah 40-year-old stand), an analysis of variance (ANOVA) was carried out on the per annum mortality rates due to wilt. The data were first transformed to the arcsin square root of the percentage.

Blackwood selective logging trial

In 1991 a trial was set up by the Forestry Commission to test the feasibility and effects of selectively logging blackwood (*Acacia melanoxylon*) from an area of riparian blackwood rainforest along the Arthur River (south of Smithton, north-west Tasmania). The area was logged in January 1991, and in March 1991 two transects (100 m x 10 m) were established; one within the coupe and the control in nearby unlogged forest. All myrtles of 15 cm DBH and above were assessed for myrtle wilt and recent damage using the categories given in Tables 2.1 and 2.2. The transects were remeasured in March 1992. Site details are given in Table 4.3, the forest type being fully described by Pannell (1992).

Replicates were obtained by dividing each transect into three sections, with equal numbers of myrtles in each section. Disease and damage incidence and per annum mortality rates due to myrtle wilt were calculated for each section. ANOVAs were carried out on the percentage of trees diseased (HSC 3-6/1-8) and damaged (DC 2-4/1-4), and on per annum mortality rates. Then an ANOVA on the percentage diseased (in 1992) was performed using the percentage damaged (in 1991) as a covariate. The data were first transformed to the arcsin square root of the percentage.

Table 4.3 Details of the blackwood selective logging trial

Site	Recorded	Forest type	Disturbed	Other notes
Arthur River	1991, 1992	Riparian blackwood rainforest (D6)	1991	Forestry Commission coupe SU 801B, selectively logged for blackwood

Disease spread into areas adjacent to logged areas and roads

Transects were run from the edge of disturbed areas into undisturbed forest. Starting positions were randomly allocated but all transects ran perpendicular to the edge of the disturbance. For road sites, transects were normally located on the same side of the road and were divided into subsections, the number of these depending on the length of the transect. The transect width was 10 m and transects were continued into the undisturbed forest until there were no longer any direct or indirect effects of disturbance (i.e. raised levels of damage or myrtle wilt) evident. All standing myrtles greater than 15 cm DBH were assessed for myrtle wilt and recent damage (Tables 2.1 and 2.2). Sufficient transects were completed at each site to give at least 200 myrtles, and with a minimum of five transects.

Within each transect subsection the proportion of trees diseased (HSC 3-6/1-8) and damaged (DC 2-4/1-4) was calculated and the distance from the disturbance noted (transects forming replicates). GENSTAT (GENSTAT 5 Committee 1987) was used to construct generalised linear models of the proportion of trees diseased and damaged against three explanatory variables:

- distance from disturbance;
- 1/distance from disturbance and
- log distance from disturbance.

These assumed a binomial distribution and used the logit link function. In each case the model giving the best fit was selected and the proportion of the deviance explained by the model was noted. Where a Student's t test on the regression coefficient of the explanatory variable showed it to be significant, the proportion of trees (diseased or damaged) was plotted.

The details of the sites investigated adjacent to logged and roaded areas are given in Tables 4.4 and 4.5 respectively. Forest types follow Jarman *et al.* (1991).

Table 4.4 Details of a site investigated adjacent to a logged coupe

Site	Recorded	Forest type	Disturbed	Other notes
Simons Road	1989	C2.1	Logged 1986 Burnt 1987	Forestry Commission coupe CD32

Table 4.5 Details of sites investigated adjacent to roads

Site	Recorded	Forest type	Disturbed	Other notes
Simons Road	1983#, 1988	C2.1	1974/75 (76)	Originally a track, road built by APPM###
Mt King William (Lyell Highway)	1984#, 1989	CT1 & T5.1/2	1973	Original road built in 1920s on site of walking track, widened and sealed in 1970s by DMR###
Scotts Peak Road	1988	CT1/T3.1	1968	Road constructed by HEC###
Sumac Spur 1B	1988	C/T	1986-88	Road constructed by Forestry Commission
Bennetts/ Esperance Link	1989	T1.1/2##	1986	Road constructed by Forestry Commission
Frodshams Pass	1989	I4.1/T1.2	1968	Road constructed by HEC###

\# Recorded by Elliott *et al.* (1987)

\## No *Eucryphia lucida* present

\### APPM - Associated Pulp and Paper Mills
DMR - Department of Main Roads
HEC - Hydro Electric Commission

The earlier of the two measurements of Simons Road and Mt King William are taken from the survey by Elliott *et al.* (1987). However, there were only three transects at each site and they did not start at the road edge. For the remeasurement these were extended to the road edge, with two additional transects being measured at each site. At the Simons Road site no trees had been recorded as damaged in 1983 and no damage assessment was undertaken in 1988. There were only four transects at Scotts Peak Road and these were paired on opposite sides of the road.

Disease levels along walking tracks

At Mt Scott and Weindorfers Forest a 10 m wide transect immediately to one side of the walking track was assessed for disease and damage incidence as above. A parallel control transect of the same length was located 100 m (Mt Scott) or 50 m (Weindorfers Forest) to one side. Transects were long enough to include approximately 200 myrtles. The number of trees in each health status class and disease incidence were tabulated for the whole track and control transects.

Track and control transects were then divided into an equal number of subsections of the same length and these formed replicates. For each subsection the incidence of disease and damage was calculated, slope of the transect was measured and altitude was estimated.

At Liffey Falls a simplified method was used, the transect being sited 5 m to either side of the track, with a control transect located in the closest, relatively undisturbed forest. Both transects were only 150 m long, but were divided into six subsections of equal length to give replicates. For each subsection the disease and damage incidence was calculated.

ANOVAs of track versus control were performed on all measured variables separately i.e. percentage diseased, percentage damaged, altitude and slope. Then an ANOVA on the percentage diseased was performed using the other variables as covariates. In all cases the data relating to the percentage of trees damaged and diseased were first transformed (arcsin square root of the percentage).

Details of the sites investigated are given in Table 4.6. Forest types follow Jarman *et al.* (1991).

Table 4.6 Details of walking tracks investigated

Site	Recorded	Forest type	Disturbed	Other notes
Mt Scott	1989	C3.1	±1940, ±1980, 1988	Possum trappers track (trees blazed ±1940), re-marked by Forestry Commission (markers nailed on trees ±1980 and 1988) Altitude 635-795 m
Weindorfers Forest (Nature Walk & part of Hounslow Heath Track)	1989	T5.2/3 & T4.3	1940-50, 1981	Parks Service track Altitude 910-950 m
Liffey Falls	1992	C1.1 & C3.1	pre 1972	Original track marked by Deloraine Walking Club 1972 Altitude 510 m

4.3 RESULTS

Rainforest logging and regeneration trials

All logging treatments resulted in increased levels of myrtle wilt. Figure 4.1 shows disease incidence against time for the three treatments and the control, from the Sumac Road trial. Figures 4.2 and 4.3 are plots of disease incidence against time for the other Smithton logging and regeneration trials, also showing the control from the Sumac Road trial. Disease incidence and mortality rates due to wilt in the Smithton logging and regeneration trials are given in Appendix 13.

Myrtle thinning trials

Raised levels of myrtle wilt were found in all the thinning trials using older myrtle stands. Figure 4.4 is a plot of disease incidence against time for the 40-year-old (Rabalga Spur 4) and the 65-year-old (Blackwater Spur 6) thinned plots and their controls. Table 4.7 is an

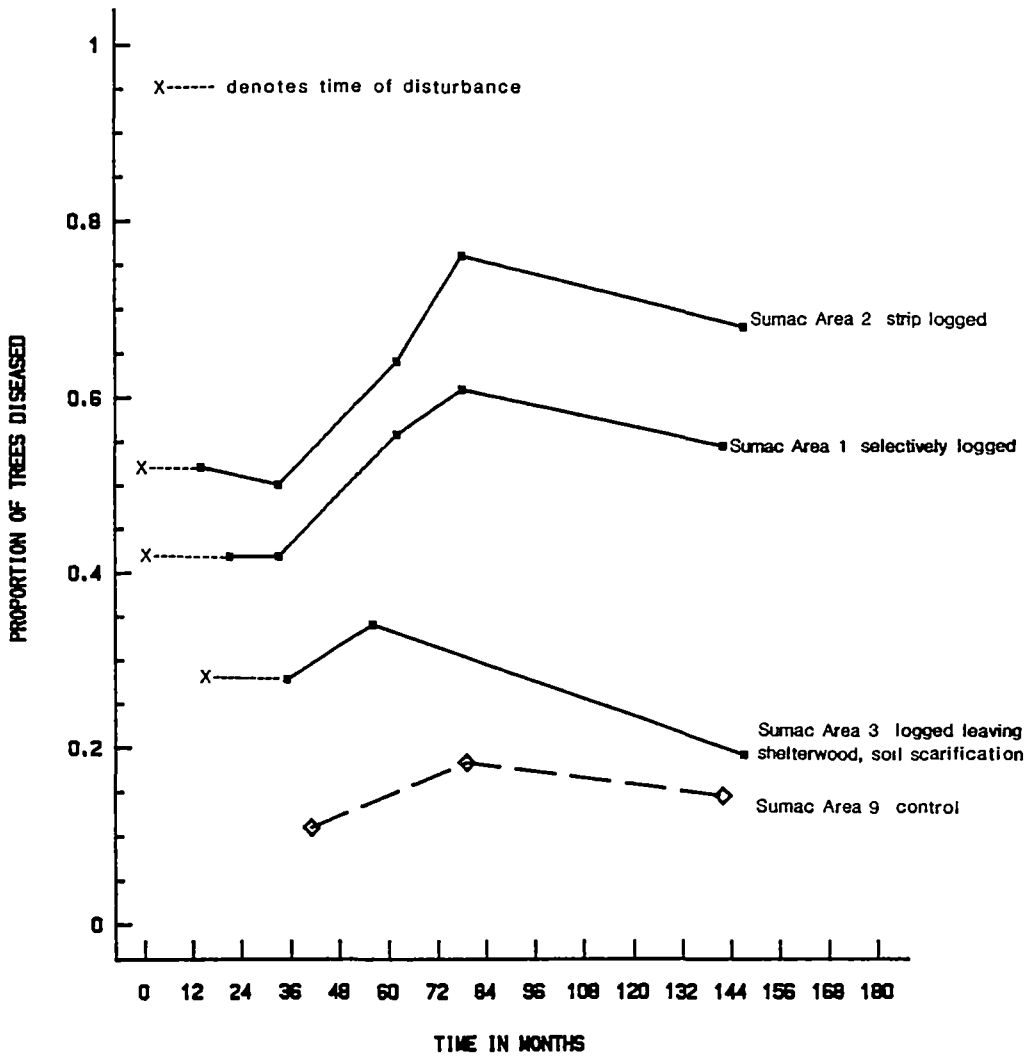


Figure 4.1 The incidence of myrtle wilt in the Sumac Road trials following different logging treatments.

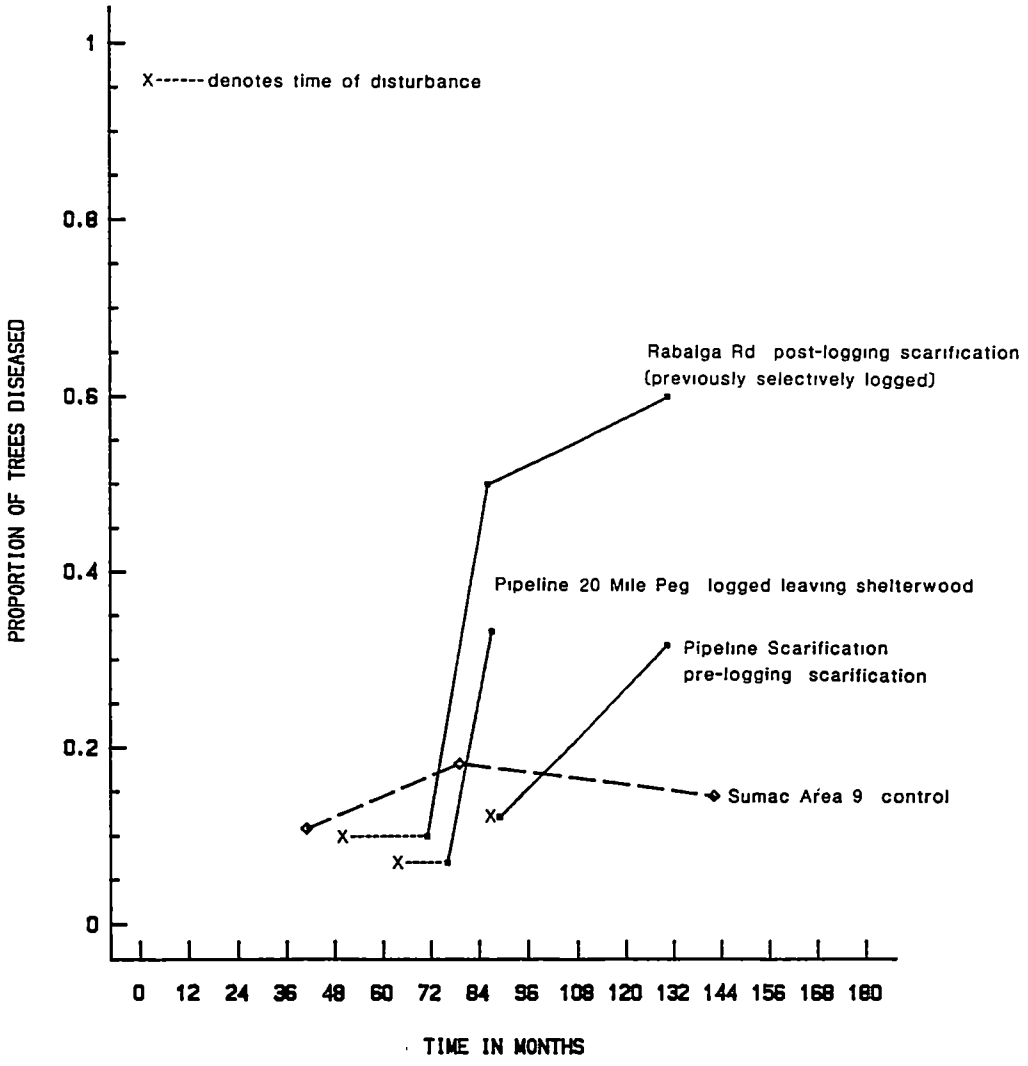


Figure 4.2 The incidence of myrtle wilt in other Smithton trials following different logging treatments.

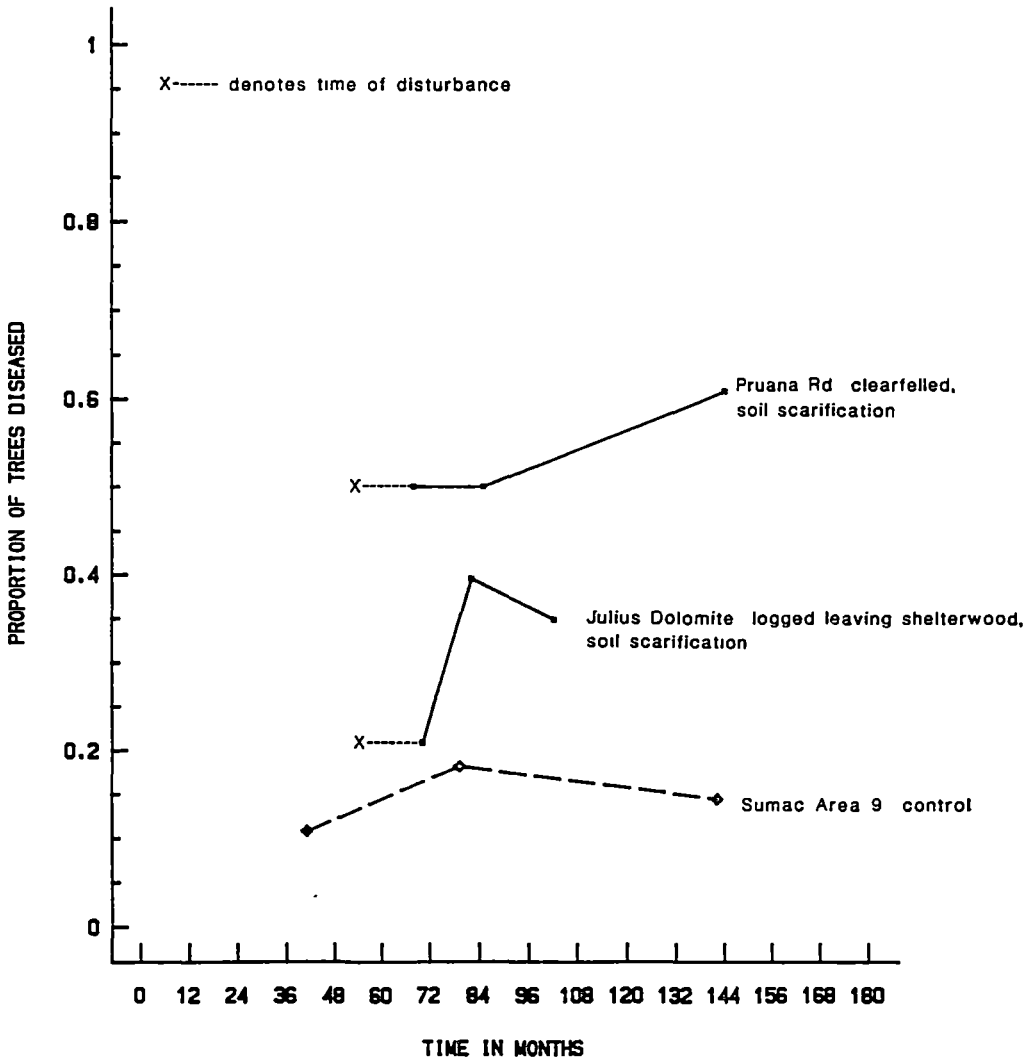


Figure 4.3 The incidence of myrtle wilt in more Smithton trials following different logging treatments.

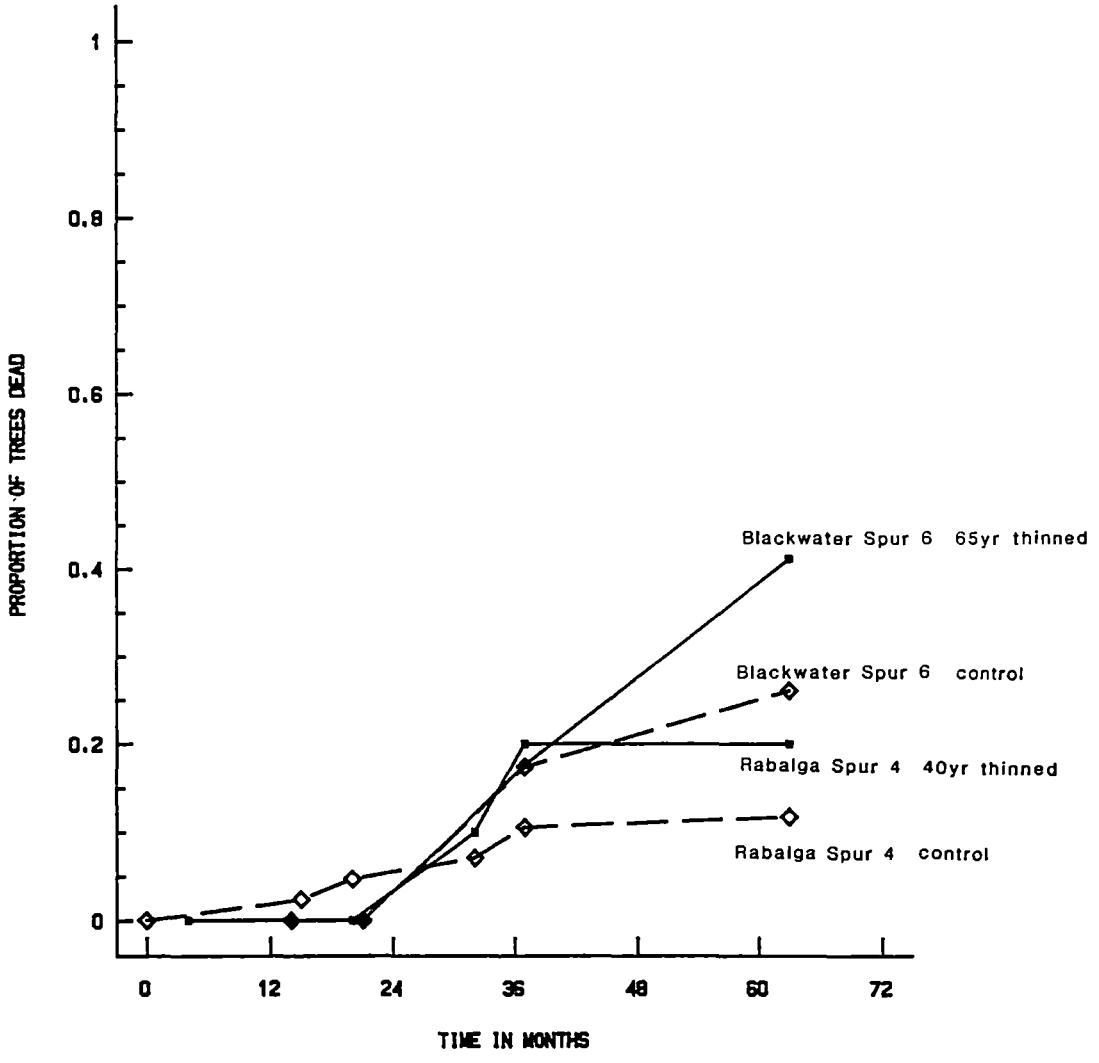


Figure 4.4 The incidence of myrtle wilt at Rabalga Spur 4 and Blackwater Spur 6, following thinning at 40 and 65 years old.

ANOVA on the per annum mortality rates due to wilt in the replicated 40-year-old thinning trial (Oonah).

There was no mortality due to wilt in either of the thinned 15-year-old stands or in their controls (0% p.a.). One tree had died (of unspecified causes) in the 18-year-old thinned stand at Pipeline 26 Mile Peg (0.01% p.a.) but there was no mortality due to wilt in the control (0% p.a.).

The disease incidence and mortality rates due to wilt in the myrtle thinning trials are given in Appendix 14. Data from the replicate plots of the Oonah 40-year-old thinning trial are given in Appendix 15.

Table 4.7 ANOVA of per annum mortality rates due to myrtle wilt in the Oonah 40-year-old thinning trial (data transformed to arcsin square root of percentage)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
BETWEEN GROUPS	207.061	1	207.061	13.155	0.011*
WITHIN GROUPS	94.441	6	15.740		
TOTAL	301.502	7			

NB In this and following tables, 5%, 1% and 0.1% significance levels are shown by *, ** and *** respectively. Non-significant values are denoted by NS.

Blackwood selective logging trial

Levels of recent damage were higher in the logged coupe than in the control. By the second measurement (14 months after logging) myrtle wilt levels were also higher, as was the per annum mortality rate due to the disease (Table 4.8). ANOVAs did not show any of these effects to be significant, although adjusting for damage (as a covariate) did decrease the significance level of myrtle wilt (as a main effect). Data from the replicates are given in Appendix 16, and the ANOVAs in Appendix 17.

Table 4.8 Myrtle wilt levels, damage levels and per annum mortality rates due to myrtle wilt in the blackwood selective logging trial

Treatment	Date	% Diseased (HSC 3-6/1-8)	% Damaged (DC 2-4/1-4)	Mortality (%p. a.)
Logged	March 91	17.9%	25.0%	
				6.5%
	March 92	28.2%	25.0%	
Control	March 91	21.4%	5.8%	
				2.9%
	March 92	21.4%	8.8%	

Disease spread into areas adjacent to logged areas and roads

At two of the six sites studied (Simons Road and the Bennetts/Esperance Link Road), the results indicated that disease had spread from the area of disturbance into the adjacent forest.

Figure 4.5 is a scatterplot of the Simons Road 1988 disease incidence data showing the fitted model (13 years after disturbance). Figure 4.6 shows the same model compared with that constructed from the 1983 data (eight years after disturbance). Figure 4.7 depicts models of both disease and damage incidence for the area adjacent to the Simons Road logged coupe (three years after disturbance).

Figure 4.8 depicts models of disease and damage incidence for the area adjacent to the Bennetts/Esperance Link Road. Figure 4.9 depicts the models of disease and damage incidence for Sumac Spur 1B; only the model for damage shows a good fit. The models for the probability of disease and damage incidence did not give good fits for the other sites.

Figure 4.10 illustrates myrtle wilt killed trees along Simons Road, and along the Cradle Mountain Link Road in north west Tasmania.

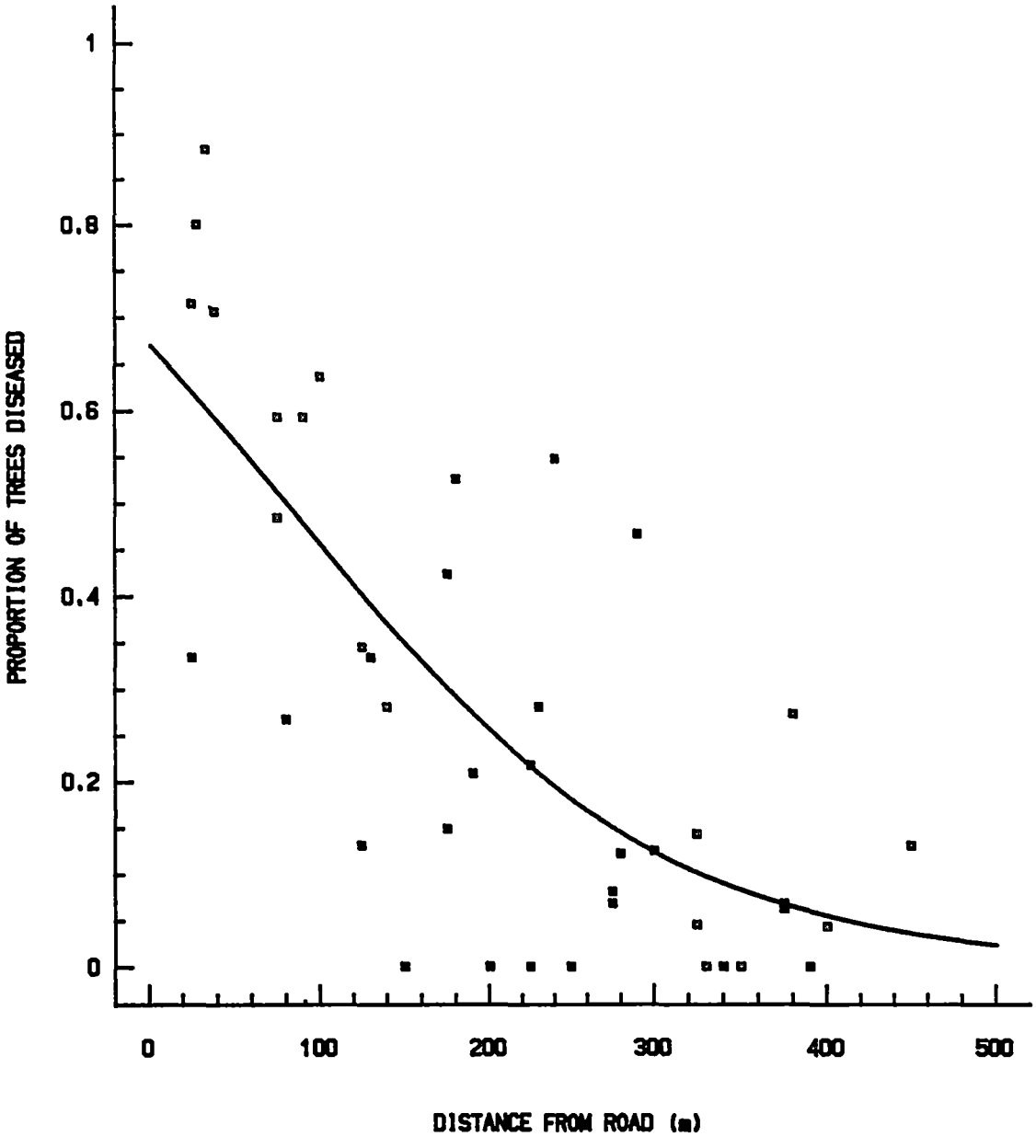


Figure 4.5 Scatterplot and fitted model of myrtle wilt incidence with distance from Simons Road in 1988 (13 years after disturbance). Reproduced from Kile *et al.* (1989).

Probability diseased shown by unbroken line.

logit (probability diseased) = 0.713-(0.008896 x distance)

% deviance explained = 53.02%

significance level of t test = 0.001

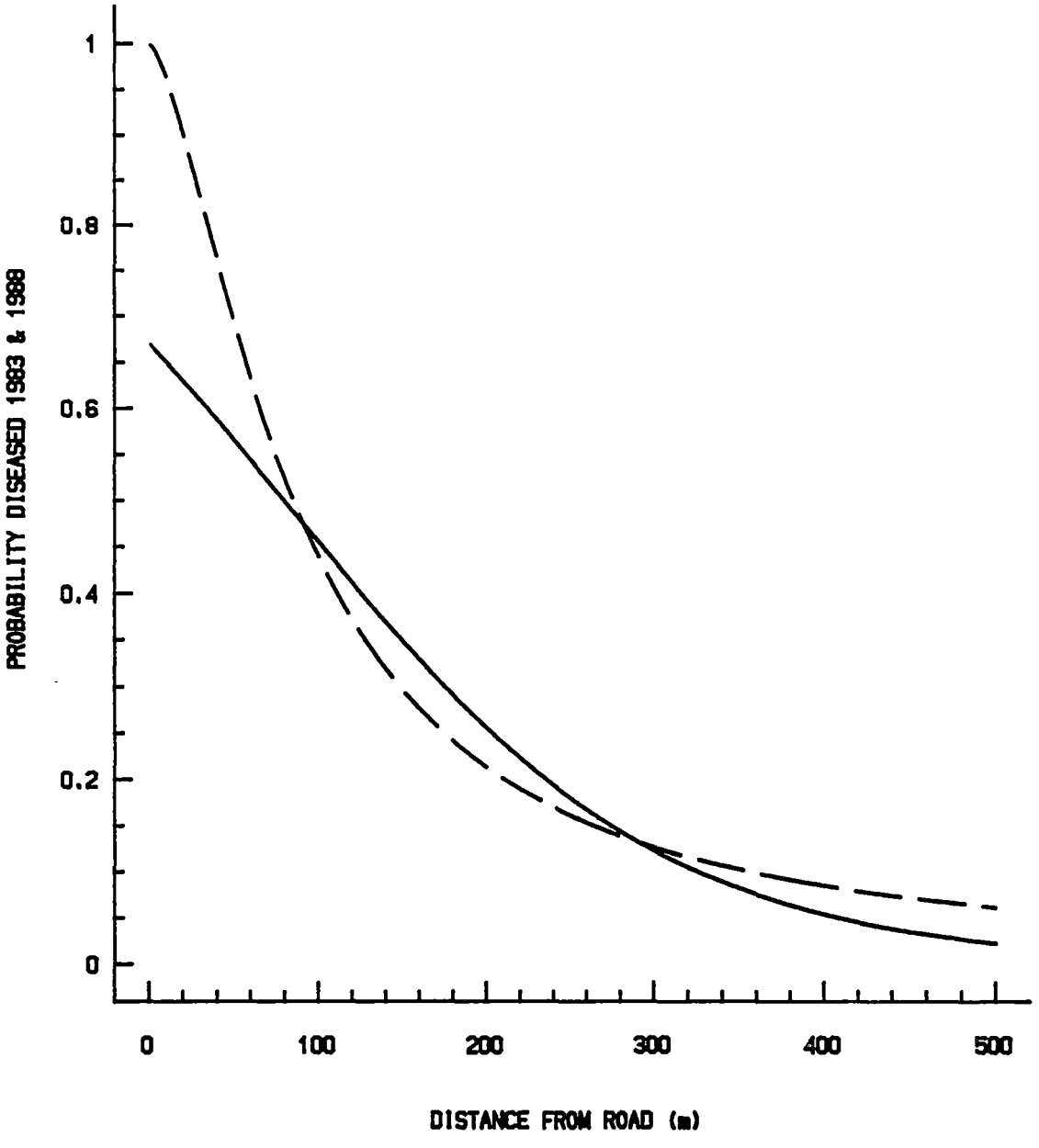


Figure 4.6 Probability of myrtle wilt incidence with distance from Simons Road, in 1983 (eight years after disturbance) and in 1988 (13 years after disturbance).

Probability diseased in 1983 shown by broken line.

logit (probability diseased) = 6.82-(1.533 x log distance)

% deviance explained = 40.68%

significance level of t test = 0.001

Probability diseased in 1988 shown by unbroken line.

logit (probability diseased) = 0.713-(0.008896 x distance)

% deviance explained = 53.02%

significance level of t test = 0.001

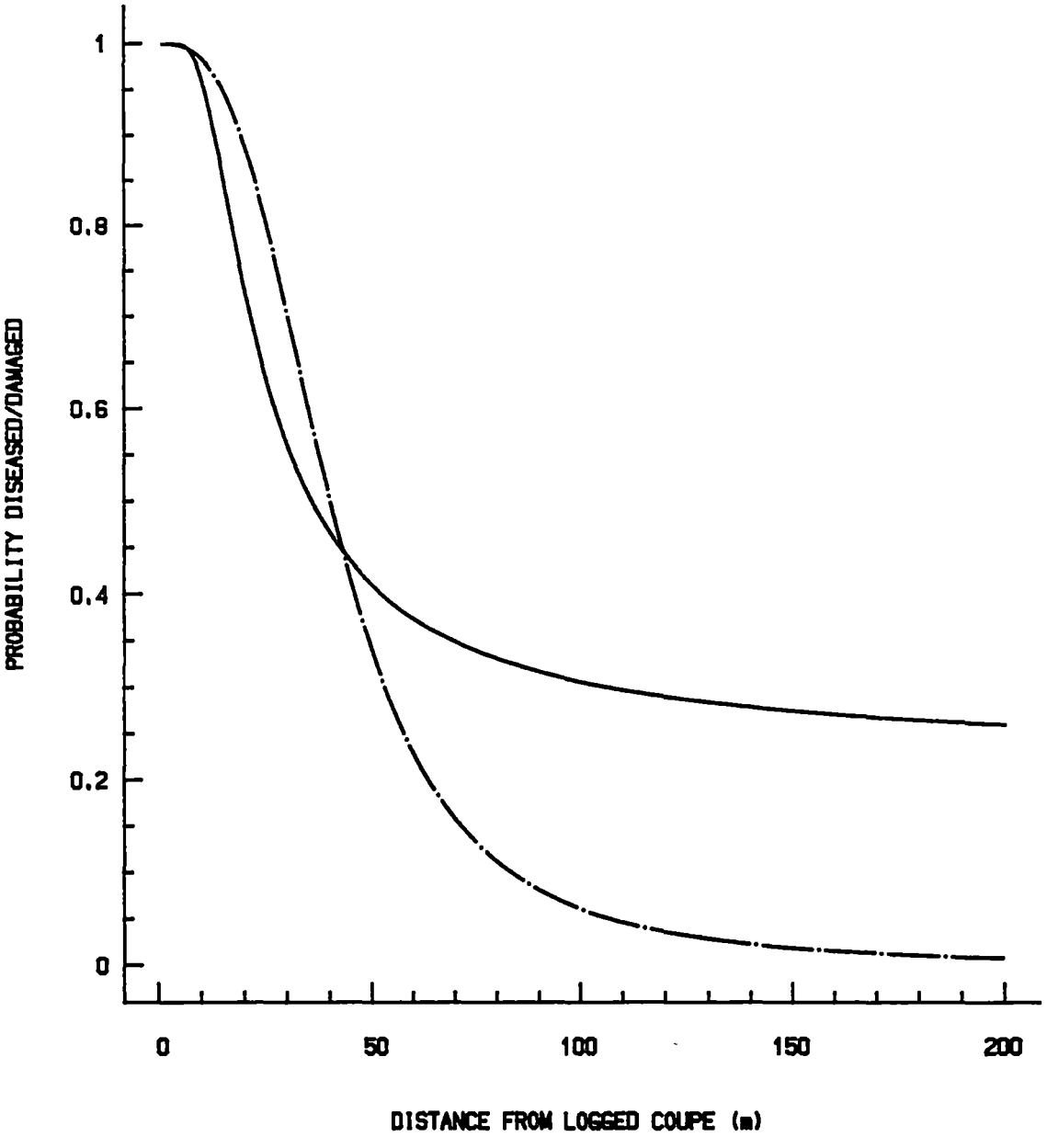


Figure 4.7 Probability of myrtle wilt and damage incidence with distance from a logged coupe at Simons Road (three years after disturbance).

Probability diseased shown by unbroken line.

logit (probability diseased) = -1.271+(45.58/distance)

% deviance explained = 40.31%

significance level of t test = 0.001

Probability damaged shown by broken line.

logit (probability damaged) = 11.04-(2.991 x log distance)

% deviance explained = 67.3%

significance level of t test = 0.001

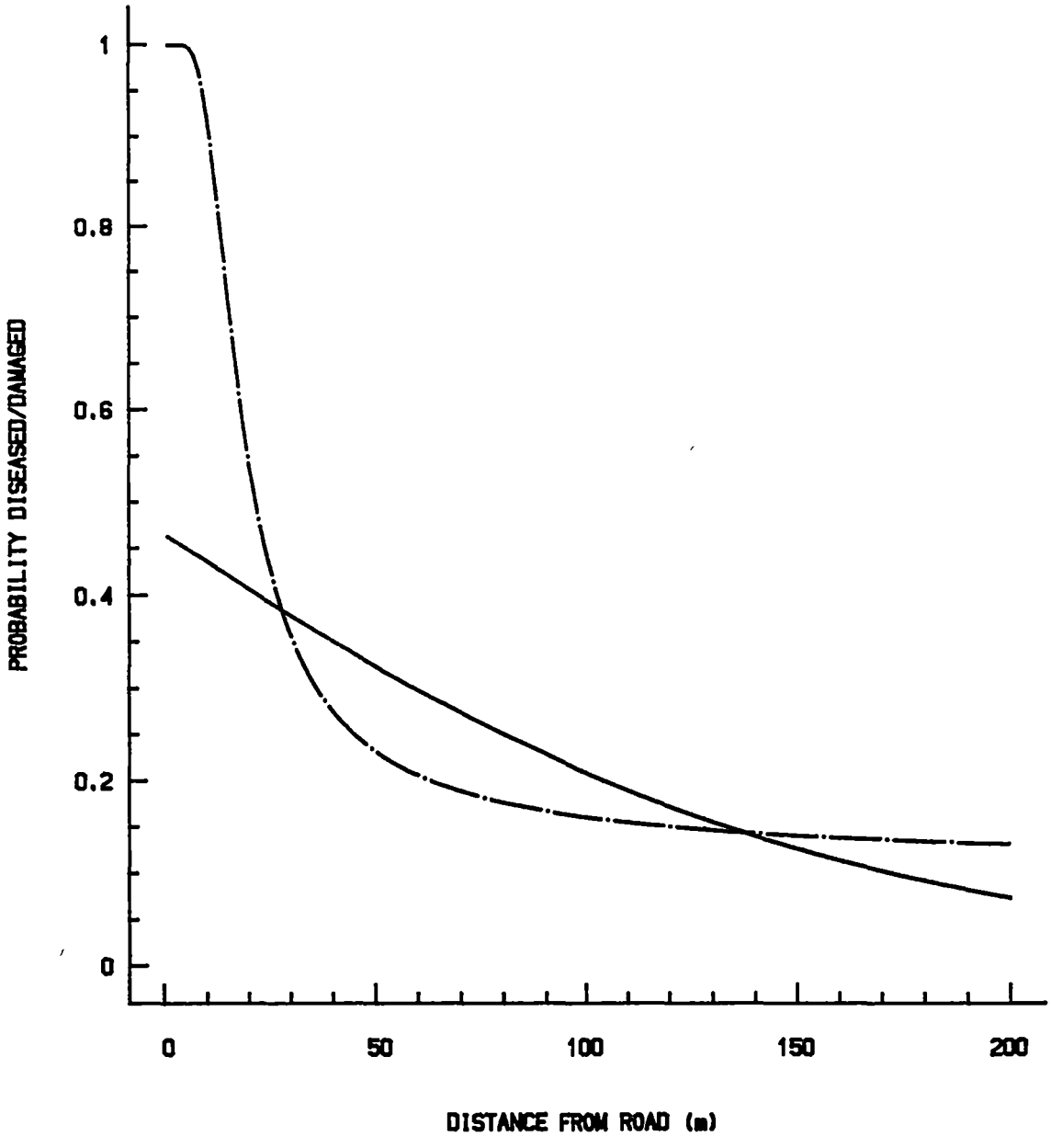


Figure 4.8 Probability of myrtle wilt and damage incidence with distance from the Bennetts/Esperance Link Road (three years after disturbance).

Probability diseased shown by unbroken line.

logit (probability diseased) = -0.136-(0.01196 x distance)

% deviance explained = 17.16%

significance level of t test = 0.005

Probability damaged shown by broken line.

logit (probability damaged) = -2.111+(45.76/distance)

% deviance explained = 34.76%

significance level of t test = 0.001

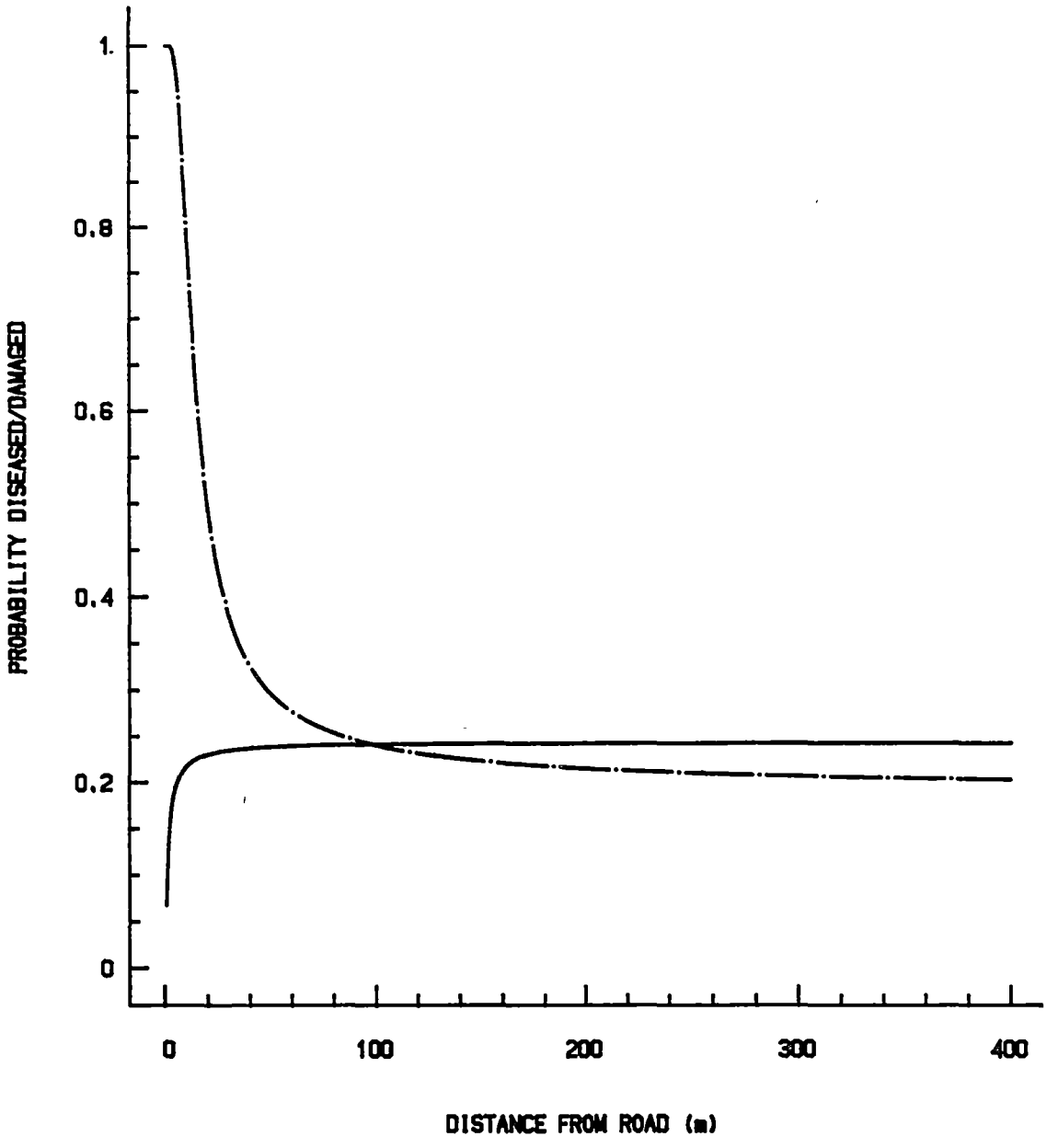


Figure 4.9 Probability of myrtle wilt and damage incidence with distance from Sumac Spur 1B (two years after disturbance).

Probability diseased shown by unbroken line.

logit (probability diseased) = -1.129-(1.49/distance)

% deviance explained = 0.05%

significance level of t test = NS

Probability damaged shown by broken line.

logit (probability damaged) = -1.434+(28.35/distance)

% deviance explained = 14.10%

significance level of t test = 0.001

a

b

Figure 4.10 Myrtle wilt along roadsides: a) Simons Road; b) Cradle Mountain Link Road.

Disease levels along walking tracks

There was no significant difference in myrtle wilt levels between track and control transects, although there was considerable variation between sites (Table 4.9). ANOVAs on myrtle wilt incidence levels are given in Appendix 18.

Table 4.9 Health status of myrtles along walking tracks and along nearby, parallel transects

Site	No. of myrtles in HSC			Total	%Diseased
	1&2	3,4,5&6	7&8		(HSC 3-6/1-8)
Mt Scott					
Track	169	63	3	235	26.81%
Control	107	53	5	165	32.12%
Weindorfers Forest					
Track	306	48	24	378	12.70%
Control	200	46	24	270	17.04%
Liffey Falls					
Track	26	31	0	57	54.38%
Control	28	15	0	43	34.88%

Tables 4.10, 4.11 and 4.12 show the ANOVAs of the (transformed) disease incidence on track and control transects at Mt Scott, Weindorfers Forest and Liffey Falls respectively, including damage incidence, altitude and slope (where measured) as covariates. Covariates (particularly damage and altitude) were significant in some instances, and in all cases inclusion of covariates in the ANOVA reduced the significance level of the main effect, explaining some of the variation in wilt levels between track and control transects (ANOVAs on damage, altitude and slope are given in Appendix 18).

Table 4.11 ANOVA of percentage of diseased trees along the Mt Scott walking track and along a nearby, parallel transect adjusted for damage, altitude and slope (data transformed to arcsin square root of the percentage diseased)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATES	3353.15	3	1117.71	3.355	0.0348*
Damage	1313 46	1	1313.46	3.943	0.0581 NS
Altitude	162.725	1	162.725	<1	0.4984 NS
Slope	208.016	1	208.016	<1	0.4452 NS
MAIN EFFECT					
Track/control	16.534	1	16.534	<1	0.8288 NS
RESIDUAL	8327.83	25	333.113		
TOTAL	11697.33	29			

Table 4.11 ANOVA of percentage of diseased trees along walking tracks in Weindorfers Forest and along nearby, parallel transects adjusted for damage, altitude and slope (data transformed to arcsin square root of the percentage diseased)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATES	704.580	3	234.860	2 054	0.1319 NS
Damage	265.627	1	265.627	2.324	0.1400 NS
Altitude	515.198	1	515.198	4.507	0.0438*
Slope	38.801	1	38.801	<1	0.5716 NS
MAIN EFFECT					
Track/control	24.493	1	24.493	<1	0.6524 NS
RESIDUAL	2858.01	25	114.321		
TOTAL	3587.09	29			

Table 4.12 ANOVA of percentage of diseased trees along the Liffey Falls walking track and along a nearby transect adjusted for damage (data transformed to arcsin square root of the percentage diseased)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Damage	37.282	1	37.282	0.248	0.6355 NS
MAIN EFFECT					
Track/control	226.361	1	226.361	1 506	0.2508 NS
RESIDUAL	1352.33	9	150.259		
TOTAL	1615.97	11			

4.4 DISCUSSION

Types of disturbance

All logging, regeneration and thinning treatments increased mortality levels due to myrtle wilt. This was shown to be significant for the replicated Oonah 40-year-old thinned site. The degree of disturbance (from an anthropocentric viewpoint) did not appear to affect mortality levels, except that strip-logging and thinning of a 65-year-old stand had rather severe effects. It was evident that thinning was only likely to be successful in younger (20 years old or less) myrtle pole stands; older stands suffered unacceptable levels of myrtle wilt (Hickey and Felton 1991).

The 0.93% per annum mortality rate for the undisturbed Sumac Area 9 (Appendix 13), was close to the (adjusted) level found by Elliott *et al.* (1987) for undisturbed Sumac Road rainforest of 0.78% per annum, and somewhat higher than their (adjusted) average for undisturbed sites of 0.61% per annum. One of the Rabalga Spur 4 unthinned control plots had a higher mortality rate than the thinned plot, but it was situated close to access roads and to the thinned plot, and could have been affected by this disturbance (S. Jennings, unpublished data). Since naturally occurring epidemics do occur, it is not particularly surprising to find similar trends in some control areas.

Elevated disease levels associated with roading activities were detected at some of the sites investigated (Figures 4.7 and 4.8). However, there was no significant difference between

myrtle wilt levels along any of the walking tracks surveyed, compared with control forest, even allowing for the differential effects of damage, altitude and slope.

The general area of the Mt Scott track had suffered disturbance from selective logging in the early 1970s and raised inoculum levels may account for the rather high levels of wilt found (Table 4.9). It is probable that in the 1940s when a number of the trees on the track were blazed, inoculum levels were lower. At Weindorfers Forest there were a number of old tracks which had been re-routed over the years so that the control forest was not undisturbed, although it had certainly been disturbed less recently than the area of the present tracks. Disease levels were low as could be expected from such a high altitude site (Elliott *et al.* 1987). At Liffey Falls the disease incidence did appear to be higher along the track, but this was not significant, probably due to the limited sample size.

Disturbance and disease incidence through time

Elevated mortality rates due to disturbance-related myrtle wilt did eventually drop in some partially logged areas, but both this effect and the time taken for it to occur were variable. Where monitoring had been in progress long enough for it to be recorded, the time taken for mortality to return to background levels, was found on average to be nine years (range 4 - 13·5 years). It will be necessary to monitor the remaining sites to see whether a reduction in mortality eventually occurs.

Ideally one should include trees recruited into the smallest diameter class in remeasurements, since failure to do this will result in an apparent increase of mortality with time (there being fewer live trees in the population) which may mask the effect being assessed. Also it is unfortunate that in many cases measurements were not made until some time after the disturbance, and it is suggested that in any subsequent work, monitoring should commence before, or as soon as possible after, the disturbance.

Van der Plank (1975) viewed epidemic and endemic disease as a continuum, with local or micro-epidemics being inevitable even in a mainly endemic situation, due to variation in local conditions. Where the myrtle forest is disturbed and local conditions are changed over a relatively large area, myrtle wilt often moves to an epidemic phase on a larger scale than is normally seen in undisturbed forest. Using other terminology (Stewart 1989), disturbance can precipitate myrtle wilt induced dieback.

Epidemics of polycyclic (compound interest) diseases often initially give rise to logarithmic (or exponential) growth in the amount of disease (however measured), since plants infected early on provide inoculum sources for later infections:

$$\frac{dx}{dt} = r_i x$$

where x is the amount of disease, t is time and r_i the logarithmic infection rate.

Later on the growth may become logistic as the environment becomes limiting (e.g. lack of susceptible trees or new infection sites):

$$\frac{dx}{dt} = rx(1-x)$$

where r is the logistic infection rate, and the maximum value of x is 1 (Van der Plank 1963).

Logistic growth gives rise to S shaped growth curves, and thus the sigmoid curves found in, for example, the Sumac Road trials (Figure 4.1) can be seen in terms of truncated para-logistic growth. True logistic growth depends on a number of stringent conditions and does not occur under many field situations (Hengeveld 1989). Such growth curves probably represent the passing of a micro-epidemic which was precipitated by disturbance, and with new infections eventually ceasing.

In the later measurements of the trials reported here, incidence levels actually declined. This was due to the loss of already infected trees by windthrow and felling, and to trees previously described as sick/dead producing healthy epicormics or coppicing. The latter indicates that at this site vegetative regeneration was not always killed by the myrtle wilt which affected the parent tree. This is not in accordance with the findings of Howard (1973a) who indicated there was no recovery from trees which died following *P. subgranosus* attack. Alternatively, it could mean that the dieback in these trees was incorrectly attributed to myrtle wilt.

Disease spread from disturbed areas

The investigations of disease levels in areas adjacent to logging and roading activities have shown that there was considerable disease spread into some of these areas, but that this did not always occur. Both callidendrous and thamnic forests were affected (Figures 4.7 and 4.8).

For all the recent disturbances studied, recent damage of live trees was elevated for about the first 50 m from the disturbance; further away it appeared to drop to background levels (Figures 4.7-4.9). It seems likely that this is usual, but is only apparent for the first few years after the disturbance. It may be due to damage from falling trees or machinery

movement in the area immediately adjacent to the disturbance. In the area next to the logged coupe much of the damage was due to scorching from the regeneration burn. This made the field identification of *C. australis* infected trees difficult, since *P. subgranosus* are known to be attracted to burnt as well as diseased material (Hogan 1944; Kile *et al.* 1992).

The effects of disturbance can extend well beyond the area of visible tree damage. For Simons Road there was an independent estimate of the background disease level, available from the rate of spread plot (Appendix 8), and this varied from 27.81% to 29.9%. If it is assumed that logging and roading disturbances have similar effects, disease spread with time can be considered for this site.

At Simons Road, the distance from the roading or logging disturbance at which disease incidence fell to the background level of 28% (probability diseased 0.28), gives a conservative estimate of the extent of disease spread. This was approximately 130 m after three years (Figure 4.7); 155 m after eight years and 180 m after 13 years (Figure 4.6). This represents a disease spread of 130 m in the first three years, 25 m over the next five years and 25 m over the last five years. Thus for this site, although there has been an overall increase in disease over the last five years (as was shown by the transect remeasurement in Table 3.6) it is evident that the rate of disease spread into undisturbed forest is now slowing (and the micro-epidemic passing). The time taken for this to occur is comparable with that found in the logging and regeneration trials.

In the three years after the construction of the Bennetts/Esperance Link Road there was considerable disease spread beyond the extent of the tree damage, probably to around 150 m (Figure 4.8). It is anticipated that here also, the rate of spread will decrease with time. At Sumac 1B there is as yet no sign of elevated wilt levels near the road (Figure 4.9). This could be due to the fact that parts of the road had only recently been completed when the assessment was made, or to a high level of nearby disturbance (e.g. old bulldozer tracks) masking any effects, or it could be that significant disease spread will not eventuate at this site.

The rapid disease spread seen at Simons Road and the Bennetts/ Esperance Link Road in the three years following logging and roading (40-50 m per year) would indicate initial spread via air or water borne inoculum, although continued, slow spread (e.g. 5 m per year at Simons Road) may well be via root grafting. Estimated rates of spread via root grafting of 1-5 m per year were given by Kile (1986) and Kile *et al.* (1989) based on similar diseases overseas and the linear growth rate of *C. australis* in myrtle stem tissue, and these would

seem to be of the right order. Disturbance related wounding may initially provide infection sites, but the consequent increase in disease incidence and inoculum levels may be very important in the rapid spread of disease into adjacent areas (Kile *et al.* 1989).

Overall, there is considerable unexplained variation in the incidence and spread of myrtle wilt following disturbance, but in many situations increased mortality due to wilt and disease spread into neighbouring forest are evident.

4.5 SUMMARY

The effect of logging and thinning on the incidence of myrtle wilt was examined in myrtle-dominated rainforest. All treatments were found to increase myrtle wilt incidence and mortality due to wilt, compared with control sites in undisturbed forest. The increase in mortality was found to be significant for the one replicated site.

There is evidence for some logged and thinned areas, that elevated mortality levels due to wilt eventually dropped to background levels. Where this happened it took an average of nine years (range 4-13.5 years).

Increased disease levels were found close to some of the roads investigated. However none of the walking tracks studied were found to be associated with significantly raised levels of myrtle wilt.

The spread of myrtle wilt into areas adjacent to disturbances (logged coupes and roads) was detectable in both callidendrous and thamnic forests, although not all sites were affected. Tree damage (providing infection sites) typically occurred up to 50 m from the coupe edge or road, but the extent of increased wilt incidence well exceeded this. In one site, increased disease levels were detectable 180 m from the road, 13 years after its construction, with spread still occurring, albeit slowly.

Initial disease spread occurred very rapidly (up to 150 m in three years). This was probably due to disturbance related wounding which provided infection sites; the consequent rise in disease incidence and air/water borne inoculum levels would then increase the probability of infection of any naturally wounded trees. The later, slower spread (25 m in five years) may have occurred via root grafting.

5. WOUNDS, ROOT GRAFTS AND MYRTLE WILT

5.1 INTRODUCTION

Wounds

Species belonging to the *Ceratocystis* complex are wound pathogens, gaining entry at sites where xylem is exposed (Upadhyay 1993b). Wounds are a prerequisite for infection of above ground plant organs (Kile 1993), and some species are associated with specific insect vectors, e.g. *Ophiostoma novo-ulmi, Ceratocystis fagacearum* (Upadhyay 1993b) and *C. fimbriata* (Dowding 1984). In other species, e.g. *Ophiostoma piceae, O. piliferum, O. minus* and *C. coerulescens,* conidial dispersal takes place in damp air or splash droplets (Dowding 1969). Some species are early colonisers of freshly cut wood (Seifert 1993), and wood moisture content is thought to be a factor determining colonisation success (Gibbs 1993). When they are inoculated into a felled tree, growth of these fungi is severely restricted close to the exposed surface due to evaporation (Dowding 1984).

Ceratocystis fagacearum is recorded as being spread by the infection of wounds resulting from pruning and logging operations (Jeffery 1953; Davies 1992). Susceptibility of trees to wound infection by *C. fagacearum* and *O. novo-ulmi* is known to be seasonal, and is highest during the spring wood formation period (Jeffery 1953; Neely 1968). *Ceratocystis fimbriata* f. *platani,* which causes flame canker of plane trees, is also spread by pruning operations (Accordi 1986). With seedlings of *Pseudotsuga menziesii* (Mirb.) Franco, wounds on fine roots are thought to be the principal infection courts for *C. wageneri* (Hessburg and Hansen 1986a).

There is now considerable evidence showing that myrtle wilt incidence is related to recent stem and crown damage in *Nothofagus cunninghamii* (Elliott *et al.* 1987; Kile *et al.* 1989; Chapter 4). Wounds are known to be infection courts for *Chalara australis,* and infection can occur through branch and twig stubs (G. A. Kile and M. F. Hall, unpublished data). It is therefore important to understand some of the factors which could influence susceptibility of wounds to infection by *C. australis.*

Root grafts

Root grafting is a widespread naturally occurring phenomenon in many higher plant species (Graham and Bormann 1966; Sykes 1984). It is less common in herbs, vines and shrubs than in trees, where root grafts may provide wind stability (Keeley 1988).

Root grafts are of three main types: self grafts occur between roots of the same tree, and are the most frequently found; intra specific grafts occur between different trees of the same species; inter specific grafts occur between trees of different species, and are much less common (Graham and Bormann 1966). Root grafts take various forms, and butt grafts are also possible (Beddie 1941).

Grafts may be complete or partial, and can transfer water, minerals, assimilates, plant growth substances or pathogens between trees (Graham and Bormann 1966; Loehle and Jones 1990). Roots are considered functionally grafted when they are connected by common bark, phloem, cambium and xylem tissues (Epstein 1978). In *Pinus strobus* L. effective root fusion only occurred between tissues with normal anatomical and physiological alignment, thus transfer across grafts necessitated tangential or radial movement. Organic substances from the phloem more readily moved through grafts than water and minerals from the xylem (Bormann 1966). It is known that assimilates and growth factors can be transferred across partial grafts, whereas water transport requires a more complete union (Loehle and Jones 1990). In some cases translocation through root grafts occurs only during times of high water stress (Schultz and Woods 1967).

Numerous factors have been found to influence the frequency of root grafting. Most of these probably operate by controlling the level of root contact which occurs, e.g. soil depth and type, slope, tree size, growth habit and stand density. The degree of genetic similarity between trees may also influence the degree of root grafting (Reynolds and Bloomberg 1982; Loehle and Jones 1990; Schuster and Mitton 1991).

Root grafting has been commonly recorded in the Fagaceae (Saunier and Wagle 1965; Graham and Bormann 1966; Sykes 1984), and intra specific root grafting is known to occur in all the New Zealand species of *Nothofagus*, with inter specific grafting having been recorded between *N. truncata* and *N. solandri* var. *solandri* (Beddie 1941).

Root grafts as a mechanism for disease spread

Epstein (1978) listed a number of significant tree pathogens which are transmitted via root grafts, and commented that the non-vector, tree to tree transmission of most vascular parasites seems limited to functional root grafts. In particular, spread by root grafts is known to be important for the local spread of vascular wilt and vascular stain diseases, in perennial hosts (Kile 1993).

Elms (*Ulmus* spp.) are frequently root grafted (Graham and Bormann 1966; Von Braun *et al.* 1978), and in many American cities and towns, the majority of *O. novo-ulmi* infections appear to result from root graft transmission rather than insect vector transmission (Neely and Himelick 1963; Cuthbert *et al.* 1975).

Henry *et al.* (1944) first suggested that *Chalara quercina* (anamorph of *C. fagacearum*) was transferred via root grafts, and this has been subsequently confirmed (Kuntz and Riker 1950). Although the importance of this transfer mechanism has been questioned (Graham and Bormann 1966), in stands of oak it is now known to represent a greater potential hazard than vector transmission (Epstein 1978; Mielke *et al.* 1983).

Ophiostoma wageneri, the causal agent of black stain root disease in pines (*Pinus* spp.) is known to spread locally via root contacts and grafts, bark beetles being suspected of long distance dissemination. Root graft transfer was successfully demonstrated by tracing the characteristic root stains through the grafts (Landis and Helburg 1976). Hessburg and Hansen (1986a; 1986b) showed that local spread could occur through root grafts, via roots in contact or close proximity to each other, and via insects which spread conidia.

Ceratocystis fimbriata f. *platani* was shown to spread from centres of infection via root grafts by inoculation and re-isolation of known strains of the pathogen (Accordi 1986). Additionally, observations on *C. coerulescens,* the causal agent of sapstreak disease in sugar maple (*Acer saccharum* Marsh.), suggested that limited spread of the disease occurred via root grafts (Houston 1991).

Mathematical models of pathogen spread through root grafts have been developed for various species (Gordon and Roth 1976; Thomson 1979), there being a number of models for *C. fagacearum* (Menges and Loucks 1984; Menges and Kuntz 1985; Appel *et al.* 1989; Bruhn *et al.* 1991).

At the start of this work root grafts had not been observed in *N. cunninghamii.* However, both their existence, and the below ground spread of myrtle wilt, had been predicted for a number of years on the basis of clumping of diseased trees and relationships between disease incidence and stand density (Candy 1982; Elliott *et al.* 1987; Kile *et al.* 1989). An attempt had been made to model the phenomenon but relevant data were lacking (Blanden 1986). It was therefore deemed necessary to confirm the existence of root grafts in myrtles, to test if *C. australis* was transferred via this route, and to assess the importance of this mechanism for disease spread.

5.2 WOUNDS AS INFECTION COURTS FOR *CHALARA AUSTRALIS*

5.2.1 BACKGROUND AND AIMS

Investigation of factors which could influence the susceptibility of wounds to infection by *C. australis,* required that suitable experimental methods be developed. Experiments with *C. fagacearum* showed that inoculation of chisel wounds in the trunk and root collar, was more effective than inoculation of cut branches or roots (Parmeter *et al.* 1956). G. A. Kile and M. F. Hall (unpublished data) had previously used a number of methods to wound and infect both *N. cunninghamii* billets (cut lengths of saplings), and living *N. cunninghamii* saplings, and the following summary of their work gives a necessary background to the present study.

Summary of the work of Kile and Hall (unpublished data)

In a field experiment, the stems of growing saplings were wounded at different heights with a chisel, the wounds being inoculated with a *C. australis* spore solution after varying amounts of time. The rate of infection of wounds by *C. australis* decreased with wound age; 100 percent of fresh wounds became infected, dropping to 38 percent of 28-day-old wounds (maximum age investigated). Further analysis indicated that the extent of the *C. australis* stained column above the wound, and the lateral extent of infection at the wound site, were significantly reduced with increasing wound age. Sapling diameter was also important. The height of wounding did not significantly affect *C. australis* growth (G. A. Kile and D. A. Ratkowsky, unpublished data). Saplings were wounded in November and inoculated in November/December.

In a parallel experiment *N. cunninghamii* billets were cut and stored outside, but under cover. One possible problem that was recognised was the potential for water loss both through the bark and through the cut ends of the billets, and the likely compounding effect this could have on infection rate. As an attempt to overcome the latter problem, one end of each billet was sealed with a silicon based sealant. The other end of each billet was inoculated with a *C. australis* spore solution after varying amounts of time. However, infection rates were much lower than in the field experiment , with no infection of billets more than three days old. Billets were cut and inoculated in December/January.

A third experiment used billets which were cut and stored indoors at room temperature, having both ends sealed with a silicon based sealant. Holes were then drilled in the billets, and these were inoculated (by spraying) at different times with a *C. australis* spore solution.

Infection rates were intermediate between the previous two experiments, and dropped with wound (hole) age, with 21-day-old wounds (the oldest investigated) giving a 52 percent rate of infection when inoculated with a spore solution of 10^6 spores/mL. The concentration of the spore solution was also found to be important. Billets were cut and inoculated in August/September.

From this work it was possible to draw up some guidelines for the design of future experiments:

- if laboratory and field based experiments were to be compared it would be necessary to use comparable methods of wounding;
- in experiments using billets, water loss appeared to be a complicating factor which could be reduced by sealing both ends of the billets. However this loss needed to be quantified, as did water loss caused by the drying out of wounds through time, compared with water loss through bark;
- spore concentration was a factor determining infectivity of wounds, and required further testing using a standardised wounding procedure;
- wound age was an important factor determining infectivity of wounds up to 28 days old, but older wounds needed investigation, preferably under field conditions.

Water loss from *N. cunninghamii* billets

Since moisture content was likely to be of major importance in determining infection rates, the aims of this experiment were to determine:

- the degree of water loss from sealed billets, up to a three month period;
- the degree of water loss from wounded, sealed billets, up to a three month period;
- whether water loss occurs mainly through the bark of sealed billets, or through wounds.

If major water loss from billets occurred mainly through the *bark,* the identification of any additional effects of *wound* age on wound infection rate would require that *billets* were the same age (moisture content) when inoculated. Conversely, with negligible water loss from billets, or water loss which occurred mainly through *wounds,* the age of billets at inoculation would be of lesser importance.

Effects of wound age and spore concentration on the infection of *N. cunninghamii* billets

The aims of this experiment were to determine the effects of wound age and spore concentration on infection rate, under laboratory conditions. The experiment of G. A. Kile and M. F. Hall (unpublished data) indicated that increasing wound age (from 0 to 21 days)

and decreasing spore concentration (from 10^6 to 10^3 spores/mL) decreased the infection rate of wounds. However this apparent effect of wound age may have been an artefact, since billets were different ages when inoculated, and the previous experiment showed that this would mask any real effect of wound age. Also the wounds were drilled holes, and it was desirable to standardise the wounding procedure.

Effect of wound age on the infection of *N. cunninghamii* saplings

The aim of this experiment was to determine the effect of wound age on infection rate under field conditions. The field experiment of G. A. Kile and M. F. Hall (unpublished data) had obtained a 36% infection rate of 28-day-old wounds and it was therefore desirable to extend the investigation to older wounds. To avoid both compounding effects of season/weather on the growth of *C. australis,* and any difference between batches of inoculum, it was necessary to inoculate all saplings on the same date.

Effect of chemical treatments on the infection of *N. cunninghamii* saplings

This experiment aimed to test the effectiveness of four chemicals with known fungicidal, insecticidal or herbicidal properties, in preventing infection of fresh wounds by *C. australis.* For the reasons outlined above, it was desirable to inoculate all saplings on the same date.

Boric acid is commonly used as a wood preservative, and in the control of dry rot and wood boring Coleoptera (Singh *et al.* 1989; Quarles 1992). It is also known to reduce the regenerative capacity of *Armillaria calvescens* Berube and Dessureault, associated with sugar maple decline (Bauce and Allen 1992). Amino isobutyric acid also limits the spread of dry rot (Dobson *et al.* 1993). Diverse carbon and nitrogen sources and variable C:N ratios affect fungal growth rates, and sodium nitrate is known to prevent growth in some species (Torres-Lopez and Hepperly 1988; Gabr *et al.* 1990). Specifically, nitrate is known to be poorly utilised by *Ophiostoma* spp. (Seifert 1993). Ammonium sulfamate is used to control regrowth from hardwood stumps, and has been found to favour the growth of some fungi which cause rapid wood decay and which ensure the rapid killing of stump roots. Since some of these fungi also compete well with the pathogen *Armillaria mellea* (Vahl ex Fr.) Kummer, ammonium sulfamate has potential as a control agent for *A. mellea* (Rayner 1976; Risbeth 1976; Shaw and Kile 1991).

5.2.2 MATERIALS AND METHODS

Water loss from *N. cunninghamii* billets

Myrtle saplings with a diameter at breast height (DBH) of 4.5-9 cm were felled and cut into 1.2 m billets. Billets were trimmed with a drop saw and all cut ends and abrasions were sealed with silicon based sealant (Silicone Aussigrip). Half of the billets were wounded by removing a 12 mm x 12 mm square of bark with a 12 mm chisel, with four wounds per billet (approximately 30 cm apart), the others were left unwounded. There were five replicates per treatment. All billets were weighed and their diameter was measured. They were then placed horizontally across a laboratory table and reweighed after 26, 28, 49 and 97 days. Billets were cut and wounded in October.

Percentage weight loss was calculated for each billet, at each date. A multifactor analysis of variance (ANOVA) was performed using time and wounding as main effects, and adjusting for diameter by using it as a covariate (the data being first transformed to the arcsin square root of the percentage weight loss).

Effect of wound age and spore concentration on the infection of *N. cunninghamii* billets

This factorial experiment used four wound ages and five spore concentrations, with four replicate billets per treatment. Billets were the same age and were inoculated at the same time, but wounded on four different dates, with five different spore concentrations.

Myrtle billets were cut, sealed and wounded using the basic methods previously described. However, the cut ends were sprayed with alcohol and flamed before sealing, and both the chisel and the immediate wound area were similarly surface sterilised before wounding. Along each billet, the four wounds were arranged in a spiral pattern, approximately 30 cm apart. Wounding dates were the start of the experiment, and after 14, 21 and 28 days. Inoculation of all billets took place 28 days after the start of the experiment, giving wound ages of 28, 14, seven, and zero days at inoculation. Billets were cut and inoculated in November/December. They were weighed at the start of the experiment, before and after wounding, before inoculation and at the final harvest.

Inoculum was prepared from six to eight-day-old colonies of *C. australis* (Isolate 1.91), growing on 3% malt agar plates, using sterilised glassware throughout. One plate was thoroughly wetted with water taken from a 200 mL flask of sterile distilled water, and a glass rod was drawn across the surface to dislodge conidia. The liquid was then poured back into

the flask, with the concentration of the resultant spore solution being determined using a Neubauer haemocytometer, and counting the number of conidia in 25 replicate cells per sample. The solution was then adjusted to a concentration of 10^6 spores/mL, using sterile distilled water. Further dilutions were made to give additional concentrations of 10^4, 10^3 and 10^2 spores/mL. Sterile distilled water (0 spores/mL) was used as a control. Details of Isolate 1.91 are given in Appendix 19.

Billets were inoculated with a sterile 1 mL syringe, using 0.1 mL of inoculum per wound, and ensuring that the wound surface was thoroughly wetted, and using only one concentration per billet. Billets were then randomised, and stood in a vertical position in the laboratory for the duration of the experiment.

At 34/35 days after inoculation wound infection of half of the replicates was assessed by sawing through billets approximately 1 cm above and below each wound, forming disks. Each disk was partially cut through and then split radially through the wound site using surface sterilised tools. Any wood staining characteristic of *C. australis* was noted. At least three fragments of split wood from each wound area were removed using a sterile scalpel and plated out onto 3% malt agar and incubated at 20°C. After 5/6, 7/8 and 17/18 days, plates were assessed for the presence of *C. australis* (characterised by the green/black coloration of the colonies, and by the presence of typical phialides).

Effect of wound age on the infection of *N. cunninghamii* saplings

This experiment at Russell Road in southern Tasmania used live *N. cunninghamii* saplings, inoculated on the same date with *C. australis* or sterile distilled water, but wounded 112, 56, 28, 14 or zero days before inoculation. Roadside *N. cunninghamii* saplings of 5-11 cm DBH were selected, randomly allocated to different treatments and colour coded accordingly. There were three replicate saplings per treatment. Saplings were wounded from October to February, and inoculated in February.

Each sapling had four stem wounds made at the same time, approximately 30 cm apart, and arranged in a spiral pattern from 0.5 to 1.4 m above ground level. Each wound was made by removing a 12 mm x 12 mm square of bark with a 12 mm chisel. Before wounding the immediate wound area was brushed with a wire brush, then sprayed with alcohol and flamed, the chisel being similarly surface sterilised.

Wounds were immediately covered with protective cages to prevent accidental infection by rainwater or *Platypus subgranosus* frass contaminated with *C. australis* (or other) spores

(Figure 5.1a). Each cage was constructed from a 3 cm length of clear PVC tubing (2 cm diameter), having one straight and one bevelled end. The straight end was covered by a 4 cm x 4 cm piece of fabric gauze (Shapenell stiffening), held in place by plastic covered garden wire (Twistee). A leather punch was used to make two opposite holes in the bevelled end, through which string was threaded. The cage was positioned over the wound, and the string tied round the sapling so that the whole cage sloped downwards to facilitate runoff. Finally the base of the cage was stuck to the stem and sealed using a silicon based sealant (Aussigrip).

Chalara australis (Isolate 1.91) inoculum with a concentration of 10^6 spores/mL was prepared using the methods previously described. Wounds were inoculated through the fabric gauze with a sterile needle and 1 mL syringe, using 0.1 mL of inoculum per wound, and ensuring that the wound surface was thoroughly wetted. For each wound age, wounds on control saplings were inoculated with sterile distilled water. After inoculation, spore viability of the remaining inoculum was checked by adding one drop to 100 mL of 1% malt extract solution (autoclaved) and incubating for 48 hours at 20°C. The percentage germination of 100 spores was then counted under a compound microscope.

Saplings were felled 70 days after inoculation and transported to the workshop for further examination. The diameter of each sapling was measured approximately 0.95 cm above ground level (i.e. in the centre of the wounded section), and wound infection was assessed by using a drop saw to section saplings at the point of each wound. Characteristic *C. australis* staining was noted, and both the lateral and radial extension of such staining was measured. The extent of the stained column above and below each wound was assessed by further sectioning. Frequently the measurement of the stained column below the wound represented a minimum value, since the disease had spread into the root system. Occasionally stained columns from two wounds merged; in such cases it was assumed that half of the resultant column had originated from each wound.

For wounds where staining was not typical of *C. australis,* and for at least one other wound per treatment, the presence of *C. australis* was verified by isolation from disks cut from the immediate wound area. Each disk was partially cut through and then split using surface sterilised tools. Three fragments of split wood from each wound area were removed using a sterile scalpel and plated out onto 3% malt agar and incubated at 20°C. The split disks were then placed on paper towels in the laboratory. After 5-12 days, plates were assessed for the presence of *C. australis* (characterised by the green/black coloration of colonies, and by the

presence of typical phialides). As a further check, disks were again assessed for the development of characteristic *C. australis* staining.

The effects of inoculation with *C. australis* and wound age on the wound infection rate were tested by a multifactor ANOVA on the proportion of wounds infected, using saplings as replicates. Inoculum type and wound age were used as factors, adjusting for sapling diameter by using it as a covariate. The data were first transformed to the arcsin square root of the percentage infected. A multiple range analysis (which gave homologous groups based on 95% confidence limits) was then performed on infection rate by wound age.

The relative importance of inoculum type, wound age and individual saplings as sources of variability in *C. australis* growth, was assessed by nested ANOVAs, the four wounds on each sapling being used as replicates. The response variates were the lateral and radial extent of staining and the extent of the disease above and below each wound.

The significance of the effects of inoculum type and wound age on the growth of *C. australis* was estimated by multifactor ANOVAs, the response variates being the lateral and radial extent of staining and the extent of staining above and below each wound. Wounds were used as replicates. Multiple range analyses (which gave homologous groups based on 95% confidence limits) were then performed on each of the response variates by wound age.

Effect of chemical treatments on the infection of *N. cunninghamii* saplings

This experiment was run in conjunction with the previous one, and used the same basic methodology throughout, but had only one wound age and four chemical treatments, there being four replicate saplings per treatment.

Treatments involved spraying freshly made wounds with different solutions, before attaching cages. Chemicals were made up with sterile distilled water and were as follows: 4% boric acid (H_3BO_3); 10% ammonium sulfamate ($NH_4SO_3.NH_2$); 5% sodium nitrate ($NaNO_3$); 1% α–amino isobutyric acid ($H_6N_2O_3S$). Wounds were inoculated the following day with a *C. australis* (Isolate 1.91) suspension of 10^6 spores/mL. Controls were not sprayed with chemicals but were wounded and inoculated the following day with *C. australis*. Saplings were felled after 68 days and examined as before.

Data analysis was as outlined for the previous experiment, except that only a single factor (chemical treatment) was investigated.

5.2.3 RESULTS AND CONCLUSIONS

Water loss from *N. cunninghamii* billets

Water loss from sealed billets was considerable; rising from approximately 11 percent of the total weight after 26 days, to 29 percent after 97 days (Table 5.1).

Table 5.1 Mean percentage weight loss from wounded billets and controls up to three months old

Treatment	Age of billets (days)			
	26	28	49	97
Wounded	11.2%	12.0%	19.3%	28.5%
Control	10.9%	12.4%	19.4%	29.3%

The ANOVA indicated that the most important factor determining weight (water) loss from sealed billets was age, the effect of wounding being non-significant. Diameter of billets was also very important, and when used as a covariate, increased the significance level of the wounding effect (Table 5.2).

Table 5.2 ANOVA of percentage weight loss of billets by age and wounding, adjusting for diameter (data transformed to arcsin square root of the percentage weight loss)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Diameter	20.616	1	20.616	63.325	0.0000***
MAIN EFFECTS	1089.083	4	272.271	836.318	0.0000***
Age of billets	1088.273	3	362.758	1000.000	0.0000***
Wounding	0.811	1	0.811	2.490	0.1247 NS
2 FACTOR INTERACTIONS					
Age x Wounding	0.843	3	0.281	0.863	0.4705 NS
RESIDUAL	10.092	31	0.326		
TOTAL	1120.635	39			

NB In this and following tables, 5%, 1% and 0.1% significance levels are shown by *, ** and *** respectively. Non-significant values are denoted by NS.

Thus water is lost primarily through the bark of sealed billets. Unless billets are the same age when inoculated, this may be a complicating factor when investigating the effect of wound age on infection rate.

Effect of wound age and spore concentration on the infection of *N. cunninghamii* billets

Although some staining of wounds occurred, *C. australis* could not be isolated from any of the wound sites, and the remaining replicates were not harvested. It was concluded that by the time of inoculation (28 days after the start of the experiment) and/or by the final harvest (62/63 days after the start of the experiment) the chisel wound sites had dried out too much to sustain *C. australis* growth.

Using bored holes as wounds thus appears to be a better laboratory method than chisel wounds. However, G. A. Kile and M. F. Hall (unpublished data) obtained only 52% infection with 21-day-old billets and holes and 10^6 spores/mL, and given that billets need to be the same age at inoculation (at least 28 days old if 28-day-old wounds are to be investigated), it is likely that any such laboratory experiment would have very limited usefulness.

Effect of wound age on the infection of *N. cunninghamii* saplings

Wound age significantly decreased the infection rate of wounds, although the significance was decreased when sapling diameter was used as a covariate. Inoculation with *C. australis* had a significant effect on the infection rate of wounds, indicating that cages had successfully prevented accidental infection of wounds on control saplings (Table 5.3). Of the wounds tested, isolations confirmed all but two of the *C. australis* identifications. The viability of the inoculum used was found to be 44%.

Table 5.3 ANOVA of proportion of wounds infected, by inoculum type and wound age, adjusting for diameter (data transformed to arcsin square root of percentage infected)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Diameter	684.224	1	684.224	9.688	0.0057**
MAIN EFFECTS	13136.429	5	2627.286	37.199	0.0000***
Inoculum type	4638.625	1	4638.626	65.677	0.0000***
Wound age	7381.059	4	1957.765	27.720	0.0000***
2 FACTOR INTERACTIONS					
Inoculum type by Wound age	8867.423	4	2216.856	31.388	0.0000***
RESIDUAL	1341.924	19	70.628		
TOTAL	24030.000	29			

Nested ANOVAs indicated that differences between individual saplings resulted in very little variability in *C. australis* growth; wound age being the most important factor (Appendix 20). The effects of wound age were significant on all *C. australis* growth measures investigated (Tables 5.3-5.7).

Table 5.4 ANOVA of lateral extent of disease, by inoculum type and wound age, adjusting for diameter

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Diameter	1.242	1	1.242	31.388	0.0000***
MAIN EFFECTS	19.389	5	3.878	98.002	0.0000***
Inoculum type	6.173	1	6.173	156.014	0.0000***
Wound age	12.128	4	3.032	76.623	0.0000***
2 FACTOR INTERACTIONS					
Inoculum type by Wound age	14.062	4	3.515	88.842	0.0000***
RESIDUAL	4.313	109	0.040		
TOTAL	39.006	119			

Table 5.5 ANOVA of radial extent of disease, by inoculum type and wound age, adjusting for diameter

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Diameter	4.214	1	4.214	57.219	0.0000***
MAIN EFFECTS	48.903	5	9.781	132.793	0 0000***
Inoculum type	14.866	1	14.866	201.842	0.0000***
Wound age	31.235	4	7.809	106.022	0.0000***
2 FACTOR INTERACTIONS					
Inoculum type by Wound age	36.991	4	9.248	125.559	0.0000***
RESIDUAL	8.028	109	0.074		
TOTAL	98.136	119			

Table 5.6 ANOVA of extent of disease above wounds, by inoculum type and wound age, adjusting for diameter

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Diameter	486.099	1	486.099	15.441	0.0001***
MAIN EFFECTS	7873.147	5	1574.629	50.020	0.0000***
Inoculum type	1811.286	1	1811.286	57.538	0.0000***
Wound age	5572.916	4	1393.229	44.258	0.0000***
2 FACTOR INTERACTIONS					
Inoculum type by Wound age	6493.020	4	1623.255	51.565	0.0000***
RESIDUAL	3431.327	109	31.480		
TOTAL	18283.592	119			

Table 5.7 ANOVA of extent of disease below wounds, by inoculum type and wound age, adjusting for diameter

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Diameter	1037.340	1	1037.340	37.123	0.0000***
MAIN EFFECTS	15301.186	5	3060.237	109.515	0.0000***
Inoculum type	3599.285	1	3599.285	128.805	0.0000***
Wound age	10756.516	4	2689.129	96.234	0.0000***
2 FACTOR INTERACTIONS					
Inoculum type by Wound age	12608.420	4	3152.105	112.802	0.0000***
RESIDUAL	3045.854	109	27.944		
TOTAL	31992.800	119			

Mean values for infection rate of wounds, for the lateral and radial extent of the disease and its spread above and below wounds of different ages, are given in Table 5.8, showing the results of the multiple range analyses. No wounds older than 14 days became infected with *C. australis.* This is in contrast to the results of G. A. Kile and M. F. Hall (unpublished data), which showed that 38% of 28-day-old wounds became infected. However, it is possible that this discrepancy was largely due to differing weather conditions experienced during the two experiments: saplings in the original study were inoculated in November/December (spring/summer); those in the present study were inoculated in the comparatively warmer and drier conditions in February (late summer).

Wound infection rate and the extent of lateral and radial growth of *C. australis,* were more sensitive to wound age than the extent of the stained column above or below wounds. Again, these results were in contrast to those of G. A. Kile and M. F. Hall (unpublished data), and the necessity of recording a number of *C. australis* growth measures is emphasised.

Table 5.8 Mean values of infection rates and growth measures of *C. australis* at different wound ages (multiple range analyses show homologous groups based on 95% confidence intervals)

Wound age (days)	Infection rate (%)	Lateral growth (cm)	Radial growth (cm)	Growth above wound (cm)	Growth below wound (cm)
0	100 *	0.896 *	1.450 *	18.604 *	25.979 *
14	33.3 *	0.200 *	0.229 *	0.688 *	1.021 *
28	0 *	0 *	0 *	0 *	0 *
56	0 *	0 *	0 *	0 *	0 *
112	0 *	0 *	0 *	0 *	0 *

NB In this and following tables, each **column** of asterisks represents one homologous group.

Effect of chemical treatments on the infection of *N. cunninghamii* saplings

Chemical application did not have a significant effect on the infection rate of wounds, the significance decreasing when sapling diameter was used as a covariate (Table 5.9). Of the wounds tested, isolations confirmed all of the *C. australis* identifications. The viability of the inoculum used was 35%.

Table 5.9 ANOVA of proportion of wounds infected, by chemical treatment, adjusting for diameter (data transformed to arcsin square root of percentage infected)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Diameter	940.010	1	940.010	5.250	0.038*
MAIN EFFECTS					
Chemical treatment	367.098	4	91.774	0.513	0.7277 NS
RESIDUAL	2506.643	14	179.046		
TOTAL	3813.750	19			

Nested ANOVAs indicated that differences between individual saplings resulted in very little variability in *C. australis* growth; chemical treatment being far more important (Appendix 21). The effects of chemical application were significant on all *C. australis* growth measures investigated (Tables 5.10-5.13).

Table 5.10 ANOVA of lateral extent of disease, by chemical treatment, adjusting for diameter

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Diameter	2.704	1	2.704	9.435	0.0030**
MAIN EFFECTS					
Chemical treatment	5.936	4	1.484	5.177	0.0010**
RESIDUAL	21.212	74	0.287		
TOTAL	29.852	79			

Table 5.11 ANOVA of radial extent of disease, by chemical treatment, adjusting for diameter

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Diameter	1.391	1	1.391	1.323	0.2537 NS
MAIN EFFECTS					
Chemical treatment	23.878	4	5.969	5.678	0.0005***
RESIDUAL	77.795	74	1.051		
TOTAL	103.064	79			

Table 5.12 ANOVA of extent of disease above wounds, by chemical treatment, adjusting for diameter

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Diameter	60.014	1	60.014	0.147	0.7067 NS
MAIN EFFECTS					
Chemical treatment	13666.739	41	3416.685	8.361	0.0000***
RESIDUAL	30239.968	74	408.648		
TOTAL	43966.722	79			

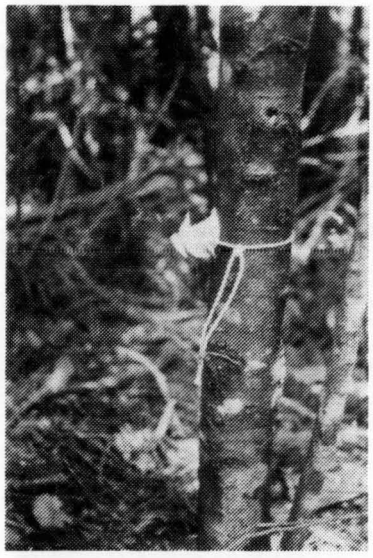
Table 5.13 ANOVA of extent of disease below wounds, by chemical treatment, adjusting for diameter

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Diameter	1298.254	1	1298.254	2.264	0.1367 NS
MAIN EFFECTS					
Chemical treatment	11134.915	4	2783.729	4.854	0.0016**
RESIDUAL	42434.866	74	573.444		
TOTAL	54868.036	79			

Mean values for infection rate of wounds, for the lateral and radial extent of the disease and its spread above and below wounds treated with different chemical treatments, are shown in Table 5.14. Although none of the chemical treatments had significant effects on the infection rate of wounds, they all had significant negative effects on one or more of the *C. australis* growth measures. Overall, 4% boric acid (H_3BO_3) and 5% sodium nitrate ($NaNO_3$) had the greatest effects, with 1% α–amino isobutyric acid ($H_6N_2O_3S$) and 10% ammonium sulfamate ($NH_4SO_3.NH_2$) being less effective.

Table 5.14 Mean values of infection rates and growth measures of *C. australis* with different chemical treatments (multiple range analyses show homologous groups based on 95% confidence intervals)

Chemical treatment	Infection rate (%)	Lateral growth (cm)	Radial growth (cm)	Growth above (cm)	Growth below (cm)
H_3BO_3	100 *	1.363 *	1.750 *	16.969 *	20.119 *
$NaNO_3$	87.5 *	1.194 *	1.844 *	10.938 *	20.688 *
$H_6N_2O_3S$	100 *	1.638 * *	2.219 *	25.438 *	31.719 * *
$NH_4SO_3.NH_2$	87.5 *	1.563 * *	2.356 * *	18.063 *	31.406 * *
Control	93.75*	2.044 *	3.288 *	48.500 *	53.031 *



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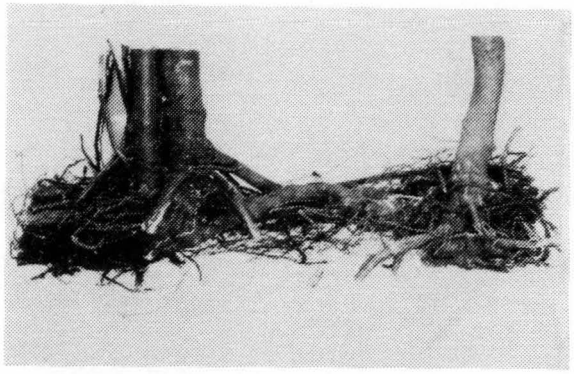

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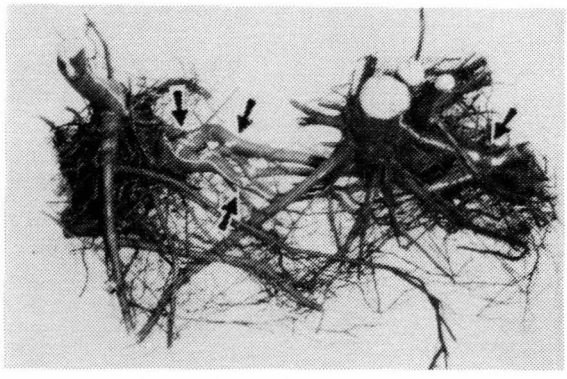

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Figure 5.1 a) Cage for minimising accidental infection of wounds.

b) Interconnected root systems of myrtle saplings.

c) Roots of myrtle saplings (arrows mark possible grafts).

d) Dye transfer via root grafts (arrows show extent of dye transfer).

5.3 ROOT GRAFTING IN NOTHOFAGUS CUNNINGHAMII

5.3.1 BACKGROUND AND AIMS

At the beginning of this project, there was only circumstantial evidence for root grafting in *N. cunninghamii.* In 1990, the root systems of two windthrown saplings growing on an old log, were examined and found to be connected. It was necessary to ascertain whether these connections were functional root grafts, whether *C. australis* could be transferred through such connections, and their significance in the spread of myrtle wilt.

Sectioning and staining of root grafts

Apparent root connections between trees do not necessarily mean that functional root grafts are present (Epstein 1978). It was necessary to section and photograph a myrtle root connection to establish whether bark between the two roots had broken down, and whether their vascular systems had fused.

Dye transfer through root grafts

Following confirmation of the existence of root grafts in *N. cunninghamii,* it was necessary to determine whether these grafts were functional; that is whether the vascular systems connected, allowing the transfer of assimilates or other substances between trees.

Traditionally such questions have been best resolved by tracking the below ground movement of radioactive isotopes from 'donor' to 'recipient' trees, the donor usually being felled to ensure a relatively stronger transpiration pull from the recipient. Beta or gamma emitters have been generally used since this radiation can be detected through soil and tree trunks. Although Phosphorus-32, Rubidium-86 and Iodine-131 have been used successfully in the past (Beckman and Kuntz 1951; De Byle 1964; Hutnik 1964; Hough *et al.* 1965; Miller and Woods 1965; Bormann 1966; Schultz and Woods 1967), safety regulations now prevent the use of these isotopes in unguarded, accessible field sites.

Fortunately, injected dyes can be used for the same purpose (Rexrode 1978), although their detection often involves the destruction of the recipient trees. The use of acid fuchsin dye has been fairly successful in this regard (Bormann and Graham 1961; Tew *et al.* 1969; Wood and Bachelard 1970).

Wilt underground transfer (WUT) plots

The final step was to establish whether *C. australis* could be transferred through root grafts, and the importance of any such transfer in the spread of myrtle wilt. Because of marked differences in myrtle wilt levels around Tasmania (Elliott *et al.* 1987), and because trees take, on average, two to three years to die from myrtle wilt (Chapter 3), it was necessary to study several sites over a number of years.

5.3.2 MATERIALS AND METHODS

Sectioning and staining of root grafts

The first saplings with connected root systems were found at Russell Road in southern Tasmania. They were trimmed, transported to the laboratory and photographed intact, using an Olympus OM-4 camera and 50 mm lens, using flashlights as necessary. One root connection was excised using a band saw and photographed with an Olympus OM-4 camera with an Olympus 80 mm macro lens and bellows. It was then cut into sections, the surfaces being sanded with a belt sander and wet and dry paper. The root sections were photographed using a Pentax K1000 camera and a Tokina AT-X 90 mm macro lens.

The area where the roots actually fused was cut out and sectioned (under steam where necessary) with a Leitz sledge microtome. Microtome sections were 15-20 µm thick, and were stained for lignin by mounting in a drop of saturated aqueous solution of phloroglucinol in 20% hydrochloric acid (Jensen 1962). They were examined and photographed under Nikon Optiphot-2 compound and Nikon SMZ-10 binocular microscopes, using a Nikon F301 camera.

Dye transfer through root grafts

For this experiment ten roadside *N. cunninghamii* saplings at Russell Road were selected. They were approximately 5-12 cm DBH, had *N. cunninghamii* neighbours at distances less than 1 m, superficial root systems, and, in some cases were evidently root grafting with their neighbours. The selected trees were felled, acid fuchsin dye being applied under pressure to the base of each stump. Warm, windy days were selected, to maximise the transpiration pull of neighbouring trees.

Dye was injected into the stumps using the method developed by G. A. Kile and M. F. Hall (unpublished data) for the injection of a *C. australis* spore suspension. Two or three holes were drilled in the base of each stump using a 13/64" drill bit. Plastic collars were removed from surgical needles; one collar being firmly set in each hole. Plastic syringes containing

the dye were then inserted into the collars. Each syringe had a pair of opposite holes drilled in the base of the outer case, and a series of holes drilled through the plunger. Each syringe was partially filled with air, partially with dye. Pressure was applied to the plunger, and a nail passed through the outer holes and the plunger so that it stayed under pressure. Syringes were refilled as necessary during the experiment.

Seven sapling stumps were each injected using three syringes containing an aqueous 0.15% solution of acid fuchsin, the syringes being finally removed after 27-41 days. Of these, the first three were injected using three 10 mL syringes; subsequently 20 mL syringes were used. One stump was of a sapling which had been felled 35 days previously, the others were freshly cut. The remaining three saplings were felled and the stumps were each injected using two syringes containing an aqueous 1.0% solution of acid fuchsin; in one case the syringes were applied close to evident root grafts. The syringes were removed after 19 days.

During the experiment, bark was removed from the surface roots and stems of the neighbouring trees to check for dye transfer. After the syringes had been removed the neighbouring trees were felled and their stumps examined for traces of dye.

Wilt underground transmission (WUT) plots

Three sites, previously disturbed and with abundant myrtle saplings, were selected for experimental work. At each site a number of healthy myrtle trees and saplings 3-30 cm DBH (and up to 41 cm with double leaders) were inoculated with *C. australis*, while surrounding trees were colour coded with plastic tape according to their disease and damage status.

Wobbly Creek is a mixed forest site in southern Tasmania, with scattered groups of myrtle saplings, probably resulting from fire. Inoculations were made in three areas at this site. Russell Road is another mixed forest site in southern Tasmania, but consists of roadside regeneration along an old track, saplings being generally small and closely spaced. Other work at the site indicated that saplings were probably 19-33 years old at the time of inoculation. Mt Michael in north east Tasmania is a high altitude callidendrous rainforest site which has been burnt following mining disturbance, resulting in an apparently even-aged forest of *N. cunninghamii* and *Atherosperma moschatum*. The area had a fairly uniform slope. Other site details are given in Appendix 22.

Chalara australis (Isolate E8) inoculum of 10^6 spores/mL was prepared as before, details of the isolate being given in Appendix 19. Inoculation used the method previously described for

the injection of dye (G. A. Kile and M. F. Hall, unpublished data). Three 20 mL syringes per tree, each containing 10 mL of inoculum, were positioned around the stem base, immediately above main roots (where these could be distinguished). With several very small saplings, only two syringes were used, each containing 5 mL of inoculum. Before inoculation, stem bases were sprayed with alcohol and flamed. Syringes were removed at the end of the day, by which time most of the inoculum had been taken up. The plastic collars were left in the saplings, the hole being sealed with petroleum jelly (Vaseline). After inoculation, spore viability of the remaining inoculum was checked by adding 1 mL to 5 mL of 1% malt extract solution (autoclaved) and incubating for 22 hours at 20°C. The percentage germination of 100 spores was then counted under a compound microscope.

Sites were monitored yearly for three years. At each date, inoculated trees and their neighbours were assessed for foliage symptoms of myrtle wilt (wilting, yellowing and browning of leaves or leaf loss), for *P. subgranosus* attack and for the development of black conidial felts of *C. australis* on the stem. Recent stem and crown damage were also noted. The direction and distance (stem to stem, at ground level) from inoculated to newly infected trees was measured. The DBH of inoculated and newly infected trees was also measured. At Mt Michael the direction of slope was noted at each inoculation area. In relation to slope, the direction of disease spread was classified as either 'uphill' or 'downhill'. Roadside disturbance, the scattered distribution of *N. cunninghamii* or largely flat topography made the other sites unsuitable for this assessment.

Inoculated saplings which did not become diseased within three years were omitted from analysis, details being given in Appendix 22. Pending further investigation, and in the absence of other obvious explanations (e.g. recent damage), it was assumed that myrtle wilt had spread from inoculated trees to neighbouring newly infected trees. For each site the infection rate (***R***) was estimated as being the average number of newly infected neighbours, per successfully inoculated tree, per year. This was calculated using the first two years' data, when the number of available neighbouring myrtles was unlikely to be limiting disease spread.

A multifactor ANOVA was performed on the numbers of infected trees resulting from each inoculation, after three years. The main factor was the site, with the DBH of inoculated trees being used as a covariate. A similar ANOVA was performed on the maximum extent of disease spread from each inoculation (after three years), with the number of infected trees being used as a second covariate. Relationships between the three variables were investigated using correlations, the data from the three sites being analysed together.

A multifactor ANOVA was performed on the DBH of infected trees, with the site being the main factor and using DBH of inoculated trees as a covariate. Correlation analysis was performed on the DBH of inoculated and infected trees and the distance between them, the data from the three sites being analysed together.

Finally, data from the three sites were combined, and means and standard errors determined for the numbers of infected trees resulting from each inoculation, and for the maximum extent of disease spread from each inoculation. These were then plotted against time.

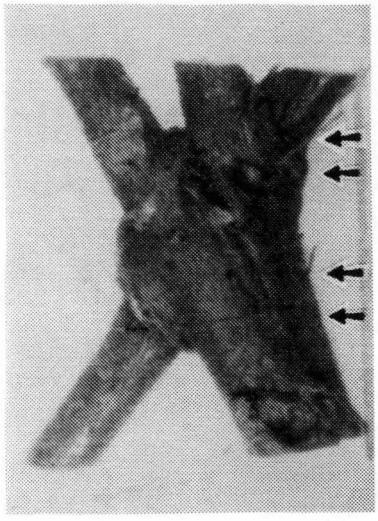
5.3.3 RESULTS AND CONCLUSIONS

Sectioning and staining of root grafts

The root systems of the two myrtle saplings were connected at various points (Figure 5.1b and c). The excised root connection proved to be a true root graft, with most of the bark between the roots having broken down (Figure 5.2), although microtome sections showed that occluded bark fragments did persist in some areas. Despite this the two secondary vascular systems had clearly fused (Figure 5.3). It can be concluded that root grafting does occur in *N. cunninghamii.*

Dye transfer through root grafts

Dye had not moved to the stems of the neighbouring trees. However in one case it had clearly moved across a root graft into the root of a neighbouring tree (Figure 5.1d). Interestingly the above ground part of this tree was actually dead, although not from myrtle wilt. Presumably its root system was still alive, grafted to those of its neighbours. In this instance 0.15% acid fuchsin was used and dye transfer across the graft was observed six days after injection, representing a movement of 55 cm. It can be concluded that functional root grafts do occur in *N. cunninghamii,* but that not all root connections are necessarily functional.



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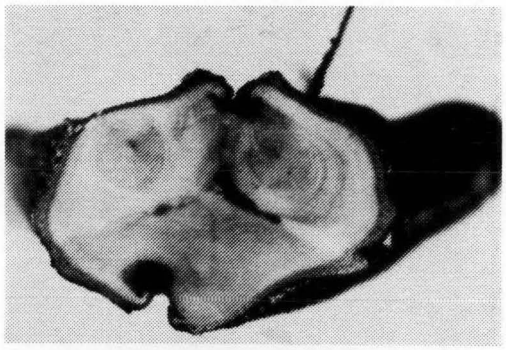

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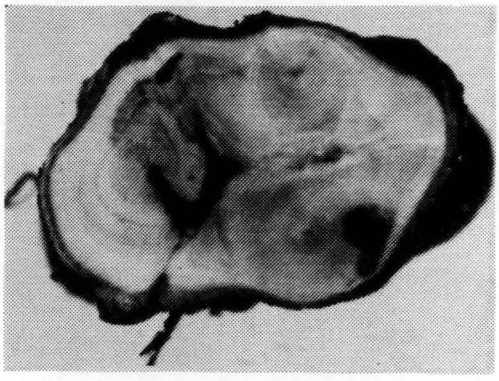

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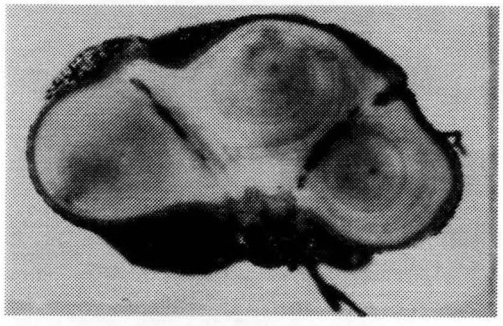

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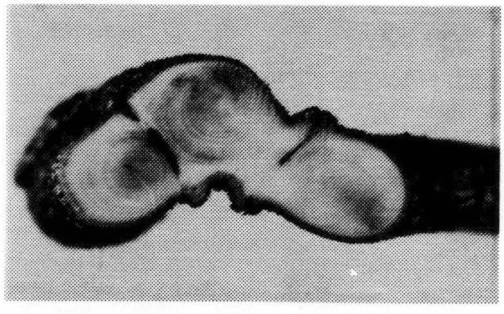

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Figure 5.2 a) Excised root graft (x ±1.5). (Arrows show positions of sections.)
b-e) Sections through root graft, showing continuity of sapwood and occluded bark fragments (x ±3).

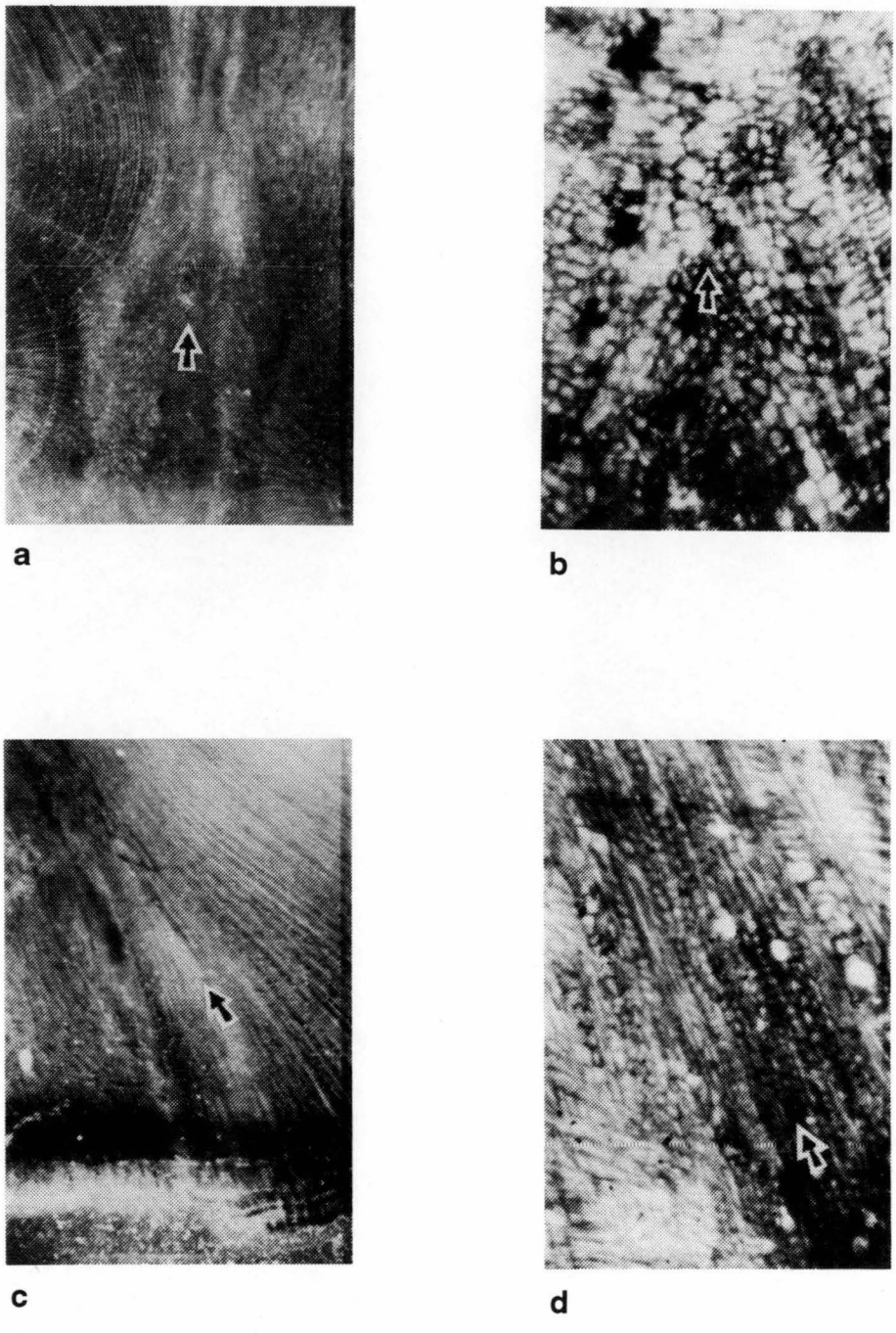


Figure 5.3 a) Transverse section through root graft. Arrow shows occluded bark fragment (x 9.5).

b) Transverse section through root graft (x 70). Arrow marks position of graft.

c) Radial longitudinal section through root graft (x 13.5). Arrow marks position of graft.

d) Radial longitudinal section through root graft (x 70). Arrow marks position of graft.

Wilt underground transmission (WUT) plots

Of the 36 trees inoculated at the three sites, 30 showed symptoms of myrtle wilt. An additional 20 neighbouring trees became infected over the following two years (26 over three years). Estimated yearly infection rates are given in Table 5.15. Details of spore viability and inoculation success rate at each site are given in Appendix 22.

Table 5.15 Yearly infection rates in the WUT plots

	No. of successful inoculations	No. of infected neighbours (2 yrs)	Infection rate ***R*** (infections/inoculation/yr)
Wobbly Creek	7	3	0.214
Russell Road	14	11	0.393
Mt Michael	9	6	0.333
Totals	30	20	0.333

Recording the foliage symptoms of myrtle wilt over time enabled an independent assessment of other characteristic symptoms of the disease. The majority of trees with myrtle wilt also had *C. australis* felts and *P. subgranosus* attack (Table 5.16). In only two instances did *P. subgranosus* attack occur on trees which did not subsequently become infected (and these were both very close to infected trees), thus confirming the accuracy of *P. subgranosus* attack as an indicator of *C. australis* infection.

Table 5.16 *P. subgranosus* attack and *C. australis* felts in myrtle wilt infected trees

	NUMBER OF TREES WITH		
	Myrtle wilt (foliage symptoms)	*P. subgranosus* attack on stem	*C. australis* conidial felts
Wobbly Creek	11	9	7
Russell Road	26	12	16
Mt Michael	19	19	19
Totals	56	40	43

Of the 26 trees which became infected, 12 either never suffered *P. subgranosus* attack, or were attacked subsequent to foliage symptoms developing. Clearly *P. subgranosus* was not acting as a vector, and since none of these trees had obvious damage (infection courts), contaminated air-borne frass could not have been a factor. This strongly implicated underground spread of *C. australis*.

At Mt Michael, of ten trees which became infected with *C. australis* during the course of the experiment, nine were classified as being 'downhill' of the inoculated tree (the tenth being situated only 4° 'uphill'). The clear implication at this site is that myrtle wilt spread mostly down the slope.

ANOVAs showed that neither the number of (uninoculated) trees which became infected, nor the maximum extent of disease spread from inoculated trees, were significantly affected by site; nor were they related to the diameter of the inoculated trees (Tables 5.17 and 5.18). However, the maximum extent of disease spread from inoculated trees was related to the number of trees which became infected, since this covariate effect was significant (Table 5.18).

Table 5.17 ANOVA of number of infected trees per inoculation, by site, adjusting for diameter of inoculated trees

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Diameter (inoc.)	4.039	1	4.039	2.266	0.1443 NS
MAIN EFFECTS					
Site	3.070	2	1.535	0.861	0.4345 NS
RESIDUAL	46.357	26	1.783		
TOTAL	53.467	29			

Table 5.18 ANOVA of maximum extent of disease spread, by site, adjusting for diameter of inoculated trees and for the number of infected trees per inoculation

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATES	109443.85	2	54721.924	25.542	0.0000***
Diameter (inoc.)	1862.75	1	1862.748	0.869	0.3699 NS
Number infected	92111 59	1	92111.594	42.995	0.0000***
MAIN EFFECTS					
Site	1547.392	2	773.696	0.361	0.7005 NS
RESIDUAL	53559.802	25	2142.392		
TOTAL	164551.04	29			

The correlation between the maximum extent of disease spread and the number of infected trees was shown to be positive and highly significant. There also appeared to be some positive correlation between the diameter of inoculated trees and the maximum extent of disease spread, although this was not significant (Table 5.19).

Table 5.19 Correlations between diameter of inoculated trees, number of infected trees and maximum extent of disease spread per inoculation

	Number infected	Maximum distance
Diameter (inoculated)	0.2749 (0.1416 NS)	0.3245 (0.0802 NS)
Number infected		0.8086 (0.0000***)

NB Figures refer to correlation coefficients, with significance levels in parentheses. Sample size = 30.

ANOVA indicated that the diameter of (uninoculated) infected trees was significantly affected by site, and that the diameter of inoculated trees was a significant covariate effect (Table 5.20). Correlation showed that the diameters of inoculated and subsequently infected trees were positively and significantly correlated. The diameter of inoculated trees, and the distance between inoculated and subsequently infected trees, were also positively and significantly correlated (Table 5.21).

Table 5.20 ANOVA of diameter of infected trees, by site, adjusting for diameter of inoculated trees

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Diameter (inoc.)	204.860	1	204.860	10.341	0.0040**
MAIN EFFECTS					
Site	258.001	2	129.001	6.512	0.0060**
RESIDUAL	435.838	22	19.811		
TOTAL	898.700	25			

Table 5.21 Correlations between diameter of infected trees, diameter of inoculated trees and the distance between them

	Diameter (inoculated)	Distance
Diameter (infected)	0.4774 (0.0136*)	0 2878 (0.1540 NS)
Diameter (inoculated)		0.4453 (0.0226*)

NB Figures refer to correlation coefficients, with significance levels in parentheses. Sample size = 26.

After three years, the mean number of trees which had become infected per inoculated tree, was slightly less than one. After an initial delay, the disease spread, on average, approximately 15 cm per year for the final two years (Figure 5.4).

5.4 *CHALARA AUSTRALIS* ISOLATIONS FROM ROOT GRAFTS

5.4.1 BACKGROUND AND AIMS

Observations and re-isolations from WUT plots

To verify the spread of myrtle wilt via root grafts, it was necessary: firstly, to demonstrate continuity between the root systems of inoculated and neighbouring infected trees; secondly, to eliminate the possibility of other means of disease transfer; thirdly, to re-isolate *C. australis* from neighbouring infected trees and root grafts, and finally to characterise the *C. australis* isolates, so that the re-isolates could be compared with that originally inoculated.

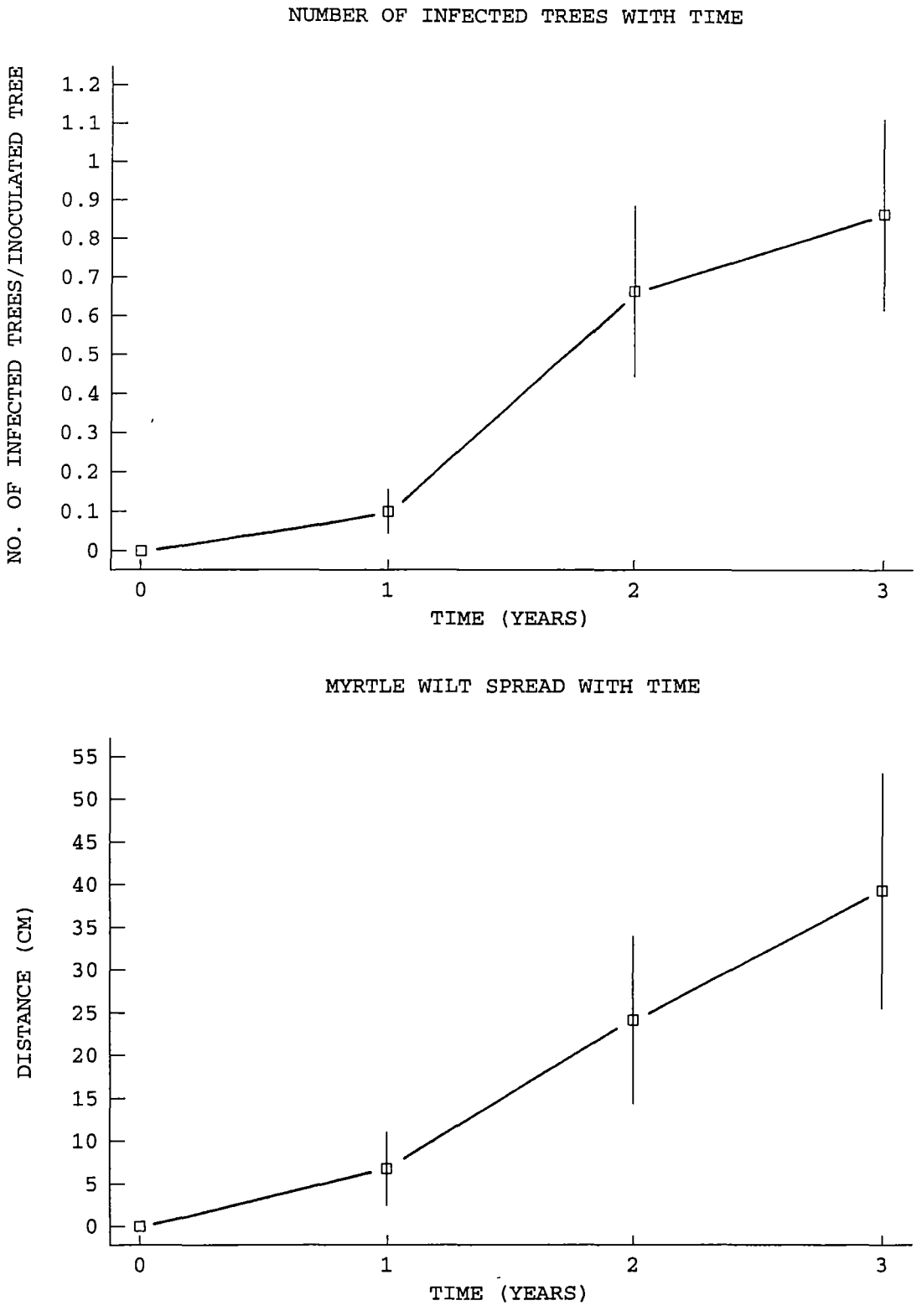


Figure 5.4 Spread of myrtle wilt from inoculated trees (means and standard errors):

number of infected trees resulting per inoculation (top);

maximum extent of disease spread from each inoculation (bottom).

Characterisation of isolates

Conidial measurements

Kile and Walker (1987) observed that *C. australis* isolates were fairly uniform in cultural characteristics although there was some colour variation. However, while some isolates had few conidia longer than 15-17 µm, others had many conidia 20-24 µm long. Conidial length was therefore an objective character by which isolates could be compared.

Isozyme electrophoresis and colony morphology

The combination of isoenzyme, comparative morphology and mating compatibility studies forms a powerful tool in fungal taxonomy. Of particular relevance to the present work, variation at a subspecific level can be detected by these methods (Zambino and Harrington 1989, 1992; Chang and Mills 1992; Yang *et al.* 1993).

Electrophoretically separated enzymes are an initial expression of the genome in tangible form, and have been successfully used to distinguish between species of *Penicillium* Fr. (Cruickshank and Pitt 1987). Zambino and Harrington (1989, 1992) have successfully used this method with *Leptographium* (a form genus including anamorphs of some *Ophiostoma* species) to identify variation between and within species.

Other information on extracellular enzyme production in the *Ceratocystis* complex is largely concerned with cell wall degrading enzymes (Fergus *et al.* 1957; Dube *et al.* 1990). The phenoloxidases produced by wood decaying fungi include laccase and peroxidase. Laccase is known to be a highly inducible enzyme; its production being stimulated by the presence of tannin, phenol derivatives and organic acids (Kaarick 1965).

Early isoenzyme electrophoresis work on *C. australis* failed to detect any variation between 15 Tasmanian isolates: pectic enzyme and amylase both gave consistent banding patterns, and ribonuclease activity was not detected (R. H. Cruickshank, unpublished data).

Compatibility tests

In fungi, vegetative incompatibility is heterogenic and allelic, with viable anastomoses being formed only between strains which have identical alleles at all of the vegetative compatibility (v-c) loci (Anagnostakis 1984). Seifert *et al.* (1993b) cite several methods suitable for the examination of *Ophiostoma* and *Ceratocystis* species. Compatibility tests have been effective in distinguishing between mating types, races and v-c groups of *O. novo-ulmi.* In *O. novo-ulmi,* perithecia production occurs with strains of opposite mating types, the v-c groups being functionally independent of mating type (Brasier 1984).

Pairings of *O. novo-ulmi* strains revealed five classes of vegetative reaction type; varying from completely compatible (no reaction), to completely incompatible (mycelial barrage formed), the large number of v-c groups suggesting multi-allelic and/or polygenic control (Brasier 1984). Pairings of another ascomycete, *Endothia parasitica* (Murr.) Anderson and Anderson, showed that strains in the same v-c group merged (many hyphal anastomoses), while those on different v-c groups formed either a zone of inhibition (few anastomoses, usually associated with dead cells) or formed two ridges of pycnidia (containing conidia) (Anagnostakis 1984).

Compatible isolates of *Leptographium wageneri* gave dense hyphal growth in the zone of confrontation, and this was linked to anastomosis. Heterokaryosis appeared to be maintained by frequent anastomoses. Multiple v-c groups were detected; these were electrophoretically distinct and usually had unique geographic ranges. (Zambino and Harrington 1990). To date there is no information on the existence (or otherwise) of v-c groups in *C. australis.*

Temperature/growth profiles

O. ulmi and *O. novo-ulmi* have different optimum and maximum temperatures for growth (based on their radial growth rates in culture) and can be successfully separated by their relative growth rates at 20°C and 33°C (Brasier *et al.* 1981). In culture, *C. australis* makes the most rapid radial growth at 20°C (8-9 mm per 24 hours), with no growth occurring below 5°C or above 25°C (Kile and Walker 1987). Although Victorian isolates from the Central Highlands and Strzelecki Ranges demonstrated comparable growth rates, and had the same temperature range for optimal growth (17.5-20°C) as Tasmanian isolates (Packham and Kile 1992), slight differences between the temperature/growth profiles of these isolates were apparent (G. A. Kile and M. F. Hall, unpublished data). Thus the comparison of temperature/growth profiles was another possible method of characterising isolates.

5.4.2 MATERIALS AND METHODS

Observations and re-Isolations from WUT plots

The root systems of selected inoculated trees and their neighbours were excavated using hand tools. Selected trees had at least one newly infected neighbour and were picked for ease of excavation. One tree each from Russell Road and Wobbly Creek, and two trees from Mt Michael were excavated.

The excavated root systems were sketched, noting the position of any root grafts, and then photographed *in situ,* using an Asahi Pentax camera and a Takumar 135 mm macro lens. Where practicable, newly infected trees were felled and sectioned into disks up the stem, noting the extent of *C. australis* staining. Cut disks from the tree base and at 50 cm intervals up the stem were similarly photographed, and transported to the laboratory for isolations to be made. Where trees were not felled wood samples from the stem were taken using a mattock.

Root grafts were excised using various hand saws, transported to the laboratory, then trimmed and sectioned using a band saw. The surfaces were sanded with a belt sander and wet and dry paper, and the sections were then photographed before being used for isolations. Continuity of the vascular systems and *C. australis* staining was noted. Photography used an Asahi Pentax camera and a Takumar 135 mm macro lens, using bellows and flashlights as necessary.

Where *C. australis* staining was clearly visible in both the connecting roots, the root graft was further sectioned into 1 cm cubes, using the band saw. The relative positions of the cubes were noted, and those in the immediate area of the graft were sectioned with a Leitz sledge microtome. Microtome sections were 15-20 µm thick, and were stained for lignin by mounting in a drop of saturated aqueous solution of phloroglucinol in 20% hydrochloric acid (Jensen 1962). Other sections were stained with cotton blue (in lactophenol) to show up any fungal hyphae. They were examined and photographed under a Zeiss Axioskop compound microscope, using an Olympus OM-4 camera.

The presence of *C. australis* was verified by isolation from root grafts, stem disks and stem wood samples. Stem disks and root grafts were partially cut through and then split using a surface sterilised mattock; stem wood samples were split using a sterile scalpel. Fragments of split wood were removed using a sterile scalpel and plated out onto 3% malt agar and incubated at 20°C. After 3-17 days, plates were assessed for the presence of *C. australis* (characterised by the green/black coloration of the colonies, and by the presence of typical phialides). Where contaminants made identification difficult, further isolations were made from the plates.

As an additional check for *C. australis,* bark scrapings were made from infected stems and root grafts where black conidial felts were apparent. These scrapings were mounted in water and viewed under a high power compound microscope, the presence of phialides being noted. It is known that the composition of the black conidial felts may be complex; the

phialides can vary in structure and *Chalara moniliformis* may also be present (Kile and Walker 1987). For this reason felts (and the presence of phialides) do not conclusively prove the presence of *C. australis,* although they are indicative of it. *P. subgranosus* tunnels were also noted.

In an attempt to verify the spread of myrtle wilt via root grafts, the *C. australis* isolates which were inoculated and re-isolated from the WUT plots were compared, using a range of methods (see below). Three control isolates taken from sites in Tasmania and Victoria were included. Brief details of the isolates used are outlined in Table 5.22, with more information being given in Appendix 19.

Table 5.22 Brief details of *C. australis* isolates used for morphological comparisons, isozyme electrophoresis and temperature profiles

Isolate	Origin	Date	Details
E8	Esperance, S Tas	14/3/89	WUT plot inoculum source. Maintained on 3% MA from 89
1C	Russell Rd, S Tas	17/3/93	WUT plot Tree 1C stem (50 cm), re-isolated onto 3% MA
1E	Russell Rd, S Tas	17/3/93	WUT plot Tree 1E root, re-isolated onto 3% MA
1H	Russell Rd, S Tas	30/3/93	WUT plot Graft 19 (Trees 1E/1H), re-isolated onto 3% MA
7A	Mt Michael, NE Tas	28/4/93	WUT plot Tree 7A stem (250 cm), re-isolated onto 3% MA
L1	Mt Michael, NE Tas	28/4/93	Control. 4.2 km from WUT plot, isolated onto 3% MA
X1	Xmas Hills, NW Tas	23/4/93	Control. Christmas Hills Rd, isolated onto 3% MA
N3	Rd 7,C H'lands, Vic	11/11/91	Control. Isolate maintained on 3% MA from 91

Characterisation of isolates

Conidial measurements

Isolates were grown on 3% malt agar at 20°C for 7 days, and then stored in a refrigerator for 5 days. Tufts of mycelium were mounted in water, and conidia were measured under a compound microscope (X1000), using an eyepiece graticule. The lengths of 25 conidia were measured for each isolate. Phialides and conidia were photographed under a Zeiss Axioskop compound microscope, using a Olympus OM-4 camera.

ANOVA and multiple range analyses (which gave homologous groups based on 95% confidence limits) were performed on conidial length, and minimum, maximum and mean

values were obtained for each isolate. Cochran's C test and Bartlett's test were used to test for homogeneity of variances.

Isozyme electrophoresis and colony morphology

For enzyme production, cultures were grown in loosely capped 5 mL Bijoux bottles, each containing 2 mL of culture medium, autoclaved at 121°C for 30 minutes, inoculated by needle point and incubated in the dark at 20-21°C for seven days. For polyphenoloxidase production the culture medium was 10% potato decoction (R. H. Cruickshank, unpublished data): 80 g of diced potato was simmered for 90 minutes in 400 mL of deionised water, mashed and strained through a double layer of muslin, and made up to 800 mL.

The method of G. A. Kile and M. F. Hall (unpublished data) was used to grow certain isolates on wood. Myrtle saplings of 5 cm DBH were surface sterilised by spraying with alcohol and flaming, sectioned into 5 cm lengths, and placed in plastic containers. The cut surfaces were inoculated with *C. australis* spore suspension and incubated at 21°C for seven days. *C. australis* was then re-isolated from the wood sections as previously described. Other isolates were grown on potato dextrose agar, or on potato dextrose agar and then on wood. Immediately prior to being transferred to potato decoction, all isolates were grown on 3% malt agar at 20-21°C in the dark.

Electrophoresis was performed in horizontal slab gels of polyacrylamide, the general procedure following Cruickshank and Pitt (1987). In all cases, deionised water was used as a control. Zymograms of gels were prepared by direct printing underwater onto high contrast Ilfospeed paper (5.1M).

Amylase and ribonuclease examination followed Cruickshank and Pitt (1987). Polyphenoloxidase was examined using 10.25% acrylamide (2.5% bisacrylamide) gels containing 0.1% TEMED and 0.1% ammonium persulphate (A. K. Mills, unpublished data). After electrophoresis, gels were incubated for 60 minutes at room temperature in a 0.1M acetate buffer, pH 5.2, filtered after the addition of 0.05 g of ortho-dianisidine dissolved in 2 mL of acetone. The gel was left in this buffer solution overnight at 4°C for the stain to develop.

The first run used the WUT plot and control isolates which had been maintained on malt agar (Table 5.22), and was carried out for the three enzyme systems. The second run was limited to polyphenoloxidase, and investigated the effect of substrate on zymogram banding; it was similar to the first run except that three additional cultures were used (Table 5.23).

These were all derived from the original inoculated isolate (E8), but previous to malt agar , they had been grown on potato dextrose agar (E8P), on myrtle wood (E8W), or on potato dextrose agar and then on myrtle wood (E8PW). The third run was also limited to polyphenoloxidase; it included E8PW, E8W, E8P, E8 and, as comparisons, 20 additional Tasmanian and Victorian isolates (of various ages and culturing histories) which had been growing on malt agar. Brief details of these isolates used are outlined in Table 5.24.

Table 5.23 Brief histories of cultures derived from the inoculated isolate (E8), and used for isozyme electrophoresis

Isolate	Cultural details
E8	WUT plot inoculum source. Maintained on 3% MA (4 years)
E8P	Grown on PDA (6 days + 1 day in fridge), then on MA (15 days)
E8W	Grown on myrtle wood (7 days), then on MA (7 days)
E8PW	Grown on PDA (6 days + 2 days in fridge), on wood (7 days), then on MA (7 days)

NB MA = 3% Malt agar
PDA = Potato dextrose agar

All the isolates used for enzyme production were also plated out onto 3% malt agar plates at 20-21^{o}C, and the colony morphology was observed after 5-15 days. The plates were back lit and photographed using one of the following: an Olympus OM-2 camera with an Olympus 80 mm macro lens and bellows; an Olympus OM-4 camera with an Olympus 80 mm macro lens and bellows; an Asahi Pentax camera with a Takumar 135 mm macro lens and bellows.

Six-day-old cultures derived from the original isolate (Table 5.23) were tested for the presence of laccase and peroxidase using the method of Stalpers (1978): a drop of 0.1 M α-naphthol in 96% ethanol applied to the mycelium gives a purplish colouration in the presence of laccase; similarly 0.1 M ρ-cresol in 96% ethanol turns orange-brown when tyrosinase is present. Cultures were assessed over a light table immediately after the solutions had been applied, and after six days.

Table 5.24 Brief histories of additional Tasmanian and Victorian isolates used for isozyme electrophoresis (Courtesy of G. A Kile and M. F. Hall, CSIRO Division of Forestry)

Isolate	Origin	Date	Details
E5	Esperance, S Tas	23/3/89	Maintained on 3% MA
E11	Esperance, S Tas	15/3/89	Maintained on 3% MA
1.55	Sumac Rd, NW Tas	21/7/80	3% MA, freeze dried 9/86, rehydrated 5/93 on 3% MA
1.66	Otways, SW Vic	15/9/80	3% MA, freeze dried 9/86, rehydrated 5/93 on 3% MA
2.81	Mt Arrowsmith, W Tas	17/4/85	3% MA, freeze dried 9/86, rehydrated 5/93 on 3% MA
2.85	Tarraleah, C Tas	17/4/85	3% MA, freeze dried 9/86, rehydrated 5/93 on 3% MA
2.88	Arve Valley, S Tas	26/6/85	3% MA, freeze dried 9/86, rehydrated 5/93 on 3% MA
2.89	Howards Rd, W Tas	17/4/85	3% MA, freeze dried 9/86, rehydrated 5/93 on 3% MA
2.90	Simons Rd, NE Tas	3/5/85	3% MA, freeze dried 9/86, rehydrated 5/93 on 3% MA
Tree 50	L. Florentine R, SW Tas	26/5/88	Maintained on 3% MA
BR1	Boyd R, SW Tas	21/9/89	Maintained on 3% MA
BR8	Boyd R, SW Tas	21/9/89	Maintained on 3% MA
BR11	Boyd R, SW Tas	21/9/89	Maintained on 3% MA
BW	Blackwater Rd, NW Tas	22/4/93	Maintained on 3% MA
N1	Deep Ck, C H'lands, Vic	11/11/91	Maintained on 3% MA
N2	Rd 7, C H'lands, Vic	11/11/91	Maintained on 3% MA
N4	Poley Rd, C H'lands, Vic	11/11/91	Maintained on 3% MA
N5	Charlie Ck, C H'lands, Vic	12/11/91	Maintained on 3% MA
N6	Strzeleckis, S Vic	13/11/91	Maintained on 3% MA
N7	Mt Erica, C H'lands, Vic	12/11/91	Maintained on 3% MA

Compatibility tests

For all tests isolates were first cultured on 3% malt agar at 20-21°C for 9 days. Agar plugs (2 mm x 2 mm) were taken from these cultures, arranged on potato dextrose agar plates and incubated at 20-21°C. Four (inverted) plugs were placed on each plate, one in the centre; the others arranged around the perimeter. This gave a maximum of six possible paired interactions per plate. In all cases the original inoculated isolate was placed centrally. The E8PW *culture* directly derived from the E8 *isolate* was used in place of E8, since it had more normal colony morphology. Sufficient plates were used to give at least two replicates of each pairing combination. Control plates consisted of pairings between the same isolate, there being one control plate per isolate.

Colony interactions were observed and photographed after 16 days, then observed again after 21 days. Plates were backlit and photographed using an Asahi Pentax camera with a Takumar 135 mm macro lens and bellows. After 28-32 days mycelial interactions were observed *in situ* using a high power binocular microscope. Perithecial initials were photographed under a Zeiss Axioskop compound microscope, using a Olympus OM-4 camera.

The isolates used in the first test were E8PW (the inoculated isolate) and the WUT plot re-isolates: 1C; 1E; 1H and 7A. The second test used E8PW and the WUT plot control isolates: L1; X1 and N3 (Table 5.22). The third test used E8PW and a selection of the Victorian isolates: Isolate 1.66 was from Youngs Creek Road in the Otway Ranges; Isolates N3, N5 and N7 were from the Central Highlands (Appendix 3, sites 3, 7/8 and 16 respectively) and Isolate N6 was from the Strzelecki Ranges (Appendix 3, site 15).

Temperature/growth profiles

The eight isolates (Table 5.22) were cultured on 3% malt agar at 20-21°C for 6 days. Mycelium was taken from the outer third of each colony with a sterile needle, and used to inoculate potato dextrose agar plates (made from 39 g of dehydrated bacto PDA in 1 L of water). The plates were then incubated in the dark for 110 hours at one of six temperatures, using incubators and controlled growth cabinets. Temperatures were recorded at intervals using a calibrated thermometer, and the mean values for the duration of the experiment were then calculated; i.e. 13.85°C, 16.00°C, 17.45°C, 19.55°C, 21.35°C and 22.75°C.

For each of the 48 isolate/temperature combinations there were five replicates. For each temperature, the petri dishes were arranged in the incubator or growth cabinet on a paper lined tray, in eight stacks of five. To minimise the effect of any vertical temperature gradients there was one replicate of each isolate at each level (i.e. vertical position in incubator/growth cabinet), the arrangement being randomised within levels.

At the end of the experiment, the plates were placed in a refrigerator to arrest growth. Each plate was placed, reverse side up, over a light box and marked into four equal sectors. The maximum extent of radial growth was measured along each of the four radii, and the mean value was then calculated (excluding measurements obviously affected by contaminants).

A multifactor ANOVA was performed on the extent of radial growth, with temperature and isolates as the main effects. Multiple range analyses (which gave homologous groups

based on 95% confidence limits) were performed on radial growth, by isolate. Cochran's C test and Bartlett's test were used to test for homogeneity of variances.

For each temperature treatment, a multifactor ANOVA was performed on the extent of radial growth, with level (i.e. vertical position in incubator/growth cabinet) and isolates as the main effects. Multiple range analyses (which gave homologous groups based on 95% confidence limits) were performed on radial growth, by isolate.

For each isolate, notional (i.e. not necessarily achievable) maximum (θ_1), notional minimum (θ_2), and optimum(T_{opt}) temperatures for growth were determined using a model proposed by Ratkowsky (1989), as utilised by Candy (1990):

$$y = \theta_4 (T - \theta_2) [1 - exp \{\theta_3 (T - \theta_1)\}] \qquad \theta_2 < T < \theta_1$$

$$= 0 \qquad \theta_3, \theta_4 > 0$$

where y is radial growth, T is temperature, and θ is a vector of unknown parameters.

To obtain initial estimates of $\theta_1, \theta_2, \theta_3$ and θ_4, the equation was solved over a range of T values from 14-23°C, until the values of θ_1 and θ_2 indicated a suitable temperature/growth curve. Using these estimates, nonlinear regression was then used to fit the temperature/growth model, with radial growth as the dependant variable.

Using the regression coefficients $\theta_1, \theta_2, \theta_3$ and θ_4, the optimum temperature (T_{opt}) was derived from the above equation by solving for:

$$dy/dT = 0.$$

The solution is given by:

$$exp \{\theta_3 (T_{opt} - \theta_1)\} [1 + \theta_3 (T_{opt} - \theta_2)] - 1 = 0.$$

Initially, values of T from 6-22°C were used to obtain an estimate for T_{opt} (i.e. the value of T giving the solution closest to zero). Utilising this estimate, the Newton-Raphson method was then used to find the precise root of the equation (T_{opt}).

Using any of the above regression coefficients, paired comparisons of the isolates can be made by calculating the value of d (the departure from the mean) as follows:

$$d = \frac{| q_a - q_b |}{\sqrt{SE_a^2 + SE_b^2}}$$

where q_a and q_b are the values of the selected regression coefficient, for isolates a and b respectively, and SE_a and SE_b are the standard errors of these values. Assuming a normal

distribution, the probabilities of obtaining absolute departures from the mean, or ***d*** values, greater than 1.96, 2.58 and 3.29, are 0.05, 0.01 and 0.001 respectively (Bailey 1981).

The coefficients θ_1 and θ_2 were selected, and using the above method, pairwise comparisons of all the isolates were made. Since a proportion of them would have been significant by chance alone, the probability figures were then adjusted for the number of simultaneous tests, using the sequential Bonferroni technique (Rice 1989).

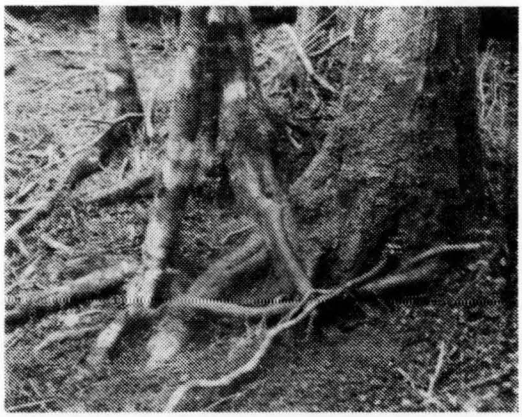
5.4.3 RESULTS AND CONCLUSIONS

Observations and re-isolations from WUT plots

Both at Russell Road and at Mt Michael, excavations revealed an inoculated tree and its neighbouring infected trees to be connected via their root systems (Figures 5.5-5.11). Sectioning showed that many of these root connections were functional root grafts. Photographs and diagrams of these systems are given in Figures 5.5 and 5.10, showing the positions of inoculation, functional root grafts, *P. subgranosus* holes, *C. australis* felts and staining, and areas from which *C. australis* re-isolations were successfully made. Possible routes of myrtle wilt transfer have also been marked. It is important to note that none of the infected trees appeared to have recent stem or crown damage (infection courts), thus infection via airborne inoculum was unlikely.

Sections of the functional root grafts (Figures 5.6, 5.7, 5.10 and 5.11) show *C. australis* staining. A non-functional root connection between *N. cunninghamii* trees from Wobbly Creek, and an apparent *N. cunninghamii/Atherosperma moschatum* connection from Russell Road are illustrated in Figure 5.12.

At Mt Michael one root from the inoculated tree (Tree 7) was clearly grafted to the buttress root of the neighbouring and infected Tree 7A (Figure 5.6). Sectioning showed a continuity of sapwood between the trees, with *C. australis* staining in both, and in the area of the root graft (Graft 1) (Figure 5.7). Sections up the stem of Tree 7A clearly indicated that the infection had originated from the base (Figure 5.6), and *C. australis* re-isolated from this area (Isolate 7A) was indistinguishable from that inoculated (Isolate E8). Myrtle wilt transfer from the inoculated tree to the neighbouring tree via the root graft is the obvious conclusion. However, Tree 7A was attacked at an early stage by *P. subgranosus,* so the possibility of direct transfer by this means cannot be completely eliminated, although it is highly unlikely.



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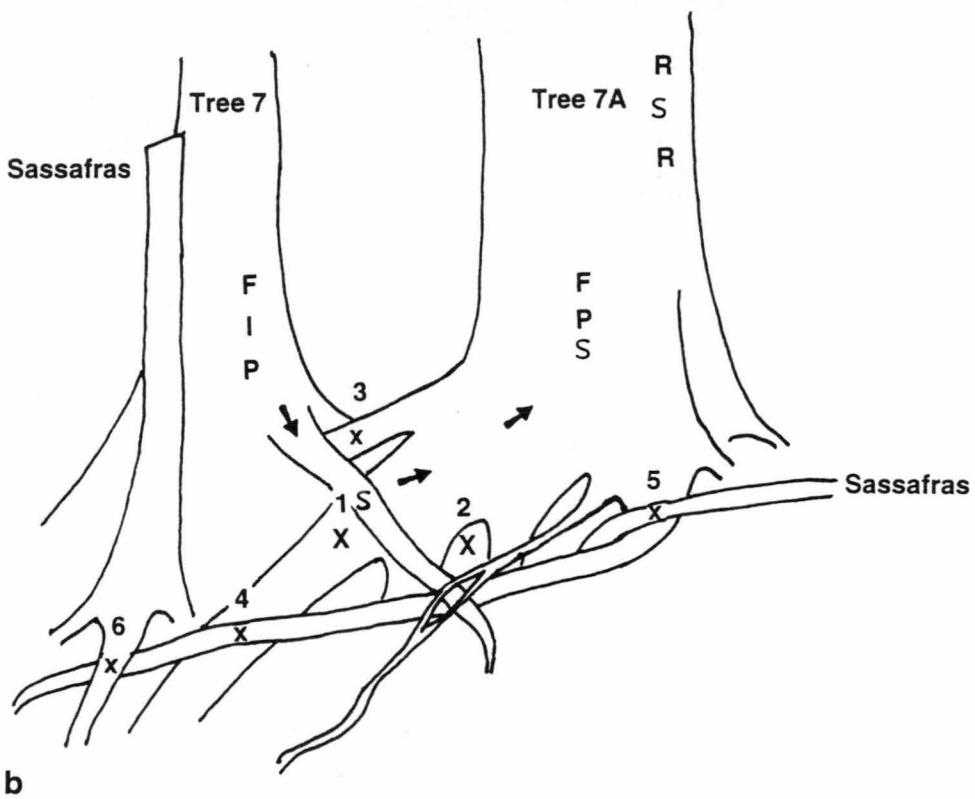
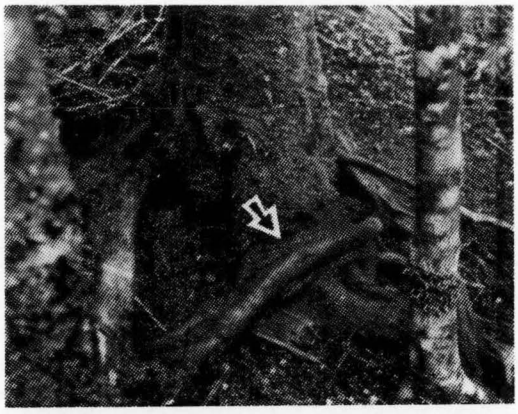


Figure 5.5 **Mt Michael WUT plot.**

a) Photograph of site.

b) Schematic diagram of plot. Areas examined/sectioned are numbered. Also shown are positions of inoculation (I), root connections (x), functional root grafts (X), *P. subgranosus* frass (P), conidial felts (F), characteristic wood stains (S), and successful re-isolations (R) of *C. australis.* (Arrows show possible routes of *C. australis* transfer.)



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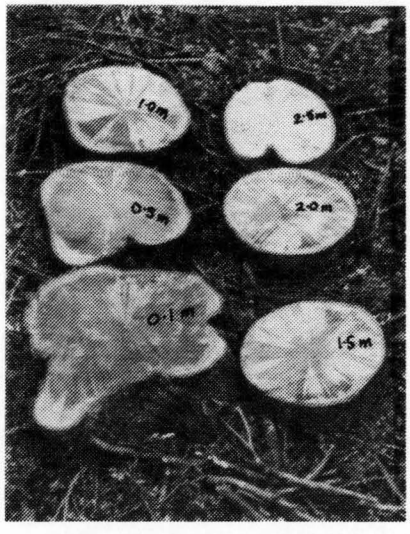


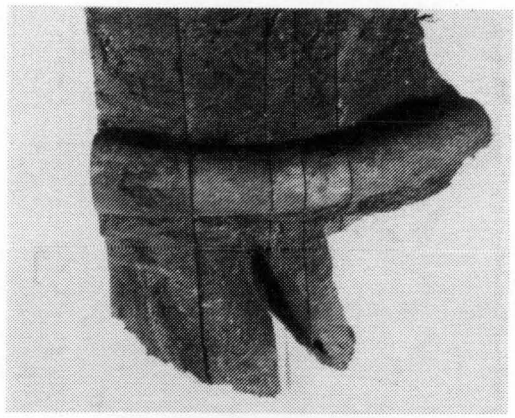
c

Figure 5.6 **Mt Michael WUT plot.**

a) Graft 1 (see arrow), connecting root of inoculated Tree 7 and buttress of Tree 7A.

b) Stump of tree 7A, showing *C. australis* stains in area of Graft 1 (excised).

c) Sections up the stem of Tree 7A; *C. australis* stains predominantly at the base.



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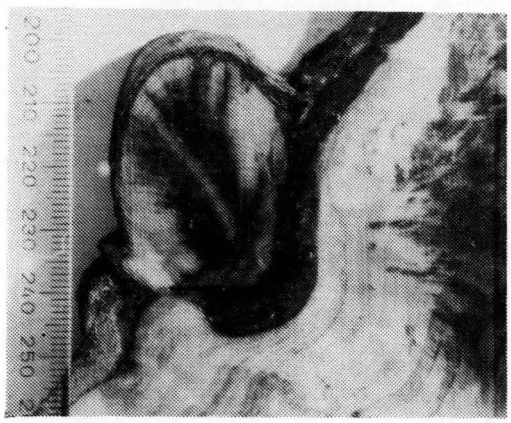

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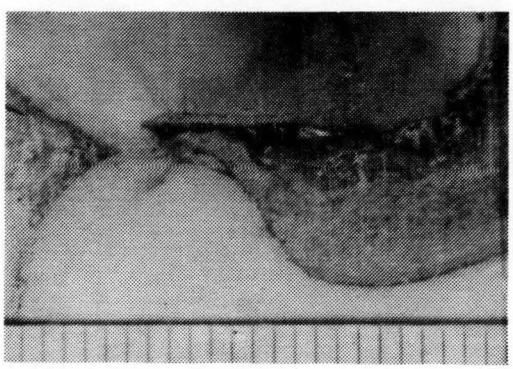


c

Figure 5.7 a) Excised graft 1 from Mt Michael WUT plot.

b) Section through Graft 1 showing *C. australis* stains (scale in cm).

c) Section through Graft 1 showing continuity of sapwood (scale in mm).

a

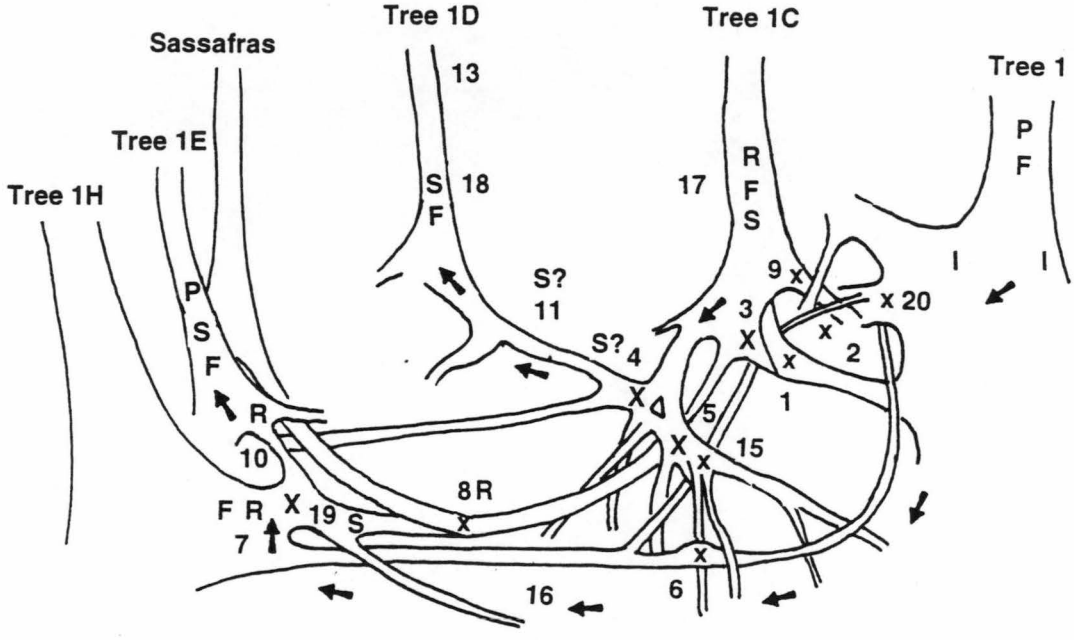
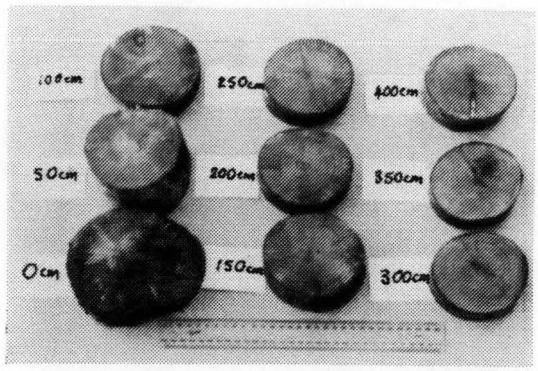


Figure 5.8 **Russell Road WUT plot.**

a) Photograph of site.

b) Schematic diagram of plot. Areas examined/sectioned are numbered. Also shown are positions of inoculation (I), root connections (x), functional root grafts (X), *P. subgranosus* frass (P), conidial felts (F), characteristic wood stains (S), and successful re-isolations (R) of *C. australis.* (Arrows show possible routes of *C. australis* transfer.)



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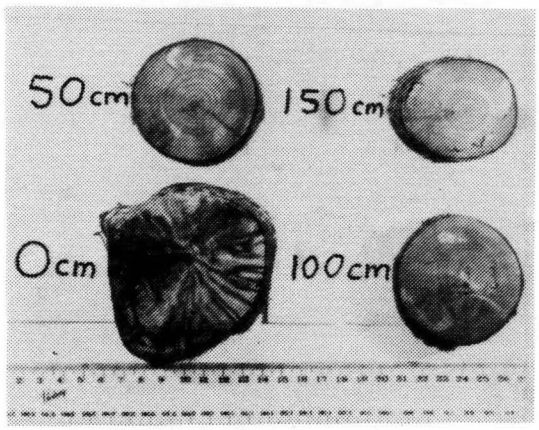
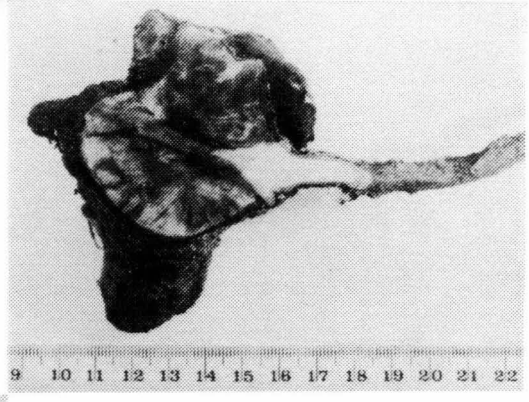


b

Figure 5.9 **Russell Road WUT plot. Sections up the stems of infected trees, with *C. australis* stains predominantly at the base: a) Tree 1C; b) Tree 1E.**

a



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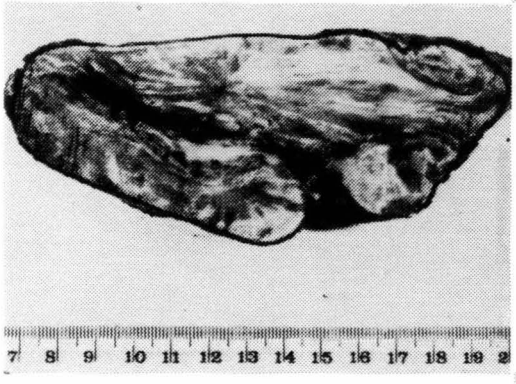

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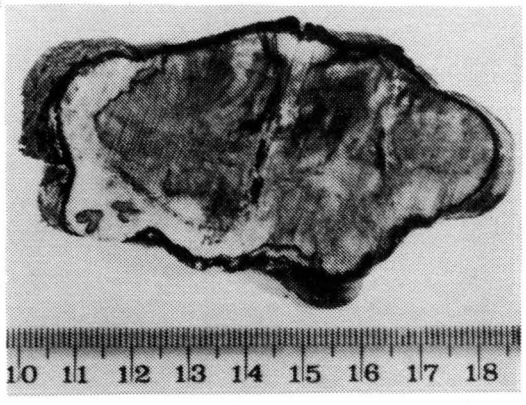
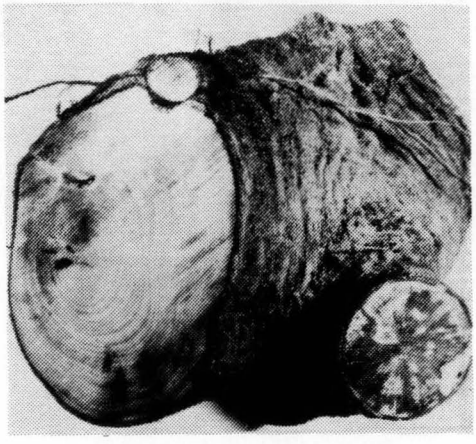


d

Figure 5.10 a) Russell Road WUT plot, Grafts 3, 4 and 5 (marked with arrows).
Sections through excised root grafts (scales in cm):
b) Graft 3; c) Graft 4; d) Graft 5.

a



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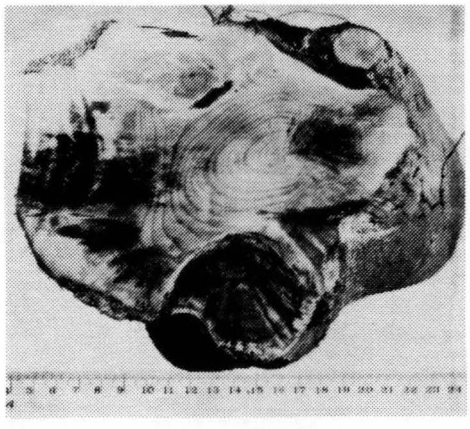
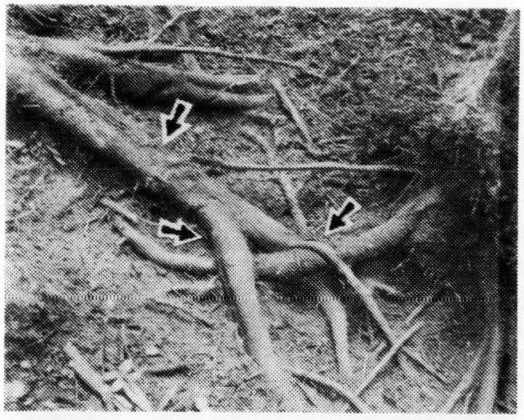


c

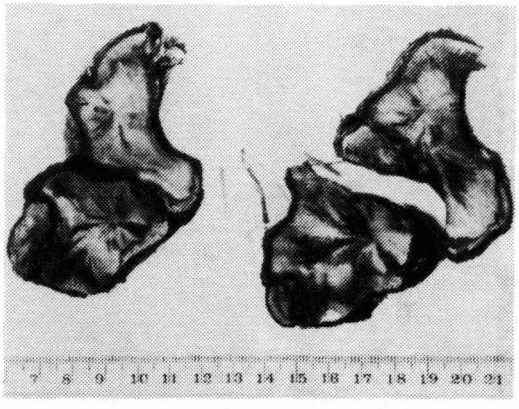
Figure 5.11 a) Russell Road WUT plot, Graft 19 (marked with arrow).

b) Section above Graft 19. A large root from Tree 1H joins a small root from Tree 1E (with *C. australis* stains).

c) Section through excised Graft 19. Roots from Trees 1H and 1E have fused. Note *C. australis* stains throughout.



a



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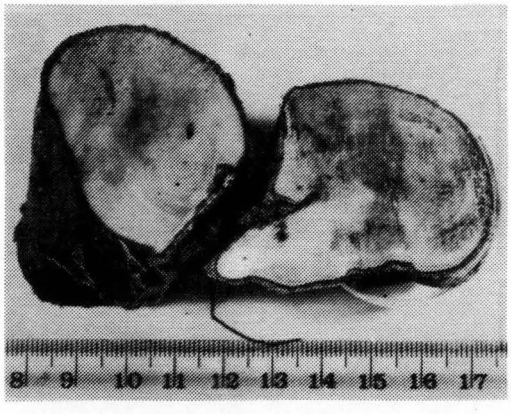


c

Figure 5.12 **Non-functional root connections (scales in cm).**

a) Wobbly Creek WUT plot, root connections 1, 2 and 3 (marked with arrows).

b) Excised root connections 2 and 3, from Wobbly Creek.

c) Excised root connection 8, from Russell Road; myrtle on left, sassafras on right.

In the case of Tree 1 at Russell Road (Figure 5.8), neighbouring Trees 1C and 1D showed clear foliage symptoms of myrtle wilt the year before *P. subgranosus* attack occurred, clearly excluding transfer by this means. Additionally, sections up the stem of Tree 1C indicated that the infection had originated in the roots (Figure 5.9a), and *C. australis* re-isolated from the stem (Isolate 1C) could not be distinguished from that inoculated into Tree 1 (Isolate E8). However, none of the apparent root connections between Trees 1 and 1C appeared to be functional, although it is possible that others existed but were not successfully excavated.

The neighbouring Tree 1D was clearly root grafted to Tree 1C (via Grafts 4 and 5), with Graft 4 showing possible *C. australis* staining (Figures 5.8b and 5.10). Because it was not possible to re-isolate *C. australis* either from Tree 1D or from Grafts G4 and G5, disease transfer to Tree 1D from Tree 1C via root grafts cannot be proven. However, with Trees 1C and 1D, both the available evidence and the elimination of other transfer mechanisms, strongly point to *C. australis* transfer via root grafts.

There is better evidence for transfer between Trees 1E and 1H, the roots of which were clearly connected in a large functional root graft (Graft 19) (Figures 5.8b and 5.11). Their joint root system was grafted to the roots of Tree 1C (via Graft 3), and was probably grafted directly to the inoculated Tree 1 (via Graft 20). Due to physical constraints it was not possible to excavate and excise the latter to confirm that it was a functional root graft. It is also highly probable that other functional root grafts existed but were not successfully excavated.

Although Tree 1E suffered early *P. subgranosus* attack, sections up the stem again indicated that the infection had originated in the roots (Figure 5.9b). Re-isolation of *C. australis* from the root graft with Tree 1H (Graft 19), and from the roots of Tree 1E below this graft, gave isolates 1H and 1E respectively, which were indistinguishable from each other, and from the inoculated Isolate E8 (Table 5.29).

At the time of excavation Tree 1H was an apparently healthy tree with no *P. subgranosus* attack. Sections through the roots of Trees 1E and 1H above Graft 19, showed *C. australis* staining only in Tree 1E, confirming that the infection in the root graft must have come from this tree. Microtome sections of this root graft clearly showed *C. australis* staining and some hyphal fragments on one side of the graft, and actually in the graft area itself (Figure 5.13). Also illustrated are longitudinal tangential and transverse microtome sections of infected roots, indicating that the dark stains of *C. australis* are largely caused by a dark brown substance which blocks both the axial conducting elements vessels and ray cells.

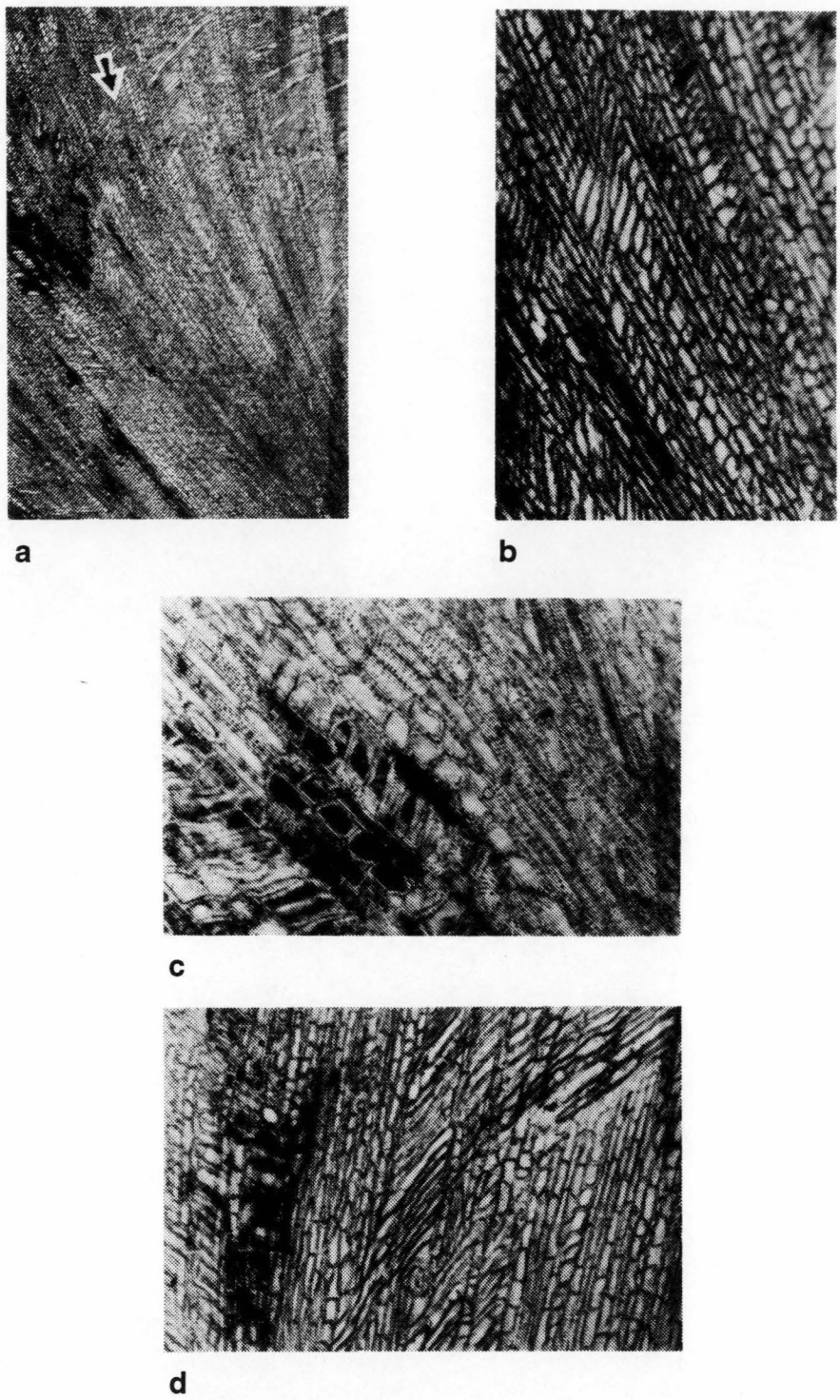


Figure 5.13 **Transverse sections through Graft 19 from Russell Road, showing characteristic dark *C. australis* stains on the edge of the graft.**

a) x 12.5 (position of graft marked with arrow).

b) x 50 (close up of above).

c) x 100 (stained with cotton blue; photographed using a blue filter).

d) x 50 (stained with cotton blue; photographed using a blue filter).

Additionally, ray parenchyma appear to give rise to tyloses in the xylem vessels (Figure 5.14). It is concluded that *C. australis* had grown from Tree 1E through the root and Graft G19, and was beginning to invade the root of Tree 1H.

Characterisation of isolates

Conidial measurements

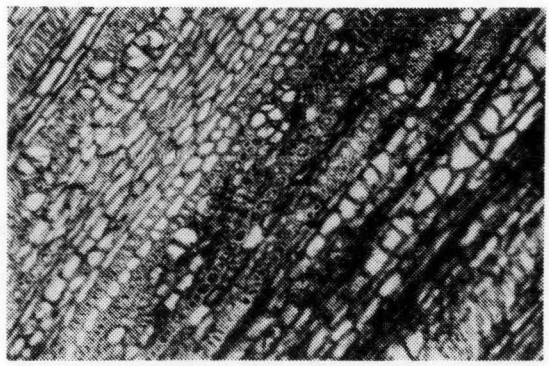
Conidial lengths were not found to differ significantly between isolates. The results of the ANOVA and tests for homogeneity of variances are given in Table 5.25. Bartlett's test indicated that variances differed significantly between isolates, and therefore results need to be interpreted with caution.

Table 5.25 ANOVA on conidial lengths of *C. australis* isolates, showing results of tests for homogeneity of variances

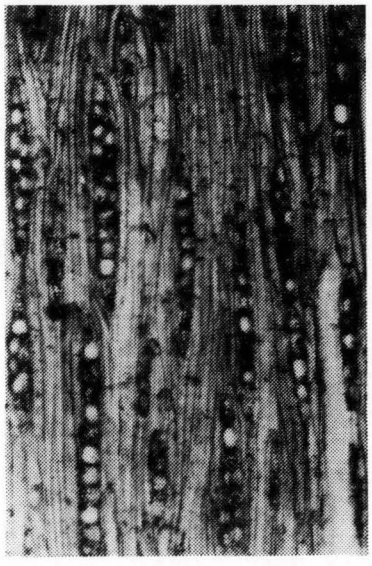
Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
BETWEEN GROUPS	43.715	7	6.245	1.338	0.2341 NS
WITHIN GROUPS	895.840	192	4.666		
TOTAL	939.555	199			

Cochran's C test:	0.172	P = 0.7201 NS
Bartlett's test:	1.124	P = 0.0024 **

Minimum, maximum and mean values for each isolate, and multiple range analyses are given in Table 5.26 . All the isolates fell into one homologous group, and none had maximum values which exceeded 19 µm. It can be concluded that all the isolates were of the same conidial type. Phialides and conidia are illustrated in Figure 5.15.



a



b

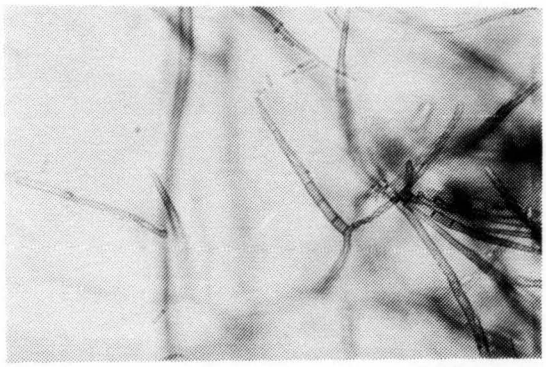
c

Figure 5.14 **Sections from the area of Graft 19 from Russell Road.**

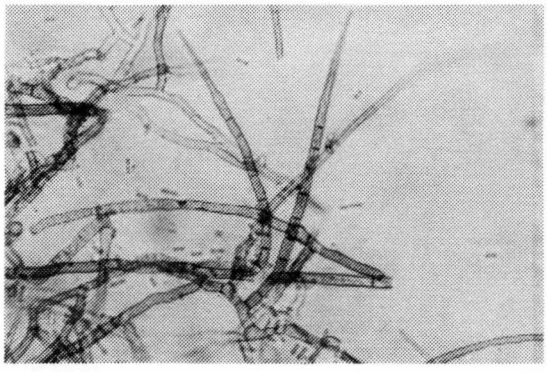
a) Transverse section showing dark substance associated with characteristic *C. australis* stains (bottom right). Lignin (grey) stained with phloroglucinol (x 50);

b) Tangential longitudinal section through healthy root, showing rays (x 100);

c) Tangential longitudinal section through root infected with *C. australis*, showing dark substance in the rays. Possible tyloses marked with arrows (x 100).



a



b

c

Figure 5.15 *Chalara australis.*

a-b) Phialides and conidia (x 200).

c) Perethecia initials (x 100).

Table 5.26 Minimum, maximum and mean values of conidial lengths for *C. australis* isolates, (multiple range analyses show homologous groups based on 95% confidence intervals)

Isolate	Conidial length (µm)			Multiple range analyses
	minimum	maximum	mean	
L1	6	18	8 20	*
E8	6	11	8.44	*
1H	6	17	8.80	*
1E	6	16	8.88	*
N3	6	11	8.88	*
X1	6	19	9.00	*
7A	7	14	9.28	*
1C	7	17	9.84	*

Isozyme electrophoresis and colony morphology

The first run detected no ribonuclease activity. Two identical bands of amylase activity were produced by all the isolates (Figure 5.16a). These results agree with those of R. H. Cruickshank (unpublished data).

With polyphenoloxidase, two identical bands were produced for all the WUT plot and control isolates. However, the original inoculated isolate (E8) produced a distinct third band (Figure 5.16b). The second run indicated that this band disappeared when the isolate was grown on potato dextrose agar (and stored in the fridge) prior to electrophoresis (E8P and E8PW). At this stage a very faint third band was also apparent for Isolate 1H (Figure 5.16c). Enzyme tests showed that laccase was produced by E8, E8W, E8P and E8PW, while tyrosinase was not produced by any of these cultures. The third run showed that the third band was again produced by E8 and E8W, but also by 1.66, 2.88 (faint), BR1, N5 and N7. Isolates 1.66 and N5 also gave a clear downward displacement of the second band (Figure 5.16d).

The cultural histories of the isolates producing the third band are varied, but the two isolates producing the displaced second band had been on malt agar for at least 18 months (Tables 5.22 and 5.24). The third band could either have resulted from a recent (and recurrent) mutation, or this phenomenon could reflect real genetic variation within the *C. australis*

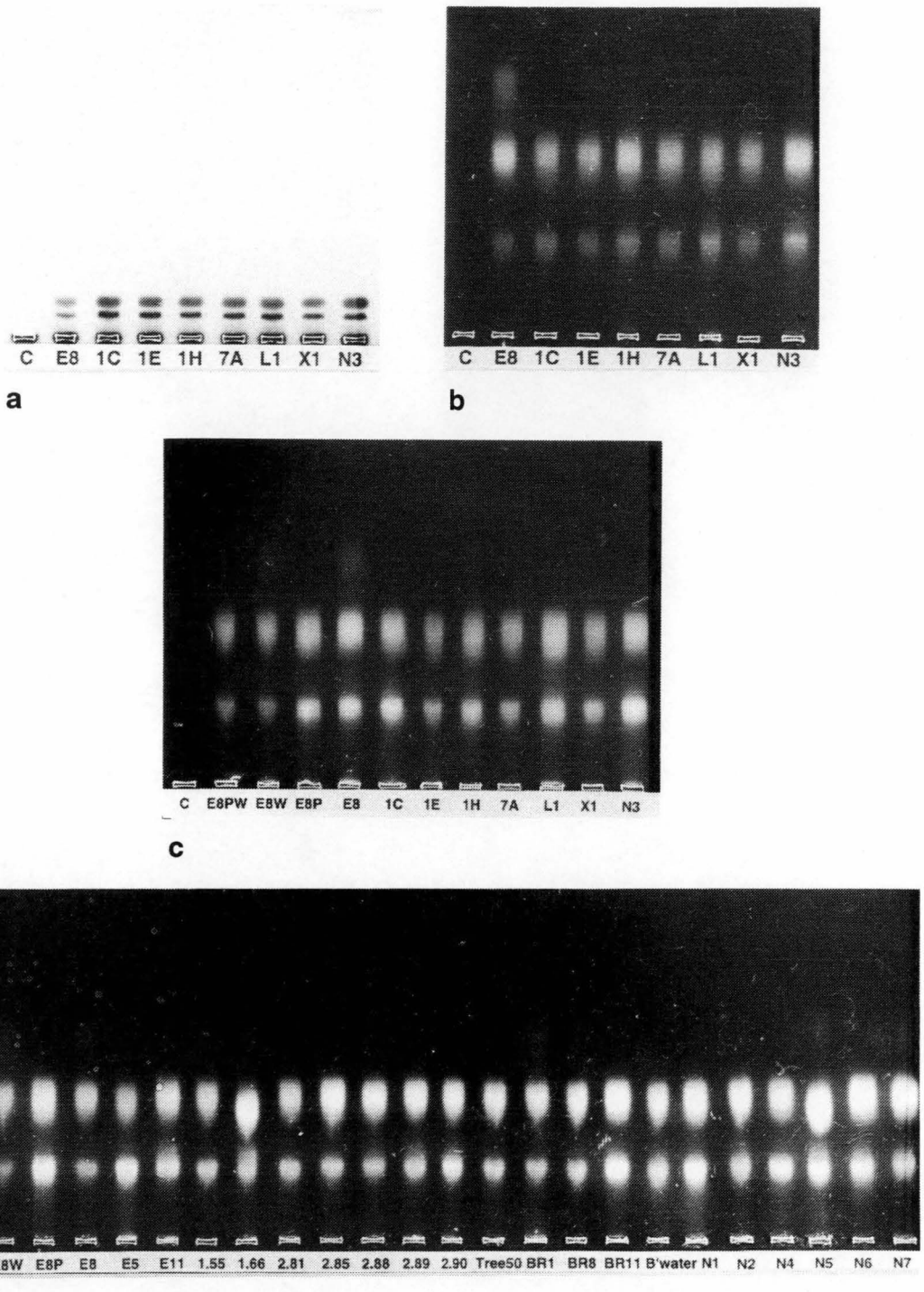


Figure 5.16 **Zymograms. Isolates are labelled; deionised water control is shown as 'C'.**

a) Run 1: WUT plot and control isolates (amylase activity).

b) Run 1: WUT plot and control isolates (polyphenoloxidase activity).

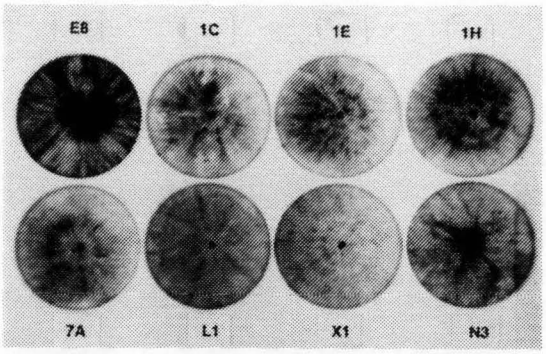
c) Run 2: effect of substrate on zymogram banding (polyphenoloxidase activity).

d) Run 3: WUT plot, Tasmanian and Victorian isolates (polyphenoloxidase activity).

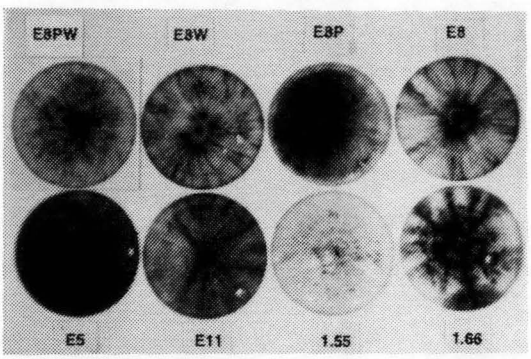
population, occurring both in Tasmanian and Victorian isolates. In either case it affected the production of polyphenoloxidase only under certain cultural conditions.

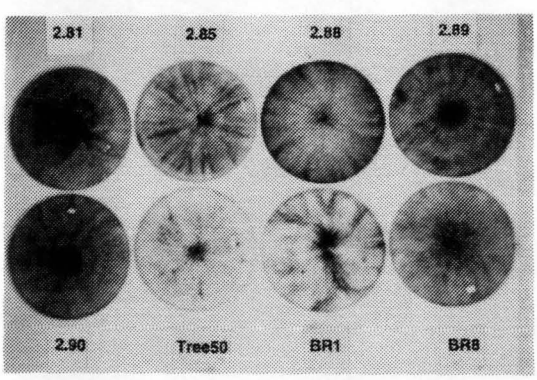
After seven days' growth the colony morphology showed distinct variation between isolates, some were producing very uneven growth, with others showing this effect to a lesser degree. After 14 days the isolates from the former group were all showing clear sectoring effects, with several others being slightly streaky (Figure 5.17). There was a good correlation between the production of the third zymogram band, and abnormal colony morphology. This is illustrated in Table 5.27, with strong and weak characteristics being represented by '**X**' and 'x' respectively.

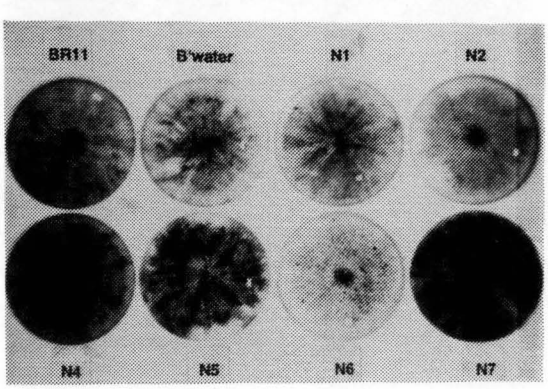
Since sectoring is characteristic of genetic change in fungal cultures (Agrios 1988), it was concluded that the appearance of the third zymogram band was the result of a recent, recurrent mutation which affected the production of polyphenoloxidase under certain cultural conditions. As such, it was not a character which could be utilised to differentiate between the WUT plot and control isolates. Although the displacement of the second band could reflect real variation in the wild population (only occurring in Victoria), this would need confirmation; it is not in any case relevant to the WUT plot and control isolates.



a







b

Figure 5.17 **Colony morphology of *C. australis* isolates on malt agar.**

a) WUT plot and control isolates (14 days old).

b) WUT plot, Tasmanian and Victorian isolates (14-15 days old).

Table 5.27 Unusual polyphenoloxidase zymogram banding and colony morphology in *C. australis*

Isolate	3rd band present	2nd band displaced	Uneven growth(7 days)	Sectoring (14 days)
E8	X		X	X
1C			x	
1E				
1H	x			
7A				
L1				
X1				
N3				
E8PW				
E8W	X		X	X
E8P				
E5				
E11				
1.55			x	
1.66	X	X	X	X
2.81				
2.85				x
2.88	x			x
2.89				x
2.90				
Tree 50				
BR1	X		X	X
BR8				
BR11				
BW				
N1				
N2				
N4				
N5	X	X	X	X
N6				
N7	X			x

Compatibility tests

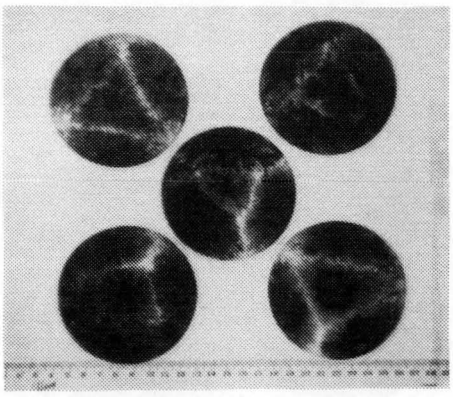
Initial observation of colony interactions after 16-21 days showed that dense hyphal tufts often formed at the point of contact between two colonies (even if they were of the same isolate), but there were no clear line or barrage reactions (Figure 5.18). After 28-32 days, hyphal anastomoses between isolates and the production of perithecia initials were observed. Where hyphal tufts formed, anastomoses were always present. Appendix 23 shows the total observed interactions (cumulative over all replicates) for the three experiments.

While hyphal anastomosis and the production of hyphal tufts were obviously mutual interactions between two isolates, the production of perithecia initials often appeared to be stimulated in one isolate when it was 'invaded' by another isolate, and the interaction was not necessarily reciprocal. In *O. novo-ulmi,* perithecia production occurs with strains of opposite mating types (Brasier 1984). Perithecia initials were formed by all the isolates tested. None of the perithecia initials developed into perithecia. Perithecia initials are illustrated in Figure 5.15.

In the first experiment, E8PW and the WUT plot re-isolates formed anastomoses in all pairings, and hyphal tufts in many pairings. Since the formation of hyphal anastomoses is a compatible reaction, *if* a v-c system exists in *C. australis*, it can be concluded that these isolates must share the same v-c group. A similar conclusion must be drawn regarding E8PW and the Victorian isolates, which also formed anastomoses in all pairings in the second experiment. However, in the third experiment, the WUT plot controls, L1 (Mt Michael) and N3 (Central Highlands, Victoria), did not produce any hyphal anastomoses or hyphal tufts. Although this could have been due to the slow growth rates (which meant half of the replicates didn't actually interact); it could also indicate that they belonged to different v-c groups. If the latter is the case, there must be more than two v-c groups, since both L1 and N3 produced anastomoses with E8PW.

Temperature/growth profiles

The ANOVA including all temperature treatments, indicated that the radial growth differed significantly between isolates. Caution is required in the interpretation of the temperature and interaction effects, since there was no randomisation within each temperature treatment. The Cochran's C and Bartlett's tests indicated that variances did not differ significantly between isolates (Table 5.28).



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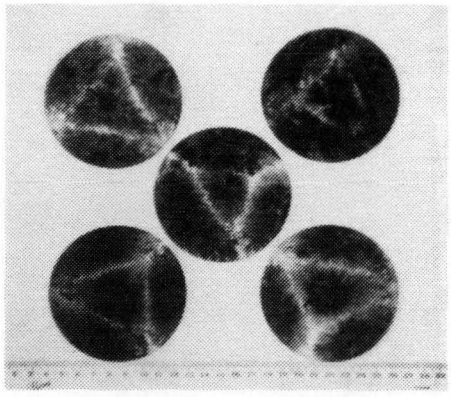

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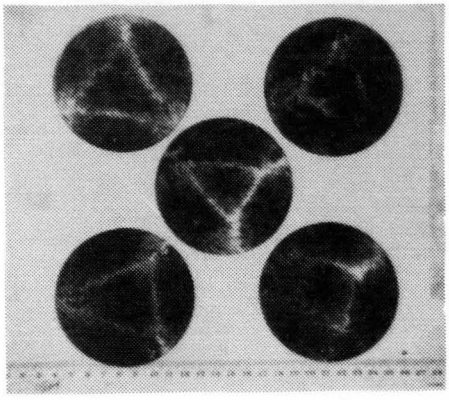

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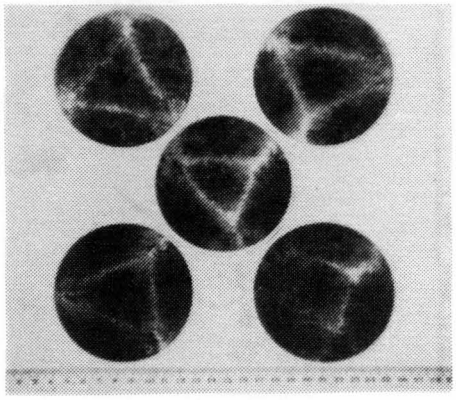


d

Figure 5.18 **Colony interactions between WUT plot and control isolates of *C. australis*, growing on potato dextrose agar (16 days old). Isolate arrangements as shown below.**

a) **E8** (x 4) **1C** (x 4)

1C
E8
1H **1E**

1H (x 4) **1E** (x 4)

b) **E8** (x 4) **1C** (x 4)

1C
E8
7A **1H**

7A (x 4) **1H** (x 4)

c) **E8** (x 4) **1C** (x 4)

1C
E8
7A **1C**

7A (x 4) **1C** (x 4)

d) **E8** (x 4) **1E** (x 4)

1E
E8
7A **1H**

7A (x 4) **1H** (x 4)

Table 5.28 ANOVA of radial growth of *C. australis* colonies, by isolate and temperature, showing results of tests for homogeneity of variances

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECTS	1546.735	12	128.895	183.087	0.0000***
Temperature	1119.765	5	223.953	318.111	0.0000***
Isolate	426.970	7	60.996	86.641	0.0000***
2 FACTOR INTERACTIONS					
Temperature by Isolate	67.652	35	1.933	2.746	0.0000***
RESIDUAL	135.170	192	0.704		
TOTAL	1749.557	239			

Cochran's C test:	0.205	P = 0.079 NS
Bartlett's test:	1.041	P = 0.233 NS

The multiple range analyses showed that the WUT plot isolates, i.e. the inoculated isolate (E8) and the re-isolates from neighbouring infected trees (1C, 1E, 1H and 7A), formed one homologous group (Table 5.29). Over the temperature range investigated, the WUT plot isolates (shown in bold), had higher rates of radial growth than all the control isolates. It is of interest that the inoculated isolate (E8) fell in the centre of this homologous group.

The differences between the WUT plot group and the controls were significant except in the case in the control isolate from Smithton (X1). Although contamination of the WUT plots from this distant source is considered highly unlikely, it does emphasise the point that the variation between Tasmanian isolates is small. However, the re-isolate from the Mt Michael WUT plot (7A) had a significantly higher rate of radial growth than the control isolate from the same area (L1). Both the Victorian (N3) and the Mt Michael (L1) controls had significantly lower growth rates than the other isolates, with the Victorian isolate having the lowest rate of radial growth.

Table 5.29 Mean radial growth of *C. australis* isolates, over a range of temperatures (multiple range analyses show homologous groups based on 95% confidence intervals)

Isolate	Mean radial growth (mm)	Standard error (mm)	Multiple range analyses
N3	9.483	0.331	*
L1	9 718	0.559	*
X1	12.283	0.455	*
1C	12.519	0.377	* *
7A	12.561	0.415	* *
E8	12.633	0.455	* *
1H	12.753	0.427	* *
1E	13.083	0.434	*

Separate ANOVAs for each temperature treatment are shown in Appendix 24 , and indicate that at each temperature, the radial growth differed significantly between isolates. The effect of level (i.e. vertical position within each incubator/growth cabinet) was only significant at the lowest temperature (13.85°C), although it also approached significance at the highest temperature (22.75°C).

Since the interaction of temperature by isolate was significant (Table 5.28), the results of the multiple range analyses are displayed for each temperature (Table 5.30). The WUT plot isolates (shown in bold) were the only isolates found in the same homologous group at all temperatures. They were distinctly different from the Mt Michael (L1) and Victorian (N3) control isolates (these controls being separable from each other at 17.45°C and 22.75°C). The Smithton control isolate (X1) was indistinguishable from the WUT group except at 22.75°C. Overall, there is a very strong indication that the WUT plot isolates were identical, having resulted from disease spread via root grafts.

Table 5.30 Mean radial growth of *C. australis* isolates, over a range of temperatures (multiple range analyses show homologous groups based on 95% confidence intervals)

Isolate	Multiple range analyses for each temperature					
	13.85°C	16 00°C	17.45°C	19.55°C	21.35°C	22.75°C
L1	*	*	*	*	*	*
N3	*	*	*	*	*	*
X1	*	*	*	*	*	* *
1C	*	*	*	*	*	* *
E8	*	*	*	*	*	* *
1E	*	*	*	*	*	*
1H	*	*	*	*	*	*
7A	*	*	*	*	*	*

Fitted values of θ_1,θ_2,θ_3 and θ_4 for the eight isolates are given in Table 5.31. Standard errors are shown in parentheses, and derived values of T_{opt} are also given. Between isolates, there were variations of 1.28°C, 3.61°C and 0.74°C respectively, in the maximum, minimum and optimum temperatures for growth. Clearly, T_{opt} (the optimum temperature for growth) varies little between isolates, and is not the best available discriminant.

Table 5.31 Notional maximum, notation minimum and optimum temperatures for growth of *C. australis* isolates

Isolate	θ_1 Maximum (°C)	θ_2 Minimum (°C)	θ_3	θ_4	T_{opt} Optimum (°C)
E8	23.79 (0.23)	6.31 (1.26)	0.69 (0.18)	1.24 (0.17)	20.35
1C	24.16 (0.36)	4.64 (2.18)	0.51 (0.17)	1.10 (0.24)	19.90
1E	23.81 (0.25)	4.89 (1.61)	0.71 (0.20)	1.13 (0.17)	20.32
1H	24.50 (0.35)	8.25 (1.09)	0.33 (0.11)	1.70 (0.33)	19.75
7A	24.39 (0.30)	7.73 (0.99)	0.40 (0.11)	1.50 (0.22)	19.98
L1	23.22 (0.13)	7.97 (1.42)	0.76 (0.22)	1.20 (0.22)	20.16
X1	23.52 (0.14)	4.80 (1.29)	0.82 (0.16)	1.07 (0.12)	20.33
N3	23.89 (0.21)	6.57 (0.86)	0.73 (0.16)	0.92 (0.09)	20.59

Comparisons between values of θ_1 (notional maximum temperature) for the different isolates are given in Table 5.32. The sequential Bonferroni technique indicated that the only comparisons which could be considered to be significant at the 5% *tablewide* level, were those which were *individually* significant at the 0.1% level. None of the comparisons of θ_2 (notional minimum temperature) were significant at the tablewide level (Appendix 25). Clearly θ_1 was the better discriminant, although even this merely showed that the Mt Michael control isolate (L1) was significantly different from both the Mt Michael WUT plot isolate (7A) and the Russell Road WUT plot isolate (1H). In practice, the most satisfactory differentiation between isolates was obtained by assessing growth response over a wide temperature range, the best individual discriminant temperature being 22.75°C (Table 5.30), this being very close to the fitted values of θ_1 (Table 5.31).

Table 5.32 Paired comparisons of θ_1 (maximum temperature for growth) for *C. australis* isolates (showing *d* values and significance levels of individual comparisons)

L1								
X1	1.57							
E8	2.16*	1.00						
1E	2.09*	1.01	0.06					
N3	2.71**	1.47	0.32	0.25				
1C	2.46*	1.66	0.87	0.80	0.65			
7A	3.58***	2.63**	1.58	1.49	1.37	0.49		
1H	3.43***	2.60**	1.69	1.60	1.49	0.68	0.24	
	L1	**X1**	**E8**	**1E**	**N3**	**1C**	**7A**	**1H**

NB The sequential Bonferroni technique indicated that only individual comparisons marked *** can be considered significant at the 5% 'tablewide' level.

5.5 DISCUSSION

Wounding and infection

It is apparent that in Tasmanian summer conditions, small stem wounds on *N. cunninghamii* saplings remain receptive to *C. australis* spores for less than 28 days, with most infections likely to occur within 14 days. There is limited evidence that this period may be extended in cooler, moister conditions. The optimum temperature for *C. australis* growth is

approximately 20°C (Kile and Walker 1987), and Tasmanian summer temperatures frequently exceed this level. Additionally, drying out of wounds is known to prevent infection, and in Tasmania less rain falls in the summer months (Tapper and Hurry 1993).

Combined evidence for myrtle wilt spread through root grafts

Functional root grafts commonly occur in young *N. cunninghamii* trees. Continuity of the vascular systems was demonstrated by sectioning, and by the transfer of dye across a root graft. Transfer across root grafts appeared to occur more frequently with *C. australis* than with acid fuchsin dye. In a comparable experiment, Rexrode (1978) pressure injected *Quercus* spp. with dye and a conidial suspension of *Chalara quercina,* and found that more root grafted neighbours became diseased than showed traces of dye.

At the Russell Road and Mt Michael sites, obvious connections between root systems indicated possible pathways by which *C. australis* may have spread from inoculated to neighbouring trees, producing symptoms of myrtle wilt. Temperature profiles indicated that although there was significant variation between *C. australis* isolates from other sites, re-isolates from the WUT plots could not be distinguished from the inoculated isolate. This, together with the lack of any obvious wounds (infection courts) on the WUT plots, is very good evidence that the neighbouring trees were infected (directly or indirectly) by the inoculated trees, rather than via airborne spores from other sources. This left two possible modes of transfer: *P. subgranosus;* or root grafts.

The work of Kile and Hall (1988) and Candy (1990) disproved a direct vectoring role for *P. subgranosus,* but it was conceded that a small percentage of the beetles may carry and transmit *C. australis.* Kile *et al.* (1989) indicated that wind-borne beetle frass (contaminated with *C. australis* conidia and phialides) could be an inoculum source, particularly during the summer months. Beetle holes could also, theoretically, provide infection courts for wind borne inoculum. However, in the trees examined at Russell Road and Mt Michael, *C. australis* staining was concentrated in the bases and did not extend right up the trunks, indicating these infections probably originated in the root systems, rather than in *P. subgranosus* holes. Additionally, at Russell Road, two trees became infected before *P. subgranosus* attack occurred. It was concluded that *P. subgranosus* could not have been responsible for all of the observed instances of disease transfer.

C. australis staining in the cross section of a root graft from Russell Road gave further proof that disease transfer could take place by this means. Similar temperature profiles of *C. australis* isolates, inoculated and then re-isolated from trees with grafted root systems,

clearly indicated that myrtle wilt had been transferred via root grafts. Further confirmation is dependent on the definite characterisation of these isolates, which are being maintained in culture. Thus, while the evidence for myrtle wilt transfer via root grafts is not yet indisputable, it is now very strong.

Problems with the characterisation of *C. australis* isolates

The most effective differentiator of *C. australis* isolates was their radial growth rate, assessed over a range of temperatures. This method clearly indicated that the original inoculated isolate was identical to *C. australis* re-isolated from neighbouring, root grafted trees. This group of isolates was distinctly different from control isolates taken from other parts of Tasmania and Victoria. The best differentiation of isolates occurred at the highest temperature tested (22.75°C).

Conidial measurements and compatibility tests did not distinguish between *C. australis* isolates. Paired isolates failed to produce mature perithecia, and thus the conclusions of T. C. Harrington and C. M. Brasier (personal communications) are supported: isolates appear to be of one mating type; the other mating type is either absent or at a low frequency in the population. However, the question of v-c groups in *C. australis* has not been satisfactorily resolved.

Zambino and Harrington (1989) discuss the causes and consequences of strictly asexual reproduction for the pathogen *Leptographium wagenerii.* This species has similarities to *C. australis* in that sexual reproduction appears to be rare or lacking; it has a weak pathogen/vector relationship and it also spreads via root contacts. One theory was that the low probability of establishing new infection centres gave a high risk of losing one mating type during any one establishment, leading to geographical isolation of mating types. This theory is supported by a study of *C. fagacearum* in Texas; the majority of infection centres contained only one mating type, while only one diseased tree yielded both mating types (Appel *et al.* 1985). However, as with *C. australis,* despite extensive sampling only one mating type of *L. wagenerii* has yet been found. An alternative theory was that under the prevailing conditions, strictly asexual reproduction was not necessarily detrimental. Thus, in situations where reproduction was predominantly asexual, the ability to reproduce sexually could be lost through mutations or by negative selection (Zambino and Harrington 1989).

In theory, isozyme electrophoresis should give better characterisation of isolates, but with the systems studied to date, *C. australis* has shown very little variation. Identical banding patterns were produced with amylase. Using polyphenoloxidase, the appearance of an extra

band was dependant on culturing history, and was correlated with unusual colony morphology, notably sectoring. Sectoring is common in cultures, but has yet to be explained genetically, although heteroploidy, mutations, heterokaryosis and parasexualism have all been implicated. It is also known that gene expression may be affected by external factors, e.g. production of cell wall degrading enzymes by pathogens may be induced by suitable substrates in the host cell wall (Agrios 1988).

Zambino and Harrington (1992) found that electrophoretic relatedness among *Leptographium* isolates generally corresponded to their morphological similarity. In contrast to the present work, they also found that electrophoretic characters (unlike culture morphology) did not degenerate with age. Cruickshank and Pitt (1987) came to similar conclusions about the electrophoretic characters of deteriorating cultures of *Penicillium* species. However, it should be noted that these workers used multiple enzyme systems to reach these conclusions whereas the present work, in effect, only used two such systems.

Because one band of polyphenoloxidase activity was non permanent, the most likely explanation is that a mutation arose in culture, affecting both morphology and gene expression (i.e. the production of polyphenoloxidase) when the isolate was grown consistently on malt agar. This 'strain' was probably eliminated or suppressed by growing the isolate on potato dextrose agar for a time. Similar reversible effects occur when a pathogen loses virulence in culture due to the selection for avirulent strains within the isolate. It may often be remedied by transferring the isolate back to the host, thus favouring the virulent strains (Agrios 1988).

While electrophoretically separated enzymes are the initial expressions of the genome in tangible form (Cruickshank and Pitt 1987), the use of DNA markers allow the exploration of the genome itself (Figdore *et al.* 1988; Hausner *et al.* 1993d). Restriction fragment length polymorphisms (RFLPs) are heritable differences in the length of DNA fragments arising after treatment with a particular restriction endonuclease (Figdor *et al.* 1988). Randomly selected cloned DNA sequences are used as hybridisation probes, and RFLPs result from changes in the DNA homologous to the cloned segment itself, or between the nearest recognition sites for the restriction enzyme (Shattuck-Eidens *et al.* 1990). Point mutations create or abolish restriction endonuclease sites while DNA rearrangements, insertions or deletions alter their relative positions. By analysis of the fragment sizes produced, the linear order of restriction sites, or 'map' of a DNA segment can be determined (Evola *et al.* 1986).

Recent work by De Scenzo and Harrington (1994) used RFLPs, and a $(CAT)_5$ DNA probe to produce DNA fingerprints (unique RFLP pattern of individuals) for *O. piliferum* and other species. The probe was found to be useful in delineating genotypes, in identifying infra specific variants, and, potentially, in quantifying genetic variation. Using this method, a limited number of *C. australis* isolates produced a single RFLP pattern, although this was distinctly different from that of the morphologically similar *Ceratocystis virescens* (Harrington *et al.* 1992).

Gumley (1993) used similar methods to examine seven isolates from Tasmania and Victoria. Six of these were the same isolates used in the present work; from Boyd River (BR8), Christmas Hills (X1) and Esperance River (E11) in Tasmania, and from Mt Erica (N7), Tarra Valley (N6) and Mt Donna Buang (N3) in Victoria (Tables 5.22 and 5.24). The Aire Valley isolate was a more recent isolation from the Otway Ranges in Victoria. The results produced four dissimilar fingerprints, indicating genetic diversity. The Tasmanian fingerprints were more variable than the Victorian ones, which suggested greater genetic diversity in the former.

An alternative explanation of the results could be due to the amount of time the isolates had been maintained in culture. Variation in fingerprints appeared to be expressed by the *absence* of certain bands (Gumley 1993). Reference to the culturing history of the isolates (Tables 5.22 and 5.24) showed that two of the three variable bands were missing only in the oldest isolates (BR8 and E11), both cultured on malt agar for four years: the first band (0.7) was missing from both; the second band (3.2) was missing only from the E11 isolate; a third band (7.1) was missing from the E11 isolate and also from the N6 isolate (cultured on malt agar for two years). The most recent isolates (X1 and Aire Valley) had the full complement of bands.

There appeared to be no correlation between the variability detected by RFLPs and that detected by isozyme electrophoresis and colony morphology. The Mt Erica isolate (N7) used by Gumley (1993) was the only isolate to produce 'abnormal' zymogram banding and colony morphology, but gave a 'normal' DNA fingerprint complete with all bands.

Gumley (1993) also used random amplified polymorphic DNA (RAPD) analysis, and again found the Tasmanian isolates to be the most variable. Unfortunately the results were not reproducible. A proportion of conidia from all isolates were found to be binucleate and it was concluded that heterokaryosis resulted in a high degree of variability within isolates, making it impossible to obtain reproducible fingerprints.

In summary, while work with isozymes and DNA markers does point to the existence of infra specific variation in *C. australis,* it has not been satisfactorily proven that this is due to actual variation in the population, as opposed to mutations occurring whilst in culture. At this stage, the work is not sufficiently advanced to reliably differentiate between isolates.

Mode of transfer through root grafts

It is evident that root grafting is relatively common in young trees and saplings of *N. cunninghamii,* but in only one of ten apparently root grafted saplings did dye transfer occur. However, it is known that translocation through root grafts may only occur during times of high water stress (Schultz and Woods 1967). In this case the transfer was relatively rapid; 55 cm in 6 days (equivalent to 31 m per year). This contrasts strongly with the rate of *C. australis* spread in the comparable WUT plots (15 cm per year), even allowing for the fact that disease symptoms take time to develop. The implication is that *C. australis* is not carried passively in the xylem, but actively grows through the root tissues from host to host.

This is in agreement with conclusions reached by other workers. In general, species of *Ceratocystis* penetrate the sapwood radially via medullary rays, and longitudinally via vessels and tracheids. The hyphae of xylem inhabiting species are typically crowded in the ray cells and occur sparsely in the lumina of the tracheids and vessels (Dowding 1984). In trees and fresh logs the stain is typically triangular or wedge shaped in cross section, as a result of host invasion involving growth along the medullary rays (Gibbs 1993). Kile and Walker (1987) first noted that *C. australis* caused brown, radial streaks across multiple growth rings, suggesting that the pathogen was not confined to the axial conductive tissue of the xylem during pathogenesis, and was not a true vascular wilt pathogen. Unlike *C. fagacearum* and *O. novo-ulmi,* it did not become systemic in the vascular system, but was generally restricted to the roots and lower to mid stem.

In *O. wageneri,* axial colonisation by hyphae is confined to the xylem tracheids, and radial development of mycelia in ray tracheids is limited (Hessburg and Hansen 1987). In contrast, Gumley (1993) found that in *C. australis* infected seedlings, tracheids and vessels were blocked with dark staining granular substances (probably tannins). Hyphae were found within tracheids but were not present in the xylem vessels or intercellular spaces, but tyloses were present in both the tracheids and vessels of stained sapwood. She concluded that *C. australis* was more like a sapstreak organism than a vascular wilt.

The present work indicated that the dark brown substance associated with infection by *C. australis,* not only blocked the axial conducting elements, but was also apparent in the ray parenchyma. Additionally, in infected roots, tyloses appeared to form in vessels associated with the ray parenchyma. Work with *C. quercina* has indicated that tylosis formation is a host response, causing a reduced transpiration stream and wilting (Beckman *et al.* 1953). With *C. australis,* the association of tyloses with the ray parenchyma, implies that the pathogen can grow through the rays. This could explain the radial spread of *C. australis* within the host, and confirms the initial observations of Kile and Walker (1987). It may also explain why the disease appears to move through root grafts more readily than dye.

Bormann (1966) proposed that effective fusion of *Pinus strobus* L. roots only occurred between tissues with normal anatomical and physiological alignment, and that any transfer across root grafts necessitated tangential or radial movement. A pathogen growing through the ray parenchyma would therefore have a much greater chance of transfer than a dye carried passively in the xylem.

Rate of spread via root grafts

In the ROS plots, the number of infected trees started to plateau after three years (Figure 5.4). It is probable that at this stage, most of the root grafted (susceptible) near neighbours were already infected. Thus root grafting between clusters of myrtles may be one explanation for the clumped distribution of symptomatic myrtle wilt trees, which have been recorded on the ROS plots and by earlier workers (Chapter 3). Below ground spread of *C. australis* was predicted by Elliott *et al.* (1987) and Kile *et al.* (1989) because of this clumping of diseased trees, and because of relationships between disease incidence and stand density. Their conclusions are corroborated by the present work.

The maximum extent of disease spread did not level off to the same degree as the numbers of infected trees, indicating that at least some trees at greater distances were still being infected after three years (Figure 5.4). The rate of disease spread found in the WUT plots (15 cm per year), was much less than the estimate (5 m per year) derived from mature forest at Simons Road (Chapter 4), and reiterates the finding that below ground spread of myrtle wilt is greater with trees of larger diameter (Table 5.21). Trees were also more likely to infect others of a comparable size (Table 5.21), although this could be largely explained by the significant effects of site (Table 5.20).

Direction of disease spread

The downhill spread of myrtle wilt at Mt Michael is a potentially significant finding which requires explanation. It also supports the observation, that in the north east of Tasmania myrtle wilt has apparently spread down river valleys (R. C. Ellis, personal communication).

The only remaining alternative to root graft transfer of *C. australis,* is transfer through the soil. *C. fagacearum* is not free living in the soil (Berry and Bretz 1963), but *O. wageneri* is known to move between adjacent but non-grafted roots, and is apparently transported short distances through the soil, although it also spreads through root grafts (Hessburg and Hansen 1986a; 1986b). Although there is no direct evidence that this occurs in *C. australis,* Kile *et al.* (1989) confirmed that conidia were air or water dispersed, having been found in rainwater. They were probably associated with airborne *P. subgranosus* frass which was infected with conidia or hyphal fragments (G. A. Kile, personal communication). Conidial dispersal via damp air or splash droplets is known to occur in *Ophiostoma piceae, O. piliferum, O. minus* and *C. coerulescens* (Dowding 1969). It is theoretically possible that *C. australis* spores (resulting from inoculation spillage) were washed downslope by rain, resulting in other trees becoming infected. Such an explanation makes the assumption that infection courts were present in each case, at or below ground level.

Nevertheless, photographic evidence from one inoculated tree at Mt Michael clearly indicated that *C. australis* was spreading by growing through root grafts (Figures 5.6 and 5.7). If it is assumed that this was generally the case at the site, there are at least two plausible explanations. Eis (1974), investigating *Pseudotsuga menziesii (*Mirb.) Franco and other species, found that for 50% of trees, larger and more numerous roots developed on the downhill side. The proportion of trees in which greater root development occurred on the uphill side or along the contour, were 20% and 30% respectively. However in *Pinus palustris* Mill., Iodine-131 had a tendency to move uphill through root connections (Hough *et al.* 1965). Reynolds and Bloomberg (1982) found that root systems in *P. menziesii* were concentrated in the slope direction, thus increasing the probability of contact between trees upslope or downslope from each other.

Since the frequency of root grafting must depend in part on the frequency of root contacts, in a forest of even density, disease spread through root grafts would be more likely to occur in the direction of the slope than along the contour (Reynolds and Bloomberg 1982). Because, on average, each tree would contact (or be contacted by) the same number of trees upslope and downslope, this does not, of itself, explain the downhill movement of *C. australis.* However, although *N. cunninghamii* trees were injected at several points around their boles,

injections were made above main roots where possible, and it now appears probable that these larger roots extended mostly downhill. Since initial spread of *C. australis* within the bole is mainly axial (Kile *et al.* 1992), these roots are the most likely to become infected (and infective). Thus, the downhill spread of myrtle wilt could be an artefact of the methodology used.

The second explanation is that the pathogen itself has a tendency to grow downwards (geotropic response). The fact that *C. australis* growth below wound sites was consistently greater than growth upwards, supports this hypothesis (Table 5.8). Additionally, Kile and Walker (1987) found that the pathogen was generally restricted to the roots and lower to mid stem of diseased myrtles. Overall, this is thought to be the most plausible hypothesis.

Root grafting - implications for epidemics

The WUT plots showed that each inoculated tree infected, on average, less than one other tree via root grafting. Within the diameter range (3-30 cm DBH) studied, this factor was not dependent on the size of the trees. An epidemic can only occur when each diseased individual infects, on average, more than one other individual during its lifetime (Van der Plank 1963). This 'threshold theorem' is given by:

$$iR > 1$$

where R is the rate of increase of disease per unit of infectious tissue, and i the period over which tissue remains infective.

iR is thus the average number of 'daughter' infections per 'parent' infection, when infection is unrestricted by any shortage of healthy tissue (Van der Plank 1975). Relating this to myrtle wilt, i is the length of time that diseased myrtles remain infective and is assumed to be equivalent to the time they take to die, previously estimated as 2.6 years (Chapter 3). i is thus taken to be 2.6. R is the yearly infection rate estimated for the three WUT plots (Table 5.15).

After three years, the number of infected trees was beginning to level off (Figure 5.4). This is an example of truncated para-logistic growth (Chapter 4), indicating that some factor, probably the number of root grafted (susceptible) near neighbours, was limiting disease spread. Therefore estimates of R have been derived from the first two years' data only.

Multiplying ***R*** by ***i*** gives empirically derived estimates of ***iR*** for each WUT plot, and an overall figure for the three sites:

- Wobbly Creek 0.556;
- Russell Road 1.022;
- Mt Michael 0.866;
- WUT plot average 0.866.

Thus at Wobbly Creek, at Mt Michael, and overall (where ***iR < 1***), the spread of myrtle wilt through root grafts alone is not likely to sustain an epidemic. However, at Russell Road, if other factors are not limiting, spread of myrtle wilt through root grafts alone would have the potential to sustain an epidemic. In this instance, the number of myrtles available for infection appeared to become limiting after three years.

If these results are indicative of other sites and of more mature forests, it can be concluded that myrtle wilt transfer through root grafting alone is unlikely to cause an epidemic, although it is theoretically possible. In most cases, sustained damage providing multiple infection courts for airborne inoculum would be a necessary precipitating factor. Such damage could be natural or anthropogenic. However, since values of ***iR*** were relatively high (average for WUT plots was 0.866), it can be concluded that the degree of root grafting is a very important factor predisposing stands to epidemics, and plays a major role in the spread of myrtle wilt.

Wounding, root grafting and management implications

Prevention and control of diseases caused by species of *Ceratocystis* and *Ophiostoma* complex has taken various forms, as detailed by Epstein (1978). Some of the techniques which have been principally concerned with reduction in vector populations are of little relevance to myrtle wilt (Martinez de Azagra *et al.* 1988; Magasi *et al.* 1993). Others, including mechanical trenching and the use of soil sterilants have aimed to control disease spread through root grafts (Himelick *et al.* 1963). Using the soil sterilant SMDC (Vapam) of root graft transmission of dutch elm disease was reduced by 56 percent (Neely and Himelick 1966).

An alternative method used to stop the underground spread of canker (caused by *C. fimbriata*) has been the injection of the herbicide glyphosate. Trees thus treated are unable to transfer the disease through their roots (Grosclaude *et al.* 1992). Chemical treatments, notably thiabendazole hypophosphite (Cerotect, Arbotect), have been used with some success as both curative and preventative treatments for dutch elm disease (Greig 1990;

Magasi *et al.* 1993), and propiconazole has been experimentally used as a preventative for *C. fagacearum* (Appel and Kurdyla 1992).

Of the stump treatments and chemical treatments tested during the present work, none were effective in preventing infection by *C. australis*, although it should be noted that the inoculum spore load was artificially high. However, all treatments reduced *C. australis* growth in saplings, and this may well have been due to the time of infection being delayed by the chemicals. *Chalara australis* infection is generally fatal, and recent attempts to find a curative treatment for the myrtle wilt have been unsuccessful (G. A. Kile, personal communication). Thus, there is a need for further testing of possible preventative treatments.

A number of management recommendations for the prevention or minimisation of wounding in *N. cunninghamii* have been previously made (Kile *et. al* 1989; Packham 1991), some of these having now been incorporated into the Tasmanian Forest Practices Code (Forestry Commission 1993). The current results only serve to re-emphasise the principle, since fresh wounds provide an infection court for *C. australis,* and disease spread via root grafts may lead to the death, not only of the wounded tree, but of many of its neighbours also. In root grafted sites where multiple wounding occurs, local epidemics may result and this is probably the explanation for the dead myrtles which flank numerous rainforest roads (Figure 4.10).

Additionally, it is evident that the probability of myrtle wilt spread in the direction of the slope is greater than that along the contour. It is also likely that downhill spread is greater than uphill spread, but this requires confirmation. The obvious application would be that disturbance immediately uphill of *N. cunninghamii* forest should be avoided or minimised.

Kile *et. al* (1989) suggested the use of buffer zones to protect core myrtle stands from the effects of adjacent disturbance. Buffers were provisionally defined as being zones in which all existing myrtles would not be killed within 50 years (by which time myrtle regeneration could form an identifiable forest). On the basis of estimated below ground spread of 1-5 m per year, buffer zones of 50-250 m were proposed. There is some evidence that such buffers may not be sufficient where initial damage has extended into a mature forest (Packham 1991; Chapter 4). They may also require adjustment on sloping sites.

The current work indicates that buffer zones may be more effective if they were to be extended in the direction of the slope (particularly downhill), and reduced in the direction of

the contour. Also, they can probably be drastically reduced in regrowth areas comprised only of myrtle saplings up to 30 cm DBH. In the areas studied, the rate of underground spread averaged only 15 cm per year, and an appropriate buffer zone may need to extend only 7.5 m beyond the extent of initial damage.

A final point concerns the translocation of silvicides through root grafts, or backflash (Bormann and Graham 1960). In species that graft freely, Eis (1972) recommended that the use of silvicides in spacing and thinning treatments be restricted to young stands before grafts are established. The present work shows that in *N. cunninghamii,* root grafts frequently establish between young saplings; silvicides could therefore prove hazardous in thinning operations.

Root grafting and observed myrtle wilt levels

If root grafting is generally important in the spread of myrtle wilt, it could explain some of the findings of Elliott *et al.* (1987). For root grafting to be an effective mechanism of transfer, trees would need to be close enough together for root contacts to exist (Lohhle and Jones 1990). In most myrtle-dominated forest this could be assumed, since root zones of neighbouring myrtles would abut or intersect. Where myrtle was a less important forest component, the chances of root contacts would be lessened.

In Tasmania, the dominance of myrtle is generally greatest in callidendrous rainforest, decreases in thamnic rainforest and reaches a minimum in implicate rainforest types (Jarman *et al.* 1984, 1991). *Eucalyptus*-dominated mixed forest types contain varying amounts of myrtle (Kirkpatrick *et al.* 1988). Elliott *et al.* (1987) found that myrtle wilt incidence was higher in callidendrous than in thamnic-implicate forests, and increased in mixed forests with both absolute and relative measures of myrtle density. Sykes (1984) commented that because intra specific grafts were common and inter specific grafts rare, that stands of mixed species could be expected to reduce the risk of transmitting pathogens by means of root grafts.

Elliott *et al.* (1987) also found that myrtle wilt incidence was also higher for trees of larger diameter. This is harder to explain in terms of root grafting because although myrtle wilt *spread* (in terms of distance) was found to be greater with trees of larger diameter (Table 5.21), the *number* of trees infected per inoculated tree was not significantly related to the size of the inoculated tree (Table 5.16), and disease incidence (and epidemic status) is directly dependant on the latter. Therefore it is likely that other factors, e.g. the increasing

probability of damage with increasing age and size, are mainly responsible for this phenomenon.

The ecological implications of root grafting and disease spread

That root grafting commonly occurs in many tree species is indisputable (Graham and Bormann 1966); why it occurs is much less obvious. Most explanations have focussed on possible competitive advantages gained by root grafted trees, but in general only intra specific competition has been considered.

A detailed study of *Pinus strobus* L. (Bormann 1966) indicated that stand development was shaped by both (intra specific) competition, and by inter tree food translocation which counteracted the effects of competition by delaying the death of individuals. Graham and Bormann (1966) suggested that the main ecological implications of root grafting were to affect the growth of surviving trees, and to influence stand development. They postulated that natural grafts among young trees could influence the pattern of spacing and the establishment of dominance in forest stands. Keeley (1988) argued that the benefits obtained by dominants from food translocation via root grafting were minimal, and that an alternative explanation for evolution and maintenance of root grafts was their potential to provide support and stability (which would always advantage dominants). Loehle and Jones (1990) considered neither was a complete explanation, and proposed that differences between species in grafting incidence resulted partly from proximate factors unrelated to adaptive significance.

Alternatively, it was suggested that a greater degree of genetic similarity should lead to more grafting between trees. Loehle and Jones (1990) proposed that with a genetically uniform (or similar) stand of trees, root grafts would be large and frequent; in such circumstances grafting could represent a significant adaptation for resisting windthrow. Schuster and Mitton (1991) confirmed that root and trunk fusion usually occurred between more genetically similar trees and discussed the possibility that root grafting had evolved through kin selection.

The ecological and adaptive significance of disease transfer via root grafts has been considered by relatively few workers. Eames (1911), cited by Graham and Bormann (1966), suggested that live stumps grafted to living trees could serve a useful function in slowing the tree to tree spread of root rots. Graham and Bormann (1966) proposed that live stumps provided infection courts for pathogens; alternatively they suggest that the fact stumps remain alive may be indicative of resistance to pathogens. Loehle and Jones (1990)

concluded that disease transfer via root grafts was a significant mortality risk and therefore an evolutionary 'cost' of root grafting, whereas Klepzig *et al.* (1991) report observations of extensive defensive reactions (within healthy roots grafted to infected roots), which were successful in preventing fungal invasion of healthy roots.

While in a stable natural environment, the relative ecological costs and benefits of root grafting may not be obvious, in instances where introduced pathogens invade extensively root grafted hosts, the results may be disastrous. High proportions of trees affected by dutch elm disease and oak wilt are known to be infected via root grafts (Neely and Himelick 1963; Cuthbert *et al.* 1975; Epstein 1978; Mielke *et al.* 1983).

While it has not reached the epidemic proportions of these diseases, myrtle wilt is the main cause of *N. cunninghamii* mortality in undisturbed Tasmanian stands (Elliott *et al.* 1987). Combined evidence indicates that root grafting is common in stands of young *N. cunninghamii,* is probably responsible for most short distance transfer of myrtle wilt, and is likely to play a major role in local, micro epidemics. The obvious question is why there has not been selection against root grafting in *N. cunninghamii.* There are two obvious possibilities:

- myrtle wilt is a recent phenomenon in evolutionary terms;
- the advantages of root grafting outweigh the disadvantage of disease spread (and both require that inter specific competition be taken into account).

Since the longevity of *N. cunninghamii* can be up to 500 years (Read and Hill 1981), the time scale necessary for selection to operate against root grafting may be thousands of years. There are no records of myrtle wilt prior to 1942 (Chapter 2), and recent attempts to assess genetic variation in the *C. australis* population have been too limited or unreliable to draw firm conclusions.

Wind stability is one possible advantage of root grafting in *N. cunninghamii.* In New Zealand, all the *Nothofagus* species are known to form root grafts (Beddie 1941). Windthrow is serious and often extensive in New Zealand beech forests, which are frequently either mono specific or dominated by one or more species of *Nothofagus* (Wardle 1984). Beddie (1941) postulated that wind action would be of very minor importance in a thick stand of sapling beeches, but conceded that on wind swept ridge tops, root grafts in, e.g. *N. menziesii,* may be of some value in resisting gales.

In Tasmania, *N. cunninghamii* rarely occurs in mono specific stands, although it is the dominant tree in many rainforest communities (Jarman *et al.* 1984, 1991). Over much of its range it occurs in mixed forests (Kirkpatrick *et al.* 1988; Hickey and Savva 1992), often at low enough densities to decrease chances of root grafting. In natural situations, windthrow is usually an isolated event, and is only a serious problem in silvicultural regeneration (Jennings 1988a). It is therefore unlikely to be a powerful selective force.

Nutrient transfer is another possible advantage of root grafting in *N. cunninghamii.* It is probable that the observed gap phase regeneration of this species (Read and Hill 1985a) facilitates the development of root grafts between trees of the same age. The diameters of inoculated and subsequently infected trees (presumed to be root grafted) were found to be positively and significantly correlated (Table 5.21). Root grafting between establishing seedlings would ensure an even distribution of nutrients, allow more seedlings to survive and facilitate gap capture. As seedlings died due to self thinning, their root systems would be taken over by survivors, resulting in a 'root gap' which had been entirely captured by *N. cunninghamii.* Since most viable *N. cunninghamii* seed falls within 20 m of its source (Hickey *et al.* 1982), establishing seedlings are likely to be closely related (kin selection). Thus, in the presence of competition from other species, nutrient transfer through root grafts is likely to be advantageous to *N. cunninghamii.*

Finally, it can be concluded that the spread of myrtle wilt through root grafts is the cause of the clumped pattern of infected trees (Chapter 3), which in turn may result in large 'canopy gaps'. The ecological and adaptive implications of such gaps are discussed in the following chapter.

5.6 SUMMARY

Wounds as infection courts for *C. australis*

In Tasmanian summer conditions, small stem wounds on *N. cunninghamii* saplings provided suitable infection courts for *C. australis* spores for less than 28 days, with most infections occurring within 14 days. Chemical treatments did not affect the infection rate of wounds, but did decrease the growth of *C. australis* within saplings.

Root grafting in *N. cunninghamii*

Functional root grafts commonly occurred in young *N. cunninghamii* trees. Continuity of the vascular systems was demonstrated by sectioning, and by the transfer of dye across a root graft. Most young myrtles experimentally inoculated with *C. australis spores* became

infected within three years, as did many of their uninoculated neighbours. Since *P. subgranosus* could not have been responsible for all of the instances of disease transfer, and because of the lack of obvious wounds (infection courts) on the neighbouring trees, it was concluded that there had been underground transfer of *C. australis.*

Underground disease spread from the points of inoculation was still occurring after three years, and was greater with trees of larger diameter. Inoculated trees were also more likely to infect others of a comparable size. Most myrtle wilt spread took place down the slope, possibly due to geotropic responses of myrtle roots and *C. australis.* While the rate of dye transfer through a root graft was relatively rapid (equivalent to 31 m per year), the rate of *C. australis* spread from inoculated trees was only 15 cm per year, implying that the fungus was not carried passively in the xylem.

C. australis isolations from root grafts

Site excavation revealed connections between the root systems of inoculated and neighbouring trees, and indicated routes by which spread of *C. australis* may have occurred. Sectioning showed a number of these connections to be functional root grafts. *C. australis* staining was present in the bases of a number of trees and in the cross section of one such graft. A dark brown substance was associated with the pathogen, particularly in the ray parenchyma. Since transfer across root grafts generally necessitates tangential or radial movement, this could explain why *C. australis* appeared to move through root grafts with relative ease.

The presence of *C. australis* staining in the cross section of a root graft indicated that the pathogen *could* grow through root tissues from host to host. To prove that spread via root grafts *had* actually occurred, re-isolations of *C. australis* were made from neighbouring trees and from the infected root graft. Attempts were made to characterise and compare the original inoculated isolate, the re-isolates, and controls from Victoria and Tasmania.

Compatibility tests between paired isolates failed to produce mature perithecia; isolates appeared to be of one mating type. With a limited number of enzyme systems, the *C. australis* isolates showed very little electrophoretic variation. The most effective differentiator of the isolates was their radial growth rate, assessed over a range of temperatures. Although temperature profiles showed significant variation in the *C. australis* population, re-isolates could not be distinguished from the inoculated isolate. This indicated that *C. australis* had been transferred via root grafts.

Chalara australis wilt transfer through root grafting alone is unlikely to cause an epidemic. In most cases, sustained damage, providing multiple infection courts for airborne inoculum, would be a necessary precipitating factor; such damage could be natural or anthropogenic. However, root grafting is a very important factor predisposing stands to epidemics, and plays a major role in the spread of myrtle wilt.

Finally, it can be concluded that the spread of myrtle wilt through root grafts is the cause of the clumped pattern of infected trees. This gives rise to patches of dead and diseased trees, which in turn result in large gaps in the forest. It is proposed that in such gaps, root grafting between establishing myrtle seedlings ensures an even distribution of nutrients, allows more seedlings to survive, and facilitates gap capture.

6. MYRTLE WILT AND RAINFOREST FLORISTICS

6.1 INTRODUCTION

The first reports of myrtle wilt noted that in both disturbed and undisturbed forest, the disease brought about a considerable departure from the normal pattern of tree death and forest structure, causing gaps in the forest (Howard 1973a, 1981). Myrtle wilt has since been shown to occur in undisturbed forest throughout Tasmania (Elliott *et al.* 1987).

Although there was a considerable amount of related information available, the effects of myrtle wilt on rainforest floristics, myrtle regeneration and future forest composition had not been specifically investigated prior to this project. The present work investigates the effects of canopy gaps on myrtle regeneration and rainforest floristics in five callidendrous and thamnic rainforest sites (both pure and mixed). The effect of myrtle wilt on stand dynamics and future forest composition has been studied at two of these sites.

Disease, canopy gaps and regeneration

In many forests canopy gaps provide an important regeneration niche. However, host specific disturbances and major disturbances affecting primarily canopy sized or mature trees, may lead to the disproportionate importance of one tree species as a gap-maker (Worrall and Harrington 1988).

A study of natural regeneration in disease centres caused by the oak wilt fungus, *Ceratocystis fagacearum,* was carried out in North American oak forests by Tryon *et al.* (1983). Regeneration in infection centres was found to be similar to that of selection cuttings in similar but disease free stands, but no comparison with closed canopy forest was made. Regeneration was not infected except in the case of sprouts from diseased stumps. It was concluded that the abundance and species of the advanced regeneration, which was site dependent, would be of major importance in forming the next stand.

In New Zealand *Nothofagus* forests, disturbances were thought to prevent the competitive exclusion of *N. fusca* by the more shade tolerant *N. menziesii;* one possible disturbance being the death of *N. fusca* canopy trees caused by *Platypus* species. However, detailed studies showed that the two species actually coexisted via different life history strategies, *N. menziesii* having lower juvenile mortality and *N. fusca* having a faster height growth rate, greater longevity and adult survivorship (Stewart and Rose 1988, 1990). Thus canopy gaps caused by *Platypus* epidemics are not essential for the survival of *N. fusca.*

Regeneration in single tree gaps

In Tasmania, parallels to the New Zealand example are found in the *N. cunninghamii* (myrtle) and *Atherosperma moschatum* (sassafras)-dominated callidendrous rainforest. Although myrtle frequently forms the canopy, sassafras is more shade tolerant and has been predicted to succeed it (Gilbert 1959; Noble and Slatyer 1978, 1980). However, further studies showed that in lowland sites sassafras does not compete well enough with myrtle to actually reach the canopy. This is due either to insufficient shade tolerance, or to the infrequency of seedling establishment (Read 1985; Read and Hill 1985a).

Gap phase regeneration of myrtle has been frequently recorded (Mesibov 1977; Hickey and Felton 1991) and is thought to be one of the factors contributing to the stability of rainforest (Ellis 1985). Read and Hill (1985a) found that Tasmanian rainforest was normally self replacing, with no major changes in species composition or dominance occurring, and with *N. cunninghamii* maintaining its dominance by seedling regeneration in canopy gaps created by the death of old myrtles.

Read and Hill (1985a) showed that both myrtle and *Eucryphia lucida* (leatherwood) seedlings were clustered on a scale comparable with that of canopy gaps (8-18 m diameter) caused by the death of single myrtles, whilst sassafras tended towards self replacement by means of vegetative reproduction in the smaller gaps (5-12 m diameter), caused by the death of the main stem of sassafras trees. Hickey (1982a) recorded that sassafras regenerated by coppicing from basal sprouts with seedling regeneration mainly confined to man-fern trunks, whilst myrtle and leatherwood regenerated primarily by seedlings within canopy gaps, with some coppicing in the case of leatherwood.

Myrtle wilt and expanding canopy gaps

Howard (1973a) reported that in undisturbed forest the death of a single tree due to myrtle wilt was often followed year by year with the death of surrounding trees. This formed gaps with the most decayed dead trees at the centre, and the newly dead trees with (recent) *P. subgranosus* attack at the periphery (Howard 1981).

Myrtle regeneration is known to occur in gaps caused by myrtle wilt (Howard 1973a, 1981; Hickey 1982a). Howard (1981) noted that in undisturbed mature myrtle forests a very high proportion of trees were overmature, and suggested that these provided a suitable site for *P. subgranosus* whilst young trees withstood attack. It has been proposed that myrtle wilt gaps

may provide a regeneration niche for myrtle, the disease being in equilibrium with other processes (Howard 1981; Jarman *et al.* 1984).

Canopy gaps and rainforest regeneration

A number of factors have been shown to limit or determine regeneration in gaps but their interactions with myrtle wilt are largely unknown.

Gap size and shade tolerance

Gap size may determine the tree species which can colonise and capture gaps. Myrtle and leatherwood, with higher growth rates in high, unfiltered light, have been shown to be more successful in larger gaps, whilst sassafras, with its greater shade tolerance, vegetatively colonised smaller gaps. Gap size is also likely to determine the proportion of myrtle and leatherwood stems of vegetative origin, since this was shown to decrease with increased levels of diffuse light. (Read 1985; Read and Hill 1985a, 1985b, 1988). The gaps studied to date however, have been relatively small (up to 500 m^2), generally resulting from the death of a single tree, while myrtle wilt gaps can be quite large, often involving a number of trees (Howard 1973a).

Dispersal mechanisms

Initial floristic composition has been shown to be an important determinant of current canopy composition, with the failure of sassafras to achieve dominance attributed to the low dispersal rate of its predominantly vegetative propagules (Read and Hill 1985b). In good light conditions seedlings are the more important regeneration method for myrtle and leatherwood (Hickey 1982a; Read and Hill 1985a) but Hickey *et al.* (1982) recorded that while some seeds may be blown up to 150 m, most of the viable seed falls within 20 m of its source. *Phyllocladus aspleniifolius* (celery top pine) is thought to have soil-stored seeds as does *Acacia melanoxylon*; *Tasmannia lanceolata* is known to be bird dispersed and to invade forest disturbances (Hickey 1982a; Read 1989).

'Seedling banks'

Mount (1979) noted that seedlings already present were stimulated by canopy opening due to myrtle wilt. Seedling, sapling and pole trees of myrtle may all be present in a gap resulting from myrtle wilt (Howard 1981). This wide age range could occur when advanced myrtle regeneration is present at the time of gap formation; it could also occur when gap formation and seedling establishment takes place over an extended period of time.

Substrate

Read and Hill (1988) recorded that branch litter from trees dying of wilt retarded seedling establishment in one site but not in another, and concluded that the effect of wilt on regeneration is dependent on site characteristics which determine the rate of litter decomposition. Bare soil or logs are frequent regeneration sites (Hickey 1982a). In shaded and very wet positions, myrtle is known to favour logs and rotting wood for establishment, rather than the forest floor (Mesibov 1977). Soil is often exposed on the rootplates or in craters formed by trees which have been uprooted, and myrtle, sassafras and blackwood often establish on these sites. Stumps do not provide such a good substrate and seedlings are more common at their bases than on the top of them (Mesibov 1977). Mount (1979) observed that wilt-killed trees generally do not fall over until their roots have rotted, thereby causing minimal soil disturbance.

Competition

Competition from other species, particularly ferns, may limit seedling establishment. Jarman *et al.* (1984) noted that in callidendrous forest a dense cover of *Histiopteris incisa* and *Hypolepis rugosula* was common along road margins and tracks or beneath canopy gaps in forest suffering severe damage from myrtle wilt. Mesibov (1977) found that where there was a dense cover of *Histiopteris incisa*, *Polystichum proliferum* or *Blechnum wattsii,* large gaps could be virtually barren of tree seedlings unless rotting wood was present . He also noted that myrtle seedlings were sparse on logs already enveloped by rhizomes of *Microsorium* and *Rumohra* species, and that on the ground they could be crowded out by *Trochocarpa, Bauera* and *Dianella* species and various grasses. Mount (1979) observed that *H. incisa* was stimulated by canopy opening due to wilt, and often smothered new germinants.

Browsing

Browsing may also be an important factor in gaps. Hickey (1982a) recorded that while sassafras and blackwood suffered heavy browsing (unless in inaccessible places or protected by other vegetation), leatherwood was moderately browsed, myrtle was only lightly browsed and celery top pine was untouched. Mount (1976) reported that browsing effects were greatest where regeneration was in small patches or was sparse and slow growing.

6.2 MATERIALS AND METHODS

Five Tasmanian sites were selected in callidendrous and thamnic rainforest, with and without eucalypt overstoreys (mixed forest and pure rainforest), and at different altitudes. Details of the sites are given in Appendix 26. At each site, 10 m x 10 m plots, subdivided into 2 m x 2

m subplots, were located in the centre of recent myrtle wilt gaps of various sizes, with control plots located in the surrounding forest. Using the health status classes (HSC) given in Table 2.1, recent gaps were defined as having a dying (HSC 4) or recently dead (HSC 5) myrtle on the edge.

A number of larger, older gaps were also investigated at the Simons Road site. These showed evidence of having been caused by wilt but the trees had been dead longer (HSC 6).

Gap size was determined by selecting four healthy trees of any species immediately on the edge of the gap, one per quadrant. Measuring tapes were run between the boles of opposite trees; the gap centre being where they crossed and the gap size estimated by multiplication of the two distances.

For each plot the aspect, slope and depth of the litter layer (organic material) were estimated. Aspect was coded according to the level of insolation:

1 (facing 90-179°);
2 (facing 0-89° or 180-269°);
3 (facing 270-359°).

The diffuse light factor was assessed by hemispherical photography of the canopy, using a Tokina 17 mm, 1:3.5 fisheye lens and the method described by Anderson (1964, 1970). Canopy photographs were taken at ±1.5 m above ground level, in the centre of each plot and with any immediately overhanging vegetation removed (Figure 6.1). For each control plot four additional canopy photographs were taken in and around the plot and the mean of the five values used. Details of the sites and plots can be found in Appendix 10.

The presence of all higher plants, ferns, filmy ferns and some clubmosses was noted in each 2 m x 2 m subplot, with germinants (less than four true leaves) being recorded separately. On a 10 m x 10 m plot basis, this generated four data sets:

- presence/absence data including germinants;
- presence/absence data excluding germinants;
- frequency data including germinants (%);
- frequency data excluding germinants (%).

Data were entered into the ecological database ECOPAK (Minchin 1986).

Figure 6.1 A canopy gap caused by myrtle wilt (photographed from below, using a Tokina 17 mm, 1:3.5 fisheye lens).

Vegetation composition in gaps

Classification of vegetation

Classification of each of the four data sets was achieved using TWINSPAN, a program for two-way indicator species analysis (Hill *et al.* 1975). The following transformation was used for the frequency data in the classification analysis:

0.1-0.9%	1;
1-4%	2;
5-24%	3;
25-49%	4;
50-74%	5;
75-100%	6.

Ordination of sites

Ordination of each of the four data sets was achieved using DECORANA, a program for detrended correspondence analysis (Hill and Gauch 1980) and KYST, a program for multidimensional scaling (Kruskal *et al.* 1973). In ordinations using DECORANA and KYST the Braun-Blanquet scale was used to transform the frequency data.

Only the first two of the four ordination axes produced by DECORANA were used. KYST was initially run using global non-metric multidimensional scaling (Kruskal 1964) in six dimensions. The two and three dimensional solutions were saved and used as the starting configurations for the final KYST runs in two and three dimensions, using global hybrid multidimensional scaling (Faith *et al.* 1987). In all KYST runs the Kulczynski coefficient (Faith *et al.* 1987) was used.

All these ordination solutions were plotted:

DECORANA	2 dimensional solution (axes 1 x 2);
KYST	2 dimensional solution (axes 1 x 2);
KYST	3 dimensional solution (axes 1 x 2, 1 x 3 and 2 x 3).

Using the four data sets this gave a total of 12 ordinations and 20 ordination diagrams. These were examined for relationships between site, gap size, forest type, environmental variables and vegetation composition.

Vector analysis of environmental variables

Vector analysis (Dargie 1984) was used to show the direction of environmental variables in relation to the ordination axes arising from DECORANA and KYST. Two ordinations were selected, using presence/absence data excluding germinants:

DECORANA 2 dimensional solution;
KYST 3 dimensional solution.
For each measured environmental variable a vector was determined, along which the ordination sample scores had maximum correlation with that variable. A Monte-Carlo approach was used to test the significance of the maximised correlations (Minchin 1990).

Canonical correlations between vegetation composition and environmental variables
Canonical correlations (Dunteman 1984) were used to examine the relationships between the measured environmental variables and trends in vegetation composition, as expressed by the ordinations arising from DECORANA and KYST. Three ordinations were selected, using two of the data sets:
DECORANA 2 dimensional solution, presence/absence data excluding germinants;
KYST 3 dimensional solution, presence/absence data excluding germinants;
KYST 3 dimensional solution, frequency data including germinants.

In each case, the first set of canonical variables (environmental variables) was plotted against the second set (vegetation trends). To examine the environmental differences between sites, the environmental variable scores from the first two canonical correlations were plotted against each other.

Vegetation composition in large old gaps at Simons Road
Ordination of plots
At Simons Road some older, large gaps were included in the study and are enclosed by the broken line in Figures 6.2-6.6. This gave sufficient plots to be able to ordinate this site on its own using KYST (as above) in only two dimensions, on presence/absence data excluding germinants. One control plot (17) was omitted as it was in an area of roadside disturbance.

Vector analysis of environmental variables
Vector analysis, as described above, was used to show the direction of environmental variables in relation to the ordinations arising from KYST.

Regeneration and vegetation structure in gaps
Additional information about regeneration and vegetation structure was recorded on a 2 m x 2 m subplot basis and summed for each plot.

For higher plants the number of seedlings (at least four true leaves) or herbaceous plantlets, epicormics (shoots arising from roots or stems lying horizontally either at or above ground

level), basal sprouts (coppice and sprouts from live trees), saplings (diameter at breast height (DBH) greater than 1 cm), trees (greater than 5 cm DBH) and dead (D) myrtle trees were recorded. Numbers were counted up to ten and thereafter estimated to be less than 100, 1000 etc. The midpoints were used for totalling i.e. 55, 550 etc. Wherever possible the origin of saplings was noted, as was the health and damage classes of myrtles.

The percentage cover of all fern and filmy fern species was also estimated. The following cover classes were used: 0%, <1%, 1-4%, 5-24%, 25-49%, 50-74%, 75-100%. Once again, midpoints were used for totalling.

For each plot the total number of seedlings and vegetative shoots (epicormics and basal sprouts) of myrtle and sassafras were calculated and fern cover was taken as the mean of the subplots (percentage of rooted cover of all ferns and filmy ferns). Separate analyses of variance (ANOVAs) were carried out on these data to compare gaps with control forest and to investigate differences between sites (fern cover being first transformed to the arcsin square root of the percentage).

Investigation of myrtle regeneration at Five Road

To assess whether myrtle regeneration at Five Road was likely to be sufficient to maintain the current forest composition, methods were adapted from those used following commercial forestry operations (Forestry Commission 1991).

Two parallel transects, 700 m long and 10 m apart were run through the forest at Five Road. Regeneration plots were sited every 20 m, and staggered between the two transects, giving a total of 72 plots. Each plot comprised a 4 m^2 circle with a 1 m^2 circle at its centre. The presence or absence of myrtle seedlings (including germinants) and the height of the tallest myrtle seedling were recorded in the 4 m^2 plots, with numbers of myrtle seedlings being additionally recorded in the 1 m^2 plots. Myrtle trees (>15 cm DBH), poles (>5 cm DBH) and saplings (>1 cm DBH), were noted when they were within 5 m of the plot centre.

The position of each 4 m^2 and 1 m^2 plot was noted as being under the canopy, in a gap, or on the edge of a gap. ANOVAs were carried out on the numbers of plots with myrtle seedlings present (stocked plots) found below gaps, gap edges and the closed canopy.

Finally, for each 4 m^2 plot the substrate (or substrates) on which seedlings were growing was recorded, the categories being: forest floor; log (bark present), log (sapwood rotted), log (heartwood rotted); uproot (crater and mound); old mound. ANOVA was used to

investigate the relative frequency of the different seedling substrates in stocked 4 m^2 plots. A multiple range analysis (which gave homologous groups based on 95% confidence intervals) was also carried out.

Future forest composition at Simons Road and Five Road

Surveys were carried out at the Simons Road and Five Road sites, using a transect method modified from that of Veblen (1985b, 1989a), Stewart and Rose (1990) and G. H. Stewart (personal communication), identifying canopy and sub-canopy trees, gap-makers (those trees which had died or were dying, causing or contributing to gaps) and potential successors (those trees most likely to succeed them). Gap-makers were classified (uproot, dead standing, stump) and their decay class noted (bark present, sapwood rotted, heartwood rotted). A potential successor was defined as being the nearest (tallest, greatest DBH) tree to a gap-maker, between 1/2 and 2/3 of the canopy height. In some instances there were no trees in this category within, or on the edges of the gap; in such cases no potential successor was recorded.

At Simons Road, three 350 m transects were run perpendicular to the road edge. At Five Road one 700 m transect was run through the undisturbed forest. All gap-makers within 5 m of the line were recorded and the potential successors identified. The extent of all extended (bole to bole) and canopy (canopy edge to canopy edge) gaps encountered along the transects was measured.

Tree composition was measured by means of point-centred quarter plots (Cottam and Curtis 1956) at 100 m intervals along approximately the same transect lines. Since a number of plots had already been recorded in gaps on this site, this merely involved recording the nearest canopy and sub-canopy tree species in each quadrant and the (paced) distance from the transect. The species, DBH and substrate (forest floor, mound, log - decay class noted) of all trees were recorded as were the health status classes of all myrtles.

Omitting felled trees, Student's t tests were carried out on the number of gap-forming, *P. subgranosus* attacked (presumed *C. australis* infected) myrtles which had snapped (leaving stumps) compared with those which had uprooted (forming rootplates). The three transects were used as replicates.

The interactions between substrate and species, and between substrate and canopy position were examined separately. In each case the percentage of trees established on each substrate was transformed to the arcsin square root of the percentage, and Student's t tests

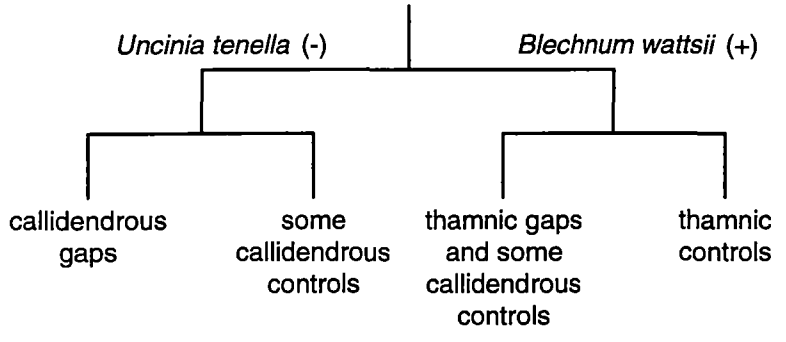
were carried out. The three transects at Simons Road were used as replicates, and the Five Road transect was sectioned to give replicates: 0-250 m; 250-450 m; 450-700 m.

6.3 RESULTS

Vegetation composition in gaps

Classification of vegetation

Classification of each of the four data sets gave a rough separation between callidendrous and thamnic plots. The primary division tended to separate the callidendrous gap plots with some controls (Simons Road) from the thamnic plots and the rest of the callidendrous controls. Subsequent divisions generally resulted in the separation of the sites. The dendrogram below illustrates the results obtained using presence/absence data including germinants.



Slightly different indicator species for the primary division were determined by the other data sets:

- presence/absence data excluding germinants
  Uncinia tenella (-) *Eucryphia lucida* (+)
  Hymenophyllum australe (+);
- frequency data including germinants (%)
  Uncinia tenella (-) *Blechnum wattsii* (+)
  Hypolepis rugosula (-) *Hymenophyllum australe* (+);
- frequency data excluding germinants (%)
  Uncinia tenella (-) *Blechnum wattsii* (+)
  Hypolepis rugosula (-) *Hymenophyllum australe* (+).

Ordination of sites

Good site separations were obtained with the ordinations and only the first two axes (dimensions) were necessary to achieve site separations, KYST and DECORANA being equally effective. Very similar configurations were found in many of the diagrams. Within each site, gap and control plots were fairly consistently found in the same relative positions, with gaps tending to be more 'callidendrous' than controls.

Examples of the results are illustrated in Figure 6.2, which was achieved using DECORANA on presence/absence data, excluding germinants, and in Figures 6.3 and 6.4, which were achieved using KYST (3 dimensional solution) on the same data set. The ordination axes represent trends in vegetation composition, with the distance between plots representing their dissimilarity. Control plots are underlined, the others were situated in gaps of various sizes.

Figure 6.2 also shows that the callidendrous and the thamnic sites are separated in the ordination, with the gaps tending to be more callidendrous than the controls. The DECORANA species scores indicate that in relation to the direction of the first ordination axis, gaps had less *Eucryphia lucida, Pittosporum bicolor, Phyllocladus aspleniifolius, Cenarrhenes nitida, Phebalium squameum* and *Prionotes cerinthoides,* and more *Acacia dealbata, Hydrocotyle javanica,* and *Hymenophyllum cupressiforme.* Additionally, in relation to the direction of the second ordination axis, gaps had less *Gnaphalium callinum* and *Cyathodes juniperina* and more *Atherosperma moschatum, Clematis aristata, Pterostylis longifolia* and *Hymenophyllum flabellatum.*

The relationship between trends in vegetation composition and gap size was not so obvious. The ordering of plots within sites according to gap size was most effectively achieved using KYST and presence/absence data excluding germinants, or frequency data including germinants. A third axis (dimension) was often necessary. Whilst within some sites plots were readily sorted according to gap size, in others this was achieved by very few or no ordinations. Success appeared to be inversely related to the mean gap size of the site, up to a mean gap size of 800 m^2 (Table 6.1). Thus trends in vegetation composition within sites were related to the mean gap size (up to 800 m^2).

Table 6.1 The relationship between the mean gap size of sites and ordination success in ordering plots according to gap size.

Site	Forest type	Mean gap size of site (m^2)	No. of ordination diagrams with plots ordered according to mean gap size (Total no.=20)
Wilson Rd	Thamnic (pure)	201 m^2	17
Arve Loop	Thamnic (mixed)	317 m^2	7
Mt Michael	Callidendrous (pure)	423 m^2	6
Simons Rd (recent gaps)	Callidendrous (pure)	677 m^2	3
Five Rd	Callidendrous (mixed)	941 m^2	0
Simons Rd (old gaps)	Callidendrous (pure)	3373 m^2	0

Vector analysis of environmental variables

Environmental vectors which were significantly correlated with ordination samples scores, were plotted and used to interpret the ordination diagrams arising from DECORANA and KYST (Figures 6.2, 6.3 and 6.4 respectively). In every case altitude was the most important vector; slope, depth of litter and diffuse light were also important. Gap size was not significant, but the direction of this vector has been shown. However, the major aspect of this variation (gap size) may be obtuse to the ordination axes, and this is explored later (Figure 6.7).

Canonical correlations between vegetation composition and environmental variables

Canonical correlations between measured environmental variables and the ordination axes (representing trends in vegetation composition) are given in Tables 6.2-6.4, the more important trends being shown in bold. Table 6.2 shows the results using DECORANA on presence/absence data excluding germinants. The analysis indicates that altitude was strongly negatively correlated to the first ordination axis, as was, to a lesser extent, diffuse light. Slope was strongly negatively correlated with the second ordination axis, as also were altitude and depth of litter.

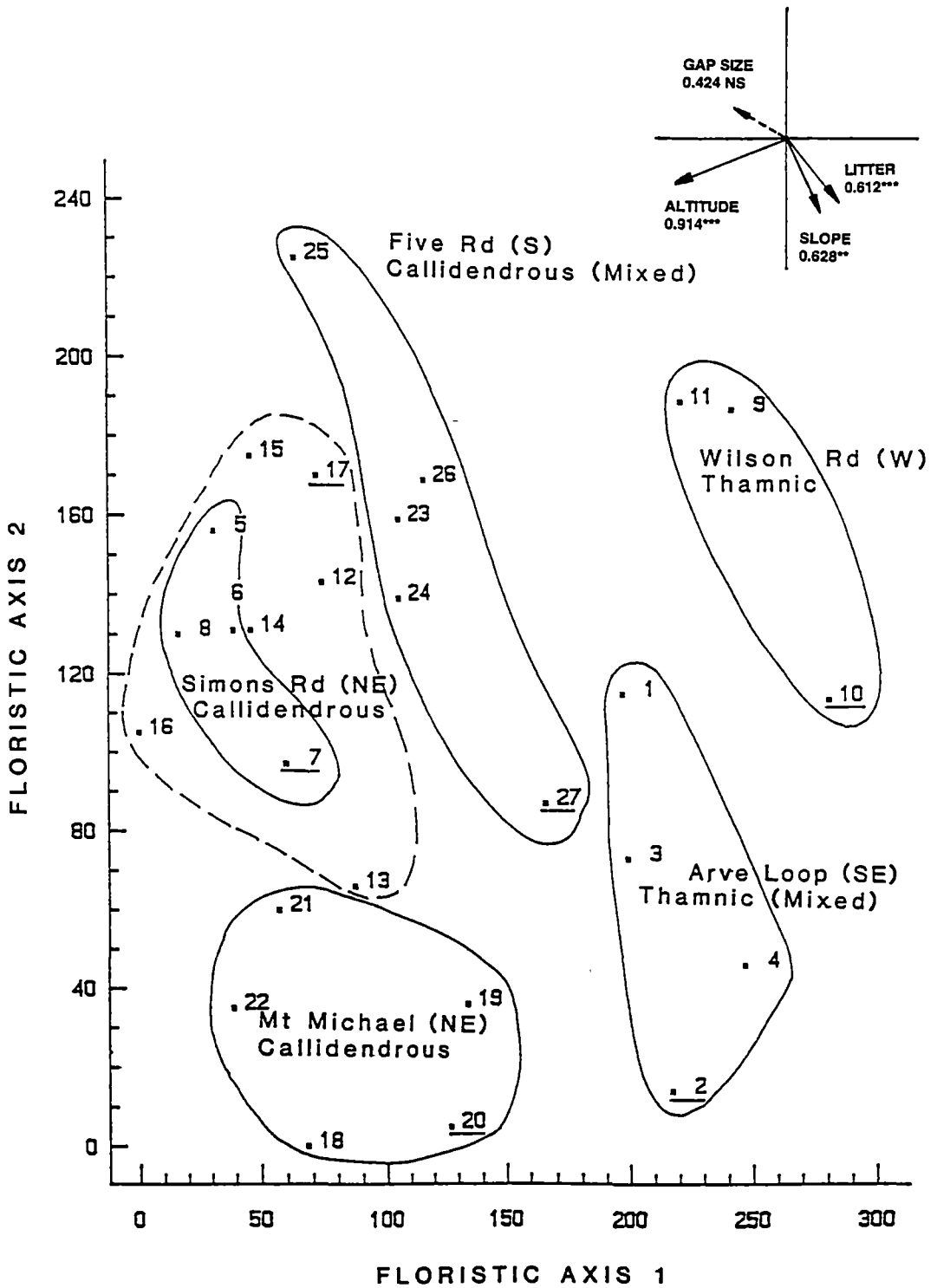


Figure 6.2 DECORANA ordination of 27 plots from five sites, located in myrtle wilt gaps with controls (underlined) in the surrounding forest. The broken line encloses the series of old gaps at Simons Road. Forest type, and the direction and magnitude of environmental vectors are shown.

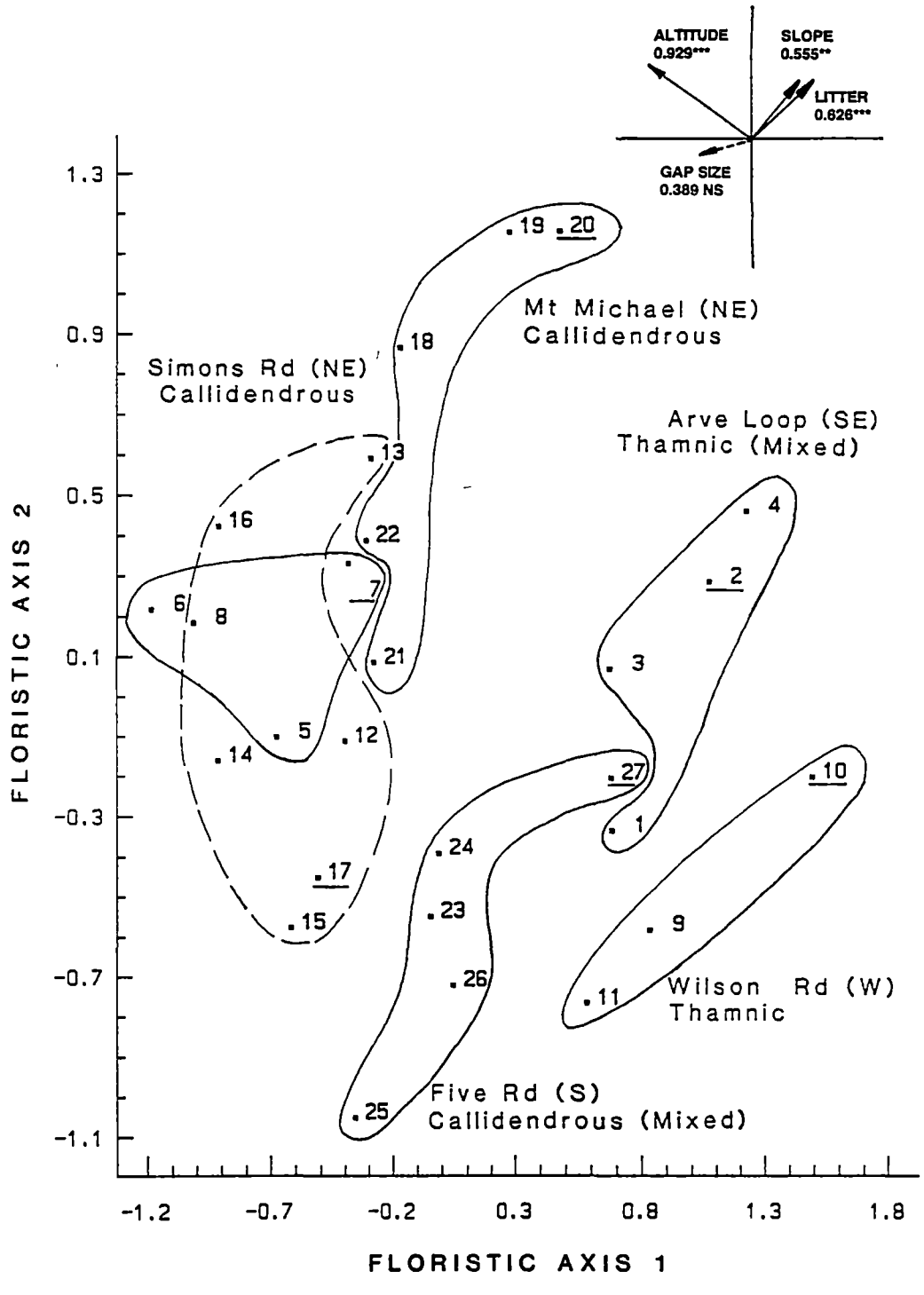


Figure 6.3 KYST (3D) ordination of 27 plots from five sites, located in myrtle wilt gaps with controls (underlined) in the surrounding forest. The broken line encloses the series of old gaps at Simons Road. The direction and magnitude of environmental vectors are shown. (Axes 1 x 2.)

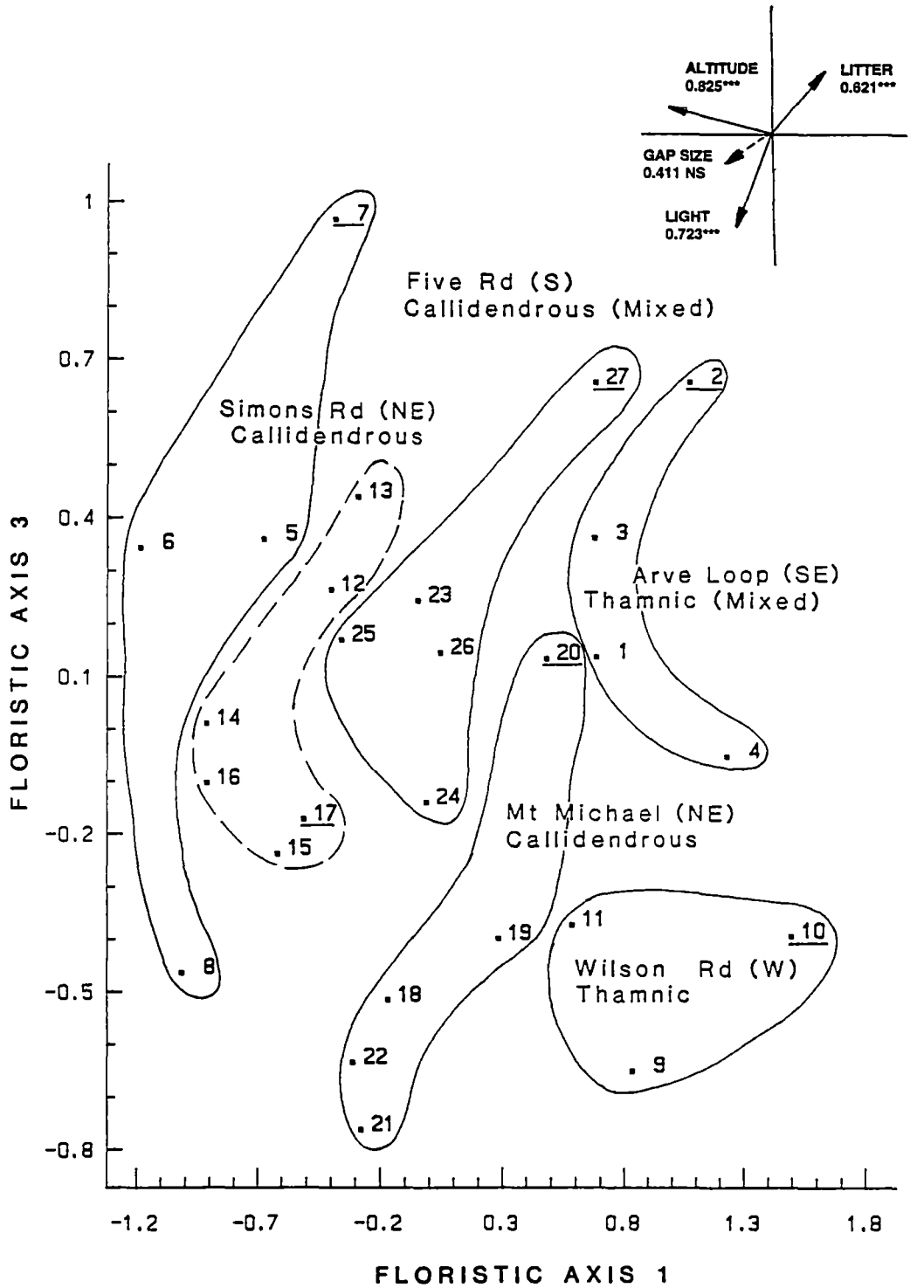


Figure 6.4 KYST (3D) ordination of 27 plots from five sites, located in myrtle wilt gaps with controls (underlined) in the surrounding forest. The broken line encloses the series of old gaps at Simons Road. The direction and magnitude of environmental vectors are shown. (Axes 1 x 3.)

Table 6.2 Canonical correlation of environmental values and trends in vegetation using DECORANA on presence/absence data excluding germinants

No	Eigen value	Canonical correlation	Wilks lambda	Chi-square	DF	Significance level
1	0.9200	0.9592	0.0442	67 045	12	**0.0000*** **
2	0.4473	0.6688	0.5527	12.747	5	**0.0259***

Coefficients for canonical variables of the first set

ALTITUDE	**-0.926**	**0.429**
ASPECT	-0.004	0.125
SLOPE	0.141	**0.660**
DEPTH OF LITTER	0.006	**0.469**
DIFFUSE LIGHT	**-0.301**	0.203
GAP SIZE	0.060	-0.238

Coefficients for canonical variables of the second set

ORDINATION AXIS 1	**1.001**	0.094
ORDINATION AXIS 2	0.200	**-0.986**

NB In this and following tables, 5%, 1% and 0.1% significance levels are shown by *, ** and *** respectively. Non-significant values are denoted by NS.

Table 6.3 shows the results using KYST on presence/absence data excluding germinants. It indicates that altitude was strongly negatively correlated to the first ordination axis, as was, to a lesser extent, diffuse light. Depth of litter was strongly correlated with the second and third ordination axes, as also was altitude, with diffuse light being negatively correlated.

Table 6.3 Canonical correlation of environmental values and trends in vegetation using KYST on presence/absence data excluding germinants

No.	Eigen value	Canonical correlation	Wilks lambda	Chi-square	DF	Significance level
1	0.9473	0.9733	0.0144	89.052	18	**0.0000*** **
2	0.6041	0.7773	0.2730	27.264	10	**0.0024** **
3	0.3104	0.5571	0.6896	7.805	4	0.9900 NS

Coefficients for canonical variables of the first set			
ALTITUDE	**-1.022**	**0.434**	-0.231
ASPECT	-0.171	0.051	-0.524
SLOPE	0.075	0.042	-0.802
DEPTH OF LITTER	-0.011	**0.704**	-0.127
DIFFUSE LIGHT	**-0.344**	**-0.473**	-0.761
GAP SIZE	0.153	0.049	0 398

Coefficients for canonical variables of the second set			
ORDINATION AXIS 1	**0.910**	0.232	-0.344
ORDINATION AXIS 2	-0.410	**0.631**	-0.659
ORDINATION AXIS 3	0.064	**0.740**	0 670

Table 6.4 shows the results using KYST on frequency data including germinants. It indicates that altitude was strongly negatively correlated to the first and second ordination axes, as to a lesser extent, was diffuse light. Aspect was strongly correlated with the third ordination axis, as also was altitude.

Table 6.4 Canonical correlation of environmental values and trends in vegetation using KYST on frequency data including germinants

No.	Eigen value	Canonical correlation	Wilks lambda	Chi-square	DF	Significance level
1	0.9403	0.9697	0.0128	91.533	18	**0.0000*** **
2	0.6645	0.8152	0.2145	32.328	10	**0.0004*** **
3	0.3607	0.6005	0.6393	9.394	4	0.0520 NS

Coefficients for canonical variables of the first set			
ALTITUDE	**-0.939**	**0.416**	0.580
ASPECT	-0.138	**1.087**	0.385
SLOPE	0.221	0.055	0.476
DEPTH OF LITTER	0.001	-0.143	0.598
DIFFUSE LIGHT	**-0.451**	-0.148	0.341
GAP SIZE	0.226	-0.241	-0.482

Coefficients for canonical variables of the second set			
ORDINATION AXIS 1	**0.825**	0.067	0.561
ORDINATION AXIS 2	**-0.565**	0.114	0.817
ORDINATION AXIS 3	0.009	**0.991**	-0.131

All the measured environmental variables were more strongly correlated with trends in vegetation composition represented by the ordination axes, than was gap size. Altitude was probably the most strongly correlated overall, whilst aspect was important only when frequency data was used.

Figure 6.5 illustrates relationships between measured environmental variables and trends in vegetation composition. It is based on the canonical variable number 1 shown in Table 6.2, the first set of canonical variables (environmental values) being plotted against the second set (vegetation trends). Sites were found to be ordered along the y axis according to decreasing altitude and along the x axis according to forest type; ranging from pure to mixed callidendrous forest through mixed and pure thamnic forest. Along the diagonal, sites were roughly ordered along an east-west gradient.

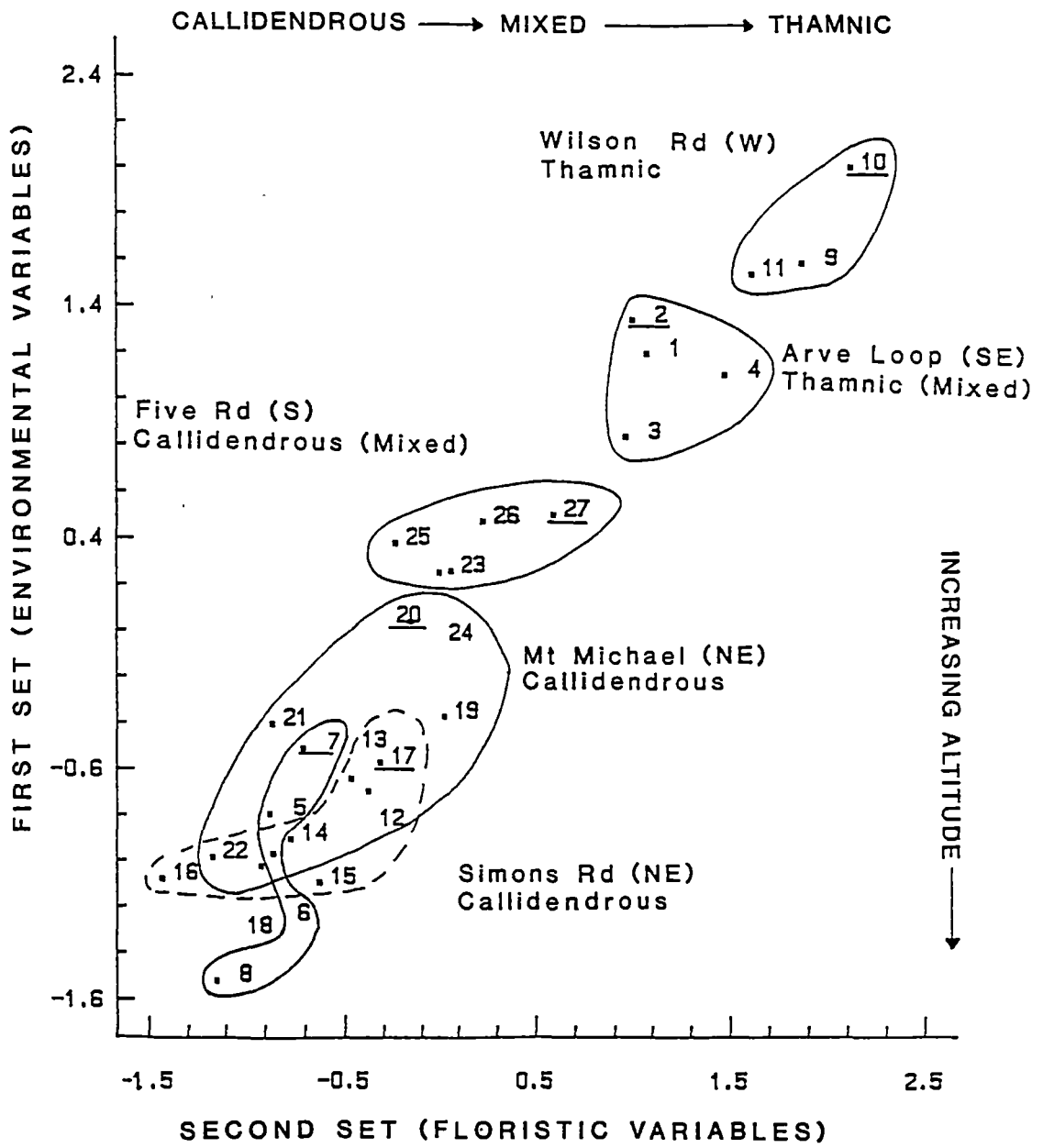


Figure 6.5 Plot of the first set (environmental values) against the second set (vegetation trends) of canonical variables. Control plots are underlined.

Figure 6.6 illustrates the environmental differences between sites. It is based on the first set of canonical variables (environmental values) shown in Table 6.2, canonical variable number 1 having been plotted against canonical variable number 2. Sites were found to be well separated by the measured environmental variables, with the exception of Simons Road and Mt Michael which were very similar.

Vegetation composition in large old gaps at Simons Road

Ordination of plots

The results of the ordination of the Simons Road plots are shown in Figure 6.7. Once again the ordination axes represent trends in vegetation composition and gap sizes have been shown. With smaller gaps a relationship between vegetation composition and gap size is evident, as shown by the ordering of sites along the diagonal line according to gap sizes. The older, large gaps however, do not fit the pattern and are more similar to the control (gap size 0 m^2) than they are to the other gaps.

Vector analysis of environmental variables

Environmental vectors which were significantly correlated with ordination samples scores, were plotted and used to interpret the ordination diagram arising from KYST (Figure 6.7). Within this site the only significant vector was gap size.

Regeneration and vegetation structure in gaps

Figure 6.8 shows the vegetation structure of a control plot at Simons Road (plot 7). This depicts numbers of higher plants and percentage cover (rooted in the plot) of ferns and filmy ferns. Figure 6.9 shows a recent gap (plot 6) and Figure 6.10 a large old gap (plot 16). In these gaps there was an increase in myrtle and sassafras regeneration, fern cover and alpha diversity. In the large old gap there was also an increase in the number of vegetative shoots of myrtle and sassafras. Figure 6.11 illustrates rainforest regeneration in a large old gap caused by myrtle wilt.

Table 6.5 shows the effect of myrtle wilt gaps on myrtle and sassafras regeneration and fern cover for the five sites based on the data set given in Appendix 27 and the ANOVA tables given in Appendix 28. Fern cover was significantly increased in gaps and myrtle seedlings showed a similar but non-significant trend. Myrtle vegetative shoots were significantly decreased in gaps. Sassafras regeneration showed no significant trends.

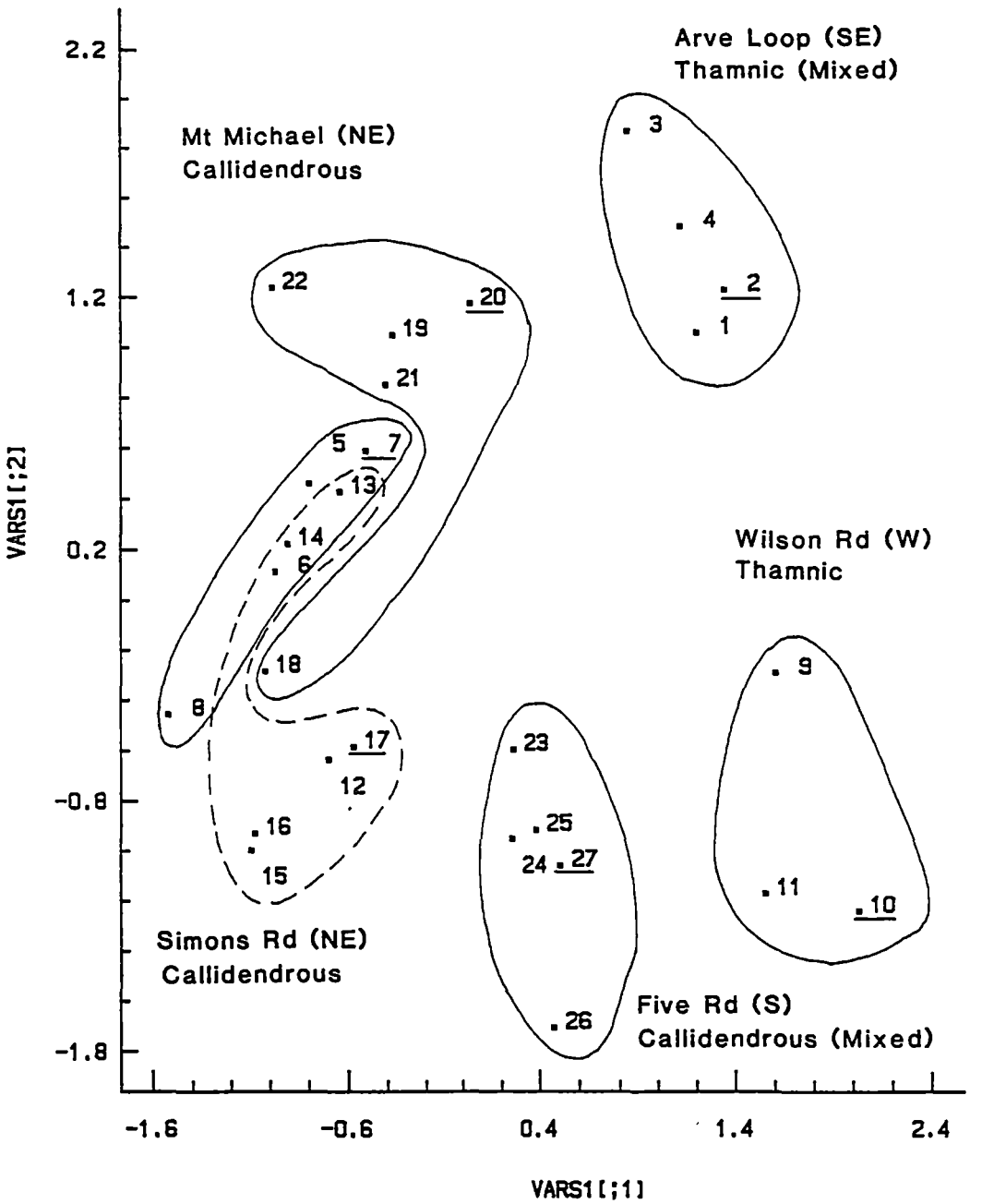


Figure 6.6 Plot of the first set of canonical variables (number 1 against number 2) representing the measured environment of each plot. Control plots are underlined.

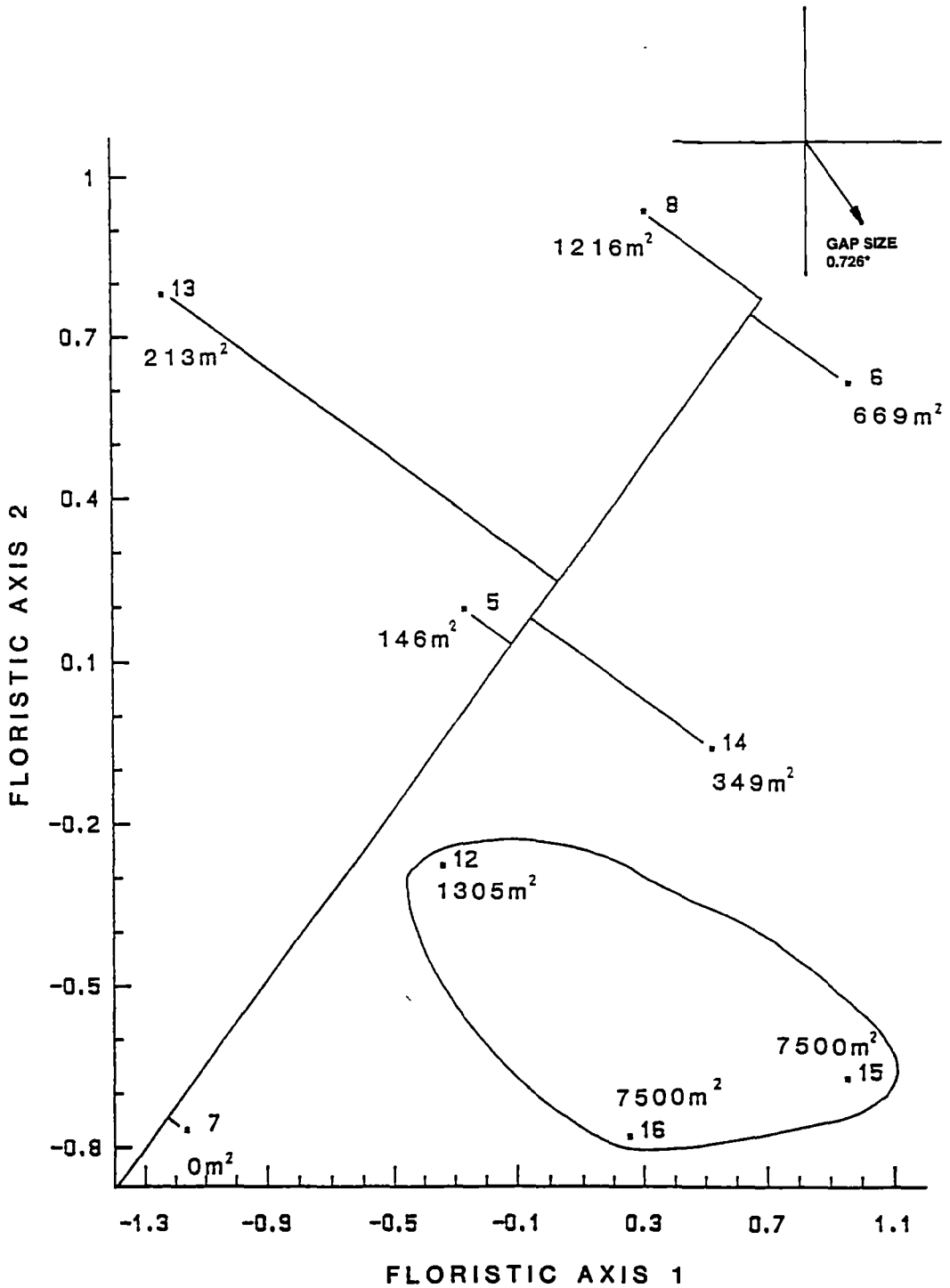


Figure 6.7 Ordination of Simons Road plots showing gap size (control 0 m^2). The direction and magnitude of environmental vectors are shown.

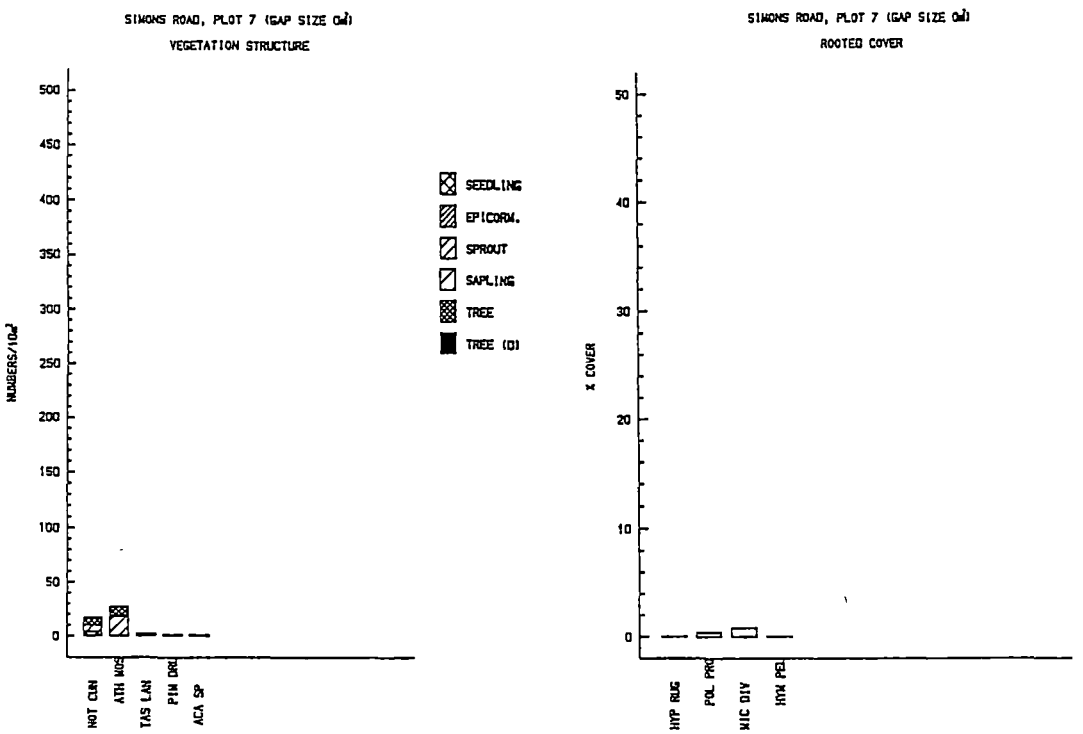


Figure 6.8 Vegetation structure and rooted fern cover of control forest at Simons Road (gap size 0 m^2).

Key to species codes

Aca sp	*Acacia sp.*	Not cun	*Nothofagus cunninghamii*
Ath mos	*Atherosperma moschatum*	Pim dru	*Pimelea drupacea*
Hym pel	*Hymenophyllum peltatum*	Pol pro	*Polystichum proliferum*
Hyp rug	*Hypolepis rugosula*	Tas lan	*Tasmannia lanceolata*
Mic div	*Microsorium diversifolium*		

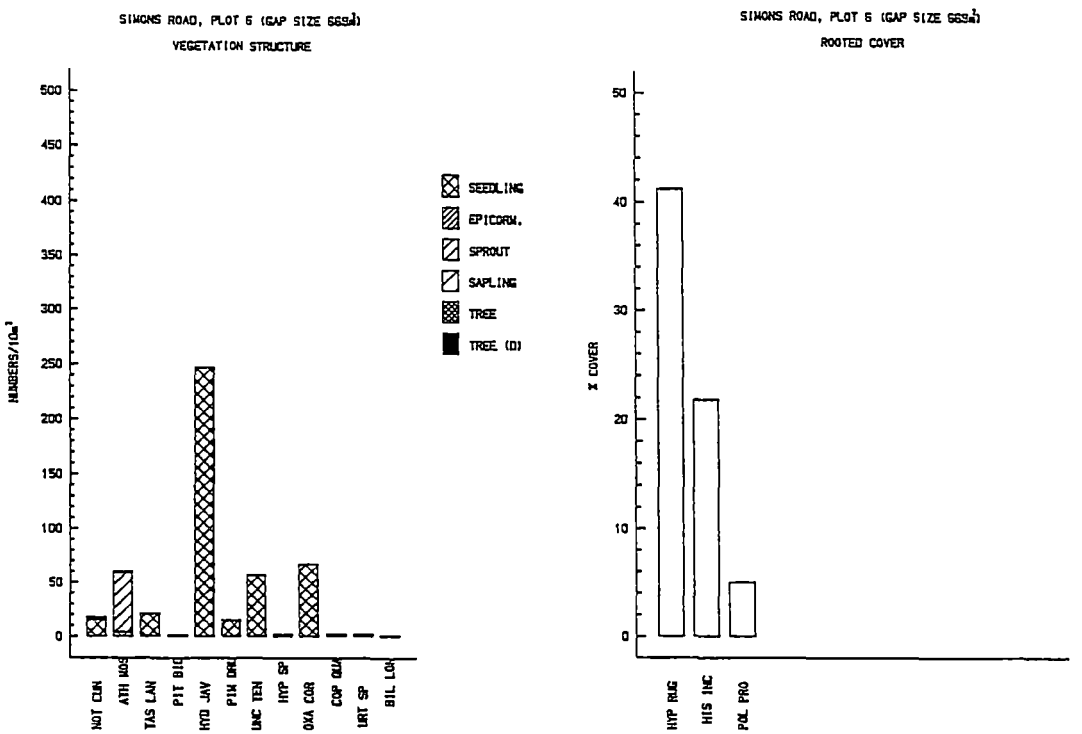


Figure 6.9 Vegetation structure and rooted fern cover of a recent gap at Simons Road (gap size 669 m^2).

Key to species codes

Ath mos	*Atherosperma moschatum*	Oxa cor	*Oxalis corniculata*
Bil lon	*Billardiera longiflora*	Pim dru	*Pimelea drupacea*
Cop qua	*Coprosma quadrifida*	Pit bic	*Pittosporum bicolor*
Hist inc	*Histiopteris incisa*	Pol pro	*Polystichum proliferum*
Hyd jav	*Hydrocotyle javanica*	Tas lan	*Tasmannia lanceolata*
Hyp rug	*Hypolepis rugosula*	Unc ten	*Uncinia tenella*
Hyp sp	*Hypochaeris* sp.	Urt sp	*Urtica* sp.
Not cun	*Nothofagus cunninghamii*		

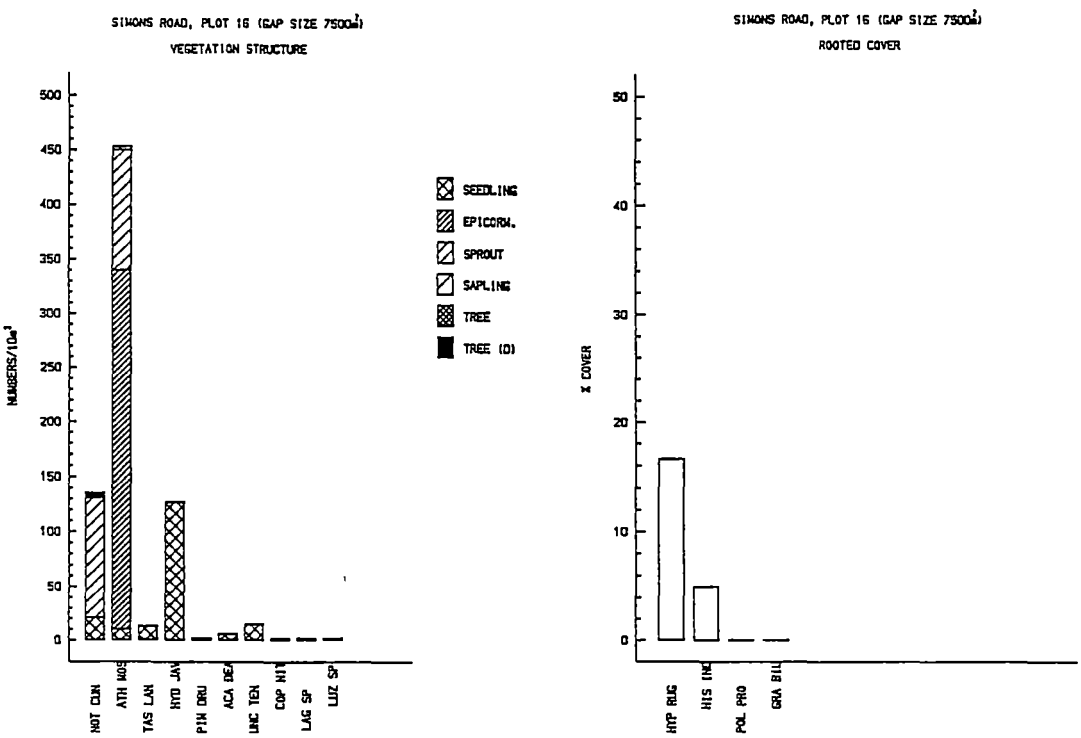


Figure 6.10 Vegetation structure and rooted fern cover of a large old gap at Simons Road (gap size 7500 m^2).

Key to species codes

Aca dea	*Acacia dealbata*	Lag sp	*Lagenifera* sp.
Ath mos	*Atherosperma moschatum*	Luz sp	*Luzula* sp.
Cop nit	*Coprosma nitida*	Not cun	*Nothofagus cunninghamii*
Gra bil	*Grammitis billardieri*	Pim dru	*Pimelea drupacea*
Hist inc	*Histiopteris incisa*	Pol pro	*Polystichum proliferum*
Hyd jav	*Hydrocotyle javanica*	Tas lan	*Tasmannia lanceolata*
Hyp rug	*Hypolepis rugosula*	Unc ten	*Uncinia tenella*

Figure 6.11 Rainforest regeneration in a large, old gap caused by myrtle wilt. Mt Michael (NE Tasmania).

Table 6.5 Myrtle and sassafras regeneration and fern cover in myrtle wilt gaps and in control forest (fern cover transformed to arcsin square root of the percentage). Means (& standard errors)/10 m^2 plot.

	Gaps	Controls	Significance level of ANOVA
Myrtle seedlings	172.8 (60.9)	30.3 (14 9)	0.216 NS
Myrtle vegetative shoots	28 6 (9 1)	102.3 (34.7)	0.006**
Sassafras seedlings	91.0 (37.0)	141.2 (129.1)	0.369 NS
Sassafras vegetative shoots	67.7 (22.5)	32.8 (27.6)	0.450 NS
% Fern cover	26.5 (5.1)	5.6 (1.6)	0.0103*

Table 6.6 shows the effect of site on myrtle and sassafras regeneration and fern cover for both gaps and controls based on the data set given in Appendix 27 and the ANOVA tables in Appendix 28. Five Road in particular had low numbers of myrtle seedlings.

Table 6.6 Myrtle and sassafras regeneration and fern cover at five sites in callidendrous and thamnic rainforest (fern cover transformed to arcsin square root of the percentage). Means (& standard errors)/10 m^2 plot.

	Arve Loop (T)	Simons Road (C)	Wilson Road (T)	Mt Michael (C)	Five Road (C)	Sig. level of ANOVA
Myrtle seedlings	36.5 (20.1)	123.9 (65.5)	29.7 (11.0)	455.8 (174.2)	11.6 (4 7)	0 0315*
Myrtle vegetative shoots	102.0 (50.7)	28.6 (19.0)	56.0 (30.9)	45.0 (14.8)	25.6 (15.2)	0.209 NS
Sassafras seedlings	278.5 (151.8)	2.2 (1.0)	25.7 (1.8)	0.4 (0.2)	308.8 (123.4)	0.0003***
Sassafras vegetative shoots	33.0 (29.0)	68.2 (42.2)	115.7 (57.3)	0.0 (0.0)	92.0 (26.9)	0.496 NS
% Fern	7.6 (3.8)	34.2 (8.4)	5.8 (1.3)	5.9 (1.6)	34.2 (8.6)	0.0071**

Myrtle seedlings were present in all gaps investigated with the exception of one plot at Five Road. In three sites, gaps resulted in increased numbers of myrtle seedlings but at Wilson Road and at Five Road this did not occur (Appendix 27). Seedling and vegetative regeneration of myrtle did not show symptoms of myrtle wilt.

Investigation of myrtle regeneration at Five Road

Myrtle seedlings (including germinants) were present in 65% of the 1 m^2 plots, and in 89% of the 4 m^2 plots. Larger seedlings (> 1 cm high) were present in 36% of the 4 m^2 plots. 75% of plots had a myrtle tree within 5 m, 10% had no myrtle trees, poles or saplings within 5 m, 10% had a myrtle pole within 5 m, and 5% had a sapling within 5 m.

Of the plots with no myrtle seedlings (unstocked plots), 100% of the 4 m^2 plots and 88% of the 1 m^2 plots had myrtle trees, poles or saplings within 5 m. The remaining 12% of unstocked 1 m^2 plots were all within 4 m^2 plots which had myrtle seedlings (stocked plots).

In summary, myrtle (seedling, sapling, pole or tree) was present within 5 m of every plot centre along the transects.

Stocked plots (both 4 m^2 and 1 m^2) were more frequently found in and on the edges of gaps, than under the closed canopy (Table 6.7). However, ANOVAs showed this trend to be non-significant.

Table 6.7 The proportion of stocked 4 m^2 plots located below gaps, gap edges and the closed canopy

	% STOCKED PLOTS		
PLOT SIZE	Gaps	Gap Edges	Canopy
4 m^2	90.5%	91.7%	82.5%
1 m^2	80.0%	75.0%	42.9%

There were significant trends with the substrates on which seedlings were growing (Tables 6.8 and 6.9). Seedlings were most frequently found on the forest floor or on logs (heartwood rotted or bark present), and least frequently found on uproots, mounds or logs (sapwood rotted).

Table 6.8 ANOVA on the relative frequency of different seedling substrates in stocked 4 m^2 plots

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
BETWEEN GROUPS	253.667	5	50.733	13.836	0.0030**
WITHIN GROUPS	22.000	6	3.667		
TOTAL	275.667	11			

Table 6.9 The relative frequency of different seedling substrates in stocked 4 m^2 plots (multiple range analyses show homologous groups based on 95% confidence intervals)

Substrate	% Frequency (mean)	Multiple range analyses
Uproot	1.5%	*
Mound	8.5%	*
Log (sapwood rotted)	8.5%	*
Log (bark present)	13.0%	* *
Log (heartwood rotted)	30.0%	* *
Forest floor	38.5%	*

NB In this and following tables, each **column** of asterisks represents one homologous group.

Future forest composition at Simons Road and Five Road

At Simons Road, a total of 125 dead trees (gap-makers) were sampled along the transects, forming 25 gaps: 115 of these trees were myrtle, seven were *Acacia dealbata* and three were sassafras. Of the 115 gap-forming myrtles, 13 had no evidence of *P. subgranosus* attack: five of these had been felled, four were windthrown and four were stumps which had died of unknown causes (HSC 7).

An investigation of all *P. subgranosus* attacked (presumed *C. australis* infected) gap-forming myrtles (omitting felled trees) revealed that 67 were dead standing (or dying standing), 16 had snapped (leaving stumps) and seven had uprooted (forming rootplates). A t test showed no significant difference between the numbers snapped and uprooted.

The relative proportions of species composing the present forest at different distances from Simons Road, together with those forming and filling gaps is given in Table 6.10.

Table 6.10 Canopy, sub-canopy, gap-maker and potential successor composition at Simons Road

Distance from road	Canopy trees	Sub-canopy trees	Gap-makers	Potential successors (PS)
0-50 m	92% myrtle 8% sassafras	50% myrtle 42% sassafras 8% *A. dealbata*	100% myrtle (76% wilt)	57% *A. dealbata* 23% myrtle 10% no PS 7% sassafras 3% *Eucalyptus* sp.
50-150 m	100% myrtle	58% sassafras 42% myrtle	95% myrtle (93% wilt) 2.5% sassafras 2.5% *A. dealbata*	43% myrtle 40% sassafras 17% no P S
150-250 m	75% myrtle 25% *A. dealbata*	58% sassafras 42% myrtle	76% myrtle (72% wilt) 24% sassafras	43% myrtle 39% sassafras 14% no PS 4% *Pittosporum bicolor*
250-350 m	58% *A. dealbata* 42% myrtle	92% myrtle 8% sassafras	73% myrtle (63% wilt) 20% *A. dealbata* 7% sassafras	53% sassafras 30% myrtle 17% no PS

At Five Road, a total of 79 dead trees (gap-makers) were sampled along the transect, forming 27 gaps: 68 of these trees were myrtle, six were *E. obliqua,* four were sassafras and one was leatherwood. Of the 68 gap-forming myrtles, three had no evidence of *P. subgranosus* attack and had died of unknown causes (HSC 7); two of these were dead (or dying) standing and one was a stump.

An investigation of all *P. subgranosus* attacked (presumed *C. australis* infected) gap-forming myrtles (omitting felled trees) revealed that 55 were dead standing (or dying standing), eight had uprooted (forming rootplates) and two had snapped (leaving stumps). A t test showed no significant difference between the numbers snapped and uprooted.

The relative proportions of species composing the present forest along the transect at Five Road, together with those forming and filling gaps is given in Table 6.11.

Table 6.11 Canopy, sub-canopy, gap-maker and potential successor composition at Five Road

Distance along transect	Canopy trees	Sub-canopy trees	Gap-makers	Potential successors (PS)
0-50 m	75% *E. obliqua* 25% *E. delegatensis*	75% myrtle 25% leatherwood	67% myrtle (100% wilt) 33% *E. obliqua*	100% myrtle
50-150 m	100%*E. obliqua*	75% myrtle 25% sassafras	87% myrtle (87% wilt) 13% *E. obliqua*	47% no PS 33% sassafras 13% myrtle 7% *A. dealbata*
150-250 m	75% myrtle 25% sassafras	75% sassafras 25% myrtle	93% myrtle (86% wilt) 7% *E. obliqua*	64% sassafras 36% myrtle
250-350 m	100% myrtle	100% sassafras	100% myrtle (100% wilt)	67% sassafras 20% no PS 13% myrtle
350-450 m	75% myrtle 25% sassafras	75% myrtle 25% sassafras	77% myrtle (69% wilt) 15% sassafras 8% *E. obliqua*	46% sassafras 46% no PS 8% myrtle
450-550 m	75% myrtle 25% sassafras	25% myrtle 75% sassafras	56% myrtle (56% wilt) 22% sassafras 11% leatherwood 11% *E. obliqua*	89% sassafras 11% leatherwood
550-650 m	50% sassafras 25% myrtle 25% leatherwood	50% myrtle 50% sassafras	100% myrtle (100% wilt)	75% sassafras 25% leatherwood
650-700 m	100% myrtle	50% sassafras 25% myrtle 25% celery top pine	100% myrtle (50% wilt)	100% sassafras

The proportions of the transects at Simons Road and Five Road which fell under canopy and expanded gaps are given in Tables 6.12 and 6.13 respectively.

Table 6.12 The proportion of transects under canopy and expanded gaps at Simons Road

Distance from road	Canopy gap	Expanded gap
0-50 m	87%	99%
50-150 m	42%	68%
150-250 m	33%	49%
250-350 m	47%	73%

Table 6.13 The proportion of the transect under canopy and expanded gaps at Five Road

Distance along transect	Canopy gap	Expanded gap
0-50 m	27%	84%
50-150 m	53%	92%
150-250 m	69%	100%
250-350 m	63%	86%
350-450 m	43%	63%
450-550 m	24%	48%
550-650 m	30%	65%
650-700 m	34%	74%

The percentage of trees of each species established on each substrate type (mean of three transects) at Simons Road and Five Road, are given in Tables 6.14 and 6.15 respectively.

Table 6.14 The proportion of trees of each species established on different substrates at Simons Road (superscripts refer to samples shown by t tests to be significantly different from each other at the 5% level)

Substrate	Myrtle	Sassafras	*A dealbata*	*P. bicolor*
Mound	33.3%	24.1%	31.4%	0%
Forest floor	55.1%[a]	44.8%	68.6%	0%
Log (heartwood rotted)	11.6%[a]	31.0%	0%	0%
Dicksonia antarctica	0%	0%	0%	100%
Total no. of trees	198	58	35	1

Table 6.15 The proportion of trees of each species established on different substrates at Five Road (superscripts refer to samples shown by t tests to be significantly different from each other at the 5% level)

Substrate	Myrtle	Sassafras	*E. obliqua*	Leatherwood
Mound	28.1%[a]	25.7%	52.2%	16.7%
Forest floor	54.4%[ab]	42.9%	47.8%	66.6%
Log (heartwood rotted)	17.5%[b]	31.4%	0%	16.7%
Total no. of trees	103	70	23	6

The percentage of trees in each canopy position established on each substrate type (mean of three transects) at Simons Road and Five Road, are given in Tables 6.16 and 6.17 respectively.

Table 6.16 The proportion of trees of all species in each canopy position established on different substrates at Simons Road (superscripts refer to samples shown by t tests to be significantly different from each other at the 5% level)

Substrate	Canopy trees	Sub-canopy trees	Gap-makers	Potential successors
Mound	45.7%^{b}	33.3%	32.8%^{a}	23.1%^{ab}
Forest floor	37 1%	37.0%	59.2%	57.4%
Log (heartwood rotted)	8.6%	29.6%	8.0%	18.5%
Manfern	0%	0%	0%	0.9%
Total no. of trees	32	27	125	107

Table 6.17 The proportion of trees of all species in each canopy position established on different substrates at Five Road (superscripts refer to samples shown by t tests to be significantly different from each other at the 5% level)

Substrate	Canopy trees	Sub-canopy trees	Gap-makers	Potential successors
Mound	34.4%^{a}	31.2%	28.2%^{c}	28.6%^{d}
Forest floor	62.5%^{b}	46.9%	60.3%^{cf}	31.7%^{f}
Log (heartwood rotted)	3.1%^{abe}	21.9%	11.5%^{cg}	39.7%^{deg}
Total no. of trees	32	32	78	73

6.4 DISCUSSION

Vegetation composition in gaps

Classification of the data gave a broad separation between callidendrous and thamnic forests, with gaps tending towards the callidendrous side and controls toward the thamnic side. *Uncinia tenella* and *Hypolepis rugosula* were the indicator species for the primary division (with all four data sets), characterising gaps in callidendrous forest and also some closed canopy callidendrous forest (at Simons Road).

Hypolepis rugosula was only an indicator species when frequency data was used, indicating that it occurred at higher levels in callidendrous gaps. It was actually present in all gaps and *Histiopteris incisa* was present in all gaps except one. With the exception of one disturbed control plot where both species were present, *H. incisa* was not present in the control plots whereas low levels of *H. rugosula* were recorded in two control plots. Both species have been previously recorded by Jarman *et al.* (1984) at high levels in wilt gaps and disturbed sites in callidendrous forest.

It is evident from the ordinations that there was no distinct set of species which characterised all gaps (or the gap plots would have been clustered around the same place on the graph, regardless of the site in which they were located); instead, the plots were positioned on the graph according to site, and vegetation composition was therefore more dependent upon site differences than on the presence or size of canopy gaps. This was supported by the results of the vector analyses.

A trend was apparent in the ordinations, with controls being specifically located within each site grouping; e.g. in Figure 6.2 they generally had higher scores on floristic axis 1 and lower scores on floristic axis 2 than the gaps. This indicated that gaps were more likely to contain the following species than controls: *Acacia dealbata, Clematis aristata, Dicksonia antarctica, Histiopteris incisa, Hydrocotyle javanica, Hymenophyllum cupressiforme, H. flabellatum, H. peltatum, Hypolepis rugosula, Oxalis corniculata, Polystichum proliferum, Pterostylis longifolia,* and *Uncinia tenella.* Many of these are typical of callidendrous forest (Jarman *et al.* 1984), and this is in agreement with the results of the classification shown above. While some of the species are typical of disturbed sites, and recognised as 'opportunists' within rainforest, introduced species were not found in any of the plots with the exception of two plants of *Hypochaeris* sp. present in a recent gap at Simons Road (Figure 6.9).

Gaps were more likely to contain sassafras and *A. dealbata* than were plots in closed canopy forest, but myrtle showed no strong preference, although it was present in gaps. This agreed with the observations of Mesibov (1977); although myrtle was primarily a 'gap seedling', it was not restricted to gaps. This, and the implications for forest succession, are discussed below, and related to regeneration and forest structure.

The mean gap size of a site proved to be important, in that only in sites with a mean gap size less than 800 m^2 was there any obvious relationship between trends in vegetation and gap size. Presumably this is related to some change in microclimate which occurs in very large gaps or it may be due to relationships between the size and age of gaps and the successional stages within them. A similar threshold gap size was implied by Runkle (1985) who reported significant differences in the response of potential canopy species to differences in gap size for naturally formed gaps of less than 1000 m^2 (and generally less than 400 m^2).

Canonical correlations and vector analyses indicated the relative strengths of the measured environmental variables in relation to trends in vegetation composition (as defined by the ordinations). Altitude was the most important single environmental correlate (presumably affecting various unrecorded environmental factors), but slope, depth of litter and diffuse light all had strong effects as related to the different ordination axes. Aspect only had a strong influence when frequency data including germinants was used (Table 6.4), indicating a correlation between aspect and species *abundance* (as compared to species composition). Gap size was, relatively speaking, weakly correlated with trends in vegetation composition. Veblen (1989b) showed the importance of altitude and forest type in determining the size of gap necessary for *Nothofagus* regeneration in South America.

A plot of the first set of canonical variables (environmental values) against the second set (vegetation trends) ordered sites according to decreasing altitude along the y axis (Figure 6.5). These sites were also ordered according to forest class along the x axis (from callidendrous through mixed to thamnic forest). Thus the callidendrous sites used were at higher altitudes than the mixed and thamnic sites, and many of the trends in vegetation composition noted with altitude, may primarily be due (at least in the proximate sense) to forest type.

The ordering of these sites along an east-west gradient (Figure 6.5) probably reflects the north west/south east boundary between the thamnic and implicate rainforest in the west of Tasmania, and the callidendrous rainforest in the east (Jarman *et al.* 1984). Studies in

South American forests showed that for a given tree species, responses to gaps may vary along an environmental gradient, with changes in associated tree and understorey species (Veblen 1989b).

A plot of the environmental values (Figure 6.6) showed that the sites were all different in terms of the measured environmental variables. The exceptions were Simons Road and Mt Michael which had very similar environmental profiles. It is interesting that they also both supported high altitude callidendrous forests C2.1 (Jarman *et al.* 1984, 1991).

Vegetation composition in large old gaps at Simons Road

The relationship between the age and size of myrtle wilt gaps was first observed by Howard (1973a), and this was supported by studies on disease spread via root grafts (Figure 5.4). The ordination and vector analysis of Simons Road data alone (Figure 6.7) indicated that within this site, trends in vegetation composition were generally related to gap size.

With the smaller gaps, this relationship was clear; however, the large, old gaps did not fit the pattern and were more similar in floristic composition to the surrounding forest than they were to the smaller gaps. With these large, old gaps, it is possible that any secondary succession which occurs within expanding gaps had come almost full cycle, with the vegetation composition reverting to that of the surrounding forest. This would be in accordance with the reported self replacing nature of Tasmanian rainforest (Ellis 1985; Read and Hill 1985a).

Since myrtle seed dispersal can be a limiting factor in rainforest regeneration (Hickey *et al.* 1982), and in view of the high proportion of dead myrtles present, it is of interest that two gaps (plots 15 and 16) situated in the area of roadside disturbance shown in Figure 4.10, were very similar to the surrounding forest in terms of floristic composition.

Regeneration and vegetation structure in gaps

Vegetation structure in gaps and control forest may be very different; in particular, myrtle and sassafras regeneration was increased in gaps, and in the large old gap there was an increase in vegetative shoots of these species. The transect studies at Simons Road indicated that for most gaps there was a sub-canopy tree in a position to fill them, but where there was no potential successor or where wilt was still active, regeneration would become important. At Simons Road there is evidence that not only seedlings but vegetative regeneration may be important in filling large old gaps.

Numbers of myrtle vegetative shoots were significantly decreased in gaps while seedling regeneration tended to increase (although not significantly). This agrees with the findings of earlier workers (Read 1985; Read and Hill 1985a, 1985b, 1988). However the lack of vegetative myrtle shoots in gaps may be due in many cases to the lack of the live myrtle trees needed to produce them. Fern cover also increased significantly in gaps but in general was not eliminating seedlings. Seedling numbers and fern cover both differed significantly between sites (Table 6.6).

The failure of gaps at Wilson Road to give rise to increased numbers of myrtle seedlings is partially explicable. At Wilson Road (thamnic rainforest) the gap size was small and a dead myrtle (evidence of an old gap) was found even in the control plot, so minimal differences between gaps and the control might have been expected.

However at Five Road the situation is less clear. At Five Road (mixed callidendrous) very large wilt gaps occurred, possibly due to damage from the occasional eucalypts which overtopped the rainforest. The canopy at Five Road was rather broken and the diffuse light measurement of control forest was the highest of all the sites (Appendix 26).

Although myrtle seedling numbers were comparatively low at Five Road, more myrtle regeneration took place in the control forest, and the effect of gaps was not obvious. Sassafras seedlings showed a similar trend with higher numbers in the control. Fern cover was lower in the control and at high levels in the gaps, and could have been out-competing seedlings, although this was not evident in other sites. In contrast to sassafras, no myrtle *saplings* were present in gaps but only in the control (Appendix 27). This agrees with the observations of Mesibov (1977); myrtle regenerates reliably on fern-poor ground under a break in a well-closed canopy, but not often under an open, species diverse and multi-levelled canopy (nor in gaps under an understorey of mainly short trees).

At Five Road sassafras appeared to be regenerating in gaps more successfully than myrtle, although some myrtle seedlings were present in all gap plots except one. In this plot there were only myrtle germinants, whereas sassafras was present as germinants, seedlings, vegetative shoots, saplings and trees. The presence of myrtle germinants indicates that the problem was one of establishment and survival rather than of seed dispersal.

However, in most cases studied myrtle wilt gaps did not result in any decrease in the number of myrtle seedlings. Usually myrtle seedling numbers were increased in gaps regardless of the increased fern cover and the myrtle regeneration did not appear to be infected by wilt.

Investigation of myrtle regeneration at Five Road

The apparent paucity of myrtle regeneration at Five Road, i.e. in large gaps in mixed forest having a callidendrous fern understorey (C1.1 - Jarman *et al.* 1984, 1991) may be exceptional, although high levels of wilt and large gaps do appear to be likely in such sites (Chapter 3). Additionally, the nearby Florentine Valley was the location where sassafras was predicted to succeed myrtle (Gilbert 1959). Thus, further investigations of the regeneration status of myrtle were necessary to elucidate the dynamics and possible outcome of succession in such areas.

Transect surveys at Five Road showed that 89% of 4 m^2 plots contained myrtle seedlings or germinants. Although this exceeds the 80% minimum acceptable level recommended for multi-aged stands following harvesting (Forestry Commission 1991), the standard is based on eucalypt forests, and its application to myrtle forests is unknown. However, 36% of 4 m^2 plots contained established myrtles (> 1 cm high), and it can be concluded that numerous regeneration sites occurred along the transects.

The effect of gaps on seedling establishment was again found to be non-significant, although there was a trend towards establishment in gaps and on gap edges (Table 6.7). At Five Road, the preferred substrates for myrtle seedling establishment were the forest floor and logs (heartwood rotted); the least suitable substrates being uproots (crater and mound). Although large gaps with a dense fern cover can be virtually barren of tree seedlings, rotting wood is known to provide a regeneration niche at such sites (Mesibov 1977). At Five Road rotting logs were an important substrate for myrtle seedlings, and despite the presence of large ferny gaps, myrtle regeneration was well distributed through the site.

Future forest composition at Simons Road and at Five Road

Causes of gaps

Transect studies at Simons Road and Five Road indicated that at both sites myrtle was the most important gap-forming species, with wilt being the most frequent cause of canopy gaps.

At Simons Road myrtle dominated the site, and its representation as a gap-maker was not much greater than as a canopy tree (Table 6.13). This agrees with the observations of Rowberry (1979) that myrtle downers (i.e. trees which have fallen down) are found in proportion to their occurrence as live standing trees. He also noted that in comparison with

myrtle, sassafras decomposes faster and rarely falls over, usually decaying whilst standing. This could have led to a slight underestimate of sassafras as gap-makers.

At Five Road the representation of myrtle as a gap-maker was disproportionately high. In parts of this site, senescent *Eucalyptus obliqua* and *E. delegatensis* overtopped a mature rainforest canopy, and the damage caused by falling eucalypt branches possibly precipitated the high myrtle wilt levels (Chapter 3). Since each eucalypt has the potential to damage a number of myrtles below it, this could also account for the high proportion of myrtle as gap-makers.

At Simons Road and Five Road the numbers of myrtle deaths due to windthrow and unknown causes were relatively unimportant. Most myrtle gap-makers remained dead standing, and there was no overall trend towards either snapping or uprooting in the remainder. The exposed mineral soil often favoured by myrtle regeneration (Mesibov 1977) is only produced by uproots, and Mount (1979) has suggested even this may be minimal in the case of wilt-killed trees.

Area under gaps

The proportion of transects under expanded gaps at Simons Road (away from the road edge), varied from 49% to 73% (Table 6.15). At Five Road the range was 48-100% (Table 6.16). This compares with values of 9-77% in North American spruce, fir, birch and other hardwood forests (Worrall and Harrington 1988), and 9-11.6% in the lowland rain forests of Chiloe Island, Chile (Veblen 1985b).

Specifically compared to other *Nothofagus* forests, Simons Road and Five Road had very high proportions of land area under expanded gaps. In South America, values ranged from 13.3% (montane *N. dombeyi* forest) to 26% (mixed *N. dombeyi*/conifer forest) to 29%(evergreen *N. dombeyi* rainforest with some *N. antarctica*) (Veblen 1985b, 1989a).

The importance of substrate

At Simons Road, there were no significant differences between species in their relative distributions between the different substrates (Table 6.14). Myrtle was significantly more likely to be found on the forest floor than on old logs. Both canopy trees and gap-makers (of all species) were significantly more likely to be established on mounds than were potential successors (Table 6.16). Since mounds are formed by uproots or by very rotten stumps it is likely that many of the trees forming the present canopy established on the exact sites of their predecessors. The fact that the present potential successors are not following the

same pattern is probably due to the fact that most of them are sassafras and actually unlikely to ever reach the canopy (see below).

At Five Road also, there were no significant differences between species in their relative distributions between the different substrates (Table 6.15). Myrtle was more frequently found growing on the forest floor than on mounds or old logs. This finding reflected that of myrtle seedlings (Table 6.9). Sassafras was also more frequently found growing on the forest floor than on mounds.

Canopy trees (of all species) were more frequently found on the forest floor or mounds than on old logs (Table 6.17). Gap-makers were most frequently found on the forest floor, and least frequently found on old logs. However, potential successors were more frequently found on old logs than on mounds. In general old logs were more likely to support potential successors than canopy trees or gap-makers, whereas the forest floor was more likely to support gap-makers than potential successors. Again, this is probably partly due to the fact that most of the potential successors were sassafras; it is also likely that the old logs rot down to mounds as potential successors become canopy trees and, eventually, gap-makers.

Simons Road - the effect of disturbance

Table 6.10 shows the canopy, sub-canopy gap-maker and potential successor composition at different distances from Simons Road. Along the road edge (0-50 m) some myrtle canopy trees had been felled and in the remainder there was a very high mortality due to wilt, as shown in Figures 4.5a and 4.10. Sassafras was present in the canopy but this was only where all the taller myrtles had died or been felled. *A. dealbata* regeneration was abundant with some eucalypts and these species are likely to form the next roadside canopy, and to be eventually succeeded by the longer lived myrtle.

Further from the road (50-250 m) and for the larger part of the transects, myrtle-dominated the canopy and sassafras was the main sub-canopy tree. Gaps were formed mostly by dead myrtles but sassafras was an important potential successor. It is known, however, that on fertile sites, sassafras does not compete well enough with myrtle to actually reach the canopy (Read 1985; Read and Hill 1985a) and autogenic replacement of myrtle by sassafras is most unlikely, having only ever been recorded at one site (Gilbert 1959). The browsing of sassafras in preference to myrtle (Hickey 1982a) may make it even less likely. Given the absence of other potential successors, myrtle will probably be self replacing in this area.

The area 250-350 m from the road was possibly the site of a fire about a century ago, and an *A. dealbata* canopy overtopped a sub-canopy of myrtle poles. Myrtles were the most important gap-makers, with some *A. dealbata* probably coming to the end of their lifespan. Although the main potential successor again appeared to be sassafras, followed by myrtle (*A. dealbata* was not regenerating under the closed canopy), in practice myrtle is likely to succeed *A. dealbata* at the expense of sassafras due to its higher growth rates in high, unfiltered light (Read 1985, Read and Hill 1985a). Mount (1979) classified such stands as mixed forest and predicted that in the event of another fire, *A. dealbata* would regenerate from ground stored seed, and myrtle from seed on the trees.

At Simons Road, with the exception of the immediate roadside area, I predict that myrtle will be self replacing and there is little reason to suggest that myrtle wilt will lead to any permanent change in forest composition.

Five Road - mixed forest

Mixed forests represent a transition in space or time (cline, sere or disclimax) between the rainforests and the wet sclerophyll (*Eucalyptus*) forests into which they grade, and in Tasmania they typically occupy sites with an intermediate moisture availability or fire frequency (Cameron 1992, Duncan and Packham 1994). According to Jackson (1968) areas with a fire frequency of 150-200 years carry mixed forest, but if mixed forest remains unburnt for 400 years it is converted to pure rainforest. This is due to there being no regeneration of eucalypts between fires, while the replacement of rainforest trees is slow and continuous. The first 150 m of the Five Road transects illustrates this point; although the eucalypts were represented as canopy trees and gap-makers, they were not potential successors. The age and fire history of this site meant that the senescent eucalypts were damaging a mature and relatively even aged myrtle sub-canopy. In such a situation, high levels of myrtle wilt and relatively low numbers of myrtle potential successors could be expected.

Within the mixed forest/rainforest dynamic there is also the possibility of a change in the composition of the rainforest component. Gilbert (1959) predicted that sassafras would autogenically replace myrtle in rainforest and mixed forest in the Florentine valley, there being poor myrtle regeneration, whereas sassafras was well represented in the smaller size classes. Although this site has now been cleared (Read and Hill 1985a), the nearby Five Road site appears to have many similarities to it, particularly the 150-450 m, and 650-700 m sections, where sassafras was the main potential successor.

For the reasons given above sassafras may not reach the canopy, myrtle being the next most important species. However, for much of this section there were no trees large enough to be clear potential successors. In such cases potential successors would eventually emerge from the gap regeneration; myrtle being favoured over sassafras. Also, since the longevity of sassafras (250 years) is much less than that of myrtle (500 years) (Read and Hill 1981), a permanent change in canopy composition would be unlikely unless all the myrtle seed trees were lost.

Noble and Slatyer (1978, 1980) used 'vital attributes' (e.g. dispersal, persistence, shade tolerance, time to maturity, longevity) of Tasmanian mixed forest species, and predicted that exclusion of fire for 400 years would lead to the loss of *Eucalyptus* spp., leaving rainforest. If this remained undisturbed for a further 100 years sassafras was predicted to succeed myrtle. Since observations show that most such stands have a mixture of myrtle and sassafras, they proposed that the presence of myrtle was due to its ability to establish after some type of disturbance.

Read and Hill (1985a) concluded that the persistence of myrtle in lowland temperate rainforest in Tasmania was due to its ability to regenerate in canopy gaps created by the fall of old trees. However, they reported that death due to myrtle wilt was usually associated with roading and logging disturbances. Further work by Elliott *et al.* (1987) conclusively showed that for undisturbed Tasmanian sites, myrtle wilt is the main cause of myrtle deaths (including canopy trees). In later work in undisturbed sites, Read and Hill (1988) concluded that the effect of myrtle wilt gaps on the differential establishment of species was uncertain, but was probably determined by site characteristics controlling the rate of litter decomposition (since branch litter from dying trees appeared to retard seedling establishment).

The present work corroborates these findings; myrtle wilt being the main gap forming process in both the sites investigated. Many of these gaps were large, presumably formed by the spread of the disease via root grafts. Although depth of litter was significantly correlated with trends in vegetation composition, in most instances myrtle wilt gaps gave rise to increased myrtle regeneration. Given the fact that large gaps favour myrtle over sassafras regeneration in callidendrous rainforest (Read and Hill 1985a), myrtle wilt adequately fulfils the role of the disturbance predicted by Noble and Slatyer (1978, 1980) to be necessary for the persistence of myrtle. This assumes at least some history of association between the disease and these forests, which may well be the case, there being no good evidence for the recent introduction/evolution of the disease (Chapter 2).

Leatherwood, an important component of many thamnic rainforests (Jarman *et al.* 1984, 1991), has a similar photosynthetic response to myrtle in full sunlight (Read 1985), and is a possible competitor with myrtle regeneration in large gaps (Read and Hill 1985a). Leatherwood (with sassafras) was found to be a potential successor to myrtle in the 450-650 m section of the Five Road transect.

The dominance of myrtle decreases in thamnic rainforest (Jarman *et al.* 1984, 1991), and myrtle wilt gaps tend to be smaller (Table 6.1), and presumably receive less direct sunlight. In intermediate light levels myrtle is known to have a higher photosynthetic response than leatherwood (Read 1985), which may explain its persistence in the smaller gaps in thamnic forest. Ordination results indicated that gaps generally had less leatherwood than control forest, so there is little reason to suppose that myrtle is likely to be out-competed in such situations. *Anodopetalum biglandulosum* (horizontal), another thamnic rainforest species, is known to invade gaps, but because standing dead myrtles cause little disturbance, it does not invade myrtle wilt gaps (Barker and Brown 1994). Implicate rainforest, by definition has a broken canopy and myrtle wilt gaps are not discrete.

In summary there is little reason to suppose that there will be any long term change in the proportions of myrtle and sassafras at Five Road. In some areas, however, myrtle wilt has caused such large gaps that it may take a further 50 years or so before an identifiable canopy reforms. Because of the senescent eucalypts and myrtles there is a high fuel load on the site, and the possibility of another fire cannot be discounted.

6.5 SUMMARY

The effect of canopy gaps caused by myrtle wilt on callidendrous and thamnic rainforest floristics was studied at five sites. Classification, ordination, canonical correlations and vector analysis of vegetation and environmental data indicated that gaps were more 'callidendrous' in nature than the surrounding forest, but that there was no distinct set of species which characterised all gaps. Whilst gap size (up to 800 m^2) may have an effect, it is relatively weakly correlated with trends in vegetation composition, site differences (described by other environmental variables) being the more important correlates.

In most cases studied, gaps in the canopy caused by myrtle wilt resulted in increased numbers of myrtle seedlings and decreased numbers of vegetative shoots, compared with control forest. Fern cover also increased in gaps but in general was not eliminating

seedlings. One undisturbed site (callidendrous fern mixed forest) had very high levels of myrtle wilt and large canopy gaps. Although myrtle regeneration was not significantly higher in gaps than under the (broken) canopy, it was well distributed through the forest. This implied that seed sources and establishment sites were not severely limiting factors, and no major change in forest composition is anticipated.

Ordination of data from one callidendrous site indicated that the vegetation composition of large, old gaps was more similar to the control forest than to that of smaller and more recent gaps. Thus, the vegetation composition in the large, old gaps appeared to be reverting to that of the surrounding forest.

Transect studies at two callidendrous sites indicated that myrtle was the main gap causing species, with myrtle wilt being the most important gap forming agent. The proportion of forest area under gaps was high compared to comparable forest types, many gaps being extensive and presumably formed by the spread of the disease via root grafts. Since large gaps are known to favour myrtle over sassafras regeneration, in undisturbed forest, myrtle wilt appears to facilitate the regeneration process of myrtle.

7. THE DECLINE AND FALL OF THE HOLEY RAINFOREST EMPIRE?

7.1 INTRODUCTION

In the previous chapters various aspects of myrtle wilt have been investigated. The present chapter aims to synthesise some of the results from this work, in order to assess the long term significance of the disease for the myrtle population.

In Tasmania, the average mortality due to myrtle wilt is now estimated to be 0.61% p.a. (Chapter 3). It is possible that due to site variations and to myrtle's ability to persist and grow very slowly under a closed canopy, this mortality rate may be compatible with the currently observed longevity of myrtle; i.e. up to 500 years (Read and Hill 1981; Runkle 1985). However, in diseased areas, the survival of myrtle is dependent on seedling regeneration, coppice regeneration also being killed by myrtle wilt (Howard 1981); also it is the larger, seed producing myrtles which are most susceptible to wilt (Elliott *et al.* 1987). Thus, the probability of survival of sufficient 'seed trees' to ensure the long-term continuity of the myrtle population, needed to be determined.

7.2 MATERIALS AND METHODS

A simple predictive model was constructed using an EXCEL spreadsheet (Microsoft Corporation 1992). This applied differential mortality rates (due to myrtle wilt) to the different size classes found in a typical uneven-aged distribution of myrtle. The model was run over a number of 60-year-periods, during which time myrtle wilt (at different levels), other mortality factors, growth and either self thinning or regeneration were applied, with survivors growing up into the next size class. Appendix 29 illustrates how the model applied average levels of myrtle wilt to a starting population at time $t = n\ (= 0)$, and calculated the resultant population at time $t = n+1$.

Assumptions and limitations of the model

Plot size

The model followed the fate of myrtles located in a hypothetical plot of callidendrous rainforest, in which myrtle was the dominant species. The plot was circular, and had an area of 1250 m^2 (0.125 ha), which was comparable to the size of the largest myrtle wilt gap found in *undisturbed* forest (Figure 6.7, Simons Road plot 12). The plot was therefore assumed to be representative of a larger myrtle population; consequently, tree numbers did not necessarily have to be integers.

The plot radius was approximately 20 m. Hickey *et al.* (1982) found that most *N. cunninghamii* seed falls within 20 m of the parent tree, although some viable seed may be transported up to 40 m by wind. Thus the assumption was made that one surviving 'seed tree' would be sufficient to provide seed for the entire plot. Although relatively young myrtles (30 years old) are known to produce seed in lowland areas (Read and Hill 1981), silvicultural practice requires seed trees to be 'well crowned' (Hickey and Felton 1991). Thus, only myrtles with a diameter at breast height (DBH) of greater than 55 cm were designated as seed trees.

Size class distribution and stand basal area

The model assumed a starting population of 100 myrtles, distributed over nine size classes from 0-175 cm DBH. The starting size class distribution was reverse J shaped (negative exponential), typical of continuously regenerating populations with a constant mortality rate (Read and Hill 1985a), and reflecting the situation in the absence of size specific mortality factors. The resultant stem density of 400 myrtles/ha, and the myrtle basal area of 83 m^2/ha (both based on myrtles >15 cm DBH), were comparable to the estimates given by Elliott *et al.* (1987) for the callidendrous rainforest at Simons Road (397 myrtles /ha and 95.1 m^2/ha). The basal area of myrtles on the plot was thus 10.37 m^2 (83 m^2/ha x 0.125 ha).

Since, at a given site, there is generally a good relationship between myrtle size and age (Read and Hill 1985a), it was assumed that all the trees in one size class (e.g. 15-35 cm DBH) would grow into the next size class (e.g. 35-55 cm DBH) within one period of 60 years. This represented an average annual growth increment of 0.33 cm DBH/year, which is probably representative of the growth of dominant myrtle trees in good quality callidendrous rainforest (J. E. Hickey, personal communication). It was assumed that young regeneration (0-15 cm DBH) would make slightly faster growth (0.4 cm DBH/year) and move into the next size class within one growth period.

Competitive interactions with other species were not taken into account, as there is little evidence that myrtle is out-competed in gaps caused by myrtle wilt (Chapter 6). Thus, it was assumed that the basal area of myrtle on the plot would remain constant (at 10.37 m^2), *provided that a seed source was present.*

Mortality due to myrtle wilt

Annual mortality rates due to myrtle wilt for each size class (>15 cm DBH) were calculated using the survey data of Elliott *et al.* (1987). For each size class the proportion of myrtles in health status classes (HSC) 3 and 4 were given. Across all sites, trees of HSC 3 and HSC 4 were shown to occur in a ratio of 3:4. Thus, for each size class the proportion of myrtles in HSC 4 (currently dying due to myrtle wilt) could be calculated. Since it was known that myrtles spend an average of 2.6 years in HSC 4 (Chapter 3), to arrive at an estimate of the annual mortality rate due to myrtle wilt for each size class, the proportion of myrtles in HSC 4 was divided by 2.6.

Because the occurrence of the disease in young myrtles is relatively rare (Howard 1973a, 1981; Elliott *et al.* 1987; Kile and Walker 1987), the annual mortality rate due to myrtle wilt in the smallest size class (0-15 cm DBH) was assumed to be zero.

Mortality due to causes other than myrtle wilt

Read and Hill (1985a) found that the size distributions of several myrtle populations were best fitted by a power function, which implied that the mortality rate decreased with increasing population age. They concluded that this was due to the high degree of competitive thinning experienced by myrtle seedlings establishing in canopy gaps.

An estimate of the total annual mortality rate for the 0-15 cm size class was obtained from a measurement plot established in an area of myrtle regeneration which had arisen following logging of callidendrous/thamnic rainforest (Jennings 1989d; S. Jennings, unpublished data). The average mortality rate of 42 myrtles (<15 cm DBH), measured over three years was estimated as 4.396% p.a.

For trees in size classes 15-155 cm DBH, mortality due to causes other than myrtle wilt, was derived from the Arve Loop and ROS plots data (Appendix 8). For each site, the annual mortality rate was calculated from the increase in the numbers of myrtles in HSC 7 (dead, but with no evidence of *Platypus subgranosus* attack) and HSC 10 (dead and down, and not previously attacked by *P. subgranosus*) between the first and last measurements. It was assumed that all HSC 10 trees were still identifiable at the last measurement. The mean value for the six sites was 0.152% p.a.

All surviving myrtles were assumed to have died of old age by 540 years. Thus the total mortality for the 155-175 cm DBH class during one 60 year period was 100%; some of this was due to myrtle wilt, the remainder (96.0%) was attributed to 'other causes'.

60 year survivorship

For each size class, the proportion of myrtles which survived the mortality factors operating in each 60 year period was given by:

60 year survival rate = (1 - total annual mortality rate)^{60}

Running the model

Mortality x Growth

Using the starting population, the mortality factors were applied over a 60 year period, and the surviving myrtles were 'grown on' into the next size class. The new basal area of the stand was then calculated, and subtracted from that of the original stand to obtain the current 'basal area status'.

Regeneration

If the resultant basal area status was positive, and if there was at least one seed tree present, then the 'spare basal area' was filled with the appropriate number of myrtles of the smallest size class (i.e. until the basal area status was reduced to zero).

Self thinning

If the basal area status was negative, self thinning was applied. This commenced with the smallest size class (assumed to be the poorest competitors) and continued sequentially to the larger size classes, until sufficient myrtles had been removed to bring the basal area status back to zero.

Iteration and graphical display

At time ***t = n+1***, after mortality, growth, regeneration or self thinning, the resultant population was calculated, and the size class structure displayed graphically, together with that of the population at time ***t = 0***. The model was then applied to the new population over another 60 year period. This was continued for 27 such periods (i.e. 1620 years, or three myrtle lifespans).

Modelled annual mortality rates

During the running of the model, the average annual mortality rate (all causes) was calculated at the end of each growth period, both for the total myrtle population and for myrtles >15 cm DBH. Because the time periods were so long, the procedure for estimating annual mortality rates by dividing the measured mortality rate by the number of years over which it was measured, was not followed in this instance. A more accurate estimate of the annual mortality rate was derived from the equation above, and is given by:

$$\textbf{annual mortality rate} = 1 - \sqrt[60]{\frac{x_{60}}{x_0}}$$

where x_0 was the original number of myrtles, and x_{60} the number surviving after 60 years.

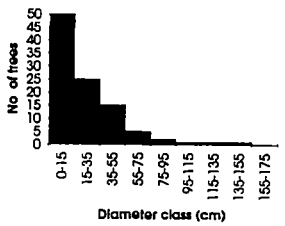
Three scenarios

The first scenario applied the average levels of myrtle wilt as described above (illustrated in Appendix 29). The second scenario applied double the average annual mortality rates due to myrtle wilt. The third scenario assumed that there was no mortality due to myrtle wilt. The three scenarios were each run for 27 periods of 60 years. At the end of each period, the number of trees in the 155-175 cm class, the DBH of the largest surviving myrtle and the *modelled* per annum mortality rates (total, and for trees >15 cm DBH) were recorded.

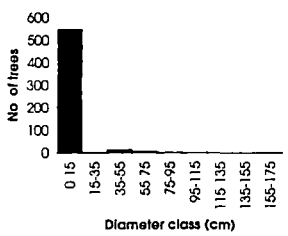
7.3 RESULTS

With both average and high levels of myrtle wilt, myrtles of the largest size class still occurred after 1620 years. However, with high disease levels the number of myrtles in the largest size classes was reduced. Minimum (modelled) mortality rates in particular were increased with high levels of myrtle wilt. With no myrtle wilt, the model predicted the extinction of myrtle after 840 years; the aging population using all the available basal area. The results are summarised in Table 7.1 and illustrated graphically in Figure 7.1.

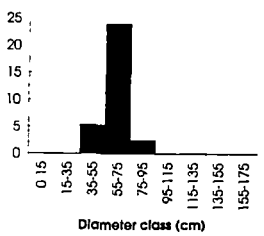
STARTING POPULATION



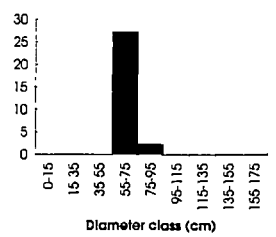
60 YEARS



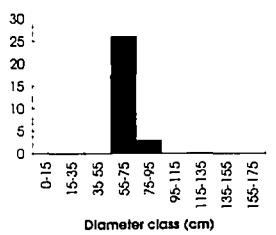
540 YEARS



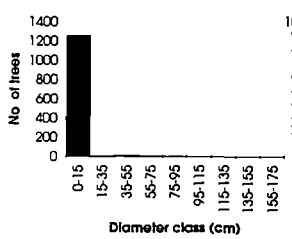
1080 YEARS

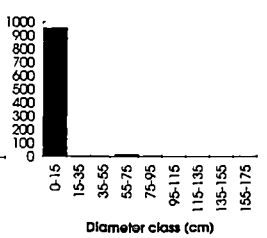


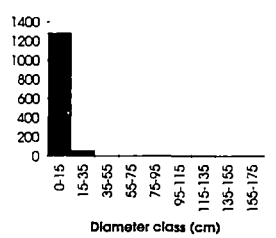
1620 YEARS

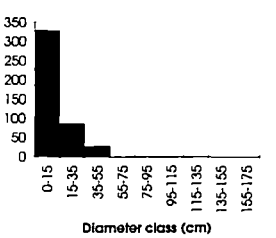


AVERAGE MYRTLE WILT LEVELS

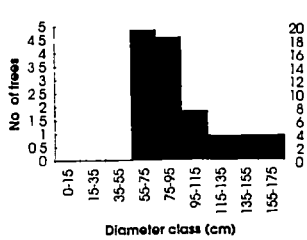


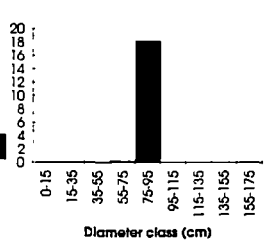


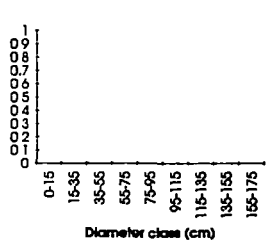


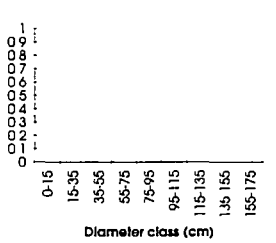


HIGH MYRTLE WILT LEVELS









NO MYRTLE WILT

Figure 7.1 Model predictions for a myrtle population, applying different levels of myrtle wilt.

Table 7.1 The effects of different myrtle wilt levels on mortality rates, and on the size and number of the largest surviving trees on the 1250 m^2 plot (ranges of values are given for each 540 year period)

	0-540 years	540-1080 years	1080-1620 years
AVERAGE MYRTLE WILT			
No. of trees at 155-175 cm	$9.59x10^{-5}$ - $6.13x10^{-2}$	0 - $1.70x10^{-3}$	0 - $1.92x10^{-3}$
DBH of largest myrtle	175 cm	135-175 cm	135-175 cm
p.a. mortality (total)	0.8-4.1%	0.9-4.3%	0.9-4.3%
p.a. mortality (>15 cm)	0.7-1.5%	0.7-2.1%	0.7-2.4%
HIGH MYRTLE WILT			
No. of trees at 175 cm	$3.57x10^{-9}$ - $3.63x10^{-3}$	0 - $5.90x10^{-8}$	0 - $8.81x10^{-7}$
DBH of largest myrtle	175 cm	135-175 cm	135-175 cm
p a. mortality (total)	1.7-4.3%	1.8-4.4%	1.7-4.3%
p.a. mortality (>15 cm)	1.2-2.9%	1.2-2.9%	1.2-2.8%
NO MYRTLE WILT			
No. of trees at 175 cm	0 -3.19	0 - 4.85 (until extinction)	
DBH of largest myrtle	55-175 cm	95-175 cm	
p.a. mortality (total)	0.2-4.3%	0.2-100.0%	
p.a. mortality (>15 cm)	0.2-2.3%	0.2-100.0%	

7.4 DISCUSSION

This simplistic model does not generate the uneven-sized stand structure often observed in Tasmanian rainforests (Read and Hill 1985a, Elliott *et al.* 1987). Probable reasons for this include the fact that the model only allows mortality and regeneration at 60-year intervals, does not take into account spatial effects (e.g. large gaps where seedlings survive because there *are* no large competing trees), and ignores the existence of the myrtle 'seedling bank' (suppressed regeneration which can survive for a long period under a closed canopy). Despite these limitations, the model clearly predicts that at current average levels, myrtle wilt will not cause the extinction of myrtle; nor will it permanently eliminate the largest size classes from callidendrous rainforest.

When compared with actual field data, the small numbers of trees predicted for the larger size classes appear to be fairly realistic. Population size structures given by Read and Hill (1988), showed that myrtles greater than 155 cm DBH, were found at only one of their 16 rainforest sites (Ballroom Forest, north-west Tasmania). The presence of large *Arthrotaxis selaginoides* (slow growing and fire-sensitive) stems at this site, indicates the absence of fire in the recent past, and this is one explanation for the presence of large myrtles. However, at this high altitude site, myrtle wilt incidence was also likely to be low (Elliott *et al.* 1987), which provides another explanation for the survival of large myrtles, and is in general agreement with the modelled results.

Higher levels of wilt do reduce the numbers of larger trees, and if extremely high levels of the disease occurred continuously over very long periods, one could expect that all seed trees would be eventually eliminated. Since this did not happen with double the average disease level over 1620 years, the possibility of the *present form* of myrtle wilt causing the extinction of myrtle is very remote. However, in some areas, there is limited evidence that myrtle wilt levels may have increased in the recent past (Chapter 2). In such areas some future change in stand structure is predicted, with a decrease in the proportion of trees in the larger size classes.

With no mortality due to myrtle wilt, the surviving trees became very large, but there was little room for regeneration. After 840 years the model predicted the simultaneous death of all the remaining seed trees, although in practice this would happen more gradually, and there would probably be some surviving regeneration. However, these results are consistent with the conclusions of Noble and Slatyer (1978, 1980), since they emphasise the need for some gap forming agent in the myrtle regeneration process. Myrtle wilt adequately fulfils this role.

Given that no overall increase in myrtle wilt levels is apparent in Tasmania (Chapters 2 and 3), in undisturbed situations there is no reason to suppose that the disease will lead to any permanent change in forest structure. However, disturbances which damage myrtles often elevate myrtle wilt levels (Chapters 4 and 5); and where continuously high levels of myrtle wilt are experienced, the loss of the largest myrtle size classes can be expected.

In summary, it may be helpful to consider the role of myrtle wilt, and specifically of *Chalara australis,* in Tasmanian rainforests. Earlier work (Kile *et al.* 1989) concluded that in *Nothofagus* forests myrtle wilt appeared to be unique, being an often severe and sustained stand-level disease caused by a primary pathogen, with disease expression apparently independent of environmental stress. The currently available information is considered with

reference to Figure 7.2, which is a modification of the flow chart presented in Chapter 1. The numbers on the flow chart correspond to those of the relevant paragraphs below.

1. In contrast to 'normal forest mortality' (refer to Chapter 1), myrtle wilt can cause the death of large numbers of neighbouring myrtles, resulting in extensive canopy gaps (Chapter 6).

2. The available evidence points to myrtle wilt being endemic in at least part (and possibly most) of its current range (Chapter 2).

3. Although young myrtle regeneration (less than 12 cm DBH) is rarely killed by myrtle wilt (Howard 1973a, 1981; Elliott *et al.* 1987; Kile and Walker 1987), Elliott *et al.* (1987) established that canopy and sub-canopy trees were affected in similar proportions.

4. Myrtle wilt levels are increased in some areas of disturbance caused by man (Chapter 4). In such cases myrtle damage acts as a precipitating factor, and the disease can be viewed as an anthropogenically induced dieback. However, myrtle wilt appears to be ubiquitous in Tasmania's extensive myrtle forests, and disease levels in areas remote from human disturbance are similar to those in undisturbed, but less remote sites (Chapter 2).

5. • Predisposing factors include: low altitude sites; callidendrous forest; high myrtle density (both absolute and relative) in mixed forest and large sizes of myrtle (Elliott *et al.* 1987). The effects of forest type and myrtle density are probably due to differences in root grafting, which could affect disease spread (Elliott *et al.* 1987, Chapter 5).

   • Precipitating factors include anything which causes damage to myrtles. Only small wounds are necessary (Chapter 5). Obvious examples are stem and crown damage from branches shed by dominant *Eucalyptus* spp. (Chapter 3), damage by other falling trees, wind and snow damage, and, possibly also possum damage (R. Mesibov, personal communication).

   • Modifying factors include wound receptivity and inoculum availability. These may all be seasonally regulated. Although conidial felts are produced mainly in the autumn and winter, there is some evidence that inoculum availability may be higher during the summer months (Kile *et al.* 1989). *P. subgranosus* frass is also produced mainly in the summer (Hogan 1948, Candy 1990), and is thought to be the most important inoculum

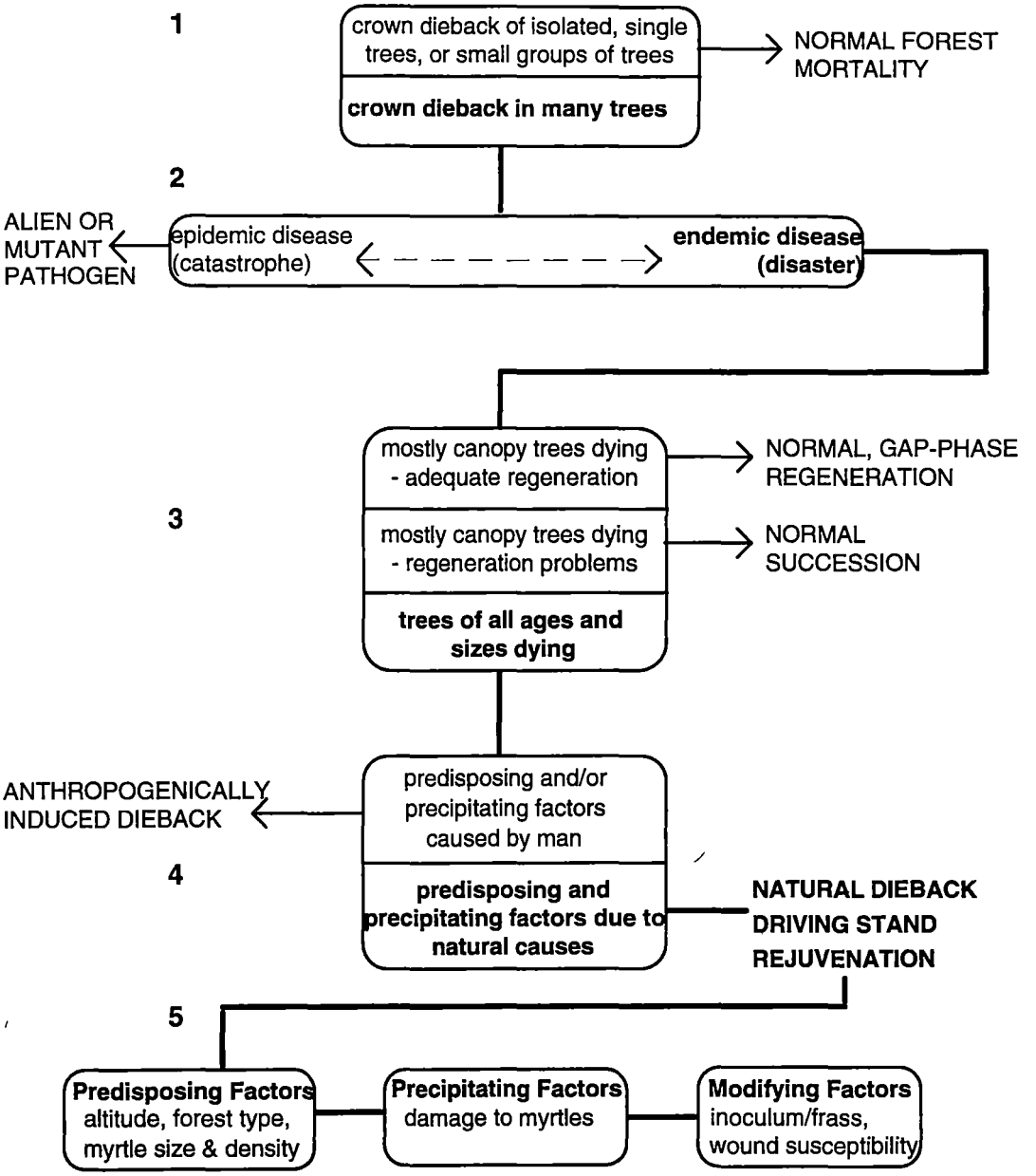


Figure 7.2 The role of myrtle wilt in undisturbed Tasmanian rainforest (outlined in bold).

source, particularly during the summer (G. A. Kile, personal communication). Conversely, there is some evidence that the susceptibility of wounds to *C. australis* infection may be reduced in the summer months (Chapter 5).

In conclusion, myrtle wilt frequently occurs in situations in which both predisposing and precipitating factors have natural origins. As such, it can be viewed as a mechanism facilitating stand rejuvenation.

7.5 SUMMARY

The probable long-term effects of myrtle wilt on Tasmanian myrtles were investigated using a simple population model. At current levels, it is unlikely that the disease will lead to any permanent change in forest structure. Thus in undisturbed Tasmanian forests, the loss of all mature myrtles due to the disease is not anticipated. However, where continuously high levels of myrtle wilt are experienced, the loss of the largest myrtle size classes can be expected.

The role of myrtle wilt in the Tasmanian rainforest ecosystem was summarised using all the currently available information. It was concluded that in undisturbed forest, the disease is primarily a mechanism which facilitates stand rejuvenation.

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APPENDICES

Appendix 1 Details of survey locations in remote areas of Tasmania

Ground surveys

Mt Ronald Cross 27.1.89
Grid Ref (1:100 000): Nive 287229 236227
Altitude: 880 m-920 m

Frankland Range (Orb Lake) 18.2.89
Grid Ref (1:100 000): Wedge 211479 209478
Altitude: 400 m-440 m

Aerial transects

Frankland Range 18.2.89
Orb Lake
Grid Ref (1:100 000): Wedge 211479 206477
Altitude: 400 m-480 m

Sanctuary Lake
Grid Ref (1:100 000): Wedge 217471 215465
Altitude: 400 m-520 m

Adamsons Peak 26.4.89
Adamsons Falls
Grid Ref (1:100 000): Huon 855985 892990
Altitude: 450 m contour

Creekton Rivulet
Grid Ref (1:100 000): Huon 893986 875002
Altitude: 180 m-800 m

Frenchmans Cap 22.3.90
Lake Gertrude (i)
Grid Ref (1:100 000): Franklin 052180 057178
Altitude: 600 m-880 m

Lake Gertrude (ii)
Grid Ref (1:100 000): Franklin 052184 057187
Altitude: 660 m-880 m

Pine Knob
Grid Ref (1:100 000): Franklin 064207 054198
Altitude: 560 m-760 m

Lake Millicent
Grid Ref (1:100 000): Franklin 053147 051155
Altitude: 460 m-620 m

Lake Cecily (i)
Grid Ref (1:100 000): Franklin 048183 045187
Altitude: 600 m-660 m

Lake Cecily (ii)
Grid Ref (1:100 000): Franklin 043188 033193
Altitude: 600 m-1020 m

Appendix 2 Details of Tasmanian aerial photographic surveys

Early surveys of main rainforest areas

Region	Mapsheet (4 miles:1")	Photo type	Area	Transect height	Run	Date	Photos showing myrtle wilt (HSC 4 or 5)
NW	Trowutta	B/W	Arthur R	11 000'	10	19.1.46	85, 90
			L Chisholm		19	19.1.46	88-96, 98-9
			Kepple Ck		21	31.1.46	800-2, 804-16
NW	Magnet	B/W	Hellyer R	13 100'	1	18.2.53	53-4
			Lyons/Keith Rs		2	18.2.53	28
			Keith/Arthur Rs		4	18.2.53	6
			Lyons/Arthur Rs		5	18.2.53	
W	Corinna & Pieman R	B/W	Wilson Rd/ Newdegate Ck	12 890'	1	6.2.47	75-6, 78
			Wilson/ Huskisson Rs		7	6.2.47	84, 84
W	King William	B/W	Hanlon Ck	13 000'	8	17.1.46	
			Hanlon Ck/ Gordon R		9	28.1.46	31, 33, 35
			Gell/Gordon Rs		10	28.1.46	98
W	Gordon	B/W	Franklin R	12 000'	1	27.11.58	99
			Gordon/Franklin Rs		2	27.11.58	
			Albert Ck (Strathgordon)		5	27.11.58	135

Region	Mapsheet (4 miles:1")	Photo type	Area	Transect height	Run	Date	Photos showing myrtle wilt (HSC 4 or 5)
S	Adamson	B/W	Craycroft R	13 000'	1	9.2.47	98
			Federation Peak		3	9.2.47	3
			New/Picton Rs		6	9 2.47	
			New R/ Precipitous Bluff		12	17.1.48	13-14, 18
E	Bicheno	B/W	Mt Henry/ Denison R	12 500'	6	2.4.49	
			Douglas/Apsley Rs		7	16.3.50	
			St Pauls/ Douglas Rs		8	16.3.50	
NE	Blue Tier	B/W	Gt Musselroe/ Ansons Rs	12 600'	3	22.5.50	
			Gt Musselroe/ Ansons Rs		4	22.5.50	
			McGoughs Lookout		5	22.5.50	68-9
			York Hill		6	22.5.50	73
			Weldborough Pass		7	22.5.50	5
			Wyniford R	12 400'	6A	5.2.52	83

Recent surveys of areas of marginal rainfall (for myrtle)

Region	Mapsheet (1:25 000)	Photo type	Area	Transect height	Run	Date	Photos showing myrtle wilt (HSC 4 or 5)
NE	Derby/ Spurrs Rivt	Colour	Frome Hill	21 000'	8E	22.10.80	71-4
SE	Taranna/ Communication	Colour	Forestier Penisula	20 700'	4	11.2.84	
			Cashs Lookout		5	11.2.84	
			Pirates Rd		6	11.2.84	
NE	Derby/ Spurrs Rivt	Colour	Frome Hill/ McGoughs Lookout	26 300'	15	28.2.92	26-32
E	Fingal/Piccaninny/ St John/Seymour	Colour	Douglas R	22 500'	18	21.2.88	99, 102
			Lookout Hill		19	21.2.88	
E	St John	Colour	St Pauls/	11 700'	36	23.1.90	
			Douglas Rs.		37	23.1.90	
					38	23.1.90	
					39	23.1.90	
E	St John	Colour	Myrtle Reserve/ Bl 35b	12 150	#	27.1.86	
E	St John	Colour	Myrtle Reserve/ Bl 34, 35c	12 150	#	23.1.90	myrtle wilt present

Photography with limited coverage

Appendix 3 Details of ground survey locations in Victoria

Central Highlands

O'Shannasy Catchment 11.11.91

1) Deep Creek (Road 7)
Grid Ref (1:25 000): Steavenson (8022-1-4) 903 388
Altitude: 795 m
Wilt: No evidence of current attack. Old stags present, one HSC 6.

2) Deep Creek (Road 9)
Grid Ref (1:25 000): Steavenson (8022-1-4) 921 385
Altitude: 765 m
Wilt: Two roadside trees both HSC 4 (DBH 8 cm/25 cm). They had both been damaged (by roadside slasher?).

3) Road 7
Grid Ref (1:25 000): Steavenson (8022-1-4) 958 375
Altitude: 700 m
Wilt: Three roadside trees, one HSC 3-4, (DBH 30 cm), the others HSC 4 (DBH 10 cm/6 cm). The latter tree had stem damage. Some other trees viewed from a distance may have died from wilt (HSC 5?).

4) Poley Road
Grid Ref (1:25 000): O'Shannasy (8022-1-3) 959 298
Altitude: 385 m
Wilt: One tree HSC 4 (DBH 40 cm) with 2 trees HSC 6.

Brittania Creek/Ada Valley 12.11.91

5) Brittania Creek
Grid Ref (1:100 000): Healesville (8022) 842 155
Altitude: 310 m
Wilt: No evidence of current attack. Old stags present, one HSC 6.

6) Dowey Spur (Dowey Spur Track)
Grid Ref (1:25 000): McCarthy (8022-2-1) 015 076
Altitude: 515 m
Wilt: *E. sieberi* forest on top of ridge with *N. cunninghamii* forest in valley below. An interesting site for future comparisons of *Chalara* species.

7) Charlie Creek (i)
Grid Ref (1:25 000): Ada River (8022-2-4) 006 118
Altitude: 590 m
Wilt: Six trees HSC 4 (DBH 8 cm-70 cm), two with crown damage and one with stem damage due to burl removal.

8) Charlie Creek (ii)
Grid Ref (1:25 000): Ada River (8022-2-4) 004 133
Altitude: 710 m
Wilt: One tree HSC 4 (DBH 80 cm)

9) Ada Big Tree
Grid Ref (1:25 000): Ada River (8022-2-4) 994 137
Altitude: 720 m
Wilt: No evidence of current attack. Old stags present, one HSC 6.

Strzelecki Ranges

Tarra-Bulga National Park 13.11.91

10) Bulga (Fern Gully)
Grid Ref (1:100 000): Traralgon (8221) 626 467
Altitude: 580 m
Wilt: No evidence of current attack. One tree was HSC 2 with crown damage but still healthy. Some dead stags in gully.

11) Tarra (Cyathea Falls Walk)
Grid Ref (1:100 000): Traralgon (8221) 597 444
Altitude: 350 m
Wilt: No evidence of current attack. One dead stag observed.

Middle Creek
13.11.91
12) Middle Creek
Grid Ref (1:100 000): Traralgon (8221) 570 430
Altitude: 500 m
Wilt: No evidence of current attack, nor of *P. subgranosus* boring on dead stags. Crown dieback probably due to *Biscogniauxia nothofagii.*

Grand Ridge Rd/Toora-Gunyah Rd 13.11.91

13) Grand Ridge Road (Pattinson's Tree)
Grid Ref (1:100 000): Foster (8120) 386 353
Altitude: 420 m
Wilt: No evidence of current attack.

14) Toora-Gunyah Road (Agnes catchment)
Grid Ref (1:100 000): Foster (8120) 407 341
Altitude: 440 m
Wilt: One tree HSC 5 (DBH 25 cm) with crown damage.

15) Toora-Gunyah Road (Franklin catchment)
Grid Ref (1:100 000): Foster (8120) 406 341
Altitude: 440 m
Wilt: One tree HSC 4 (DBH 25 cm), having had one of its leaders felled. Within 10 m another tree was HSC 4-5 (DBH 60 cm) with no damage. 15 m away a third tree was HSC 3 with crown damage.

Central Highlands

Mt. Erica (Ash Roading) 14.11.91

16) Buckle Spur Road (Middle Tyres River crossing)
Grid Ref (1:100 000): Matlock (8122) 404 024
Altitude: 600 m
Wilt: Two roadside trees HSC 4 (DBH 60 cm) with stem damage caused by roading disturbance. Another roadside tree HSC 3 (DBH 30 cm) with stem damage. 100 m from the road one undamaged tree HSC 3-4 (DBH 15 cm).

Mt Donna Buang/Cement Creek/Acheron Way 16/11/91

17) Mt Donna Buang (1½ km below Myrtle Gully Reserve)
Grid Ref (1:100 000): Healesville (8022) 793 248
Altitude: 980 m
Wilt: One roadside tree HSC 4 (DBH 25 cm) with crown damage.

18) Mt Donna Buang (1 km above Myrtle Gully Reserve)
Grid Ref (1:100 000): Healesville (8022) 809 254
Altitude: 1030 m
Wilt: One tree HSC 4.

19) Mt Donna Buang (headwaters of Walker Creek-100 m west of Summit Road.)
Grid Ref (1:100 000): Healesville (8022) 829 250
Altitude: 1130 m
Wilt: No evidence of current attack.

20) Mt Donna Buang (carpark below summit)
Grid Ref (1:100 000): Healesville (8022) 832 252
Altitude: 1175 m
Wilt: Two undamaged trees HSC 4 (DBH 25-30 cm).

21) Cement Creek (walking track downstream of Acheron Way)
Grid Ref (1:100 000): Healesville (8022) 861 253
Altitude: 630 m
Wilt: One tree HSC 4 (DBH 50 cm) with stem damage.

22) Acheron Way
Grid Ref (1:100 000): Healesville (8022) 881 311
Altitude: 630 m
Wilt: One roadside tree HSC 4 (DBH 25 cm) with stem damage.

Otway Ranges
Aire 11-14.4.92
23) Youngs Creek
Grid Ref (1:25 000): Lavers Hill (7520-1-2) 168 179
Altitude: 390 m
Wilt: Trees HSC 3-6 (DBH 50-70 cm) present. Site of old infection with recent damage from nearby logging. Some evidence of myrtle regeneration.

24) Youngs Creek (Triplet Falls)
Grid Ref (1:25 000): Lavers Hill (7520-1-2) 168 164
Altitude: 290 m
Wilt: Trees HSC 4 and numerous HSC 6 (DBH 50-70 cm) present. Site of old selective logging. Surrounding stands of *E. regnans* likely to be causing current damage (branch shedding). Regrowth myrtles present.

25) Browntown Track
Grid Ref (1:25 000): Lavers Hill (7520-1-2) 150 184
Altitude: 510 m
Wilt: None apparent. Some old growth myrtles (DBH 60 cm) with regrowth myrtle poles/saplings in a stand dominated by regrowth blackwood (120-130 yrs).

26) Beech Forest/Lavers Hill Road (Arkins Creek)
Grid Ref (1:25 000): Lavers Hill (7520-1-2) 139 183
Altitude: 515 m
Wilt: None apparent. A few old growth, multi-stemmed myrtles (DBH≤1 m). Site of previous clearing/fire dominated by open grown blackwood. No myrtle regeneration.

27) Youngs Creek Road (Aire Crossing Track)
Grid Ref (1:25 000): Lavers Hill (7520-1-2) 154 137
Altitude: 195 m
Wilt: Trees HSC 4-6 (DBH 70-80 cm) present. Large canopy gaps caused with no other canopy species. Myrtle regeneration (various ages) present.

28) Aire Crossing
Grid Ref (1:25 000): Lavers Hill (7520-1-2) 153 131
Altitude: 170 m
Wilt: None apparent. Mature myrtle and *A. dealbata* (30 m tall) along river. One young myrtle dying of unknown causes.

Barham 15.4.92
29) Turtons Track
Grid Ref (1:25 000): Skenes Creek (7620-4-2) 339 191
Altitude: 470 m
Wilt: One tree HSC 4-5 (DBH 50 cm), others healthy. Regrowth *E. regnans/A. dealbata*, with mature myrtles forming an understorey in gullies. Myrtle regeneration present.

Aire 15.4.92
30) Binns Road (landslip site)
Grid Ref (1:25 0000): Aire Valley (7620-4-3) 239 113
Altitude: 260 m
Wilt: Trees HSC 4-6 present. Selectively logged old growth eucalypts with rainforest gully. From photographic information myrtle wilt known to have been present since 1980 or earlier (I. Roberts, personal communication). Myrtle regeneration status unknown.

31) Maits Rest
Grid Ref (1:25 000): Cape Otway (7620-3-4) 219 072
Altitude: 230 m
Wilt: Trees HSC 3 & 6 present with HSC 4 numerous (DBH 80 cm). Old-growth myrtle in an over mature rainforest gully, where wilt is advancing. Some younger age classes of myrtle but no other canopy trees present.

Appendix 4 Myrtle numbers recorded in the 1964 and 1990 measurements of the Mt Maurice transect

SITE	TREES LIVE 1964			TREES DS 1964		TOTALS 64		TOTALS 90	
	Live 1990	DS 1990	NF/WT 1990	DS 1990	NF/WT 1990	Live	DS	Live	DS
Old growth myrtle	25	3 (3)	0	1 (1)	1	28	2 (1)	25	4 (4)
Re-growth myrtle	48	7 (3)	5	1 (1)	0	60	1 (1)	48	8 (4)
Myrtle woodland	20	1 (1)	0	2 (2)	0	21	2 (2)	20	3 (3)
Transect totals	93	11 (7)	5	4 (4)	1	109	5 (4)	93	15 (11)

Key

DS	Dead standing tree or stump
NF/WT	Not found or windthrown (no stump remaining in ground)
( )	Number of trees with *P. subgranosus* attack (HSC 5&6)

Appendix 5 Recording methods and assumptions used in surveys.

1) If in doubt, the healthiest part of the tree (or stem) was chosen to record.

2) If different parts of a tree were in 3 HSC categories, the middle category was chosen.

3) #With categories HSC 2 and HSC 3, which were not clearly recorded as separate at the first 3 measurements, if the disease didn't progress, it was recorded as HSC 2 (old attack).

4) With double leaders etc. the diameter of the largest was recorded (except where this had died and/or the smaller was recorded for HSC due to it being more healthy).

5) For buttressed trees, the diameter of the bole rather than the buttress was recorded.

#Only applies to Arve Loop plot.

Appendix 6 Details of Arve Loop and ROS plots

Location: Arve Loop	**Forest Type:** *E. regnans* with understorey of T1.1/T3.1	
Map (1:100 000): Huon	**Soil Type:** Deep light to medium stony gritty clay - yellowish red (5YR 4/6) gradational on Jurassic dolerite	
	Land System: Blue Hill, 572243	
Grid Ref: 796242	**Altitude:** 440 m-480 m	
Establishment date:	March 1982	
Plots measured	**Slope (degrees)**	**Aspect**
1	25-30	ENE facing slope

Location: Pipeline 18 Mile Peg	**Forest Type:** T1.1	
Map (1:100 000): Arthur River	**Soil Type:** Stony, friable, reddish brown (5YR 4/4) gradational soil on Tertiary basalt	
	Land System: Guildford 882321	
Grid Ref: 583273	**Altitude:** 500 m	
Establishment date:	23/5/89	
Plots measured	**Slope (degrees)**	**Aspect**
1	20	NW facing slope
3	25	W facing slope

Location: Frodshams Pass	**Forest Type:** I4.1/T1.2	
Map (1:100 000): Wedge	**Soil Type:** Quaternary deposits (shallow litter/peat over clay loam/light clay subsoil) on Cambrian dolomite	
	Land System: Marsden 828251	
Grid Ref: 496573	**Altitude:** 540 m	
Establishment date:	7/12/89	
Plots measured	**Slope (degrees)**	**Aspect**
1	45	N facing slope

Location: Lake Chisholm	**Forest Type:** *E. ovata, E.nitida* with understorey of T3.1/CT1	
Map (1:100 000): Arthur River	**Soil Type:** Brownish yellow (10YR 6/6) to yellowish brown (10YR 5/6) gradational soil on Precambrian slaty mudstone	
	Land System: Milkshake Hills 714131	
Grid Ref: 370444	**Altitude:** 120 m-180 m	
Establishment date:	21/11/88	
Plots measured	**Slope (degrees)**	**Aspect**
1	20	W facing slope
2	20	NW facing slope
3	45	NNW facing slope
4	50	SSW facing slope

Location: Simons Road	**Forest Type:** C2.1	
Map (1:100 000): Forester	**Soil Type:** Stony, strong brown (7.5YR 5/8) gradational soil on Devonian granite or granodiorite	
	Land System: Burns Creek 641341	
Grid Ref: 442207	**Altitude:** 820 m	
Establishment date:	23/1/89	
Plots measured	**Slope (degrees)**	**Aspect**
1	5	WSW facing slope

Location: Five Road	**Forest Type:** C1.1 with some *E. obliqua* overstorey in places	
Map (1:100 000): Wedge	**Soil Type:** Brown/grey gradational soil or alluvium on Ordivician limestone	
	Land System: Florentine 635221	
Grid Ref: 526694	**Altitude:** 440 m	
Establishment date:	6/1/89	
Plots measured	**Slope (degrees)**	**Aspect**
2	0	-
3	0	-

Forest types after Jarman *et al.* (1991). Soil types after Richley (1978), Pinkard (1980), Pinkard (1988), and Pemberton (1989).

Appendix 7 Disease classification methods used on the 1982 Arve Loop plot data

Elliott *et al.* (1987) Basis of current method	Candy (82) Clayton (82) Diggle (83a)	Jennings *et al.* (unpublished data)	Elliott, Kile and Madden (1982)
1 Healthy	1 Healthy	a	1 Healthy tree, no beetle attack
2 Healthy - old *Platypus* attack			2 Healthy tree, fresh beetle attack
3 Healthy - current *Platypus* attack	2 Recently dead, brown leaves	b	
4 Dead - current *Platypus* attack			3 Dead<2yrs
5 Dead <3 yrs - *Platypus* attack	3 Most leaves gone, branches	c	
6 Dead >3 yrs - old *Platypus* attack	4 Main branches present only	d	4 Dead>2yrs
	5 Dead for many years	e	
7 Dead - no *Platypus* attack	Not recorded		Not recorded
8 Alive - crown dieback, cause unknown	Not recorded		Not recorded

NB <---> implies equivalence

NB Elliott *et al.* (1982) have included old attack in their fresh attack class, which is therefore inflated.

Candy (1982) has taken anything with *P. subgranosus* holes to be already dead.

Appendix 8 Health status through time and estimated annual mortality rates from the Arve Loop and ROS plots

Arve Loop plot

Date of assessment	No. of myrtles in health status class 1	2	3	4	5	6	7	8	9	10	% Diseased (HSC 3-6/1-8)	Mortality (% p. a.)
Feb 1982	222	20	8	8	15	51	3	0	0	0	25.08%	
												1.43%
Apr 1984	216	21	9	4	17	56	3	0	1	0	26.38%	
												1.15%
May 1986	206	21	6	11	18	60	4	0	1	0	29.14%	
												2.19%
Aug 1988	194	23	3	12	13	69	3	1	7	2	30.50%	
												0%
Aug 1990	223	5	1	8	7	76	6	1	10	3	28.14%	
												1.90%
Aug 1992	215	6	1	6	8	81	7	3	10	3	29.36%	

Pipeline 18 Mile Peg (totals from two ROS plots)

Date of assessment	No. of myrtles in health status class 1	2	3	4	5	6	7	8	9	10	% Diseased (HSC 3-6/1-8)	Mortality (% p. a.)
May 1989	188	4	6	1	4	37	3	0	0	0	19.75%	
												0%
Jun 1991	188	5	2	4	3	39	3	0	0	0	19.26%	
												0.75%
Jun 1993	187	5	0	4	6	39	3	0	0	3	20.08%	

Frodshams Pass (one ROS plot)

Date of assessment	No. of myrtles in health status class 1	2	3	4	5	6	7	8	9	10	% Diseased (HSC 3-6/1-8)	Mortality (% p. a.)
Dec 1989	144	12	1	4	2	23	2	2	0	0	15 79%	
												1.55%
Dec 1991	148	8	0	0	5	24	2	3	1	0	15.26%	
												0.92%
Jan 1994	143	9	1	0	5	22	2	6	3	0	14.89%	

Lake Chisholm (totals from four ROS plots)

Date of assessment	No. of myrtles in health status class 1	2	3	4	5	6	7	8	9	10	% Diseased (HSC 3-6/1-8)	Mortality (% p. a.)
Nov 1988	198	19	1	10	13	104	0	2	0	0	36.89%	
												1.09%
Apr 1991	193	17	0	12	8	112	0	4	5	0	38.15%	
												2.48%
Apr 1993	183	17	5	6	9	115	0	4	12	1	39.82%	

Simons Road (one ROS plot)

Date of assessment	No. of myrtles in health status class 1	2	3	4	5	6	7	8	9	10	% Diseased (HSC 3-6/1-8)	Mortality (% p. a.)
Jan 1989	195	21	5	12	9	63	6	7	0	0	27.81%	
												2.58%
Jan 1991	190	20	4	7	13	66	7	6	5	0	28.75%	
												0.91%
Jan 1993	184	20	4	9	13	67	8	6	7	0	29.90%	

Five Road (totals from two ROS plots)

Date of assessment	No. of myrtles in health status class 1	2	3	4	5	6	7	8	9	10	% Diseased (HSC 3-6/1-8)	Mortality (% p. a.)
Jan 1989	198	10	5	26	21	126	2	4	0	0	45.41%	
												4.79%
Nov 1990	181	10	8	19	30	134	2	3	6	0	49.35%	
												0.64%
Jan 1993	184	10	4	17	25	143	1	4	13	0	48.71%	

Appendix 9 t tests on disease incidence, absolute and relative myrtle densities in callidendrous and thamnic/implicate pure rainforest. Data from Elliott *et al.* (1987)

a) Disease incidence (arcsin square root of percentage diseased)

Forest type	No. of samples	Mean	Standard deviation	t value	Significance level
Callidendrous	5	23.78	2.69		
				0.194	0.849 NS
Thamnic/Implicate	10	22.45	9.98		

b) Density of myrtle (stems/ha)

Forest type	No. of samples	Mean	Standard deviation	t value	Significance level
Callidendrous	5	305.6	83.43		
				2.655	0.0198*
Thamnic/Implicate	10	216.5	48.24		

c) Relative density of myrtle (arcsin square root of percentage)

Forest type	No. of samples	Mean	Standard deviation	t value	Significance level
Callidendrous	5	57.56	6.98		
				2.529	0.0241*
Thamnic/Implicate	10	48.35	6.33		

NB In this and following tables, 5%, 1% and 0.1% significance levels are shown by *, ** and *** respectively. Non-significant values are denoted by NS.

Appendix 10 t tests on disease incidence, absolute and relative myrtle densities in mixed forest and pure rainforest. Data from Elliott *et al.* (1987)

a) Disease incidence (arcsin square root of percentage diseased)

Forest type	No. of samples	Mean	Standard deviation	t value	Significance level
Mixed forest	5	32.64	15.15		
				1.619	0.123 NS
Pure rainforest	15	22.89	10.25		

b) Density of myrtle (stems/ha)

Forest type	No. of samples	Mean	Standard deviation	t value	Significance level
Mixed forest	5	131.4	39.98		
				-3.301	0 00377**
Pure rainforest	15	246.2	73.32		

c) Relative density of myrtle (arcsin square root of percentage)

Forest type	No. of samples	Mean	Standard deviation	t value	Significance level
Mixed forest	5	39.58	9.654		
				-2.705	0.0145*
Pure rainforest	15	51.19	7.888		

Appendix 11 Chi squared tests of estimated and actual mortality at three sites to test the null hypotheses that trees remain in HSC 4 for one, two, three or four years

Chi squared test of estimated and actual mortality rates

(null hypothesis: trees remain in HSC 4 for 1 year)

Data source	Arve Loop Plot			Simons Rd	Tombstone Ck
	82-84	84-86	86-88	83-88	84-89
OBSERVED MORTALITY	8	5	10	14	2
EXPECTED MORTALITY (from HSC 4)	16	8	22	45	10
O-E	-8	-3	-12	-31	-8
$C=(O-E)^2/E$	4	1.125	6.545	21.355	6.4
$X^2=39.425^{***}$	5 DF				

Chi squared test of estimated and actual mortality rates

(null hypothesis: trees remain in HSC 4 for 2 years)

Data source	Arve Loop Plot			Simons Rd	Tombstone Ck
	82-84	84-86	86-88	83-88	84-89
OBSERVED MORTALITY	8 + 5 = 13		10	14	2
EXPECTED MORTALITY (from HSC 4)	8 + 4 = 12		11	22.5	5
O-E	1		-1	-8.5	-3
$C=(O-E)^2/E$	0.0833		0.091	3.211	1.8
$X^2=5.185$ NS	4 DF (NB 2 classes combined so E value >5)				

Chi squared test of estimated and actual mortality rates

(null hypothesis: trees remain in HSC 4 for 3 years)

Data source	Arve Loop Plot			Simons Rd	Tombstone Ck
	82-84	84-86	86-88	83-88	84-89
OBSERVED MORTALITY	8 + 5 = 13		10	14 + 2 = 16	
EXPECTED MORTALITY (from HSC 4)	5.3 + 2.7 = 8		7.3	15 + 3.3 = 18.3	
O-E	5		2.7	-2.3	
$C=(O-E)^2/E$	3.125		0.999	0.289	
X^2=4.413 NS	3 DF (NB classes combined so E value >5)				

Chi squared test of estimated and actual mortality rates

(null hypothesis: trees remain in HSC 4 for 4 years)

Data source	Arve Loop Plot			Simons Rd	Tombstone Ck
	82-84	84-86	86-88	83-88	84-89
OBSERVED MORTALITY	8 + 5 = 13		10	14 + 2 = 16	
EXPECTED MORTALITY (from HSC 4)	4 + 2 = 6		5.5	11.3 + 2.5 = 13.8	
O-E	7		4.5	2.2	
C=(O-E)2/E	8.167		3.681	0.351	
X^2=12.199**	3 DF (NB classes combined so E value >5)				

Appendix 12 Treatments, soil and vegetation types and per annum mortality due to myrtle wilt in the rainforest logging, regeneration and thinning trials

SITE AND TREATMENT	PARENT ROCK, SOIL & FOREST TYPE & MORTALITY DUE TO WILT		
	Tertiary Basalt	Cambrian Volcanic	Pre-Cambrian Dolomite
Pipeline 22 Mile Peg 15-year-old stand undisturbed	Myrtle pole stand Callidendrous/thamnic 0% p.a.		
Sumac Spur 1 15-year-old stand undisturbed		Deep yellow/brown gradational soil Myrtle pole stand Callidendrous/thamnic 0% p.a.	
Pipeline 26 Mile Peg 18-year-old stand undisturbed	Stony brown clay loam soil Myrtle pole stand Callidendrous/thamnic 0% p.a.		
Oonah 40-year-old stand undisturbed	Myrtle pole stand Callidendrous/thamnic 1.6% p.a. (mean)		
Blackwater Spur 6 65 yr stand undisturbed		Myrtle pole stand Callidendrous/thamnic 6.4% p.a.	
Sumac Area 9 undisturbed		Yellow/brown clay loam 184 trees (126 myrtles)/ha (1980) CT1 0.93% p.a.	

SITE AND TREATMENT	PARENT ROCK, SOIL & FOREST TYPE & MORTALITY DUE TO WILT		
	Tertiary Basalt	Cambrian Volcanic	Pre-Cambrian Dolomite
Rabalga Road 40-year-old stand undisturbed (general area selectively logged in 1960s)	Deep brown friable gradational clay Myrtle pole stand C1.1 2.2% p.a. (mean)		
Pipeline 22 Mile Peg 15-year-old stand thinned	Myrtle pole stand Callidendrous/thamnic 0% p.a. (mean)		
Sumac Spur 1 15-year-old stand thinned		Deep yellow/brown gradational soil Myrtle pole stand Callidendrous/thamnic 0% p.a.	
Pipeline 26 Mile Peg 18-year-old stand thinned	Stony brown clay loam soil Myrtle pole stand Callidendrous/thamnic 0.011% p.a.		
Pipeline pre-logging scarification	Deep, stony, brown clay loam 174 trees (122 myrtles)/ha (1984) C1.1 4.0% p.a.		

SITE AND TREATMENT	PARENT ROCK, SOIL & FOREST TYPE & MORTALITY DUE TO WILT		
	Tertiary Basalt	Cambrian Volcanic	Pre-Cambrian Dolomite
Rabalga Road post-logging scarification (selectively logged in 1960s)	Deep brown, friable gradational clay 91 trees (25 myrtles)/ha C1.1 6.1% p.a.		
Sumac Area 1 selective logging for sawlogs		Yellow/brown clay loam 210 trees (110 myrtles)/ha in 1978 CT1 3.1% p.a.	
Pipeline 20 Mile Peg logging for sawlogs & pulpwood leaving a shelterwood	On basalt 101 trees (21 myrtles)/ha C1.1 7.0% p.a.		
Julius Dolomite logging for sawlogs & pulpwood leaving a shelterwood, also soil scarification			0 5 m black/dark brown peat over dolomite 68 trees (54 myrtle/ha) T1.1, CT1 & C1.1 2.0% p.a.
Sumac Area 3 logging for sawlogs & pulpwood leaving a shelterwood, also soil scarification (some ring-barking)		Yellow/brown clay loam 137 trees (88 myrtles)/ha CT1 1.0% p.a.	

SITE AND TREATMENT	PARENT ROCK, SOIL & FOREST TYPE & MORTALITY DUE TO WILT		
	Tertiary Basalt	Cambrian Volcanic	Pre-Cambrian Dolomite
Sumac Area 2 strip logging (some ring-barking)		Yellow/brown clay loam CT1 2.9% p.a.	
Pruana Road clearfelling & soil scarification leaving seed trees	Stony brown soil 16 myrtles/ha CT1 4.9% p.a.		
Oonah 40-year-old stand thinned	Myrtle pole stand Callidendrous/thamnic 5.8% p.a. (mean)		
Blackwater Spur 6 65-year-old stand thinned		Myrtle pole stand Callidendrous/thamnic 10.1% p.a.	
Rabalga Road 40-year-old stand thinned (general area selectively logged in 1960s)	Deep brown, friable, gradational clay Myrtle pole stand C1.1 **4.1% p.a.**		

NB Data refer to: soil type; tree and myrtle density; rainforest classification (after Jarman *et al.* 1991) and per annum mortality rate due to myrtle wilt (averaged over the whole of the measurement period). Treatments have been arranged in assumed order of increasing disturbance.

Appendix 13 Disease incidence and mortality due to wilt in the rainforest logging and regeneration trials

Sumac Area 1 - selectively logged for sawlogs in November 1976

Date	NUMBER OF TREES/CLASS Healthy	Sick	Dead	Windthrown etc.	%Diseased (sick+dead)/total	Mortality % p.a. (wilt)
Nov. 1976			14			
						7.0% p.a.
Aug. 1978	46	11	22	0	41.8%	
						7.0% p.a.
Aug. 1979	46	7	26	0	41.8%	
						3.9% p.a.
Jan. 1982	35	13	31	0	55.7%	
						3.1% p.a.
May 1983	31	15	33	0	60.8%	
						2.0% p.a.
Sept. 1988	36	5	38	0	54.4%	

Overall mortality due to wilt for 142 months = 36.9% (3.1% p.a.)

Sumac Area 2 - strip logged in November 1976

Date	NUMBER OF TREES/CLASS Healthy	Sick	Dead	Windthrown etc.	%Diseased (sick+dead)/total	Mortality % p.a. (wilt)
Nov. 1976						
Jan. 1978	24	10	16	0	52.0%	
						5.6% p.a.
Aug. 1979	25	6	19	0	50.0%	
						4.0% p.a.
Jan. 1982	17	10	22	1	64.0%	
						16.7% p.a.
May 1983	11	10	28	1	76.0%	
						0% p.a.
Feb. 1989	14	7	27	2	68.0%	

Overall mortality due to wilt for 133 months = 32.4% (2.9% p.a.)

NB In Sumac Area 2, nine trees were ringbarked in July 1981. Although these trees have been included with the rest of the data they have also been analysed separately below. The mortality rate between the 1983 and 1989 remeasurements for the ringbarked trees was higher than for the transect as a whole.

Sumac Area 2 - strip logged in November 1976 and ringbarked July 1981

Date	NUMBER OF TREES/CLASS Healthy	Sick	Dead	Windthrown etc.	%Diseased (sick+dead)/total	Mortality % p.a. (wilt)
Nov. 1976						
Jan. 1978	6	3	0	0	33.0%	
						0% p.a.
Aug. 1979	9	0	0	0	0%	
						0% p.a.
Jan. 1982	7	2	0	0	22.2%	
						16.7% p.a.
May 1983	2	5	2	0	77.8%	
						6% p.a.
Feb. 1989	3	2	2	4	44.4%	

Overall mortality due to wilt for 133 months = 22.2% (2.0% p.a.)

Sumac Area 3 - logged in February 1978 for sawlogs and pulpwood leaving a shelterwood, also soil scarification

	NUMBER OF TREES/CLASS					
Date	Healthy	Sick	Dead	Windthrown etc.	%Diseased (sick+dead)/total	Mortality % p.a. (wilt)
Feb. 1978						
Oct. 1979	34	9	4	0	27.7%	
						4.0% p.a.
July 1981	31	9	7	0	34.0%	
						0.33% p.a.
Feb. 1989	22	1	8	16	19 1%	

Overall mortality due to wilt for 112 months = 9.3% (1.0% p.a.)

In Sumac Area 3, seven trees were ringbarked in July 1981. These trees have been included with the rest of the data and have not been analysed separately since they have all remained healthy (0% p.a. mortality due to wilt).

Sumac Area 9 - undisturbed rainforest (control)

	NUMBER OF TREES/CLASS					
Date	Healthy	Sick	Dead	Windthrown etc.	%Diseased (sick+dead)/total	Mortality % p.a. (wilt)
April 1980	49	2	4	0	10.9%	
						1.9% p.a.
June 1983	42	3	7	3	18.2%	
						0.4% p.a.
Sept. 1988	44	0	8	3	14.5%	

Overall mortality due to wilt for 101 months = 7.8% (0.93% p.a.)

Pipeline scarification - pre-logging soil scarification, February 1984

	NUMBER OF TREES/CLASS					
Date	Healthy	Sick	Dead	Windthrown etc.	%Diseased (sick+dead)/total	Mortality % p.a. (wilt)
Feb 1984						
April 1984	36	1	4	0	12.2%	
						4.0% p.a.
Sept. 1987	26	4	9	2	31.7%	

Overall mortality due to wilt for 41 months = 13.5% (4.0% p.a.)

Pipeline 20 Mile Peg - logged in March 1982 for sawlogs and pulpwood leaving a shelterwood

	NUMBER OF TREES/CLASS					
Date	Healthy	Sick	Dead	Windthrown etc.	%Diseased (sick+dead)/total	Mortality % p.a. (wilt)
March 1982						
March 1983	27	2	0	0	6.7%	
						10.1% p.a.
Feb. 1984	20	7	3	0	33.3%	
						6.8% p.a.
Dec. 1987	20 (healthy + sick)					

Overall mortality due to wilt for 57 months = 33.3% (7.0% p.a.)

Julius Dolomite - logged in May/June 1981 for sawlogs and pulpwood leaving a shelterwood, also soil scarification

	NUMBER OF TREES/CLASS					
Date	Healthy	Sick	Dead	Windthrown etc.	%Diseased (sick+dead)/total	Mortality % p.a. (wilt)
May/June 1981						
Sept./Oct. 1982	30	7	2	4	20.9%	
						5.4% p.a.
Sept./Oct. 1983	20	13	4	6	39.5%	
						0% p.a.
May/June 1985	22	11	4	6	34.9%	

Overall mortality due to wilt for 32 months = 5.4% (2.0% p.a.)

Rabalga Road - post-logging soil scarification in December 1980 (area selectively logged in 1960s)

	NUMBER OF TREES/CLASS					
Date	Healthy	Sick	Dead	Windthrown etc.	%Diseased (sick+dead)/total	Mortality % p.a. (wilt)
Dec. 1980						
Oct. 1982	9	1	0	0	10.0%	
						0% p.a.
Jan. 1984	5	5	0	0	50.0%	
						8.2% p.a.
Sept. 1987	3	3	3	1	60.0%	

Overall mortality due to wilt for 59 months = 30.0% (6.1% p.a.)

Pruana Road - clearfelled in March 1981 with soil scarification leaving myrtle seed trees

Date	NUMBER OF TREES/CLASS				%Diseased (sick+dead)/total	Mortality % p.a. (wilt)
	Healthy	Sick	Dead	Windthrown etc.		
March 1981	48?					
						1.6% p.a.
July 1982	13	22	1	10	50.0%	
						8.1% p.a.
Dec. 1983	11	18	5	12	50.0%	
						9.1% p.a.
Nov. 1988	6	10	18	12	60.9%	

Overall mortality due to wilt for 92 months = 37.5% (4.9% p.a.)

Appendix 14 Disease incidence and mortality due to wilt in the myrtle thinning trials

Oonah - thinned 40-year-old stand, April 1984 (totals from four plots)

	NUMBER OF TREES/CLASS				
Date	Alive	Dead	Windthrown	%Dead	Mortality % p.a. (wilt)
April 1984	154	0	0	0%	
					5.6% p.a.
May 1986	136	18	0	11.70%	

Overall mortality due to wilt for 25 months = 11.7% (5.6% p.a.)

Oonah - undisturbed 40-year-old stand (totals from four plots)

	NUMBER OF TREES/CLASS				
Date	Alive	Dead	Windthrown	%Dead	Mortality % p.a. (wilt)
Feb. 1983	224	0	0	0%	
					0.96% p.a.
May 1986	217	7	0	3.1%	

Overall mortality due to wilt for 39 months = 3.1% (0.96% p.a.)

Rabalga Spur 4 - thinned 40-year-old stand, June 1981

	NUMBER OF TREES/CLASS				
Date	Alive	Dead	Windthrown	%Dead	Mortality % p.a. (wilt)
June 1981	10	0	0	0%	
					0% p.a.
April 1982	10	0	0	0%	
					0% p.a.
Oct. 1982	10	0	0	0%	
					10.0% p.a.
Oct. 1983	9	1	0	10.0%	
					26.7% p.a.
March 1984	8	2	0	20.0%	
					0% p.a.
May 1986	8	2	0	20.0%	

Overall mortality due to wilt for 59 months = 20.0% (4.1% p.a.)

Rabalga Spur 4 - undisturbed 40-year-old stand (totals from two plots)

	NUMBER OF TREES/CLASS				
Date	Alive	Dead	Windthrown	%Dead	Mortality % p.a. (wilt)
Feb. 1981	85	0	0	0%	
					1.9% p.a
May 1982	83	2	0	2.4%	
					5.8% p.a.
Oct. 1982	79	4	2	4.7%	
					2.5% p.a.
Oct. 1983	77	6	2	7.1%	
					9.4% p.a.
March 1984	74	9	2	10.6%	
					0.6% p.a.
May 1986	73	10	2	11.8%	

Overall mortality due to wilt for 63 months = 11.8% (2.2% p.a.)

Blackwater Spur 6 - thinned 65-year-old stand, April 1982

	NUMBER OF TREES/CLASS				
Date	Alive	Dead	Windthrown	%Dead	Mortality % p.a. (wilt)
April 1982	17	0	0	0%	
					0% p.a.
Nov. 1982	15	0	2	0%	
					15.0% p.a.
March 1984	11	3	3	17.6%	
					16.8% p.a.
May 1986	6	7	4	41.2%	

Overall mortality due to wilt for 49 months = 41.2% (10.1% p.a.)

Blackwater Spur 6 - undisturbed 65-year-old stand

	NUMBER OF TREES/CLASS				
Date	Alive	Dead	Windthrown	%Dead	Mortality % p.a. (wilt)
April 1982	23	0	0	0%	
					0% p.a.
Nov. 1982	23	0	0	0%	
					13.0% p.a.
March 1984	19	4	0	17 4%	
					4 9% p.a.
May 1986	17	6	0	26.1%	

Overall mortality due to wilt for 49 months = 26.1% (6.4% p.a.)

Appendix 15 Data from the replicated, 40-year-old myrtle thinning trial at Oonah, thinned April 1984

Thinned plots

		NUMBER OF TREES/CLASS				
Plot	Date	Alive	Dead	Windthrown	%Dead	Mortality % p.a. (wilt)
1	April 84	51	0	0	0%	
						5.6%
1	May 86	45	6	0	11.8%	
2	April 84	31	0	0	0%	
						7.7%
2	May 86	26	5	0	16.1%	
3	April 84	40	0	0	0%	
						2.4%
3	May 86	38	2	0	5.0%	
4	April 84	32	0	0	0%	
						7.5%
4	May 86	27	5	0	15.6%	

Control plots

Plot	Date	NUMBER OF TREES/CLASS Alive	Dead	Windthrown	%Dead	Mortality % p.a (wilt)
1	Feb. 83	69	0	0	0%	
						2.7%
1	May 86	63	6	0	8.7%	
2	Feb. 83	63	0	0	0%	
						0%
2	May 86	63	0	0	0%	
3	Feb. 83	32	0	0	0%	
						0%
3	May 86	32	0	0	0%	
4	Feb. 83	60	0	0	0%	
						0.6%
4	May 86	59	1	0	1.7%	

Appendix 16 Myrtle wilt and damage levels in the blackwood selective logging trial, logged January 1991

Logged coupe

No. myrtles in HSC								% Diseased	No. myrtles in DC				% Damaged	Mortality
1	2	3	4	5	6	7	8	(HSC 3-6/1-8)	1	2	3	4	(DC 2-4/1-4)	(%p. a.)
Replicate 1			**(Mar. 91)**											
6	2	0	0	0	4	0	1	30.8%	2	4	1	2	77.8%	
			(Mar. 92)											12.5%
4	1	0	2	0	4	1	1	46.2%	1	4	2	2	88.9%	
Replicate 2			**(Mar. 91)**											
9	0	0	1	0	2	1	0	23.1%	9	1	0	0	11.1%	
			(Mar. 92)											10.0%
7	0	1	1	1	2	1	0	38.5%	9	1	0	0	10.0%	
Replicate 3			**(Mar. 91)**											
13	0	0	0	0	0	0	0	0%	13	0	0	0	0%	
			(Mar. 92)											0%
13	0	0	0	0	0	0	0	0%	13	0	0	0	0%	
Totals			**(Mar. 91)**											
28	**2**	**0**	**1**	**0**	**6**	**1**	**1**	**17.9%**	**24**	**4**	**1**	**2**	**25.0%**	
			(Mar. 92)											**6.5%**
24	**1**	**1**	**3**	**1**	**6**	**2**	**1**	**28.2%**	**23**	**5**	**2**	**2**	**25.0%**	

Control

	No. myrtles in HSC							% Diseased	No. myrtles in DC				% Damaged	Mortality
1	2	3	4	5	6	7	8	(HSC 3-6/1-8)	1	2	3	4	(DC 2-4/1-4)	(%p. a.)
Replicate 1			**(Mar. 91)**											
11	0	0	0	0	3	0	0	21.4%	10	1	0	0	9.1%	
			(Mar. 92)											0%
11	0	0	0	0	3	0	0	21.4%	10	1	0	0	9.1%	
Replicate 2			**(Mar. 91)**											
10	0	0	1	0	3	0	0	28.6%	11	0	0	0	0%	
			(Mar. 92)											9.1%
10	0	0	0	1	3	0	0	28.6%	11	0	0	0	0%	
Replicate 3			**(Mar. 91)**											
12	0	0	0	0	2	0	0	14.3%	11	1	0	0	8.3%	
			(Mar. 92)											0%
12	0	0	0	0	2	0	0	14.3%	10	2	0	0	16.7%	
Totals			**(Mar. 91)**											
33	**0**	**0**	**1**	**0**	**8**	**0**	**0**	**21.4%**	**32**	**2**	**0**	**0**	**5.8%**	
			(Mar. 92)											**2.9%**
33	**0**	**0**	**0**	**1**	**8**	**0**	**0**	**21.4%**	**31**	**3**	**0**	**0**	**8.8%**	

NB It was not possible to assess long dead trees (HSC 6 and some HSC 7) for recent damage

Appendix 17 ANOVAs on myrtle wilt and damage levels and per annum mortality due to myrtle wilt in the blackwood selective logging trial, logged January 1991

ANOVA of percentage of diseased trees along transects in the logged coupe and in a control area in March 1992 (data transformed to arcsin square root of the percentage diseased)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
BETWEEN GROUPS	0.147267	1	0.147267	0.001	0.9833 NS
WITHIN GROUPS	1159.25	4	289.811		
TOTAL	1159.39	5			

ANOVA of percentage of damaged trees along transects in the logged coupe and in a control area in March 1991 (data transformed to arcsin square root of the percentage damaged)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
BETWEEN GROUPS	368.950	1	368.950	0.671	0.4669 NS
WITHIN GROUPS	2199.54	4	549.885		
TOTAL	2568.49	5			

ANOVA of percentage per annum mortality rates due to myrtle wilt along transects in the logged coupe and in a control area (data transformed to arcsin square root of the percentage)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
BETWEEN GROUPS	77.6161	1	77.6161	0.670	0.4673 NS
WITHIN GROUPS	463.446	4	115.862		
TOTAL	541.062	5			

ANOVA of percentage of diseased trees along transects in the logged coupe and in a control area in March 1992, adjusted for the percentage of trees damaged in March 1991 (data transformed to arcsin square root of the percentage)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
COVARIATE					
Damage	475.485	1	475.485	2.391	0.2198 NS
MAIN EFFECT					
Logged/control	87.3367	1	87.3367	0.439	0.5614 NS
RESIDUAL	596.570	3	198.857		
TOTAL	1159.39	5			

Appendix 18 ANOVAs on variables measured on track and control transects at Mt Scott, Weindorfers Forest and Liffey Falls

ANOVA of percentage of diseased trees along the Mt Scott walking track and along a nearby, parallel transect (data transformed to arcsin square root of the percentage diseased)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECT					
Track/control	50.233	1	50.233	<1	0.7345 NS
RESIDUAL	11647.1	28	415.967		
TOTAL	11697.3	29			

ANOVA of percentage of damaged trees along the Mt Scott walking track and along a nearby, parallel transect (data transformed to arcsin square root of the percentage damaged)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECT					
Track/control	489.728	1	489.728	2.064	0.1619 NS
RESIDUAL	6643.63	28	237.273		
TOTAL	7133.36	29			

ANOVA of altitude measurements along the Mt Scott walking track and along a nearby, parallel transect

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECT					
Track/control	6720.03	1	6720.03	4.382	0.0455*
RESIDUAL	42936.9	28	1533.46		
TOTAL	49656.9	29			

ANOVA of slope along the Mt Scott walking track and along a nearby, parallel transect

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECT					
Track/control	140.833	1	140.833	0.855	0.3729 NS
RESIDUAL	4613.87	28	164.781		
TOTAL	4754.70	29			

ANOVA of percentage of diseased trees along walking tracks in Weindorfers Forest and along nearby, parallel transects (data transformed to arcsin square root of the percentage diseased)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECT					
Track/control	95.515	1	95.515	<1	0.3982 NS
RESIDUAL	3491.57	28	124.699		
TOTAL	3587.08	29			

ANOVA of percentage of damaged trees along walking tracks in Weindorfers Forest and along nearby, parallel transects (data transformed to arcsin square root of the percentage damaged)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECT					
Track/control	401.063	1	401.063	7.090	0.0127*
RESIDUAL	1583.99	28	56.571		
TOTAL	1985.06	29			

ANOVA of altitude along walking tracks in Weindorfers Forest and along nearby, parallel transects

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECT					
Track/control	634.80	1	634.80	12.265	0.0016**
RESIDUAL	1449.2	28	51.757		
TOTAL	2084.0	29			

ANOVA of slope along walking tracks in Weindorfers Forest and along nearby, parallel transects

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECT					
Track/control	4.800	1	4.800	0.275	0.6096 NS
RESIDUAL	488.400	28	17.443		
TOTAL	493.200	29			

ANOVA of percentage of diseased trees along the Liffey Falls walking track and along a nearby transect (data transformed to arcsin square root of the percentage diseased)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECT					
Track/control	262.548	1	262.548	1.940	0.1939 NS
RESIDUAL	1353.42	10	135.342		
TOTAL	1615.97	11			

ANOVA of percentage of damaged trees along the Liffey Falls walking track and along a nearby transect (data transformed to arcsin square root of the percentage damaged)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECT					
Track/control	335.598	1	335.598	2.334	0.1576 NS
RESIDUAL	1438.016	10	143.802		
TOTAL	1773.614	11			

Appendix 19 Details of *C. australis* isolates used in various experiments

(#Courtesy of G. A Kile and M. F. Hall, CSIRO Division of Forestry)

Wounding experiments

#Isolate 1.91	1.91 (DAR 50145) (Kile and Walker 1987)
Origin	Peak Rivulet Road, Esperance Valley, southern Tasmania
Grid reference	(1:100 000) Huon (8221) 4913 52055
Sampling date	9/82
Details	Isolated from a recently dead *N. cunninghamii*.

WUT plots and re-isolations, morphological comparisons, isozyme electrophoresis and temperature profiles

#Isolate E8

Origin	Peak Rivulet Road, Esperance Valley, southern Tasmania
Grid reference	(1:100 000) Huon (8221) 4913 52055
Sampling date	14/3/89
Details	Isolated from a recently dead (two to three months) *N. cunninghamii,* estimated DBH 20 cm. Current *P. subgranosus* attack. Infection derived from stem wound.

Isolate 1C

Origin	WUT plot, Russell Road, southern Tasmania
Grid reference	(1:100 000) Tyenna (8212) 4825 52485
Sampling date	17/3/93
Details	Re-isolated from the stem of Tree 1C (50 cm above ground level). This tree was only 35 cm from the inoculated tree (but root connections between them did not appear to be functional). It was undamaged and dying, and its DBH was 5.5 cm.

Isolate 1E

Origin	WUT plot, Russell Road, southern Tasmania
Grid reference	(1:100 000) Tyenna (8212) 4825 52485
Sampling date	17/3/93
Details	Re-isolated from the root of the dying Tree 1E, well below the root graft with Tree 1H. Tree 1E was 155 cm from the inoculated tree, had a DBH of 10.6 cm and was undamaged.

Isolate 1H

Origin	WUT plot, Russell Road, southern Tasmania
Grid reference	(1:100 000) Tyenna (8212) 4825 52485
Sampling date	30/3/93
Details	Re-isolated from the root graft (Graft 19), which connected the apparently healthy Tree 1H with the dying Tree 1E. Tree 1H was undamaged, 32 cm from Tree 1E, 170 cm from the inoculated tree, and had a DBH of 29.7 cm.

Isolate 7A

Origin	WUT plot, Mt Michael, north east Tasmania
Grid reference	(1:100 000) Georges Bay (8515) 5838 54402
Sampling date	28/4/93
Details	Re-isolated from the stem of Tree 7A (250 cm above ground level). This tree was directly root grafted to the inoculated tree and was dying. It was undamaged and had a DBH of 17.6 cm.

Isolate L1

Origin	Lottah Road, Mt Michael, north east Tasmania
Grid reference	(1:100 000) Georges Bay (8515) 5840 54360
Sampling date	28/4/93
Details	Isolated from an undamaged, dying *N. cunninghamii,* estimated DBH 30 cm.

Isolate X1	Christmas Hills (CHT) (Gumley 1993)
Origin	Christmas Hills Road, Smithton, north west Tasmania
Grid reference	(1:25 000) Roger (3244) 3263 54547
Sampling date	23/4/93
Details	Isolated from an undamaged, dying *N. cunninghamii,* estimated DBH 20 cm.

Isolate N3	Mount Donna Buang (MDBV) (Gumley 1993)
Origin	Road 7, O'Shannasy Catchment, Central Highlands, Victoria (Site 3, Appendix 3)
Grid reference	(1:25 000) Steavenson (8022-1-4) 958 375
Sampling date	11/11/91
Details	Isolated from a stem damaged, dying *N. cunninghamii,* estimated DBH 6 cm.

Appendix 20 Nested ANOVAs of *C. australis* growth measures, by inoculum type, wound age and sapling

Lateral extent of growth

Source of variation	Sum of squares	DF	Mean square	Variance component	Percentage breakdown
Inoculum type	5.764	1	5.764	0.036	9.60
Wound age	28.913	8	3.614	0.296	79.45
Sapling	1.142	20	0.057	0.005	1.45
ERROR	3.188	90	0.035	0.035	9.49
TOTAL	39.006	119			

Radial extent of growth

Source of variation	Sum of squares	DF	Mean square	Variance component	Percentage breakdown
Inoculum type	13.534	1	13.534	0.066	7.14
Wound age	76.373	8	9.547	0.785	84.37
Sapling	2.497	20	0.125	0.015	1.64
ERROR	5.733	90	0.064	0.064	6.84
TOTAL	98.136	119			

Growth above wounds

Source of variation	Sum of squares	DF	Mean square	Variance component	Percentage breakdown
Inoculum type	1786.408	1	1786.408	2.558	1 53
Wound age	13063.392	8	1632.924	131.471	78.61
Sapling	1105.417	20	55.271	7.350	4.39
ERROR	2328.375	90	25.871	25.871	15 47
TOTAL	18283.592	119			

Growth below wounds

Source of variation	Sum of squares	DF	Mean square	Variance component	Percentage breakdown
Inoculum type	3499.200	1	3499.200	5.304	1.79
Wound age	25447.642	8	3180.955	261.479	88.41
Sapling	864.083	20	43.204	4.740	1.60
ERROR	2181.875	90	24.243	24.243	8 20
TOTAL	31992.800	119			

Appendix 21 Nested ANOVAs of *C. australis* growth measures, by chemical treatment type, and sapling

Lateral extent of growth

Source of variation	Sum of squares	DF	Mean square	Variance component	Percentage breakdown
Chemical treatment	6.611	4	1.653	0.092	21.18
Sapling	2.631	15	0.175	0.000	0.00
ERROR	20.610	60	0.344	0.343	78.82
TOTAL	29.852	79			

Radial extent of growth

Source of variation	Sum of squares	DF	Mean square	Variance component	Percentage breakdown
Chemical treatment	23.923	4	5.981	0.356	22.20
Sapling	4.238	15	0.283	0.000	0.00
ERROR	74.903	60	1.248	1.248	77.80
TOTAL	103.064	79			

Growth above wounds

Source of variation	Sum of squares	DF	Mean square	Variance component	Percentage breakdown
Chemical treatment	13722.175	4	3430.544	206.166	30.44
Sapling	1978.359	15	131.891	0.000	0.00
ERROR	28266.188	60	471.103	471.103	69.56
TOTAL	43966.722	79			

Growth below wounds

Source of variation	Sum of squares	DF	Mean square	Variance component	Percentage breakdown
Chemical treatment	11360.586	4	2840.146	154.492	19.62
Sapling	5524.080	15	368.272	0.000	0.00
ERROR	37983.370	60	633.056	633.056	80.38
TOTAL	54868.036	79			

Appendix 22 Details of wilt underground transfer (WUT) plots

Location: Wobbly Creek	**Forest Type:** Mixed forest - fire regrowth
Map (1:100 000): Huon	**Grid Ref:** 933070
Altitude: 110 m	**Aspect:** flat/SE facing slope
Inoculation date:	November 1989
Assessment dates:	October 1990, October 1991, October 1992
No. trees inoculated: 7	**No. successful inoculations:** 7
Spore viability: 86%	**Inoculum concentration:** 10^6 spores/ml

Location: Russell Road	**Forest Type:** Mixed forest - roadside regeneration
Map (1:100 000): Tyenna	**Grid Ref:** 825485
Altitude: 550 m	**Aspect:** S facing slope
Inoculation date:	November 1989
Assessment dates:	October 1990, October 1991, October 1992
No. trees inoculated: 18	**No. successful inoculations:** 14
Spore viability: 98%	**Inoculum concentration:** 10^6 spores/ml

Location: Mt Michael	**Forest Type:** High altitude callidendrous rainforest - fire regrowth
Map (1:100 000): Georges Bay	**Grid Ref:** 838402
Altitude: 710 m	**Aspect:** N facing slope
Inoculation date:	November 1989
Assessment dates:	October 1990, October 1991, October 1992
No. trees inoculated: 11	**No. successful inoculations:** 9
Spore viability: 96%	**Inoculum concentration:** 10^6 spores/ml

Appendix 23 Mycelial interactions between paired isolates of *C. australis*

Mycelial interactions between E8PW and the WUT plot re-isolates

Isolate	Produces reaction in isolates				
	E8PW	**1C**	**1E**	**1H**	**7A**
E8PW	A	AP	A	A	A
1C	AP	APH	APH	AP?H	APH
1E	A	APH	A	AP	AH
1H	AP	APH	A	AH	AH
7A	A	APH	AH	AH	AP

Mycelial interactions between E8PW and the WUT plot controls

Isolate	Produces reaction in isolates			
	E8PW	**L1**	**X1**	**N3**
E8PW	AP?	AP	AP	APH
L1	A	APH	A	
X1	A	AP	APH	APH
N3	AH		AH	AP

Mycelial interactions between E8PW and Victorian isolates

Isolate	Produces reaction in isolates				
	E8PW	**N5**	**N6**	**N7**	**1.66**
E8PW	AP?	A	AH	AP	APH
N5	A	AP	A	AP	AP
N6	AH	A	APH	AH	APH
N7	A	AP	APH	APH	APH
1.66	APH	AP	AH	APH	APH

NB A = Anastomoses

P = Perithecia initials

P? = Possible perithecia initials

H = Hyphal tufts

Appendix 24 Multifactor ANOVAs of the radial growth of *C. australis* at different temperatures, by level and isolate

Radial length at 13.85°C

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECTS	68.663	11	6.242	13.811	0 0000***
Level	16.181	4	4.045	8.950	0.0001***
Isolate	52.482	7	7.497	16.588	0.0000***
RESIDUAL	12.655	28	0.452		
TOTAL	81.319	39			

Radial length at 16.00°C

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECTS	94.440	11	8.767	14.365	0.0000***
Level	3.073	4	0.768	1.259	0.3095 NS
Isolate	93.367	7	13.338	21.854	0.0000***
RESIDUAL	17.089	28	0.610		
TOTAL	113.530	39			

Radial length at 17.45°C

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECTS	83.772	11	7.616	18.252	0.0000***
Level	2.736	4	0 684	1.639	0.1922 NS
Isolate	81.036	7	11.577	27.745	0.0000***
RESIDUAL	11.683	28	0.417		
TOTAL	95.455	39			

Radial length at 19.55°C

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECTS	72.623	11	6.602	9.367	0.0000***
Level	2.310	4	0.577	0.819	0.5238 NS
Isolate	70.313	7	10.045	14.251	0.0000***
RESIDUAL	19.736	28	0.705		
TOTAL	92.359	39			

Radial length at 21.35°C

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECTS	74.473	11	6.770	9.582	0.0000***
Level	2.823	4	0.706	0.999	0.4247 NS
Isolate	71.650	7	10.236	14.487	0.0000***
RESIDUAL	19.784	28	0.707		
TOTAL	94.256	39			

Radial length at 22.75°C

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECTS	133.139	11	12.104	17.173	0.0000***
Level	7.366	4	1.841	2.613	0.0567 NS
Isolate	125.773	7	17.967	25.493	0.0000***
RESIDUAL	19.734	28	0.705		
TOTAL	152.873	39			

Appendix 25 Paired comparisons of θ_2 (minimum temperature for growth) for *C. australis* isolates (showing *d* values and significance levels of individual comparisons)

1H								
L1	0.16							
7A	0.35	0.14						
N3	1.21	0.84	0.88					
E8	1.16	0 87	0.89	0.17				
1E	1.73	1.43	1.50	0.92	0.69			
X1	2.04*	1.65	1.80	1.14	0.84	0.04		
1C	1.48	1.28	1.29	0.82	0.66	0.09	0.06	
	1H	**L1**	**7A**	**N3**	**E8**	**1E**	**X1**	**1C**

NB The sequential Bonferroni technique indicated that none of the individual comparisons can be considered significant at the 5% 'tablewide' level.

Appendix 26 Details of sites and plots used in the gap survey

Site: Arve Loop
Map (1:100 000): Huon
Grid Ref: 796242
Forest Type: *E. regnans* with understorey of T1.1/T3.1
Soil Type: Deep light to medium stony gritty clay - yellowish red (5YR 4/6) gradational on Jurassic dolerite
Land System: Blue Hill, 572243
Altitude: 440-480 m

Details of plots:

PLOT NO.	GAP SIZE (m^2)	GAP OLD OR RECENT	ASPECT (DEGREES)	SLOPE (DEGREES)	DIFFUSE LIGHT (%)	DEPTH OF LITTER (cm)
1	209	recent	65	30	26.0	3.5
2	0		75	23	6.6	7.0
3	186	recent	75	30	55 0	6.0
4	554	recent	70	25	28.6	7.0

Site: Simons Road
Map (1:100 000): Forester
Grid Ref: 442207
Forest Type: C2.1
Soil Type: Stony, strong brown (7.5YR 5/8) gradational soil on Devonian granite or granodiorite
Land System: Burns Creek 641341
Altitude: 820 m

Details of plots:

PLOT NO.	GAP SIZE (m^2)	GAP OLD OR RECENT	ASPECT (DEGREES)	SLOPE (DEGREES)	DIFFUSE LIGHT (%)	DEPTH OF LITTER (cm)
5	146	recent	180	5	28.1	5.0
6	669	recent	230	7	45.0	2.5
7	0		315	5	4.1	5.0?
8	1216	recent	195	3	84.9	0?
12	1305	old	200	5	22.1	1.0
13	213	old	235	6	17.1	5.0
14	349	old	265	10	42.5	2.0
15	±7500	old	220	5	69.2	1.0
16	±7500	old	280	5	66.7	0?
17	0		220?	5?	8.9	1.0?

Site: Wilson Road/ Newdegate Creek

Forest Type: T3.1/CT1

Map (1:100 000): Pieman

Soil Type: Strong brown (7.5YR 5/8) to brownish yellow (10YR 6/6) to yellowish brown (10YR 5/6) gradational soil on Precambrian basalt

Land System: Bernafai 712141

Grid Ref: 370797

Altitude: 120 m

Details of plots:

PLOT NO.	GAP SIZE (m^2)	GAP OLD OR RECENT	ASPECT (DEGREES)	SLOPE (DEGREES)	DIFFUSE LIGHT (%)	DEPTH OF LITTER (cm)
9	190	recent	270	5	49.6	5.0
10	0		270	5	14.4	2.0
11	212	recent	270	5	52.6	1.0

Site: Mt Michael

Forest Type: C2.1

Map (1:100 000): Georges Bay

Soil Type: Stony, brownish yellow (10YR 6/8) gradational clay loam on Devonian granite or granodiorite

Land System: Poimena 741341

Grid Ref: 848403

Altitude: 710 m

Details of plots:

PLOT NO.	GAP SIZE (m^2)	GAP OLD OR RECENT	ASPECT (DEGREES)	SLOPE (DEGREES)	DIFFUSE LIGHT (%)	DEPTH OF LITTER (cm)
18	408	recent	0	0	74.0	2.5
19	140	recent	210	15	39.6	5.0
20	0		205	20	12.1	5.0
21	551	recent	220	20	48.4	2.5
22	592	recent	205	20	94.7	2.5

Site: Five Road

Map (1:100 000): Wedge

Grid Ref: 526694

Forest Type: C1.1 with some *E. obliqua* overstorey in places

Soil Type: Brown/grey gradational soil or alluvium on Ordivician limestone

Land System: Florentine 635221?

Altitude: 440 m

Details of plots:

PLOT NO.	GAP SIZE (m^2)	GAP OLD OR RECENT	ASPECT (DEGREES)	SLOPE (DEGREES)	DIFFUSE LIGHT (%)	DEPTH OF LITTER (cm)
23	447	recent	0	0	54.4	4.00
24	1757	recent	0	0	58.2	3.0
25	553	recent	0	0	45.0	3.0
26	1006	recent	0	0	37.8	0?
27	0		0	0	16.2	2.5

Forest types after Jarman *et al.* (1991). Soil types after Richley (1978), Pinkard (1980), Pinkard (1988), and Pemberton (1989).

Appendix 27 Myrtle and sassafras numbers and percentage fern cover in 27 (10 m^2) plots at five sites.

Site & plot no	Myrtle					Sassafras					% Fern cover
	Ger	See	Veg	Sap	Tre	Ger	See	Veg	Sap	Tre	
Arve Loop											
1	P	16	113	4	2(2)	P	382	6	5	0	15%
2	**A**	**0**	**238**	**0**	**3(0)**	**P**	**36**	**5**	**0**	**0**	**1%**
3	P	92	55	0	1(2)	P	662	1	0	0	14%
4	P	38	2	1	1(8)	P	34	120	8	3	1%
Simons Road											
5	P	20	0	0	4(0)	A	3	55	0	1	25%
6	P	12	0	0	1(2)	A	4	55	0	1	68%
7	**P**	**14**	**6**	**0**	**7(0)**	**A**	**0**	**18**	**1**	**8**	**1%**
8	P	688	1	1	0(3)	A	0	0	0	0	90%
12	P	133	0	0	0(0)	P	2	59	3	0	27%
13	A	87	0	3	1(0)	A	0	0	0	0	30%
14	P	183	0	0	1(0)	A	2	0	0	1	32%
15	A	76	0	0	0(1)	P	1	55	0	2	38%
16	P	21	110	0	2(3)	P	10	440	3	0	22%
17	**P**	**2**	**169**	**0**	**2(0)**	**A**	**0**	**0**	**7**	**4**	**9%**
Wilson Road											
9	A	37	110	22	2(0)	P	23	1	1	0	4%
10	**P**	**44**	**55**	**2**	**3(1)**	**P**	**25**	**171**	**6**	**3**	**6%**
11	P	8	3	0	0(3)	P	29	175	8	5	8%

NB Numbers refer to seedlings, vegetative shoots, saplings and trees (and dead myrtle trees).

Presence or absence of germinants is noted and control plots are shown in **bold.**

Site & plot no	Myrtle					Sassafras					% Fern cover
	Ger	See	Veg	Sap	Tre	Ger	See	Veg	Sap	Tre	
Mt Michael											
18	P	680	22	3	5(2)	A	0	0	0	0	2%
19	P	1029	71	7	11(5)	A	1	0	0	0	3%
20	**P**	**97**	**77**	**7**	**6(0)**	**P**	**0**	**0**	**1**	**0**	**6%**
21	P	276	55	0	1(2)	A	0	0	0	0	11%
22	P	197	0	0	0(1)	P	0	0	0	0	6%
Five Road											
23	A	8	3	0	2(0)	P	293	110	1	3	42%
24	P	20	0	0	0(2)	P	97	64	1	1	40%
25	P	0	0	0	0(0)	P	16	171	1	2	20%
26	P	5	56	0	0(2)	P	204	110	0	0	58%
27	**A**	**25**	**69**	**6**	**4(0)**	**P**	**786**	**9**	**2**	**4**	**10%**

NB Numbers refer to seedlings, vegetative shoots, saplings and trees (and dead myrtle trees).

Presence or absence of germinants is noted and control plots are shown in **bold**.

Appendix 28 ANOVAs of myrtle and sassafras regeneration and fern cover in 27 gap and control plots at five sites.

ANOVA of myrtle seedling numbers/10 m^2

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECTS	740821	5	148164	3.142	0.0345*
Gap/control	77814	1	77814	1.650	0.2162
Site	646090	4	161522	3.425	0.0315*
2 FACTOR INTERACTIONS					
Gap/control x Site	118999	4	29750	0.631	0.6472
RESIDUAL	801680	17	47158		
TOTAL	1661499	26			

ANOVA of myrtle vegetative shoot numbers/10 m^2

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECTS	41262	5	8252	3.415	0.0256*
Gap/control	23332	1	23332	9.655	0.0064**
Site	15905	4	3976	1.645	0.2089
2 FACTOR INTERACTIONS					
Gap/control x Site	13638	4	3410	1.411	0.2727
RESIDUAL	41083	17	2417		
TOTAL	95984	26			

ANOVA of sassafras seedling numbers/10 m^2

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECTS	518515	5	103703	8.080	0.0005***
Gap/control	11382	1	11382	0.887	0.3694
Site	506793	4	126698	9.872	0.0003***
2 FACTOR INTERACTIONS					
Gap/control x Site	351689	4	87922	6.851	0.0018**
RESIDUAL	218184	17	12834		
TOTAL	1088388	26			

ANOVA of sassafras vegetative shoot numbers/10 m^2

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECTS	42886	5	8577	0.805	0.5618
Gap/control	6623	1	6623	0.621	0.4497
Site	37530	4	9382	0.880	0.4963
2 FACTOR INTERACTIONS					
Gap/control x Site	16554	4	4139	0.388	0.8141
RESIDUAL	181185	17	10658		
TOTAL	240626	26			

ANOVA of rooted fern cover (arcsin square root of percentage)

Source of variation	Sum of squares	DF	Mean square	F ratio	Significance level
MAIN EFFECTS	3962	5	792	5.900	0.0024**
Gap/control	1034	1	1034	7.700	0.0130*
Site	2724	4	681	5.070	0.0071**
2 FACTOR INTERACTIONS					
Gap/control x Site	682	4	171	1.270	0.3199
RESIDUAL	2282	17	134		
TOTAL	6927	26			

Appendix 29 EXCEL model of the changes in a myrtle population over 60 years, applying average levels of myrtle wilt

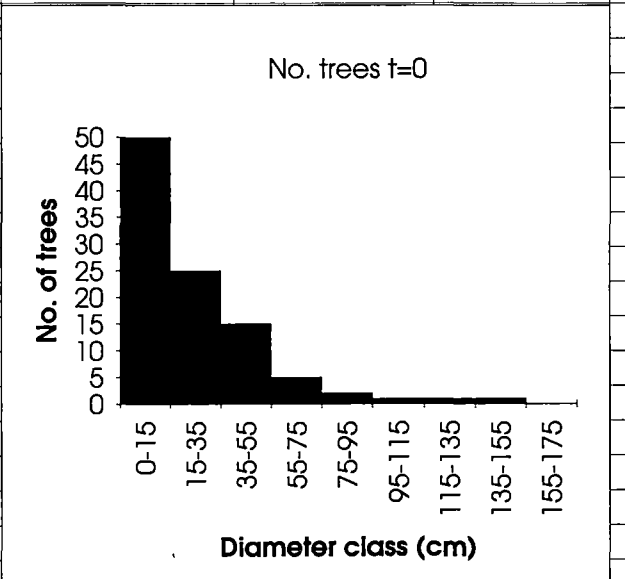
MYRTLE WILT MODEL				
CONSTANTS				
Diam class (cm)	No. trees t=0	BA/tree	BA t=0	
0-15	50	0.0044	0.22	
15-35	25	0.0491	1.2275	
35-55	15	0.159	2.385	
55-75	5	0.3318	1.659	
75-95	2	0.5675	1.135	
95-115	1	0.8659	0.8659	
115-135	1	1.2272	1.2272	
135-155	1	1.6513	1.6513	
155-175	0	2.1382	0	
			10.3709	
p.a. mort (wilt)	p.a. mort (other)	60yr survival		
0	0.043956	0.067402005		
0.005054942	0.0015	0.673955497		
0.007032963	0.0015	0.597993957		
0.012747245	0.0015	0.422746452		
0.02307691	0.0015	0.224689053		
0.020219769	0.0015	0.267791412		
0.048791182	0.0015	0.045230171		
0.04395602	0.0015	0.061341809		
0.039999978	0.960000022	0		
MORTALITY X GROWTH				
No. trees t=n	Survivors 1	BA survivors 1	BA status 1	Seed tree no.s
50				
25	3.370100252	0.165471922		
15	16.84888742	2.6789731		
5	8.969909361	2.976215926		
2	2.11373226	1.199543057		
1	0.449378106	0.389116502		
1	0.267791412	0.328633621		
1	0.045230171	0.074688581		
0	0.061341809	0.131161057		
	p.a. mort (total)			
	0.018746932	7.943803766	2.427096234	11.90738312
	p.a. mort (>15cm)			
	0.009177084			

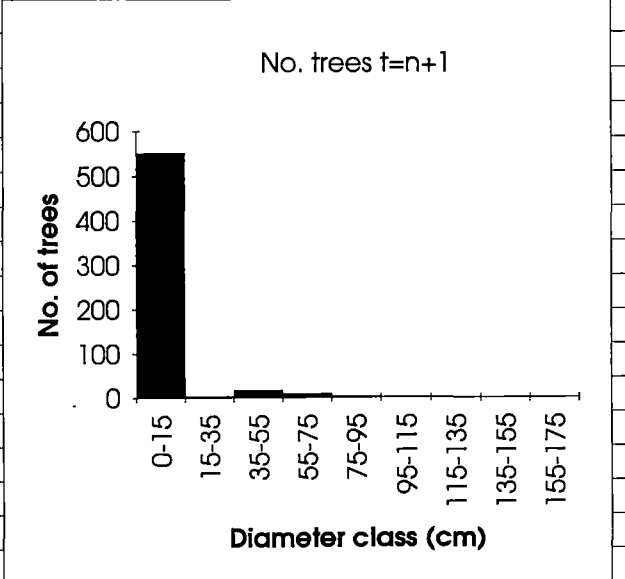
REGENERATION 0-15				
Final 0-15	Survivors 2	BA survivors 2	BA status 2	
551.6127804	551.6127804	2.427096234		
	3.370100252	0.165471922		
	16.84888742	2.6789731		
	8.969909361	2.976215926		
	2.11373226	1.199543057		
	0.449378106	0.389116502		
	0.267791412	0.328633621		
	0.045230171	0.074688581		
	0.061341809	0.131161057		
		10.3709	0	
SELF THINNING 15-35				
Final 15-35	Survivors 3	BA survivors 3	BA status 3	
	551.6127804	2.427096234		
3.370100252	3.370100252	0.165471922		
	16.84888742	2.6789731		
	8.969909361	2.976215926		
	2.11373226	1.199543057		
	0.449378106	0.389116502		
	0.267791412	0.328633621		
	0.045230171	0.074688581		
	0.061341809	0.131161057		
		10.3709	0	
SELF THINNING 35-55				
Final 35-55	Survivors 4	BA survivors 4	BA status 4	
	551.6127804	2.427096234		
	3.370100252	0.165471922		
16.84888742	16.84888742	2.6789731		
	8.969909361	2.976215926		
	2.11373226	1.199543057		
	0.449378106	0.389116502		
	0.267791412	0.328633621		
	0.045230171	0.074688581		
	0.061341809	0.131161057		
		10.3709	0	

SELF THINNING 55-75				
Final 55-75	Survivors 5	BA survivors 5	BA status 5	
	551.6127804	2.427096234		
	3.370100252	0.165471922		
	16.84888742	2.6789731		
8.969909361	8.969909361	2.976215926		
	2.11373226	1.199543057		
	0.449378106	0.389116502		
	0.267791412	0.328633621		
	0.045230171	0.074688581		
	0.061341809	0.131161057		
		10.3709	0	
SELF THINNING 75-95				
Final 75-95	Survivors 6	BA survivors 6	BA status 6	
	551.6127804	2.427096234		
	3.370100252	0.165471922		
	16.84888742	2.6789731		
	8.969909361	2.976215926		
2.11373226	2.11373226	1.199543057		
	0.449378106	0.389116502		
	0.267791412	0.328633621		
	0.045230171	0.074688581		
	0.061341809	0.131161057		
		10.3709	0	
SELF THINNING 95-115				
Final 95-115	Survivors 7	BA survivors 7	BA status 7	
	551.6127804	2.427096234		
	3.370100252	0.165471922		
	16.84888742	2.6789731		
	8.969909361	2.976215926		
	2.11373226	1.199543057		
0.449378106	0.449378106	0.389116502		
	0.267791412	0.328633621		
	0.045230171	0.074688581		
	0.061341809	0.131161057		
		10.3709	0	

SELF THINNING 115-135				
Final 115-135	Survivors 8	BA survivors 8	BA status 8	
	551.6127804	2.427096234		
	3.370100252	0.165471922		
	16.84888742	2.6789731		
	8.969909361	2.976215926		
	2.11373226	1.199543057		
	0.449378106	0.389116502		
0.267791412	0.267791412	0.328633621		
	0.045230171	0.074688581		
	0.061341809	0.131161057		
		10.3709	0	
SELF THINNING 135-155				
Final 135-155	Survivors 9	BA survivors 9	BA status 9	
	551.6127804	2.427096234		
	3.370100252	0.165471922		
	16.84888742	2.6789731		
	8.969909361	2.976215926		
	2.11373226	1.199543057		
	0.449378106	0.389116502		
	0.267791412	0.328633621		
0.045230171	0.045230171	0.074688581		
	0.061341809	0.131161057		
		10.3709	0	
SELF THINNING 155-175				
Final 155-175	Survivors 10	BA survivors 10	BA status 10	
	551.6127804	2.427096234		
	3.370100252	0.165471922		
	16.84888742	2.6789731		
	8.969909361	2.976215926		
	2.11373226	1.199543057		
	0.449378106	0.389116502		
	0.267791412	0.328633621		
	0.045230171	0.074688581		
0.061341809	0.061341809	0.131161057		
		10.3709	0	

Diam class (cm)	No. trees t=n+1
0-15	551.6127804
15-35	3.370100252
35-55	16.84888742
55-75	8.969909361
75-95	2.11373226
95-115	0.449378106
115-135	0.267791412
135-155	0.045230171
155-175	0.061341809





Of making many books there is no end,
and much study wearies the body.
Ecclestiastes 12:12

There is nothing new under the sun.
Ecclestiastes 1:9

Attributed to King Solomon, c. 935 BC.