GENOTYPIC VARIATION IN RESISTANCE TO PHYTOPHTHORA CINNAMOMI IN THE PINUS RADIATA SPECIES.

by

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SUMMARY

The aim of this study was to examine the variability in resistance to *Phytophthora cinnamomi* within the *Pinus radiata* species, to investigate its inheritance, and to determine a strategy for including the disease resistance character into Western Australian populations of radiata pine.

At the provenance or major population level, seedlings from Cambria showed the greatest degree of resistance and Monterey was similar, while seedlings from Ano Nuevo and the two island populations of Guadalupe and Cedros generally had a susceptible reaction. The variation in disease response was equally as large between the world's major domestic populations as it was among the natural populations. *Pinus radiata* from South Africa was consistently less affected by *Phytophthora cinnamomi* infection than was *Pinus radiata* from Australia and New Zealand.

Techniques for the glasshouse test have been defined, and refined to give the maximum expression of the *Phytophthora cinnamomi* disease for each family. The development of the empirical scale to represent the continuous variation in response of *Pinus radiata* seedlings to infection with *Phytophthora cinnamomi*, was a major improvement.

Two field tests have shown that the family response to *P. cinnamomi* is similar in the glasshouse and in the field. Family rankings for the incidence of disease were very highly correlated in both test environments. The glasshouse test can be used as a reliable and rapid method for determining the *Pinus radiata* family response to *Phytophthora cinnamomi.*

The genetic gain of 60% in seedling survival has been calculated for using only genotypes that have a tolerant or moderately-tolerant response, as compared with using a seed population that ignores this *Phytophthora* tolerance character. The effect of sub-lethal infections of *P. cinnamomi* on the growth of plantation trees of *Pinus radiata* has been quantified. The field tests, FP1 and FP2, have indicated that height growth was reduced by 9% when compared with the growth of a population that included tolerance to *Phytophthora cinnamomi* as a major character.

Variability within the *P. cinnamomi* pathogen population has been found to be considerable, and this has been shown to be independent of the variation in the host.

Heritability of the response of seedlings and trees of *Pinus radiata* to *Phytophthora cinnamomi* root-rot was very high and consistent, both at the family and individual level. Open-pollinated families of *Pinus radiata* have shown a remarkable consistency in their response to *Phytophthora cinnamomi* infection, indicating that cytoplasmic inheritance may be significant. Confirmation of this is required as it has a profound effect on the constitution and development of sublines within the Western Australian breeding population.

Resistance to *P. cinnamomi* disease in *Pinus radiata* is under strong genetic control. This has been shown to be constant in a diverse range of environments and for a wide range of pathogen isolates. The level of disease expression has varied according to the conditions of the environment and the virulence of the *Phytophthora cinnamomi* isolates used in the tests. However, ranking of families for their response to the pathogen has remained unchanged and in particular, the variation in disease expression in the host appears to be independent of the variation in the pathogen. Both of these forms of variability are rarely encountered with disease resistance that is controlled by major genes. Indications are that there are a number of genes in *Pinus radiata*, each having a small effect, acting additively to give general resistance to *Phytophthora cinnamomi.*

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CHAPTER 1

GENERAL INTRODUCTION

In Western Australia the majority of the timber supply has been provided from native hardwood forests. Demand for sawn timber is expected to increase from the current supply of 1.0 million cubic metres, to between 1.2 to 1.7 million cubic metres by 2050 (Conservation and Land Management, 1987). Within this time, the original hardwood forest will have been harvested and the industry will have to adjust to efficiently process the new regrowth forests. Areas of indigenous forest available for timber production will be further reduced by alienation for conservation and recreation areas. Designated timber production areas could also be lost through the spread of the jarrah dieback disease and release of State Forest for other government utilities. The present yield from the hardwood forests cannot be sustained because of the slow growth rates and the impact of the dieback disease (Forests Department of W.A., 1975). Hardwood yield will need to be reduced by one half to ensure any degree of continuity in hardwood log supplies.

There is the need in Western Australia to supplement the indigenous hardwood timber supply with local, plantation grown softwood. Plantations of pine trees have been planted in Western Australia from around the turn of the century to the present (Figure 1). The area of pine plantations established is 60,512 ha, with 55% of the area as *Pinus radiata* and 45% as *P. pinaster*. *Pinus radiata* is the preferred species because of its inherent faster growth rate, although it has more stringent site and fertiliser requirements to achieve its potential. Sawlogs are the principal objective of the plantations, to make up for the sawlog deficit from the hardwood forests. Short rotation plantations of fast growing softwoods are technologically better than hardwoods for this need. A continuing programme of pine planting is required to provide for the future timber demands of this state.

The limited area of land available, suitable for pine planting has been a major restriction to the programme. The policy has been to use repurchased farmland when it is available, but this has been insufficient to meet the plantation requirement.

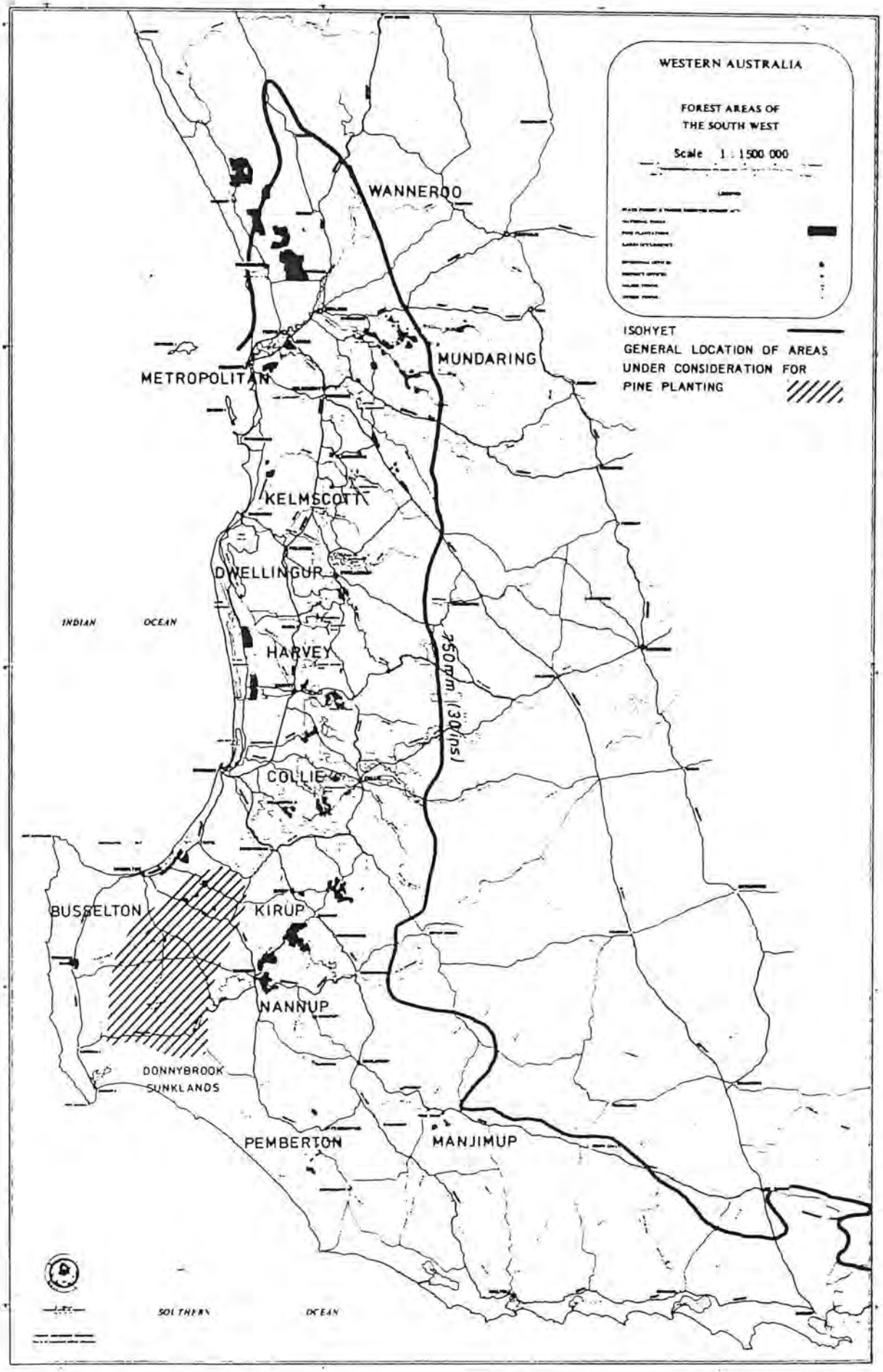

FIGURE 1

Location of major pine plantation areas in Western Australia.

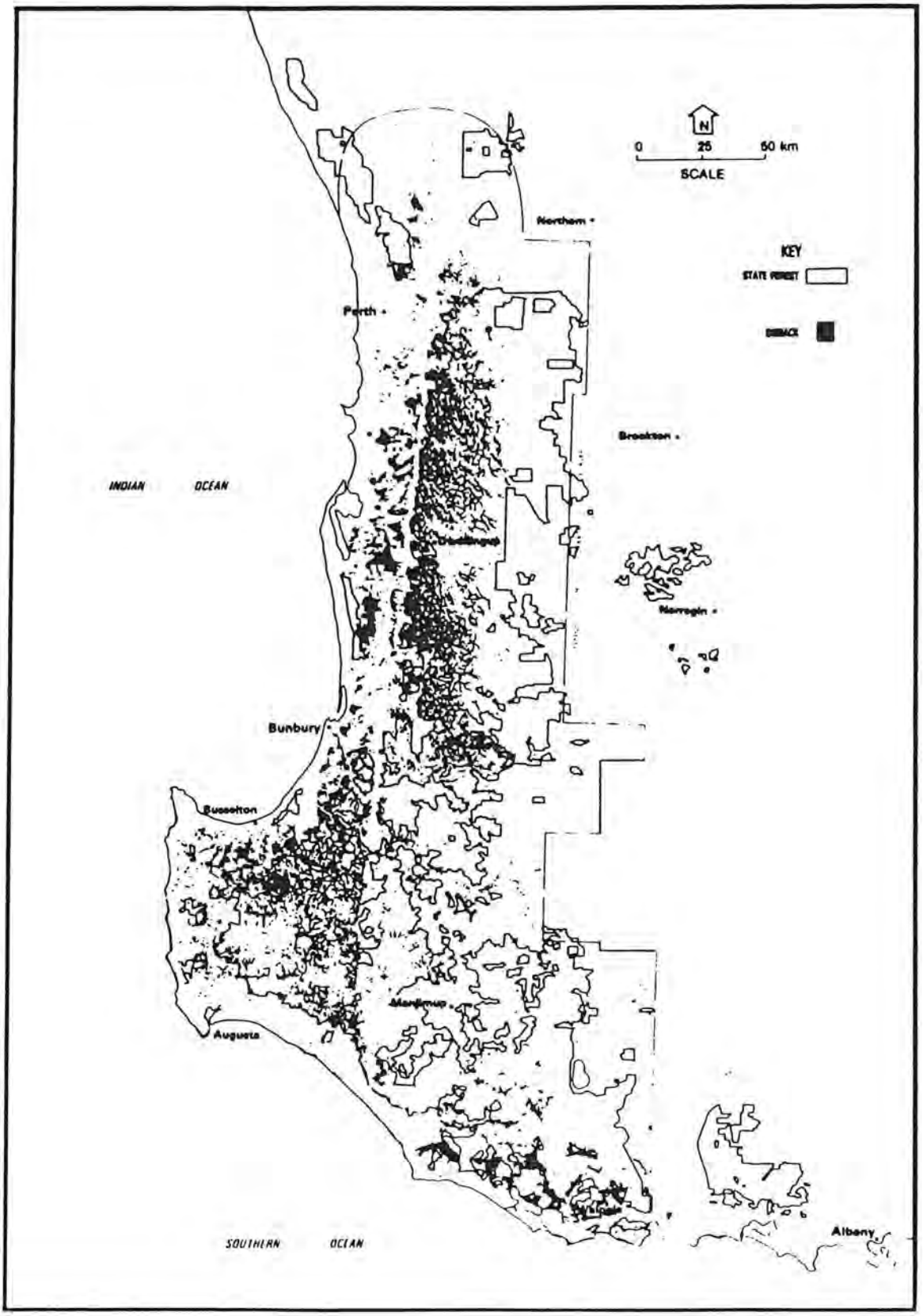

FIGURE 2

General distribution of *Phytophthora cinnamomi* in Western Australia.

In 1975 (Forests Department of W.A.), it was proposed to develop a 60,000 ha pine re-afforestation programme in the Donnybrook Sunkland, by conversion of a native hardwood forest, predominantly of *Eucalyptus marginata* (jarrah), into productive pine plantations. The jarrah dieback disease caused by *Phytophthora cinnamomi* (Podger, 1972) is prevalent in the area. In 1973, 16% of the Sunkland forest was recorded as diseased and because of its extreme susceptibility, it was seen as inevitable that some 60% of the forest would be affected by the extension of existing infections (Forests Department of W.A., 1975). The preferred species for this programme was the highly productive *Pinus radiata,* although there had been reports in the literature citing *P. radiata* as susceptible to *Phytophthora cinnamomi* (Newhook, 1959). Extensive planting of the Sunkland commenced in 1974. Chevis and Stukely (1982) reported deaths of established pine trees that were constantly associated with root infection by *Phytophthora* spp. (usually *P. cinnamomi* A2). Butcher *et al.* (1984) reported that there was considerable variation within *Pinus radiata* in tolerance to *Phytophthora cinnamomi*, that this was under strong genetic control, and that the resistance also operated in the field. Immediate steps were taken to screen all parents in the Western Australia breeding population for tolerance to *P. cinnamomi.* Based on these results, part of the West Manjimup seed orchard was culled of susceptibile genotypes to produce a *Pinus radiata* seed source with tolerance to the *Phytophthora cinnamomi* disease.

A Government policy change in 1984, not to clear any more native hardwood forests for pine plantations, ended the Sunkland project. The area of pine plantations established was 10,600 ha (December, 1987), principally of *Pinus radiata.*

Government policy is to maintain a State plantation establishment rate of 2,000 ha per annum of *P. radiata* until the early part of the twenty-first century. The intention is to establish 1,000 ha near Albany, 500 ha in the Southern Forest Region and 500 ha in the Central Forest Region (Conservation and Land Management. 1987). *Phytophthora cinnamomi* is prevalent over most of this area (Figure 2). It is accepted that *Pinus radiata,* with tolerance to *Phytophthora cinnamomi* as a principal character, will be required for the majority of these plantations.

1.1. Objectives of the Study.

The paper of Butcher *et al.* (1984) made two very worthwhile contributions to the *Pinus radiata / Phytophthora cinnamomi* root-rot system. Firstly, it established very clearly that resistance in *Pinus radiata* to *Phytophthora cinnamomi* was under strong genetic control and secondly, that genetic control of this resistance as demonstrated in the glasshouse experiments, also operated in the field. This original work gave the potential to reduce disease in the field by the selection of host genotypes. However, whether such a selection would result in reduced disease or in epidemics depended on factors for which there was little current knowledge. The aim of this study was to acquire that knowledge.

The specific objectives were to :

1. Define the most efficient procedures of selection for resistance to *P. cinnamomi* root-rot in *Pinus radiata.*
2. Evaluate the variation in resistance to *Phytophthora cinnamomi* within the natural populations of *Pinus radiata.*
3. Evaluate the variation in resistance to *Phytophthora cinnamomi* within the world's major, improved domestic populations of *Pinus radiata.*
4. Study the inheritance of disease resistance.
5. Examine the stability of the host / pathogen interaction.
6. Examine the genetic variability in disease resistance in the field, and the effects of sub-lethal infections on the growth of *P. radiata* trees.
7. Predict genetic and realised gains from breeding *Phytophthora cinnamomi* resistance in *Pinus radiata.*
8. Achieve a better understanding of the epidemiology and genetics of the *Pinus radiata / Phytophthora cinnamomi* system, for the planning of disease control stategies.

1.2. List of experiments conducted to achieve objectives.

NP1. Natural population, glasshouse test No.1.
Pinus radiata populations from Ano Nuevo, Monterey (coastal and inland), Cambria and Cedros Island.

NP2. Natural population, glasshouse test No.2.
Pinus radiata populations from Ano Nuevo, Monterey (coastal and inland), Cambria, Cedros Island and Guadalupe Island.

DP1. Domestic population, glasshouse test No.1.
Pinus radiata families from South Africa (n = 22), South Australia (n = 18), New Zealand (n = 5) and W.A. standard response families.

DP2. Domestic population, glasshouse test No.2.
Different *Pinus radiata* families from South Africa (n = 12), South Australia (n = 12), New Zealand (n = 20) and W.A. standard response families.

FP1. Field population, test No.1.
Pinus radiata families from the Western Australian breeding population (n = 26) planted on a natural *Phytophthora cinnamomi* infested site, and with artificially applied inoculum.

FP2. Field population, test No.2.
Different *Pinus radiata* families from the Western Australian breeding population (n = 36) planted on a disease-free site, and on a natural *Phytophthora cinnamomi* infested site with artificially applied inoculum.

HP1. Host *x* pathogen, glasshouse test No.1.
2 tolerant, 1 average and 2 susceptible disease response families of *Pinus radiata,* with 8 isolates of *Phytophthora cinnamomi* A2.

HP2. Host *x* pathogen, glasshouse test No.2.
3 tolerant and 5 susceptible disease response families of *Pinus radiata;* with 8 isolates of *Phytophthora cinnamomi* A2, a single isolate of *P. cinnamomi* A1 and an uninoculated control.

CHAPTER 2

REVIEW OF LITERATURE

2.1 PINUS RADIATA : HOST

2.1.1 Natural occurrence

The natural forests of *Pinus radiata* D.Don. are restricted to very small areas in coastal California and to two small islands off the Baja peninsula in Mexico (Figure 3). The small area of some 7,000 ha (Forde, 1966) belies its importance as the world's most important softwood crop. More than 2.5 million hectares of plantation have been established in the Southern Hemisphere.

Pinus radiata occurs in three separate places on the coast of central California, covering a range of about 300 kilometres (Figure 3). In these areas the pines are never more than 7 km from the sea and mostly much less. They are found almost entirely on gently sloping land lower than 300m above sea level. The three mainland populations are at Point Ano Nuevo near Swanton, 37° 06' (area of about 500 ha), Monterey, 36° 35' (area of 5,000 ha) and Cambria, 35° 35' (area of 1,000 ha). These natural stands have been discussed in detail by Lindsay (1932), Forde (1966) and Roy (1966). The most striking feature of the species distribution is its discontinuity, being restricted to headlands and ridges which were probably islands in the Pliocene or Pleistocene periods (Scott, 1960).

The two small island natural stands form disjunct and isolated populations of genetically interesting radiata pine. Guadalupe Island, at latitude 29° 10', is 800 km south of the most southerly mainland population. Libby *et al.* (1968) counted only 383 living pines during their expedition to the island in 1964. The other island occurrence is Cedros Island, some 300 km southeast of Guadalupe Island, at latitude 28° 16'. Here the pines occur in two major stands, occupying about 100 ha in the north of the island and 50 ha inland on the central part of the island (Libby *et al.*, 1968).

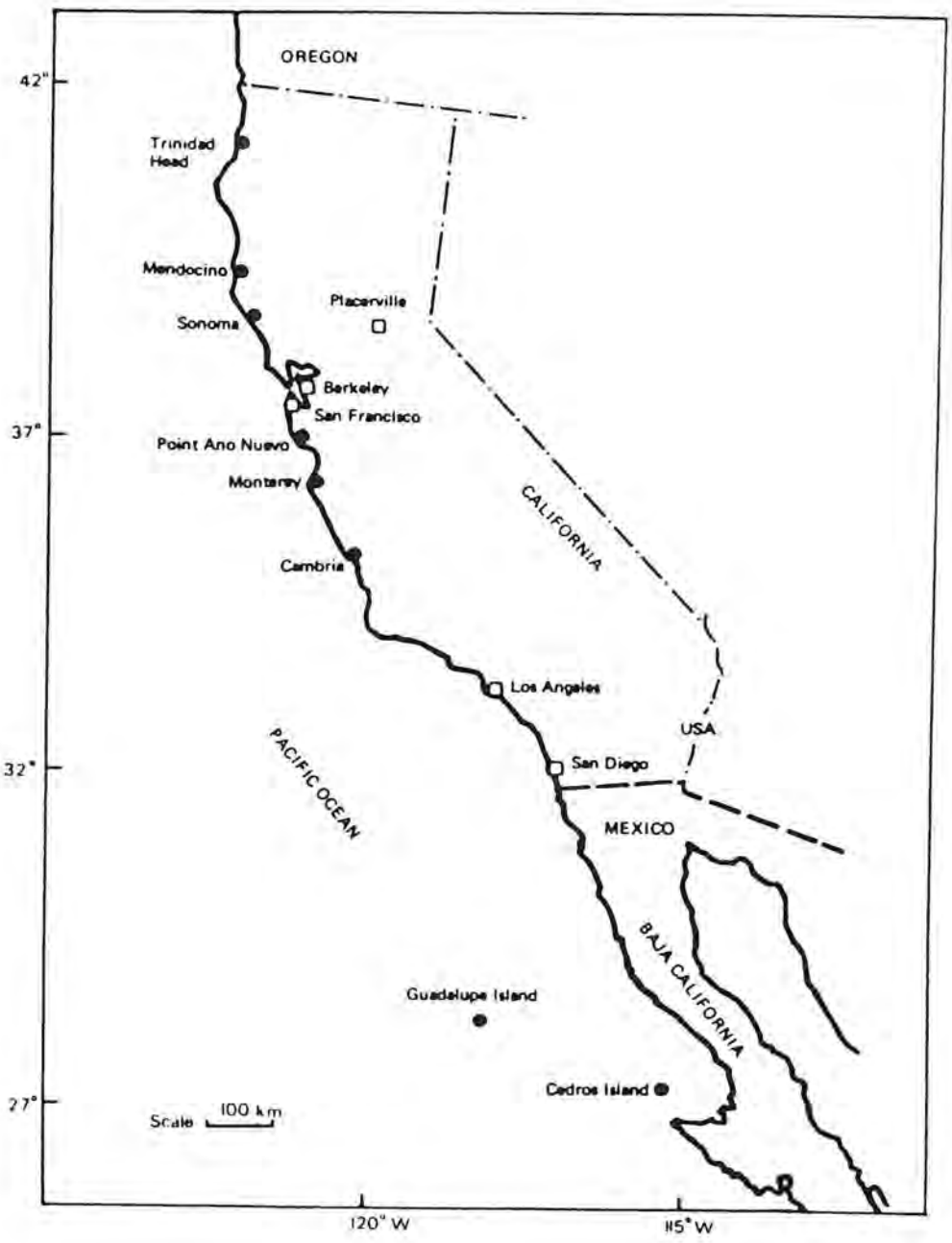

FIGURE 3

Natural distribution of *Pinus radiata*.

The central Californian coast has a Mediterranean type climate. Rainfall is concentrated in the winter months and summers are usually dry, although fogs are frequent. Temperatures are moderate and are relatively uniform throughout the year. Lindsay (1932) compiled extensive climatic records for this general area. Climate of the individual forest stands was reviewed by Scott (1960) and Forde (1966). Mean annual rainfall approximates 700 mm at Ano Nuevo, which is 50% greater than at the two southern stands. However, appreciable additional moisture is received from condensation of heavy summer fogs (Forde, 1966). Monterey pine is not found growing naturally outside the fog

However, appreciable additional moisture is received from condensation of heavy summer fogs (Forde, 1966). Monterey pine is not found growing naturally outside the fog belt; this source of moisture is essential to its survival in the southern stands. Eldridge (1983) indicated that the summer fog was even more critical to the survival of radiata pine on the high ridges of Cedros and Guadalupe Islands where rainfall is much lower.

TABLE 1

Identification and description of natural population seedlots of *Pinus radiata*. (After Eldridge, 1978a, and unpublished)

Population (rainfall)		Sub-population	Latitude	Longitude	Altitude m
Ano Nuevo	01/1	coastal strip	37^{0}06'	122^{0}17'	15- 60
	01/2	inland, central	37^{0}06'	122^{0}16'	120-330
(700 mm)	01/3	inland, Swanton	37^{0}04'	122^{0}14'	60-240
	01/4	inland, northern	37^{0}08'	122^{0}18'	60-210
Monterey	02/1	coastal dunes	36^{0}37'	121^{0}57'	5- 60
	02/2	Del Monte	36^{0}35'	121^{0}52'	20- 60
(450 mm)	02/3	Huckleberry Hill	36^{0}35'	121^{0}55'	60-210
	02/4	Jack's Peak	36^{0}33'	121^{0}52'	60-300
	02/5	Point Lobos	36^{0}30'	121^{0}57'	15- 60
	02/6	Carmel Highlands	36^{0}30'	121^{0}55'	80-580
Cambria	03/1	Pico Creek	35^{0}37'	121^{0}09'	30-120
(500 mm)	03/2	Cambria town	35^{0}34'	121^{0}06'	30-120
	03/3	Scott Rock	35^{0}35'	121^{0}04'	60-180
Guadalupe Island		(L)	29^{0}10'	118^{0}18'	580-1160
(150 mm, sea level)					
Cedros Island		(T)	28^{0}16'	115^{0}14'	290-610
(150 mm, sea level)					

Pinus radiata occurs on a very wide range of soil types in its native habitat. Detailed soisurveys are available for Monterey (Cooke, 1978) and Ano Nuevo (Bowman and Estrada, 1980) but there is little published information on soils in the other three areas. Shallow soils restrict the growth and occurrence of the tree and it does not occur appreciably on poorly drained soils. The best growth is achieved with a one metre deep sandy-loam (Scott, 1960). Lindsay (1932) reported that *P. radiata* also grew on clay-loams

Monterey, from silica-feldspar sands, coarse sandy loams through to shaley clay-loams. He divided the Monterey population into six sub-populations (Table 1) and a fair degree of uniformity existed in soil type for each sub-population .

The distinctive feature of the soils of the Cambrian forests is their relative uniformity compared with those of Monterey and Ano Nuevo. The pines are confined to sandy loam soils and do not extend onto the surrounding heavier soils or the small areas of coastal dune sand. However, Eldridge (personal communication, 1984) has described the Cambrian stands as "....the soggiest, poorest drained sites of the mainland populations".

Eldridge (1983) reported that the pines of Cedros and Guadalupe Islands are located high on exposed ridges of shallow skeletal soil. Guadalupe Island is an old oceanic volcano and its soils are well-drained basaltic loams. Cedros Island soils are mostly derived from sedimentary and metamorphic rocks (Libby *et al.* 1968).

2.1.1 Introduction as an exotic

Pinus radiata is the most important conifer for commercial afforestation that has been introduced into the Southern Hemisphere. Eldridge (1983) reported that about 2.5 million hectares of fast growing plantations of this species have been established in Australia, New Zealand, South Africa and Chile. *Pinus radiata* is adapted to the Mediterranean climate of California and thrives best as an exotic when introduced into a similar habitat. The total world area suitable for *P. radiata* is very limited, being confined to parts of two climatic regions and to sites lying between the latitudes of 30° and 46°. Of these regions, the Mediterranean is most favourable provided that soil conditions are reasonably good and the rainfall is over 500 mm and preferably over 750 mm. The West-Maritime region is also suitable provided that very low temperatures, unseasonable frosts, or prolonged summer rains do not occur (Rawlings, 1957).

As seed from the improved domestic populations forms part of the major thesis studies, a description of the regions of origin follows:

Pinus radiata in Australia

Pinus radiata was probably first introduced into Australia about 1857 where it was recorded as being planted in the Sydney and Melbourne Botanical Gardens, and in 1866 in the Adelaide Botanical Gardens (Fielding, 1957). In a review of Australian literature, records and from the recollections of older foresters, Eldridge (1983) concluded that no radiata pine plantations were established from seed imported directly from California. Early plantations were made from seed collected from park or shelterbelt trees and in later years, from Australian and New Zealand plantations.

The similarity of the appearance of trees and particularly the cone morphology, between Australian trees and the native Monterey stand was noted by Fielding (1961) and he concluded that Monterey was probably the original seed source. Research by Eldridge (1978b) indicated that the early seed collections in California were from a small number of coastal trees at Monterey and Ano Nuevo. This was later confirmed by Moran and Bell

(1983), using the gel electrophoresis technique. They found no material from Cambria, Guadalupe or Cedros Islands in the Australian breeding stock, and concluded that the original source of Australian plantations was the Monterey population, but considered that some Ano Nuevo material could be present.

In Western Australia, *P. radiata* was first planted at Bunbury on the coastal plain sands in 1897. These were a failure because of the poor sandy soils (Forests Department of W.A., 1969). The first *P. radiata* plantations on deep fertile loams were planted at Mundaring in 1922, using seed imported from South Australia. The seed source for all of the early plantations was from South Australia, although some seed was obtained from New Zealand, Victoria and New South Wales, during the period 1950 to 1960. Seed was collected from Western Australian plantations for local use after 1968, and from seed orchards after 1974.

Most of the older plantations in Western Australia have been established on naturally fertile loamy soils of basic origin. Suitable land has been obtained by repurchasing farmland, notably in the Blackwood Valley. However, funds for land purchase were very limited and there was considerable opposition to this policy from rural communities, and particularly from local government. This led to the development in the 1970's of the Sunkland pine project, under which a total of 60,000 ha of poor quality, diseased jarrah forest growing on duplex sandy soils was to be converted to pines over a thirty year period. This programme was halted in 1983 when Government policy reverted to the purchase of agricultural land.

TABLE 2

Plantation areas of *Pinus radiata* in Australia (March 1983) and the Australian plantings for the year 1982. (Data from Bureau of Agricultural Economics, 1984)

State	Plantation area (ha) total planted	annual planting
New South Wales	183,472	10,952
Victoria	173,153	7,925
South Australia	74,801	2,598
Tasmania	57,314	2,970
Western Australia	40,494	1,787
Australian Capitol Territory	13,324	0
Queensland	3,532	12
Australian total	546,090	26,244

An area of 546,090 ha of *P. radiata* plantation was established in Australia by 1983 (Bureau of Agricultural Economics, 1984). The current planting rate was given as 26,244 ha per annum. Individual State details are listed in Table 2.

Pinus radiata in Australia has been an outstanding success as a plantation species. It is a hardy tree, easy to establish on suitable sites, where it makes exceptionally fast growth to produce a high yield of timber. The wood is suitable for a wide range of uses, from sawtimber to paper pulp.

Pinus radiata in New Zealand

The tree was most likely introduced to New Zealand as individual specimens although Bannister (1965) traced a record of one settler coming to New Zealand via California in 1865 with seed and established a single plantation of some 4,000 trees on his farm. Other introductions were recorded by Weston (1957). A single *P. radiata* tree produces copious amounts of seed with

the result that large numbers of trees can originate from small numbers of parents. For example, Bannister (1965) reported that Dr. S.P. Jamieson in 1914, collected enough seed from a single tree to plant one half of his 20 ha plantation. The new Zealand Forest Service has regularly collected seed for its own use from this plantation. Seed from this plantation has also been sold to Australia, including Western Australia for afforestation. The extensive pine forests established in the 1920's and 1930's were grown from seed collected from the early shelterbelts and block plantings (Wilcox, 1983b).

The origin of the New Zealand *P. radiata* stock is not known but Burdon (1985) suggests that it is a fusion, in varying proportions, of two components :

1. A narrowly based element from the Monterey population, introduced piecemeal, mainly up to the mid-1860's; much of this material would have come via Australia but all might have come directly or indirectly from Britain.

2. A more broadly based element from the Ano Nuevo population which may have come in from the late 1860's and which may represent several initial collections.

A close resemblance between the two New Zealand populations (Kaingaroa - North Island, Nelson - South Island) and the Ano Nuevo population with respect to many characters was found by Burdon and Bannister (1973). They reported that Nelson appeared to contain an appreciable mixture of Monterey genes (about 40%) but Kaingaroa had less (about 20%). Further, they deduced that Cambria was probably not represented in New Zealand stock.

Man-made exotic forests in New Zealand now cover 0.9 million ha and 90% or 0.8 million ha is *P. radiata.* Each year about 40,000 ha of unproductive land, including uneconomic farmland, are converted to new *P. radiata* forest and another 16,000 ha are replanted following the logging of mature plantations. It involves the annual planting of about 70 million trees (Wilcox, 1983b).

Pinus radiata in South Africa

The provenance of seedlings used for afforestation in South Africa can not be traced to any particular locality within the natural range of the species (King, 1925). At no time was seed ever imported direct from California or neighbouring islands (Poynton, 1957). The early plantations used seed collected locally and was later supplemented by seed imported mainly from New Zealand and from agents in Europe, Australia and the United States. Most of the seed used for commercial planting in South Africa was collected from cultivated trees of either unknown or unspecified provenance. Poynton (1977) reasoned that the Monterey district was the most accessible of the three mainland localities in the early days and was therefore most likely to have been the source of practically all of the first seed collections. His inference was that the Monterey provenance predominates in South Africa.

Pinus radiata has been planted mainly in the winter and uniform rainfall areas, where it thrives on cool, relatively gentle, southerly and southeasterly slopes on the coastal belt. It is exacting in its edaphic requirements, needing a moderately fertile, deep, well drained soil for its optimum development. The tree is not thrifty on sites where root penetration and drainage are impeded and on these sites it can be severely attacked by *Diplodia pinea* (Poynton, 1977). However, even on marginal sites *P. radiata* is planted in preference to other species on the principle that the yield from an unthrifty stand of *P. radiata* is likely to be better than that which would otherwise be obtained (Poynton, 1977). An area of 48,000 ha had been planted to *P. radiata* as at March 1974 (Poynton, 1977).

Pinus radiata in Chile

Pinus radiata was introduced into Chile in 1885; large scale planting began in a number of places about 1935 and increased to a peak of 16,000 ha per annum in 1947-49 (Scott, 1960). The area currently being planted is about 45,000 ha per annum. The country's total artificially afforested area amounts to 1.20 million hectares, of which 1.04 million are radiata pine (Chilean Forestry News, 1987).

In Chile the Mediterranean region provides an almost exact replica of California. Generally, Chile is more suitable for MIP. radiata than are the native habitats because the soils are usually more favourable, there is little wind and the Californian mistletoe, fungi and insects are absent (Rawlings, 1957). The Chilean stands are specially healthy and vigorous, even more so than in New Zealand (Scott, 1960).

2.1.3 Diseases and Pests

One reason that is often given for the outstanding success of *P. radiata* as an exotic tree is its growth in similar habitats without the presence of injurious pests and diseases. Offord (1964) listed 72 pathogens associated with *P. radiata* stands in California and a further 86 fungi reported on exotic *P. radiata*. Ohmart (1981) recorded 319 species of insects from

P. radiata growing in the three native populations and other non-native plantings in California. The susceptibility of *P. radiata* to insects and fungi can not be ignored and the extensive monoculture of the species make it extremely vulnerable (de Gryse, 1955).

In its native habitat, a tree may be considered to have achieved a reasonably stable equilibrium in which its inherent physiological abilities are in harmony with all of the adverse and beneficial factors of the habitat (Rawlings, 1957). In an exotic habitat the climate or the soil may not be suitable, mycorrhizal fungi may be absent or there may be a biotic factor to which the tree does not have natural immunity or resistance. Introduced lethal factors may be even more serious on an exotic than on an indigenous species.

Pathological problems arise mainly as the result of unfavourable climatic or edaphic conditions. *Pinus radiata* remains healthy with a dry summer and wet winter. Summer rainfall favours attack by rust fungi. For example, large plantations of *P. radiata* were destroyed by *Dothistroma* in Brazil, Zimbabwe and east Central Africa (Zobel and Talbert, 1984). *Dothistroma* is present in the native forests in California but it is not a problem (Cobb and Miller, 1968). Infection by *Diplodia* precludes the planting of P. *radiata* in Queensland (Bryan, 1954), Kenya and Brazil (Gibson, 1972), Natal and the Transvaal in South Africa (Poynton, 1977) and Zimbabwe (Barrett and Mullin, 1968). Although present in California *Diplodia pinea* is not a serious pathogen. Offord (1964) attributes this to the absence of hailstorms in the coastal areas. *Diplodia pinea* is a more aggressive pathogen in Australia than in its own native environment (Newhook, 1964).

Numerous disorders are associated with unfavourable soil conditions. Because forestry has long crop rotations and is generally less economic than agriculture, plantations are usually established on sites that are unsuitable for agriculture, and soil conditions may be a limiting factor to healthy tree growth, predisposing trees to pathological problems. In the native stands, the best growth and the most disease-free conditions are found on sites where the soils are well drained, deep and of sandy loam. *Pinus radiata* does not thrive on clay soils; these usually infer poor drainage and restricted aeration. These factors favour the development of root diseases and reduced vigour of trees.

The creation of large uniform areas of a single species and age class is extremely hazardous and is conducive to disease and insect outbreaks (Shea, 1971). Foresters and pathologists involved with the radiata monoculture, have long been aware of this risk. The potential disease situation has been the subject of frequent review, for example :

Rawlings, (1957)	pathology of radiata pine as an exotic.
Scott, (1960)	*Pinus radiata,* diseases and pests.
Offord, (1964)	diseases of Monterey pine.
Newhook, (1964)	forest disease situation in Australasia.
Gilmour, (1966)	pathology of forest trees in New Zealand.
Magnani, (1966)	fungal diseases of *P. radiata*.
Hepting, (1971)	diseases of forest trees in America.
Allen, (1973)	pests and diseases of radiata pine.
Old, (1979)	pathology of *P. radiata* in California.
Ohmart (1981)	list of insects associated with *P. radiata* in California.

Some of the more important diseases of *Pinus radiata* are briefly introduced in the following sections. Their relevance in the natural forest and in the planted exotic plantations are discussed.

Foliage disease

Naemacycleus niveus was listed by Offord (1964) as being the most widespread and damaging of the foliar diseases on the three mainland areas of native pine. The same disease, associated with needlecaste of *P. radiata,* has been observed in Australian plantations for many years (Stahl, 1966) with the severity of the outbreaks varying from year to year. Newhook (1964) reported that it is widespread and common in Australia and New Zealand, but it was insignificant as a disease. However Van der Pas *et al.* (1984) showed significant growth reductions associated with the disease.

Needle-blight caused by *Dothistroma pini* was not listed as a disease of the native stands by Offord (1964), or of exotic stands in Australasia by Gilmour (1964). Attention was first drawn to the damaging effects of this disease in Kenya by Gibson *et al.* (1964). It was first identified in the Central North Island of New Zealand in 1964 (Gilmour, 1967) and has since spread over the rest of the country (Gadgil, 1984), and across into Australia. The first confirmed detection in Australia was in the Barrington Tops State Forest (NSW), in October 1975 (Matheson, 1985). The disease has now been reported in the northeast of Victoria and northwest Tasmania. *Dothistroma* has not been found in southern Victoria, the southeast of South Australia or Western Australia (Matheson, 1985).

Branch diseases

Diplodia pinea, the twig-blight of exotic Monterey pine is present in the native forests but has not been a serious pathogen under Californian conditions. *Diplodia pinea* was described by Gilmour (1964) as probably one of the few major tree disease organisms that was common in the radiata pine plantations in New Zealand. It has generally been considered to be a parasite of wounded or weakened trees (Gilmour, 1966), although it can parasitize young undamaged shoots (Chou, 1976). Stem malformation and tree volume losses can be quite significant.(Currie and Toes, 1978). The fungus is widespread throughout Australia (Stahl, 1968) and it is considered to be the major factor limiting the use of radiata pine in sub-tropical, summer rainfall areas such as southern Queensland and northern New South Wales (Wright and Marks, 1970). In South Africa, *P. radiata* is so susceptible that *Diplodia pinea* (= *Sphaeropsis sapinea*) has virtually restricted its cultivation to the South Western Cape where hail is rare (Swart *et al.*, 1985).

Stem diseases

The western gall rust (*Peridermium harknessii Endocronartium harknessii*) occurs at damaging levels in the Cambria, Monterey and Ano Nuevo stands and is one of the major factors limiting the value of this species in California (Offord, 1964). The rust stimulates the formation of witches-brooms, retards the growth of the infected stem and can even cause the death of a small tree. In the Cambria and Monterey areas the gall rust, together with the dwarf mistletoe (*Arceuthobium campylopodum*) are the most prevalent and destructive pathogens of Monterey pine (Offord, 1964). The North American experience

shows that the gall rusts may be vigorous and damaging diseases under a wide range of climatic conditions. The western gall rust disease has not yet been observed in Australia or New Zealand (Parmeter & Newhook, 1967). Tests have shown that the New Zealand radiata pine is just as susceptible to the disease as are the native California pines. Old (1981a) regards the gall rust as the greatest threat to radiata pine in Australia.

Root diseases

The world wide fungus *Armillaria mellea* is prevalent in the natural *P. radiata* stands, although it appears to be contained by natural biological agents (Offord, 1964). Raabe (1982) reported that the pine was rarely infected and more rarely killed by the fungus in coastal California, although it was easy to infect and kill young seedlings in the glasshouse. In New Zealand, MacKenzie and Shaw (1977) reported that *Armillaria* root-rot had caused damage to many recently established plantations of *P. radiata* on sites freshly cleared of cutover podocarp forest. In Australia it attacks many species of eucalypts and causes deaths of individuals or of small groups of trees (Gilmour *et al.*, 1975).

Fomes annosus is the most important root disease in the native stands and *P. radiata* is highly susceptible (Bega, 1962). Most of the tree deaths appear to be associated with shallow, poorly drained, heavy soils where the trees were unthrifty.

Fomes annosus has not been recorded from Australia but is present in New Zealand, apparently as a relatively innocuous fungus (Newhook, 1964). Even though ideal opportunities have existed in the past 30 years, no attack of *P. radiata* has been recorded.

Phytophthora cinnamomi has been found on nursery stock from southern California (Zentmyer and Munnecke, 1952; Munnecke and Bricker, 1976), but it has not been reported from the native stands of the Monterey pine (Offord, 1964). In the far north of New Zealand, this fungus was associated with dying and unthrifty *Pinus radiata* (Newhook, 1959), and in the state of Parana in Brazil, it was considered to be a lethal agent of *P. radiata* (Rawlings, 1957). The fungus is prevalent in Western Australia (Podger, 1972) and was associated with the death of *P. radiata* by Batini and Podger (1968). Rawlings (1957) made the important statement "... In humid-temperate regions, peaty or clay soils with poor drainage may pre-dispose trees to attack by root fungi such as *Armillaria mellea* and *Phytophthora cinnamomi*, and damage and mortality will render the management and use of *Pinus radiata* extremely difficult."

2.1.4 Crop losses in production due to disease

Dothistroma needle-blight of *P. radiata* has made the growing of *P. radiata* impracticable in Kenya and Brazil, and serious losses have been reported from Chile (Gibson, 1972). It is regarded by Wilcox (1982) as the most serious threat yet to the health of New Zealand's

forests of *P. radiata*. *Dothistroma* is now established in eastern Australia, although Edwards and Walker (1978) argue that many plantations may be little affected by reason of climate or nutrition. However, its recent discovery in Tasmania suggests that it is a potential threat to many areas in Australia (Matheson, 1985).

In New Zealand, Gadgil (1984) reports that 125,000 ha of susceptible age classes of *P. radiata* are infected by *Dothistroma pini;* tree mortality as a result of infection is rare but the major consequence is the loss of volume increment. A loss of 30-40m^3ha^{-1} of wood at the end of the rotation for unsprayed trees can be expected. Woollons and Hayward (1984) reported 6m^2ha^{-1} more basal area over a period of five years in a *P. radiata* stand that had been annually sprayed for protection against *Dothistroma*. However, their data suggests that the growth of dominant trees in unsprayed areas was not significantly affected and the cost-effectiveness of chemical spraying for sawlog and pine plantations was questioned.

Cyclaneusma (*Naemacycleus*) *minus* is a common cause of needlecaste of *P. radiata* wherever the tree is planted. The impact is usually only minor, but the severity does vary from year to year and the periodic serious outbreaks are of concern to managers. In Western Australia, only the single outbreak has occurred. This was in 1968 following abnormal autumn rainfall and most of the *P. radiata* trees in the Mungalup plantation near Collie were severely infected by *Cyclaneusma* (Butcher, unpublished data). The disease is more severe in the New Zealand climate and annual infections can significantly reduce increment. Van der Pas *et al.* (1984) calculated a reduction in revenue at clearfelling of $600-$700/ha for a persistently heavily infected stand. Genetic variation in resistance has been recorded in progeny tests (Wilcox *et al.* 1975) and it has a moderately high heritability. Wilcox (1982) has suggested that there is a positive genetic correlation between susceptibility to the *Dothistroma* and *Cyclaneusma* diseases.

Diplodia pinea causes extensive tree death of *P. radiata* after hailstorms in the summer rainfall areas of South Africa (Luckhoff, 1964). It is important in Australia and New Zealand in causing malformation of trees and significantly reducing commercial volumes. Dead topping of trees in Victoria was shown by Wright and Marks (1970) to reduce tree growth by 40%. Severe tree malformation in a heavily infected *P. radiata* stand in New Zealand reduced the potential merchantable volume by 63%. Research priorities in South Africa, to reduce the impact of *Sphaeropsis sapinea* (formerly *Diplodia pinea*), were listed by Swart *et al.* (1985). These were to evaluate host resistance, and forest management practices to reduce stress conditions and injury to the trees. Genetic variation in resistance to inoculation was shown in progenies of *P. radiata* by Burdon *et al.* (1982).

Armillaria root-rot is the most damaging disease in New Zealand of *P. radiata* established on freshly cleared indigenous forest sites (Van der Pas *et al.*, 1983). Pine mortality after two years can range from 5-27%, and may leave nearly one-third of the planted area as basically production-less openings (Shaw and Calderon, 1977). On a severely diseased site, growing costs were increased by 40%. Shaw and Toes (1977) reported that 8 to 10-year-old *P. radiata* trees lost 17-73% of annual diameter growth when infected with *Dothistroma* compared with non-infected trees, and 14-24% annual loss when infected by *Armillaria* alone. Growth reduction in trees heavily infected by both fungi was greater than the sum of the losses attributable to heavy infection by each fungus alone.

2.1.5 Monoculture

The outstanding success of *P. radiata* as an exotic in the southern hemisphere has led to the establishment of more than 2.5 million hectares of plantations. *Pinus radiata* has an inherent capacity for growing with extraordinary vigour and often is more productive on marginal sites than a less demanding, alternative species, despite poor soils and pathological problems. Plantation forestry prior to 1960 sought to choose the most adapted species for growing on a site. However, since that time the reverse has applied. The site is now modified to suit the growth of *P. radiata,* on the promise that poor growth of P. radiata is superior to healthy but slower growth of an alternative species. The trend is to increase the monoculture of *P. radiata*, despite early warnings from Professor Zobel, repeated again during his visit to Australia in 1977 (Eldridge, 1978a).

Considerable efforts have been made in Australia and New Zealand to diversify the exotic forests, and this has cost a fortune (Fenton, 1978); very few second best species have been found. Only in Western Australia are significant areas of another pine being planted. Species diversification is successful in Western Australia because poorer quality sites are planted to *P. pinaster*. Productivity is similar, and *P. pinaster* is resistant to the root-rot pathogen *Phytophthora cinnamomi*. In New Zealand, the second-best species *Pseudotsuga menziessii* has pest and disease problems, and *Pinus ponderosa* and *P. nigra* are no longer planted because of their high susceptibility to *Dothistroma* (Chou, 1981). If diversity is necessary to combat the risk of disease, it would be better on economic grounds to achieve biological diversity within *P. radiata,* rather than use numerous species. Cobb and Libby (1968) have shown that radiata seedlings from the Island populations of *P. radiata* were more resistant to *Dothistroma pini* disease than the mainland populations. Useful quantitative genetic variance in resistance to infection in the New Zealand breeding population was shown by Wilcox (1982). A *Dothistroma* resistant breed of *P. radiata* is being developed in New Zealand for planting on areas where chemical spraying for control would normally be deemed necessary (Carson, 1985).

Pinus radiata in its native habitat is in equilibrium with a considerable number of fungal diseases and insect pests. It has a very small natural range and consequently is not as genetically variable as a more widespread species. Moran and Bell (1983), using isozyme analysis, found that native *P. radiata* had about half the average value of genetic diversity recorded for other gymnosperm species. Most variation was recorded within the Monterey population, whilst the least variable was the very small, isolated Guadalupe Island population. Less genetic diversity was found within the Australian breeding population than the natural populations. Monterey and Ano Nuevo populations were probably the major source of the original introductions to Australia (Moran and Bell, 1987), and it is probable that the Cambria and Island populations are not represented in the breeding populations.

The origin of *Pinus radiata* in Australia is uncertain. However, it is certain that the vast gene pool developed as the Australian population was derived from very few Californian genes, and these genes were unlikely to be a representative sample from the five natural populations known to exist. Bannister (1959) suggested that there are many genes in the native populations that have never reached or arisen in New Zealand and that many of these genes, if introduced to the gene pool, might confer benefits on the species. With this in mind, there is still the possibility of increasing the genetic diversity of *P. radiata* and thereby extending the range of sites on which it can be grown successfully.

The restriction of the genetic base may lead to losses or even to catastrophes; for example, the potato blight caused by *Phytophthora infestans*, described in Russell (1978). Vulnerability increases with the uniformity of the crop (Ledig, 1986). Certain *Cryptomeria* tree clones that had been planted in Japan for 400 years can no longer be used because of the risks of fungal and insect attack (Toda, 1974). In the Dutch elm breeding programme, Heybroek (1982) reported that another pathogen appeared, which did not previously play a role, in an elm clone resistant to the Dutch elm disease. An effective way of minimizing the risk is to use a diverse range of genotypes (Heybroek, 1982). Forest crops require from 25 to 100 years to mature and are liable to attack from a multitude of pathogens. Genetic disease resistance is the great hope of the forest managers (Schmidt, 1980) and used wisely so that genetic diversity is maintained. it is a valuable tool for disease management in the forest.

The true situation related to genetic diversity in forest trees is generally misunderstood. Trees are almost always highly heterozygous genetically and recent studies have indicated that forest trees have the greatest genetic variability of any organism (Zobel, 1982a). Because of this, and intensive breeding programmes, the next plantation crop 20-30 years hence will be genetically dissimilar from the previous crop planted on a given site. This should negate the fear of a supervirulent pathogen evolving to destroy the new seedling and tree crop.

The importance of *Pinus radiata* to the economy of several countries is so high that it is of some concern to read comments such as "... exotics are not all fore-doomed to failure, but for every exotic the chance of failure is much greater than the chance of success" by Boyce (1954) and by Popovich (1980) "... Monterey pine was heavily planted in New Zealand and Australia, often to the exclusion of other species with greater disease resistance. Now these stands are vulnerable to severe disease attacks." On the other hand, Peace (1957) defended the practicality of more intensively managed plantations, and stressed their silvicultural and management benefits; he recognized the potential disease threat to the planted monocultures and noted that unnatural was not necessarily the same as unhealthy. Van Emden and Williams (1974) commented "... although pest outbreak is often regarded as a general consequence of reducing diversity through monoculture, it is surprisingly hard to find documented examples." Bain (1981) refuted the belief that monocultures court disaster more than a mixed species plantation, and holds that the risk can be greatly reduced by planting the right species on the right site and carefully tending it. A literature survey by Chou (1981) also showed little to support the notion of increased disease and pest risks associated with monocultures.

Even with the existing and impoverished gene pools of *P. radiata* in Australia and New Zealand, and more than 50 years of intensive management of large scale plantations, productivity losses are remarkably low, especially by comparison with native forests. Complacency must be avoided. There is a need for an even greater vigilance now, to structure breeding populations for the future that will be durable to all possible pathogens. Potentially the most serious threat to the Australasian plantations is the western gall rust (Parmeter and Newhook, 1967). The gall rust is very damaging to *P. radiata* in its native range and it is one of the main factors limiting the value of this species in California. It is likely to cause serious losses in *P. radiata* plantations if introduced into the southern hemisphere (Old, 1981a). In the event of introduction, the future of *P. radiata* will depend on the use of genetically controlled resistance and suitable management practices (Old *et al.*, 1986).

2.2 PHYTOPHTHORA CINNAMOMI : PATHOGEN

2.2.1 Origin and distribution

Phytophthora cinnamomi was first described by Rands (1922) as a pathogen on the cinnamon tree in Sumatra. The fungus is now widespread through the warm temperate, sub-tropical regions of the world (Figure 4), attacking a diverse range of plants (Zentmyer, 1980).

Information on the origin of the *P. cinnamomi* fungus is fundamental to the management of forest systems. If the pathogen is indigenous then control based on practices to exclude the pathogen are irrelevant, whereas vulnerable plant communities can be identified and protected by quarantine and hygiene if the pathogen is introduced. The question assumes great importance if areas are to be cleared for the establishment of plantations of a potentially susceptible species. Genetic resistance programmes are enhanced if gene centres for plants are associated with the pathogen (Lippik, 1970).

Zentmyer (1977) concluded that *P. cinnamomi* was not indigenous to the soils of southern California or Mexico. His studies included many soil and root samples from undisturbed native chaparral situated above possible sources of infection. No root-rot developed, neither was the fungus isolated from avocado seedlings planted in the soils which were kept very wet. In Zentmyer's Mexican and Latin American sampling of native *Persea*, growing in undisturbed areas remote from cultivation, no *Phytophthora cinnamomi* was recovered even though many of the samples were taken in areas of very high rainfall and in soils containing considerable clay. These were sites where *P. cinnamomi* would be expected to develop and to be easily cultivated if it was present.

Contrary opinions as to the length of time *P. cinnamomi* has been resident in Australia have been argued by Shepherd (1975) and by Podger (1975). The argument favours the recent introduction in forests of Western Australia, South Australia and Victoria, as its presence is usually associated with disease

In Queensland it has the characteristics of an endemic pathogen as it is widely distributed but is not associated with significant disease. The jarrah dieback disease due to the root-rotting fungus *Phytophthora cinnamomi* (Podger *et al.*, 1965) is a serious and widespread problem in southwest Western Australia affecting large areas of crown land and private property (Figure 2). The extensive occurrence of the pathogen is the result of human activity in a susceptible plant community, and environmental factors suitable for the pathogen (Batini, 1973; Shea, 1975).

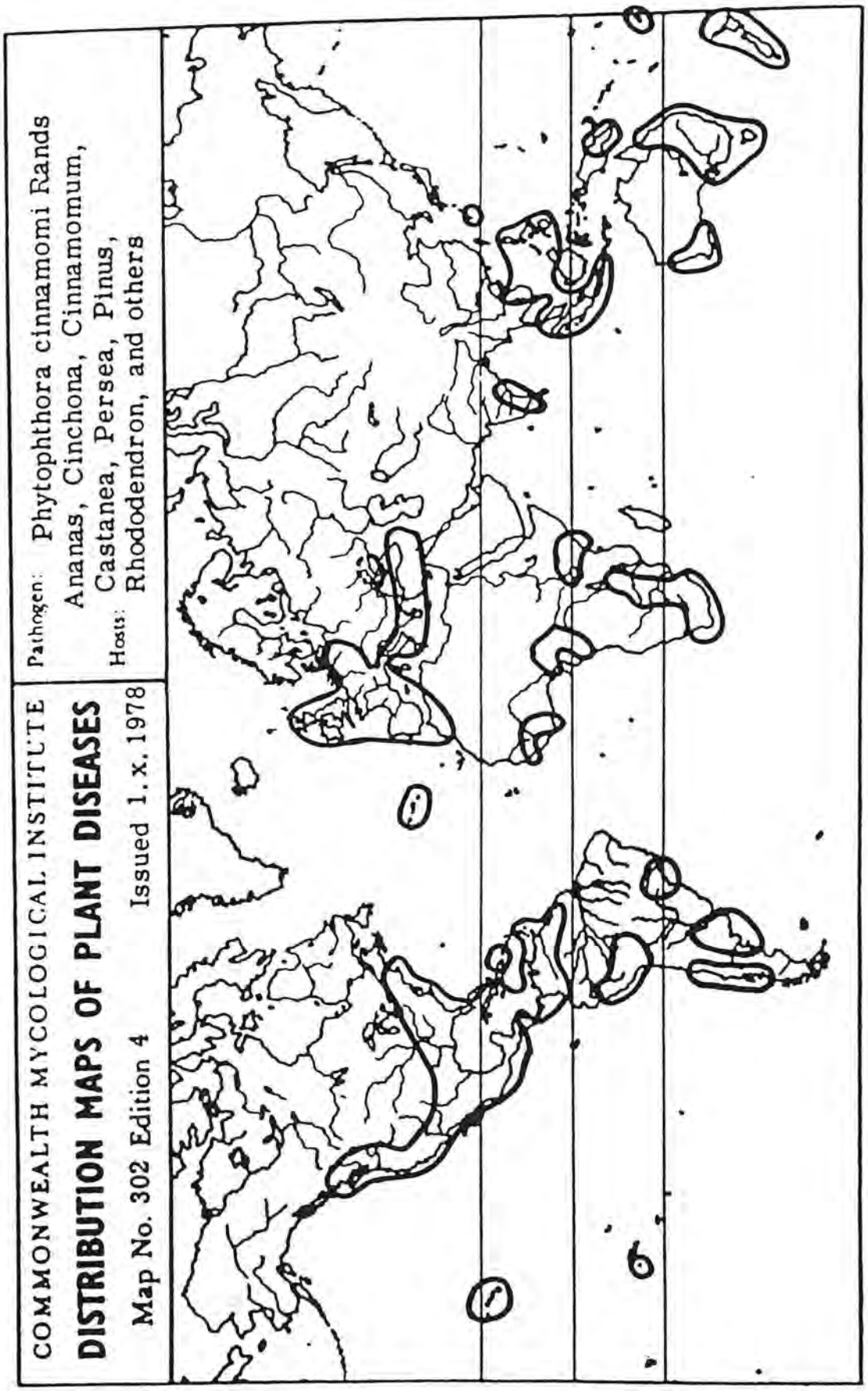

FIGURE 4

Commonwealth Mycological Institute Map 302 showing current distribution of *Phytophthora cinnamomi.*

lack of environmental stress in a mild climate with well distributed rainfall, and / or the action of soil factors on reproduction of the fungus (Podger and Newhook, 1971). Tolerance in plant communities is evidence of evolution and gives support to the theory of an indigenous pathogen, rather than a recent introduction (Robertson, 1970).

Von Broembsen (1984b) has recovered *P. cinnamomi* from all major rivers of the South-Western Cape Province in South Africa. Native vegetation in the mountains contains many hosts, some of which are highly susceptible to the pathogen (Von Broembsen and Brits, 1985) but there were little destructive effects on the plant communities. The fungus was also recovered by Von Broembsen (1984a) from dying *Pinus radiata* trees in several forest plantations and from native vegetation in mountain fynbos areas of this region. Von Broembsen (1985) postulated that it was unlikely for the pathogen to have spread from the forestry and agricultural areas without being destructive to the native plant communities; she suggests that *Phytophthora cinnamomi* is an indigenous component of the native ecosystems of these mountains.

2.2.2 Morphology

Phytophthora cinnamomi varies less morphologically than many other plant pathogens, including *Fusarium* and *Rhizoctonia*, and less than a number of species of *Phytophthora* (Zentmyer, 1980). The predominant growth form , especially for the A2 mating type, is a characteristic rosette growth pattern on PDA.

Phytophthora cinnamomi produces four spore stages: sporangia, zoospores (which are released from sporangia under certain conditions), chlamydospores and oospores. These spore types are distinctly different in form and function, and are variously affected by environmental and nutritional factors (Zentmyer, 1980). The most significant spore stage is the sporangia; these have the the capacity for greatly increasing the inoculum potential in a very short time through zoospore production thus accelerating the infection rate. Zoospores appear to form the main infective agent in non-sterile soils (Marks, 1979). Zoospores and mycelium have a very brief life-span. Chlamydospores and oospores function as the survival propagules and their formation appears to be an adaptation to considerably drier moisture conditions than those that permit sporangium production (Gisi, 1983). Oospores are probably the most resistant structures produced and serve to maintain the population when the pathogen has almost ceased to grow because of adverse environmental factors (Weste, 1983).

Phytophthora cinnamomi is heterothallic and requires the presence of opposite mating types known as A1 and A2 for the formation of the sexual stage of the life cycle (Savage *et al.*, 1968). Heterothallic species are unique in that sexual reproduction readily occurs even between morphologically and physiologically distinct species, suggesting that chemical stimulation may be involved in oospore formation during mating, a hypothesis originally proposed by Ashby (1929). *Phytophthora citricola* and *P. megasperma* are homothallic and are capable of completing their life cycle by single isolates. *Phytophthora cinnamomi* can also function as a homothallic fungus when the proper chemical stimulus is provided to the A2 mating type (Savage *et al.*, 1968). The infrequent occurrence of the A1 mating type may be related to the inability of the A1 type to form oospores

homothallically (Zentmyer, 1980). The A2 type has apparently become adapted to producing oospores by various mechanisms not requiring the A1 type and this may be a survival mechanism (Zentmyer, 1980).

2.2.3 Strains and races

Zentmyer (1980) reported that no definite races of *P. cinnamomi* had been identified, although there were many reports in the literature of variation in pathogenicity and morphology. His results indicate significant differences between isolates in pathogenicity to different hosts and in virulence on the same host. Shepherd and Forrester (1977) have found that *P. cinnamomi* is a more variable organism than previously perceived. Chee and Newhook (1965) tested many isolates from New Zealand and Australia and found some variation in temperature response but no significant differences in reaction on different hosts. In tests of 49 different isolates, all but one were able to kill seedlings of *Eucalyptus marginata* in Western Australia (Podger, 1972). Growth rates of 361 isolates, on cornmeal agar showed considerable variation (Shepherd and Pratt, 1974)

Isolates of *P. cinnamomi* have been separated into two compatibility types, A1 and A2, on the basis of their mating behaviour (Haasis *et al.*, 1964). Zentmyer (1980) reported the A2 type as the common form, geographically and in terms of the hosts infected. Both compatibility types showed similar temperature optimums and similar pathogenicity to lupin and young *Pinus radiata* seedlings (Shepherd *et al.*, 1974). New tissue culture techniques and isoenzyme analysis are being used to study variability in *P. cinnamomi*, on *Eucalyptus marginata* clones (Old, personal communication). He is finding that *P. cinnamomi* is genetically quite uniform in Australia and that the A1 mating type is more widespread than previously thought. *Phytophthora cinnamomi* from New Guinea was more different than the variation within Australia.

Phytophthora is a large and diverse genus (Gallegly, 1970). Many different species have been isolated in Australia and many have been associated with the death of *Pinus radiata* seedlings and trees; for example, *Phytophthora cryptogea* (Davison and Bumbieris, 1973; Gerrettson-Cornell, 1977), *P. megasperma* var. *sojae* (Davison and Bumbieris, 1973) and *P. drechsleri* (Heather and Pratt, 1975). Chevis and Stukely (1982) isolated *P. cryptogea* from a number of dead seedlings of *Pinus radiata* in the Donnybrook Sunklands, and *Phytophthora citricola* and *P. megasperma* var. *sojae* only rarely. However, they reported that *P. cinnamomi* A2 was by far the most prevalent *Phytophthora* spp. in the Sunkland plantations.

2.2.4 Epidemiology

Factors affecting epidemiology of *P. cinnamomi* disease were excellently described for *Pinus radiata* by Newhook (1959, 1960, 1970). He used the extreme comparison of *P. radiata* trees growing in single-row shelterbelt plantings, with massed trees in a forest plantation, to illustrate epidemic developments. The outcome of infection was

determined by shifting balances between shoot-root ratios, changing susceptibility with age, the timing of infection in relation to seasonal patterns of shoot growth, rootlet regeneration and evaporative stress, and the overriding importance of excessive rainfall (Newhook and Podger, 1972).

Newhook (1959) associated *Phytophthora* spp., including *P. cinnamomi*, with the extensive killing of mature shelterbelt and ornamental *Pinus radiata* trees in various parts of New Zealand. His survey and sampling indicated that *P. radiata* was highly susceptible as seedlings and up to 4-5 years of age. Trees were then resistant until the mature phase of growth was reached at 20-30 years. This difference was probably due to the root regeneration capacity (Newhook, 1970). He associated the major change in resistance around 20 years of age, with the general change in host physiology of *P. radiata.* It was also at this age that *P. radiata* becomes resistant to *Dothistroma pini* (Gilmour, 1967), and heartwood formation begins.

Sutherland *et al.* (1959) found that the susceptibility of *P. radiata* increased with increasingly poor soil drainage. In Western Australia, Batini and Podger (1968) reported that *P. radiata* shelterbelts had been virtually eliminated by *Phytophthora cinnamomi* on irrigated, poorly drained soils of the coastal plain. Deterioration and death of *Pinus echinata* also resulted from *Phytophthora cinnamomi* infection of fine roots under wet soil conditions (Campbell and Copeland, 1954). Experimental and field evidence in New Zealand indicated that prolonged saturation of the soil is of importance mainly because it provides requisite conditions for infection and sporulation (Newhook, 1960a).

Symptom expression was seen by Newhook (1959) to depend on the destruction of the balance between transpiration demand and absorptive capacity of the root system. If rootlet mortality is high in autumn and spring, trees will suffer from physiological drought in summer at a time when the water demand increases rapidly. Large crowned trees rapidly reach a state of permanent wilt and die (Newhook, 1959).

The difference between the shelterbelt epidemics and the "littleleaf" symptoms of an infected plantation or closed canopy forest relate to soil moisture and temperature conditions. In New Zealand, Newhook (1970) reported that the years of shelterbelt epidemics were characterised by abnormally long periods of rainfall when soil temperatures favoured infection, followed by wet winters when rootlet regeneration was poor. These large-crowned trees, with a large transpiration requirement in summer, wilted rapidly and died. On the other hand, trees in plantations usually develop "littleleaf" symptoms as a result of *Phytophthora* infections of the root system. Soil water and temperature conditions under the closed pine canopy limit the growth and sporulation of *P. cinnamomi*, mainly through the increased water and radiation interception of the canopy. Unhealthy trees with small crowns do not have a large transpiration demand and these rarely die. The importance of transpiration and interception was also shown by Newhook (1970) when plantations with "littleleaf" symptoms further deteriorated following the removal of understory shrubs.

Zentmyer (1980) defined the optimum temperatures for *P. cinnamomi* growth in the range of 24-27^{o}C, with few isolates growing below 10^{o}C or above 36^{o}C. Lethal temperatures were reported in the range from 35-44^{o}C (White, 1937; Shepherd and Pratt, 1974). Munnecke and Bricker (1976) reported that growth of *Pinus radiata* plants was greatly reduced within 2 weeks from inoculation with *Phytophthora parasitica*, but was similar to uninoculated seedlings following a very hot period in which soil temperatures

in pots reached 38°C, indicating that the pathogen had probably been killed. A warm water treatment at 41°C for 25 minutes eradicated *P. cinnamomi* from *Pinus radiata* seedlings without significantly affecting root growth capacity and mycorrhizae (Theron *et al.*, 1982). Old (1981b) has used clear plastic sheeting to generate high soil temperatures by covering nursery beds for the successful eradication of *Phytophthora cinnamomi.*

Soil-borne *P. cinnamomi* requires a high soil moisture for production and dispersal of zoospores (Gisi, 1983). Reproduction on the surface of the host is strongly influenced by environmental changes such as temperature and moisture (Weste, 1983). *Phytophthora cinnamomi* has a short latent period (Byrt and Holland, 1978), and in optimal environment conditions can reproduce rapidly on diseased roots and so increase the pathogen population and subsequently the incidence of disease.

In Western Australia, soil moisture levels are too low in the summer months for asexual reproduction, and survival outside large roots is limited (Shea *et al.*, 1980). During winter, fungal spores can survive but soil temperature levels are too low for asexual reproduction. Hence the periods during which the pathogen can reproduce are restricted to relatively brief periods in spring and autumn when there there is a coincidence of favourable soil moisture and temperature conditions (Shea *et al.*, 1980).

Generally sporangium production is sparse in flooded soils (Zentmyer, 1980). Mitchell and Zentmyer (1971) reported that production of sporangia by species of *Phytophthora* was inhibited by low oxygen and also by high carbon dioxide concentrations. Nesbitt *et al.* (1979) also suggested that poor sporulation in waterlogged soils may have been due to a low oxygen concentration.

2.2.5 Losses in production

Jackson (1945) reported that a conspicuous symptom of the littleleaf disease of *Pinus echinata* was the gradual decrease in diameter growth that accompanied the progressive foliar decline of the affected trees. The average radial growth of the diseased trees amounted to only 52% of the healthy trees (Jackson, 1951). Campbell and Copeland (1954) reported that *Phytophthora cinnamomi* was the primary cause of the littleleaf disease but other factors such as poor aeration, low fertility and periodic moisture stresses were also important. They indicated that the littleleaf area occupied some 6 million hectares of the commercial shortleaf growing area east of the Mississippi River and was causing sufficient losses on one third of the area to seriously interfere with forest management plans. Annual mortality was estimated at 3-5%. Hepting (1949) estimated that the annual stumpage loss from the disease would approximate five million dollars.

Small areas of jarrah forest were reported as having dieback disease in 1920. The dieback disease spread very rapidly after the Second World War, as a consequence of the widespread introduction of vehicles to the forest. Batini (1973) reported that 5% of the 750,000 ha of jarrah forest mapped was affected by *P. cinnamomi*, noting also that the saleable volume in the unaffected forest was 36 m^3ha^{-1} compared with 15 m^3ha^{-1} in the diseased forest. In 1976, Shea estimated the total area affected by dieback to be

282,000 ha. The forest quarantine system was implemented in 1976. Currently about 719,000 ha of forest are under quarantine in Western Australia (McKinnell, 1981).

In a New Zealand survey of 1,970 *Pinus radiata* shelterbelts, Sutherland *et al.* (1959) reported that half of these were seriously damaged by *Phytophthora cinnamomi*. Newhook (1960) stated that *Phytophthora* species were present in commercial pine plantations, and that they must be considered as a limiting factor in forest production.

2.2.6 Association of Phytophthora cinnamomi with Pinus radiata

Zentmyer (1977) has reported that *Phytophthora cinnamomi* was not indigenous to southern California. The introduced pathogen was first isolated in 1942 by Wager, on dying avocado trees in irrigated orchards. Several avocado and ornamental nurseries were found by Zentmyer and Munnecke (1952) to have *P. cinnamomi* infections in their container-grown and bare-root stock. The fungus has apparently spread to other areas from the dissemination of diseased nursery stock, but this has now ceased (Zentmyer, 1980).

In Offord's (1964) review of the diseases of Monterey pine, the only reference to *P. cinnamomi* in California was the already cited nursery stock occurrence on *Pinus radiata,* grown for ornamentals (Zentmyer and Munnecke, 1952). However, he stressed a concern as to the possible introduction of the disease into forest nurseries and the native stands of Monterey pine. The same reference was the only one given in Hepting's (1971) review of diseases of forest trees in the United States. Munnecke and Bricker (1976) reported numerous isolations of *Phytophthora parasitica* and *P. cinnamomi* from diseased roots of *Pinus radiata*, from nine Christmas tree farms in southern California. Zentmyer (personal communication, 1983) stated that no *Phytophthora cinnamomi* disease has been reported from the native forest areas of *Pinus radiata.*

Shelterbelt mortality of *Pinus radiata*, caused by *Phytophthora cinnamomi* has been severe in New Zealand (Newhook, 1959). *Phytophthora cinnamomi* has been found in association with *Pinus radiata* plantations showing "littleleaf" symptoms on poorly drained clay soils (Hepting and Newhook, 1962). These trees were restored to full vigour by the application of superphosphate (Weston, 1956). However, the potential growth could not be achieved because the population of the pathogen was unaltered (Newhook, 1970). *Phytophthora* species are present in New Zealand's indigenous forests (Newhook, 1960a). They are present in commercial plantations and must be considered as a limiting factor in forest production (Newhook, 1960b). Fertilizer application and improved drainage are likely to have limitations as control measures, and large scale application of fungicides is impracticable (Newhook, 1959). Breeding or selection for resistance was considered to be a very long term project. Newhook's solution to the problem was to change from the traditional use of *Pinus radiata*, to a species that was resistant to *Phytophthora* attack. *Pinus radiata* remains as the principal plantation tree on the Auckland clay sites (Shelbourne *et al.*, 1986). A multinodal breed is being developed for these sites that has specific adaptation to poorly drained, phosphate-deficient soils.

Phytophthora root-rot of 25-30 year old trees in a *Pinus radiata* plantation in New South Wales was reported by Hartigan (1964). The outbreak occurred in summer following very heavy rainfall in late spring. Jehne (1971) found that the high mortality in four to six-year-old *P. radiata* plantations on the south coast of New South Wales, was associated with *Phytophthora cinnamomi* on shallow, less permeable, high bulk density soils. *Phytophthora cinnamomi* was also found to be widely distributed throughout the central Tablelands plantations of New South Wales (Keirle, 1979).

Davison and Bumbieris (1973) sampled sites which had a history of *Pinus radiata* tree death and low productivity in the Adelaide Hills, and Southeast plantations of South Australia. These sites were often situated on a sandy soil overlying clay, liable to waterlogging in winter and drying out in summer. *Phytophthora cryptogea* was isolated with high frequency from the Kuitpo forest reserve in the Adelaide Hills and *P. cinnamomi* was isolated from a dead tree in the Mt. Bold seed orchard. Recovery of *Phytophthora* (*P. megasperma* var. *sojae*) from the Southeast plantations was very infrequent and may indicate that it is rare. Waterlogging was seen by Bumbieris (1976) as an important factor in the decline of young *Pinus radiata* infected with *Phytophthora cryptogea.* A survey by Bumbieris and Boardman (1979) established the presence of *P. cinnamomi* in the Adelaide Hills pine plantation, where it appears to have been introduced only recently. High first year planting mortalities of *Pinus radiata*, caused by *Phytophthora cinnamomi*, were reported in 1986 (Boardman, personal communication) in this plantation.

Phytophthora cinnamomi was associated with the shelterbelt deaths of old *Pinus radiata* trees growing on waterlogged sites of the Swan coastal plain in Western Australia (Batini and Podger, 1968). Chevis and Stukely (1982) found that deaths of established trees in the Donnybrook Sunkland were consistently associated with root infection by *Phytophthora* spp., usually *P. cinnamomi* A2, and reported a higher frequency of pine deaths in areas previously affected by jarrah dieback disease. Mortality rates of *Pinus radiata* were highest on the silty-loams of the valley floors (Chevis, 1984).

Donald and Von Broembsen (1977) reported that some 30% of the *Pinus radiata* seedlings, raised in boxes in the major forest nursery in the South West Cape Province of South Africa, were killed by *Phytophthora cinnamomi* in 1971, while only 43% were saleable in 1972. Over 500 tons of sandy-loam soil was imported into the nursery each year for the boxes, from the surrounding plantations and forest areas. *Phytophthora cinnamomi* has been isolated from the roots and soil of 10 to 20-year-old *Pinus radiata*, growing on a poor site in the same plantation area (Wingfield and Knox-Davies, 1980). Von Broembsen (1984a) reported that *Phytophthora cinnamomi* was well distributed throughout the South West Cape Province on cultivated crops. She also recovered the fungus from dying *Pinus radiata* in several forest plantations and from the mountain fynbos areas of this region.

2.3 CONTROL OF DISEASE

2.3.1 Cultural methods

The most direct method of avoiding production losses from disease is to identify infected sites and sites with the potential to become infected and exclude them from the planting

of susceptible species. Disease can be avoided by planting a resistant species on a potentially infected site.

A major pine re-afforestation project in Western Australia was proposed for the Donnybrook Sunkland (Forests Department of W.A., 1975). The plan was to convert a native hardwood forest, in which *Phytophthora cinnamomi* was prevalent, into productive pine plantations. The preferred species for the programme was the highly productive *Pinus radiata.* However, there were many reports in the literature citing *P. radiata* as susceptible (e.g.. Newhook, 1959; Batini and Podger, 1968; Jehne, 1971). The main alternative species, *P. pinaster* is resistant to *Phytophthora cinnamomi* (Batini, 1978), although the pathogen has been associated with the deaths of mature trees on an irrigated, riverine, alluvium site (Batini and Podger, 1968). Growth of the improved *Pinus pinaster* is comparable to *P. radiata* but additional breeding would be required to further improve the stem form and branching quality of the tree on these sites to make it a suitable alternative (Butcher, unpublished data).

Phytophthora cinnamomi is widespread throughout the Southwest and southern areas of Western Australia (Figure 2). Intensive site preparation is a prerequisite to plantation establishment, and this exponentially increases the chances of infecting new areas with the pathogen. The threat can be reduced by adherence to prescribed hygiene measures (Shea, 1975).

Phytophthora cinnamomi has been associated with diseased *Pinus radiata* seedlings in nurseries in California (Zentmyer and Munnecke, 1952), South Africa (Donald and Von Broembsen, 1977), New Zealand (Bassett and Will, 1964) and Australia (Keirle, 1979). Experiments by Boughton and Crane (1984) have shown that *Phytophthora cinnamomi* can infect and kill *Pinus radiata* seedlings growing in pots containing the Nannup. nursery's soil; Nannup nursery is the principal forest nursery in Western Australia. Limited sampling within the nursery has not revealed any *Phytophthora*, although *P. cryptogea* was isolated from a dying ten-year-old *Pinus radiata* tree growing next to the creek supplying the nursery's irrigation water (Stukely, personal communication). Nurseries must be kept free of infection to prevent the dissemination of the pathogen to the field.

Root-rots are considerably more difficult to control by chemical fungicides than are foliar diseases. Chemical control may be justifiable in high value, agricultural and horticultural crops but it is not economically feasible, or environmentally desirable in extensive forest plantations. Repeated applications would also be necessary for pathogen control as fungal spores occur within infected large roots and would be protected from the applied chemical.

Biological control offers another opportunity for the control of the *Phytophthora* root-rot of *Pinus radiata.* Ectomycorrhizae of *P. patula* acted as biological deterrents to *Phytophthora cinnamomi* (Marais and Kotze, 1976). Ross and Marx (1972) found that deaths in seedlings of *Pinus clausa*, caused by *Phytophthora cinnamomi*, occurred more rapidly when lateral roots were unprotected by a mycorrhizal sheath. Preliminary investigations by Malajczuk (personal communication, 1984) indicated extensive ectomycorrhiza of *Rhizopogon*, completely enveloping short roots of a tolerant family 20058 seedling of *Pinus radiata*, whereas a susceptible family did not have this protection.

Organic matter amendment is another factor in biological control that has been effective for control of the avocado root-rot disease (Zentmyer, 1980). Mixing *P. radiata bark* with soil had an inhibitory effect on *Phytophthora cinnamomi* (Gerrettson-Cornell *et al.*, 1976); polyphenols in the bark may have been involved in this inhibition. This could indicate that soils beneath a *Pinus radiata* plantation may become suppressive to *Phytophthora* with increasing age and site occupancy by the plantation.

A single application of 600 kg of superphosphate per hectare produced a spectacular improvement in the health and growth of *Pinus radiata* trees infected with *Phytophthora cinnamomi*, on P-deficient clay soils in New Zealand (Weston, 1956). The response, which persisted for 16 years, was not due to the destruction of *P. cinnamomi,* as population levels remained high (Newhook, 1970). Subterranean clover is routinely grown in the Sunkland plantations to improve the nutrition of the *Pinus radiata* tree. Boughton (1983) has found that roots of clover, growing in Sunkland soil, can be infected by *Phytophthora cinnamomi*, but it is not clear whether this will increase the disease level in stands of *Pinus radiata*, growing with clover under an agroforestry regime.

The physical manipulation of the environment offers the greatest potential for minimising timber production losses from existing plantations. Newhook (1970) has shown that the severity of the *P. radiata* host reaction to infection with *Phytophthora* depends not only on the variations in site conditions, but also on the degree to which transpiration reduces plant moisture reserves before rootlet regeneration restores the root / shoot balance. Deep, exposed crowns of vigorous shelterbelt trees rapidly depleted water reserves, leading to severe defoliation or death, whereas mortality was negligible in littleleaf areas because transpiration loss from sparse crowns was low (Newhook, 1970). Maintaining maximum canopy cover in the jarrah forest gave temperature and moisture conditions on upland sites that were unsuitable for sporulation and disease development (Shea, 1975). He also found soil environmental conditions under the closed canopy of a seven-year-old *Pinus radiata* stand to be unfavourable for infection by *Phytophthora cinnamomi* during the critical spring months. Pine canopies are capable of intercepting large amounts of rainfall, depending on the density of the plantation. Butcher (1977) has shown for a *Pinus pinaster* plantation in Western Australia, that interception can range from less than 10% in an open, parkland forest to more than 30% in a dense plantation stand. Soil profiles under the dense pine stand were rapidly depleted of moisture by transpiration and were close to wilting point in November.

The management model for growing existing *P. radiata* plantations on potentially infested sites should aim at complete site occupancy. Thinning operations should be moderate and carried out in middle to late summer. The timing of the operation could be critical. Summer operations will reduce the opportunity for spread of disease by heavy machinery involved in the harvesting, and soil damage will also be minimised. Depleted soil moisture and the physiologic condition of trees at this time, should push the micro-organism balance in favour of competitive saprophytes, rather than the facultative parasites. *Phytophthora cinnamomi* persists in the root system without an apparent effect on the health of the pine tree but once the tree is harvested, the tree resistance is removed and the pathogen can spread through the pine-stump root system, provided moisture conditions are suitable for sporulation and growth. With dry site conditions, *P. cinnamomi* will not proliferate and stump-root systems should be invaded by saprophytic fungi. Thinning in winter / spring could provide the conditions for a significant build-up of *P. cinnamomi* inoculum; if rains persisted into early summer, conditions would be optimum for an epidemic, with death of the retained high value crop trees.

2.3.2 Resistance methods

Intensive management of *Pinus radiata* plantations, particularly for a sawlog objective, can result in a real increase in the *Phytophthora cinnamomi* disease. Species, site selection, site preparation, nursery stock, mycorrhizal inoculation, fertilisation, scrub control, pruning and thinning can all have a significant effect on the *P. cinnamomi* disease.

Breeding for disease resistance is compatible both with more efficient management and increased productivity per hectare of land by reducing losses to disease. It has the advantage that, once obtained, resistance is relatively permanent and more resistance can be added (Zobel and Talbert, 1984). In a forest plantation, a genetically poor tree will continue to produce below the potential of the land and climate resources for as long as it occupies the site (Libby *et al.*, 1969).

Usable levels of disease resistance occur in many tree species. Many reports of additive inheritance suggest that resistance can be manipulated in the same way as other traits and that long-term effectiveness can be had (Dinus, 1982).

Disease of forest trees can be grouped into two categories: those caused by obligate and those caused by facultative parasites. Obligate parasites (or biotrophs) are restricted to living tissue whereas facultative parasites (or necrotrophs) are able to colonise both living and dead tissue (Tarr, 1972). Necrotrophy, which is very close to and sometimes almost indistinguishable from saprotrophy, occurs when living tissues of an organism are first killed by the fungus and then utilized (Cooke and Rayner, 1984). Most of the obligate parasites have a highly specialised physiological relationship with their hosts and have no saprophytic ability. They attack a narrow range of plants and resistance is usually specific involving only major genes. Examples are the white pine blister rust (Bingham, 1983) and poplar rust (Heather and Chandrasheker, 1982).

Trees are long lived perennials, consisting of a large and increasing proportion of dead tissues, which can provide a substrate for the saprophytic build-up of inoculum of unspecialized facultative pathogens close to living tissues. In addition to this, *Pinus radiata* used in tree plantations, are often grown on marginal sites which reduces tree vigour and renders them more susceptible to attack from facultative pathogens.

Phytophthora cinnamomi is a facultative parasite that is pathogenically unspecialized on a broad and diverse host range (Zentmyer, 1980). It causes necrosis and death of the host, can both grow and reproduce in the absence of the host, and under suitable conditions of temperature and moisture can compete to a limited extent with other soil micro-organisms for organic substrate (Weste, 1983). Erwin (1983) has reported that there is no evidence for physiologic races of *P. cinnamomi*. Given both these sets of information, it is extremely unlikely that there will be any specific relationship between races of the host and the pathogen. There will be no pressure on the pathogen to produce more virulent forms. A generalised field resistance based on a polygenic system can be assumed.

Research on the *P. cinnamomi* littleleaf disease of shortleaf pine by Zak (1955), Bryan (1965), and Ruehle and Campbell (1971), has shown that there was variation in the

degree of resistance, in individual selections of *Pinus echinata*. Bryan (1973) later reported very little variation in the height growth of his control pollinated progenies, indicating that the uniformly high growth rate reflected high vigour related to littleleaf resistance.

Preliminary glasshouse and field tests of Butcher *et al.* (1984) have shown that there is wide variation between *P. radiata* families in *Phytophthora cinnamomi* disease resistance. Very high and consistent family heritabilities were calculated, suggesting strong genetic control. A seed source based on resistance to *P. cinnamomi* is being developed.

2.4 BREEDING FOR DISEASE RESISTANCE

Genetic resistance to diseases has proven useful in many agricultural crops but applications in forestry are still in the early stages of development. Good progress has been reported for loblolly pine to fusiform rust (Zobel, 1982b), blight resistant chestnut trees (Bazzigher, 1981) and blister rustresistant western white pine (Bingham, 1983). These are but a few examples of the numerous disease resistance breeding programmes now existing. Disease-resistant varieties of trees are attainable and only when these are used can forestry come close to realising full site potential. Optimal production of forest products per unit of land can then be achieved. In a recent review on the need for resistant trees, Zobel (1982a) made the concluding statement "....breeding for resistance to pests in forestry is absolutely mandatory".

However, disease resistance breeding is not yet fully accepted by forest managers, nor is it completely understood by the scientists. Many terms have been used to describe host reaction to disease and the inheritance of it; these have often been contradictory and have confused both the manager and the tree breeder. In the majority of cases where genetic resistance is being used, there is little precise knowledge about the genetics of resistance. There is concern about the permanence of resistance and whether this will be overcome during the long tree crop rotation by a more virulent race of the pathogen, and the possibility of inducing epidemics through the narrowing of the tree species gene pool. A greater knowledge of the ways by which plants resist these diseases and of the inheritance of resistance is needed.

2.4.1 Resistance / tolerance terminology

Communication in the scientific world for the transmission of ideas and scientific information depends on the meaning of the terms used. As it is usually in the written word, unless the terms are adequately defined confusion can result. The terminology used to describe the general reaction of a host to a pathogen is particularly confusing. The looseness of the use of the term resistance is an example. In scientific literature, Calvert (1982) has found that two different meanings of resistance are used, often interchangeably, without regard to the meaning conveyed. The general view of resistance is that a resistant plant will not suffer yield loss or be damaged by a given pathogen.

However, in the biological sense, resistance is not due to the genes of the host alone but also to the genes in the pathogen. As Calvert (1982) so succinctly stated, "....resistance in the anthropocentric view refers to the host alone but in using resistance in the biological view, refers to the interaction of the genotypes of the host and pathogen". The use of these terms should be clear to resolve any problems.

The concept of plant resistance to pathogens has been given a number of interpretations (Harris and Frederiksen, 1984). Plant pathologists usually consider resistance present when the parasite is shown to be hindered in some way. Resistance should be considered separately from immunity where there is no development of a parasite on the host. It is convenient to arbitrarily characterise disease resistance genes into two major classes even though resistance and susceptibility are merely extremes of a broad continuum of host / parasite interactions (Nelson, 1982). The host response can be either a resistant or susceptible reaction. Resistance genes will enable the host to restrict the successful establishment of infection sites and also retard the colonization and reproduction of the parasite. In many cases a host may have some degree of resistance even though the host displays a susceptible reaction type, if the host supports less pathogen development. This is the area of grey between the black and white extremes of resistance and susceptibility. Partial resistance, field resistance, dilatory resistance, etc., and tolerance are terms that have been used to describe this intermediate response.

The concept of tolerance suggests endurance. To Politowski and Browning (1978), the tolerant plant yields better than another while supporting the same amount of the pathogen. In an extensive review on tolerance to plant disease, Schafer (1971) defined tolerance "....as that capacity of a cultivar resulting in less yield or quality loss relative to disease severity or pathogen development, when compared with other cultivars or crops". The National Academy of Science (1968) defines tolerance as the ability of a host plant to survive and give satisfactory yields, at a level of infection that causes economic loss to other varieties of the same host species. Tolerance has also been designated as an intermediate level of resistance somewhere between immunity and full susceptibility, but this meaning is erroneous according to Caldwell *et al.* (1958). They noted that plants with intermediate resistance are subject to the loss of resistance in the presence of new elements of the pathogen population.

Providing the habitat for the pathogen is seen by Mussell (1980) as an important aspect of tolerance. There is a general rule, says Manion (1981), that the pathogen most fit to survive has the minimum number of virulence genes. If all races of a pathogen have an equal chance of reproduction, then the probability of the appearance of a new or more virulent race is greatly reduced. In plant breeding, any characteristic that can be introduced into the host plant to make them more tolerant will lead to long term stability because these traits do not provide a competitive advantage to more virulent races of the pathogen. Mussell (1980) further argued that the converse should be true, with selection favouring less virulent characteristics in the pathogen population. The use of racespecific resistant cultivars to control *Phytophthora* root-rot of soybean has resulted in the build-up of compatible races, whereas the use of tolerant, race-non-specific cultivars should not favour the build-up of one race over another (Walker and Schmitthenner, 1984). Tolerance should offer a more permanent type of protection against losses from disease because it does not impose selective pressure on the pathogen population (Caldwell *et al.*, 1958).

A major disadvantage of tolerance is that it is unlikely to provide protection as good as that provided by more complete forms of resistance; for example, hypersensitive reaction

giving immunity. Yields will certainly be reduced and the infected tolerant plant can act as a reservoir of the pathogen. However, the use of disease tolerance offers protection against the development of any epidemic disease situation. Harlin (1976) argues for strategies in plant breeding to stabilize the host-pathogen relationship; modest annual losses would be far better than the occasional disastrous epidemic. The combination of tolerance and resistance has been suggested by Stakman and Christensen (1960) as a strategy for disease management : tolerance would provide the long term stability, and resistance would provide the short term superior protection. However, Schafer (1971) reported that adequate combinations were not being produced.

2.4.2 Genetic concepts of resistance

Whereas the preceding section attempted to qualify the use of the terms resistance and tolerance, this section is concerned with the genetical aspects of resistance. Van der Plank (1968) advanced resistance theory by dividing resistance into two basically different types, vertical and horizontal. He defined vertical resistance as effective against some but not all races of a pathogen, while horizontal resistance is spread against all races. Browning *et al.* (1977) used the functional concepts of general and specific resistance to describe the reaction. General resistance is present if there is no known differential interaction among genotypes of the host and genotypes of the pathogen; the term is roughly synonymous with horizontal, race-non-specific, partial or field resistance. Specific resistance is present where known genetic interactions exist between host genotypes and pathogen genotypes (Browning *et al.*, 1977). Synonyms are vertical or race-specific resistance. Browning *et al.* (1977) provide a list of terms that are frequently used to express the genetic concepts of resistance.

The mode of inheritance of the disease resistance character can be controlled by a single gene (monogenic), few genes (oligogenic), or many genes (polygenic), or there may be some cytoplasmic control of inheritance. Resistance that is conditioned by many genes has usually been more durable and less race specific, than resistance that is controlled by only a few genes (Russell, 1978), because the pathogen population must change at various loci to adapt to the host and the more loci involved, the harder it is for the pathogen population to adapt.

Specific resistance is, as a rule, monogenically inherited. Resistant and susceptible plants are easily distinguished as the differences are qualitative and clear-cut. Resistance genes can usually be identified and named, and Mendelian ratios and concepts can be stated (Van der Plank, 1982). Complete resistance to a pathogen is based on a hypersensitive reaction by the host. Hypersensitivity seems to involve a specific relationship between particular genes of the host and pathogen. This was described as the gene-for-gene concept by Flor (1956, 1971). Gene-for-gene pathogens are diverse and have an abundance of specific races, but all have a common feature in that they are biotrophic in the infection process (Van der Plank, 1982). In biotrophy (susceptibility), the host accepts the pathogen without reacting against it. Resistance is when the host recognizes and reacts to the pathogen, and defends itself. Variation is essentially discontinuous and this greatly facilitates breeding for resistance. Although the nature of hypersensitivity is not fully understood this kind of resistance has been widely used by plant breeders, mainly because it is easy to exploit in a breeding programme (Russell, 1978).

Some spectacular gains in disease resistance in agricultural crops have been achieved with single-gene inheritance. However, this increases the potential for selection pressure by the cultivar genotype on the constitution of the races in the pathogen population (Scott *et al.,* 1978). Such selection pressure has been considered responsible for some striking breakdowns of resistance of cultivars to obligate pathogens (Watson, 1970).

General resistance is thought of as polygenically inherited. According to Van der Plank (1968), horizontal resistance is based on independent gene action between host and parasite. However, Parlevliet and Zadoks (1977) argue that polygenically controlled resistance most likely operates on a gene-for-gene basis with minor genes in the parasite. Whether or not a gene-for-gene relationship exists for general resistance to diseases caused by *Phytophthora* spp. is not yet proven (Umaerus *et al.*, 1983).

Polygenic inheritance is synonymous with continuous variation (Van der Plank, 1982). Resistance varies from high to low values without breaks in the distribution and intermediate values are the most common. Resistance is usually measured as metric traits and it is then subjected to the statistical methods of quantitative genetics for analysis. Additive variance is the main component of genetic variance but dominance variance may also be large, particularly where there is a maternal effect. However, general combining ability (additive genetic variance) information can be used to evaluate parental potential and be used as the basis for selecting resistant parents for use in a breeding programme. Non-specific resistance is not simply inherited, and consequently some parents will show more resistance than others, in the same way that some yield better than others (Watson, 1970).

Genetic systems concerned with general resistance have been proposed as those most likely to result in permanent resistance. They are characterized by having no affinity with specific resistance, which is recognized by hypersensitivity (Watson, 1970). On theoretical grounds, Watson regarded non-specific resistance to be valuable in helping to reduce population shifts in plant pathogens because it involves in the one host, combining a series of different genetic systems responsible for controlling the disease expression. Maintaining a high degree of heterozygosity in the plant population removes the selection pressure on the pathogen and ensures that the original pathogen, plus many unknown future pathogens, will have considerable difficulty in developing diseases of epidemic proportion. A truly mutual tolerance between pathogen, plant and man can be developed through the use of quantitative resistance (Manion, 1981).

One method that has been strongly advocated as a universal solution for breeding for resistance is the accumulation of resistance, by selection among progeny of crosses between cultivars that have been rated as susceptible to the disease. The argument is that this resistance will be race-non-specific and long lasting. However Johnson (1984) disagrees; it cannot be assumed that these observed low levels of resistance will be race-non-specific. In any case, he sees little reason to avoid the use of resistance where it already exists, in favour of developing resistance afresh.

Resistance is the expression of the interaction between the host and the pathogen. Plants that are resistant to a disease under one set of environmental conditions are not necessarily equally resistant under other conditions. If a hypersensitive reaction is involved, the same expression of disease will result for different environments. Varying the environmental conditions can be used as a method to identify types of disease based on major genes (Russell, 1978). Resistance based on many genes will be influenced in its

expression by the environment. Walker (1965) emphasised two points of difference between monogenic and polygenic resistance. Firstly, polygenic resistance is usually influenced more by environmental factors and secondly, monogenic resistance is often only effective against a single or a limited range of pathogenic races, while polygenic resistance has a broader spectrum.

2.4.3 Ontogenetical resistance

The disease pyramid, defined by Browning *et al.* (1977), is an interactive model of the major components of disease: host, pathogen, environment and time. For a long-lived tree species, tree age can be substituted as the fourth factor in the model. Changes in any of these factors can affect the expression of disease. The tree ages from a seedling, juvenile tree to canopy closure, mature tree, through to an old senescent tree. There are many examples in the literature citing differential disease response for different stages of development of the tree crop.

Bell (1980) listed a number of diseases, including the *Phytophthora* root-rot disease, that are most severe in seedling hosts, but with aging, the hosts became increasingly resistant. Pratt and Mitchell (1976) reported that defence against *Phytophthora* root-rot of alfalfa increased more rapidly as it aged in resistant, than in susceptible cultivars.

A definite increase in resistance with increasing age from four to eighty years was shown by Patton (1961) for western white pine to the white pine blister rust. Progeny of resistant parents were more susceptible in their juvenile stages. Screening in the most susceptible phase was favoured by Patton because if resistant progeny were found, resistance may be expected to increase rather than break down with increasing age. Similarly, ontogenetic variation has been shown in *Pinus radiata* to *Diplodia pinea* (Chou, 1977), *Dothistroma pini* (Power and Dodd, 1984) and *Endocronartium harknessii* (Zagory and Libby, 1985). In each case, resistance increased with increased age of the seedling / tree.

Newhook (1959) reported that *P. radiata* was highly susceptible as seedlings to *Phytophthora cinnamomi*, and in the juvenile stage to four to five years. Trees were then resistant until they became susceptible again at the age of twenty to thirty years. The results of Batini and Podger (1968), Jehne (1971) and Chevis and Stukely (1982), also indicate this range of susceptibility in *Pinus radiata.* Screening tests of Butcher *et al.* (1984) were conducted when *P. radiata* was in its most susceptible growth phase. Under these conditions, certain genotypes that could be resistant to *Phytophthora cinnamomi* for most of the thirty-year rotation may be eliminated.

Although one of the strongest, and perhaps the most variable form of resistance, ontogenetic resistance unfortunately may be the least useful (Kinloch, 1982), since it is not fully expressed until after a tree crop has passed its most vulnerable growth stage.

2.4.4 Cytoplasmic resistance

Determination of the mode of inheritance of the disease character is paramount to the breeding strategy. Whether the resistance is controlled by one or many genes, or if there is an influence of the cytoplasm, will point to the breeding method to use. The normal

mode of inheritance is related to the nucleus or the chromosome segregation and assortment. The cytoplasm of the cell can modify the effect of the genes, just as the environment can cause a different expression of the genetic character. The expression of a character for a male genotype placed in one cytoplasm may be quite different for the same male genotype placed in a different cytoplasm (Strickberger, 1968). If there is a strong maternal effect, then expression of the character may be the same for a number of different male genotypes.

Cytoplasmic inheritance is controlled by the cytoplasm, as well as the chromosomes, and involves a greater contribution from the female parent. The expression of the trait in the offspring is that of the maternal parent while the paternal parent exerts little, if any, influence. The test for cytoplasmic inheritance of a trait is to make reciprocal crosses between two parents that differ in the expression of the trait. If the phenotype expression of the trait differs in the reciprocal crosses, and the expression is associated with the maternal parent, this is indicative but is not positive proof for cytoplasmic inheritance (Hooker, 1974).

Reciprocal cross effects in *Pinus radiata* were reported by Wilcox (1983a) to be significant for seed size and early seedling growth. Maternal effects could not be analysed because the reciprocal crosses were a random assemblage of full-sib crosses. The maternal phenotype evidently played a major role in the variability of seed coat characteristics and seed size, which in turn strongly influenced the early growth of the embryo.

Libby (1983) in his concluding summary to a radiata breeding conference remarked "....The surprising reliability of open-pollinated families, in which the male contribution was not controlled, compared to full-sibs or clones, has led us to wonder whether the female contribution may be much more important than that of the male; it is possible that mitochondrial and / or chloroplast DNA may have a greater proportional effect than nuclear DNA on the characteristics of interest". Open-pollinated families of *P. radiata* have shown a remarkable consistency in their response to *Phytophthora cinnamomi* infection (Butcher *et al.*, 1984), indicating that cytoplasmic inheritance may be significant. Cytoplasmically inherited characteristics have not been utilized in tree breeding, but they are commonly used in hybrid corn breeding (Manion, 1981).

2.4.5 Summary

By taking an introduced host that is susceptible, an introduced facultative pathogen that is aggressive, and puting them together on a seasonally moist site that is condusive to disease development, it is not surprising that disease of epidemic proportion could result. Although the innate capability of a host plant to respond to the presence of a pathogen is determined genetically, its disposition to disease can be shifted by environmental factors toward increased or decreased susceptibility (Barnett, 1959). Environmental stresses, particularly in temperature and the availability of water and nutrients, usually increase susceptibility to disease (Schoeneweiss, 1975). The status of water in the environment, and within host tissue has a major influence on the growth and reproduction of *Phytophthora*. Factors of the environment are investigated in the following chapter to set the experimental conditions for the study of the *Pinus radiata* / *Phytophthora cinnamomi* genetic system.

CHAPTER 3

DEVELOPMENT OF EXPERIMENTAL TECHNIQUES AND ASSESSMENT

3.1 Introduction

In the original study of Butcher *et al.* (1984) on the genetic variation of *Pinus radiata* to the *Phytophthora cinnamomi* root-rot disease, some possible deficiencies in the glasshouse study were recognised. However, these were not expected to be significant because of the very high and consistent heritabilities calculated, and the validation of the glasshouse results with field test results.

Paramount to the study of host reaction to disease is an understanding of the factors responsible for changes in the pathogen population. Disease caused by *P. cinnamomi* depends on the population density of the pathogen; increasing the population density increases the level of disease. However the disease potential can fluctuate independently because of changes in the environment or changes in host susceptibility.

An objective of this thesis study is to also define the experimental techniques, environmental conditions and assessment methods, for the more efficient study of the genetic variation in *Pinus radiata* host resistance to the *Phytophthora cinnamomi* pathogen population.

3.2 Single-family / Multi-family treatment pots

The studies of Butcher *et al.* (1984) used single-family pots as the experimental plot, i.e. seven seedlings from the same family. Equivalent amounts of *P. cinnamomi* inoculum were placed in each pot but it is probable that there was a greater buildup of inoculum in pots containing the more susceptible families. This difference in inoculum potential

could serve to increase the variance between families for disease-resistance and give inflated heritability estimates or at worst, could indicate significant genetic differences in tolerance where in fact none actually existed, i.e. the observed difference was due to the inoculum load.

To evaluate this potential source of error, the efficiency of the family treatment pot type was examined in two different tests. The first compared identical single-family, multi-family pot studies and the second assessed the incidence of disease adjacent to a susceptible family seedling, used as a bait.

In a glasshouse screening study of 49 *P. radiata* families, each family was represented by 28 seedlings in single-family pots (4 pots, each containing 7 seedlings from the same family), and by 28 seedlings in multi-family pots (28 pots, each containing 1 seedling from the specified family and 6 other different family seedlings). Data was collected for seedling deaths and expressed as plot mean percent mortality. Noncontiguous family plots of 7 seedlings were used for the multi-family pots. The plot mean percentage of seedling deaths were transformed to arcsin values for the analysis of variance (Table 3).

TABLE 3

Effect of pot type and its interaction with family mortality. (Abbreviated analysis of variance table for *Pinus radiata* percent seedling mortality, transformed into arcsin square root, 180 days after inoculation with *Phytophthora cinnamomi*).

Source	d.f.	Variance	Significance
Main plots			
Design-pots (D)	1	15931	**
Replication (R)	3	844	
error	3	339	
Sub plots			
Family (F)	48	2884	***
FxD	48	355	*
error	288	250	
Total	391		

* significant at $P < 0.05$, ** significant at $P < 0.01$, *** significant at $P < 0.001$.

The average mortality for the single-family pot was 53% which was significantly higher ($P < 0.001$) than the multi-family pot (40%). The interaction of the host genotype with the pot type was also significant (Table 3, $P < 0.05$). This is explained by the higher

seedling mortality of tolerant families in multi-family pots relative to the single-family pot due to the presence of susceptible family seedlings in the same pot. The converse was also true; susceptible family seedlings in multi-family pots had less seedling deaths than in the single-family pot.

The 2 x 2 contingency table (Table 4) of the top one third and the bottom one third groupings of the 98 (family x pot types), ranked on seedling mortality, clearly illustrates the strength of this interaction. The calculated chi-square value of 15.75, corrected for continuity, is highly significant ($P < 0.001$), showing that seedling mortality differs between the single-family and multi-family pot groups.

TABLE 4

Chi-square test of the grouping of *Pinus radiata* family mortality, caused by *Phytophthora cinnamomi*, related to a multi-family or single-family pot type.

Character group	Multi- pot	Single- pot	Total
Highest mortality group (top 1/3 of population)	7	26	33
Lowest mortality group (bottom 1/3 of population)	22	11	33
TOTAL	29	37	66

calculated chi-square, corrected for continuity = 15.75
tabular chi-square = 10.83 (d.f. 1, $P < 0.001$). (Fisher and Yates, 1963).

The second procedure was to examine the incidence of disease adjacent to a susceptible family seedling. A single susceptible family seedling was planted near the centre of each pot as a bait for the *Phytophthora cinnamomi* inoculum to reduce the amount of sampling required at the end of an experiment (Chapter 3.3).

Data from a single-family pot test of 49 families with 21 seedlings per family (3 pots, 7 seedlings of the same family and 1 seedling of susceptible family 60017) were used to illustrate the increased disease intensity associated with susceptible host genotypes. This particular test was chosen as it was conducted in a glasshouse environment not suited to the development of the *P. cinnamomi* disease. Trial average mortality was 20%. Differences in inoculum intensity were more likely to have an effect in this environment. Data for each seedling in the treatment pot is a mortality code: this is an index scale of disease intensity (Chapter 3.8).

TABLE 5

Incidence of *Phytophthora cinnamomi* disease associated with the position of the *Pinus radiata* seedling in the experimental pot.

Rank	Position in Pot	Mortality code
1	4	3.33 a
2	5	3.16 ab
3	2	3.10 bc
4	3	2.95 bc
5	6	2.85 bc
6	7	2.84 bc
7	1	2.68 c

values followed by the same letter are not statistically different at the 95% confidence level.

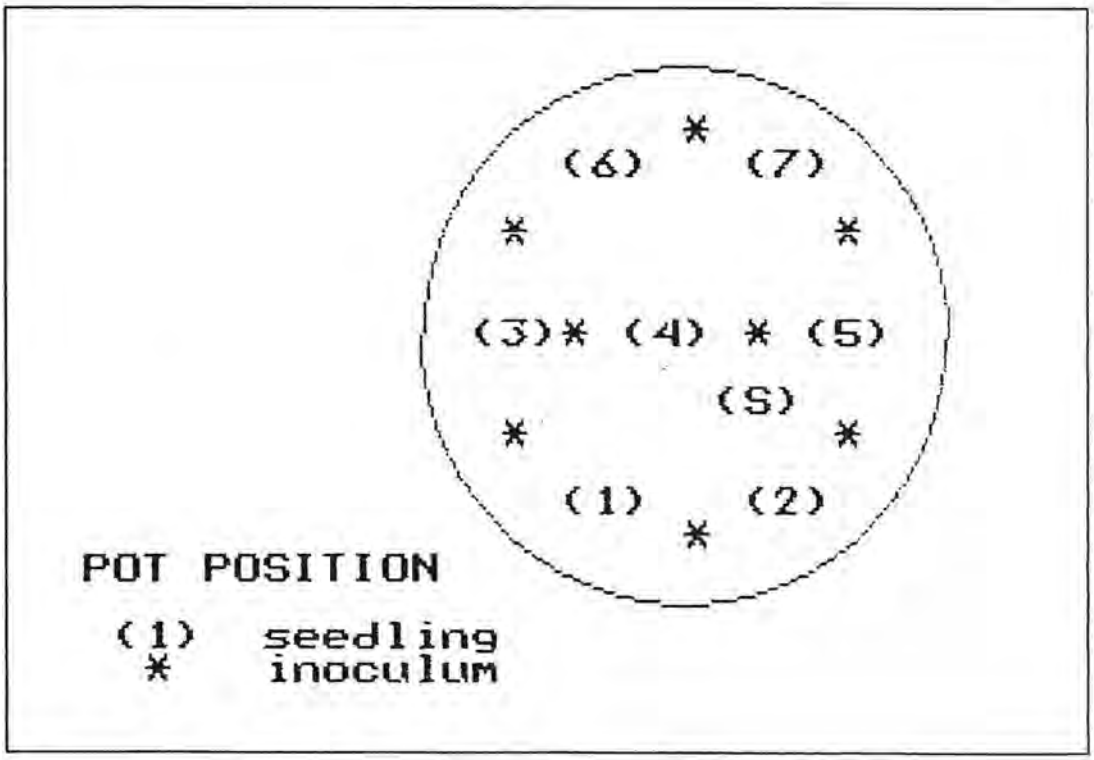

FIGURE 5

Standard experimental pot positions for different family seedlings of *Pinus radiata* and different isolates of *Phytophthora cinnamomi*.

The analysis of variance has shown significant differences in seedling mortality codes for the planting position in the experimental pot ($P < 0.05$). Higher mortality codes, which infer a greater incidence of disease, were associated with pot positions 4, 5 and 2 (Table 5). It is these positions (Figure 5) that surround the seedling of susceptible family 60017 (Butcher *et al.*, 1984). Inoculum potential in this area of the pot has been increased by the presence of a susceptible host genotype. Baker (1978) has reported that infections increase in proportion, producing a logarithmic increase in disease symptoms.

Both tests have shown that *P. cinnamomi* inoculum was significantly increased with susceptible host genotypes in the pot. In single-family pot studies, family differences in disease tolerance will be exaggerated leading to inflated heritability estimates. The multi-family pot, tests each seedling genotype with a similar level of *P. cinnamomi* inoculum and gives the best estimate of genetic variation.

For a number of screening trials using both single-family and multi-family pots, the Spearman test (Siegal, 1956) on family ranks was calculated. Coefficients of correlation were each highly significant ($P < 0.001$). The ranking of families on tolerance to disease was similar in both the single- and multi-family pots. This verifies the family rankings reported in Butcher *et al.* (1984).

3.3 Single-inoculum / Multiple-inoculum pots

The screening tests of Butcher *et al.* (1984) used only the same single isolate of *Phytophthora cinnamomi* A2, although the field test did show that resistance was effective against a broad spectrum of the natural *P. cinnamomi* population. Variation within the pathogen population is to be expected in the same way as there is variation in the host (Russell, 1978). Zentmyer (1980) concluded that there were significant differences between isolates of *P. cinnamomi* in pathogenicity to different hosts and in virulence on the same host.

Chapter 5 of this manuscript is devoted to the host (*Pinus radiata*) x pathogen (*Phytophthora cinnamomi*) interaction. This will report that there was considerable variation in *P. cinnamomi* virulence, but no evidence of any host x pathogen interaction.

This section on inoculum pot type reports on the development of the experimental technique. Information is drawn from two tests using two inoculum sources as major treatments in the design, and from the first inoculum screening test. Results from two tests with split-plot designs, each using two isolates of *P. cinnamomi* cultured from *Pinus radiata* trees, are presented in Table 6. Percentages of plot mortality were transformed to the arcsin of square roots for the analysis of variance. The difference in seedling mortality between the two *Phytophthora cinnamomi* isolates used was significant ($P < 0.05$) in one test. More importantly there were no significant differences in mortality in the host x pathogen (I * F in Table 6) interaction, in either test. Families were ranked similarly for either isolate of *P. cinnamomi*. Spearmans non-parametric statistical test of

TABLE 6

Variance analysis for the effects of two different isolates of *Phytophthora cinnamomi* used in glasshouse tests No. 3 and 4. (*Pinus radiata* percent seedling mortality, transformed into arcsin square root values).

Source	d. f.	Test 3 variance	Sig.	d. f.	Test 4 variance	Sig.
Main plots						
Inoculum (I)	1	6163	*	1	4690	
Block(B)	2	874		3	567	
error	2	241		3	982	
Sub plots						
Family (F)	99	1293	***	34	1451	***
I*F	99	265		34	541	
error	396	398		204	635	
Total	599			279		

Summary

Inoculum source	Test 3 mortality	Inoculum source	Test 4 mortality
P. cinnamomi (1)	26 *	*P. cinnamomi* (1)	41
P. cinnamomi (2)	20	*P. cinnamomi* (318)	33

* significant at 95% probability level, *** $P < 0.001$.

the family ranks gave a correlation coefficient of $r_s = 0.6$, representing very high correlation of the family rankings ($P < 0.001$).

It has been shown that isolates of *P. cinnamomi* vary in their pathogenicity to *Pinus radiata* seedlings. Only virulent isolates should be used in screening for resistance. Tests on a number of isolates indicate a broad range of virulence, and also a loss of pathogenicity for one of the *Phytophthora cinnamomi* isolates previously used. Data for seedling percent mortality in Table 7 shows isolate (6) to be highly virulent in the first test but in the test in the following year, the same isolate appears to have lost its pathogenicity, killing very few seedlings and it did not significantly affect the height growth of survivors. The second

year test was conducted in a different moisture / temperature glasshouse environment to the first test. Whether the pathogenicity of isolate (6) was affected by the different environment, or has altered through storage and subculturing is uncertain. Isolates are stored at 20°C on a cornmeal agar slope. Shepherd and Pratt (1974) found that the rate of growth and growth optimum for *P. cinnamomi* varied considerably among the isolates tested for different temperatures. Caten (1971) reported that continued culturing can result in a loss of virulence of the pathogen.

TABLE 7

Death of *Pinus radiata* seedlings, 180 days after inoculation with four different isolates of *Phytophthora cinnamomi.*

Inoculum source	HP1	HP2
P. cinnamomi A2 (1)	14%	48%
P. cinnamomi A2 (2)	18	33
P. cinnamomi A2 (6)	26	2
P. cinnamomi A2 (7)	16	34

Data suggests that multiple isolates of *P. cinnamomi* should be used in genetic variation tests to cover such eventualities as variable pathogenicity, loss of virulence associated with different environmental test conditions, or storage of the culture. Current tests use from six to eight different *P. cinnamomi* isolates. Inoculum for each isolate is grown separately, as described in Butcher *et al.* (1984). Single branch-plugs of each isolate are buried in standard positions in each pot (Figure 5). Isolate positions are recorded so that it is possible to examine the effect of different isolates on seedling health.

3.4 Glasshouse environment

Studies reported in Butcher *et al.* (1984) incorporated watering treatments in the experimental design. Waterlogging alone did not affect seedling survival or height growth. Seedling deaths in *P. cinnamomi* inoculated, waterlogged pots were lower than in *P. cinnamomi* inoculated pots that were allowed to dry periodically. This test was carried out over the summer months (inoculated in October, test ended in June) in a glasshouse where temperature control was limited to covering with shadecloth and opening of all vents. As the waterlogging treatment occupied half of the surface area of the glasshouse, this no doubt had a moderating influence on temperature.

Based on the result of their first trial, Butcher *et al.* (1984) eliminated the waterlogging regime in the second reported test. The standard watering regime adopted was to water pots to approximate field capacity, then to allow pots to dry before more water was added.

This strategy was slightly modified for the next series of tests to impose a greater environmental stress on the *Pinus radiata* seedlings, on the supposition that this could give an earlier separation of susceptible and tolerant genotypes. Water was added at three to four day intervals, giving approximately 500 ml per pot; during the course of the experiment, this was progressively reduced to compensate for dead seedlings. Glasshouse temperatures were controlled by covering with 60% sarlon shadecloth and by the use of evaporative coolers. The coolers were set for automatic operation (30°C) only on weekends, otherwise they were operated manually, switching on only when glasshouse temperatures reached 35°C. Thermohygrograph records were maintained for these tests. This low water / high temperature regime (Figure 6) was not successful. Seedling mortality for susceptible family 20011 averaged 42%, compared with the 94% mortality achieved in the high water / high temperature regime.

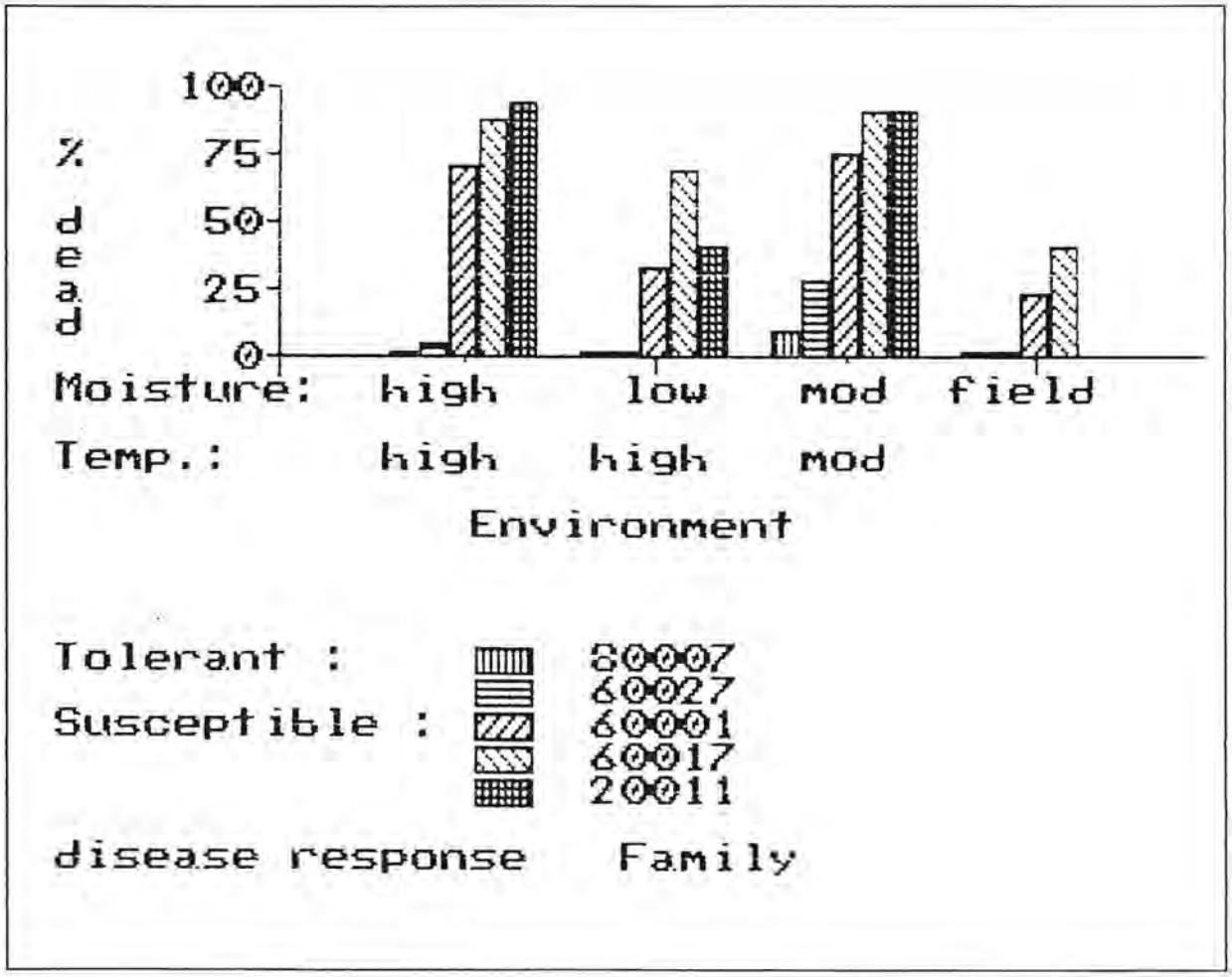

FIGURE 6
Effect of the glasshouse and field environments on the *Phytophthora cinnamomi* death of standard test families of *Pinus radiata*.

The effects of different moisture / temperature regimes on *Phytophthora cinnamomi* caused deaths of *Pinus radiata* seedlings, are shown in Figure 6. The disease pattern was essentially the same for the standard response families, although the scale was halved in the low water / high temperature regime histogram. Imposing water deficit conditions significantly reduced the pathogenicity of *Phytophthora cinnamomi.* The tests were most efficient when carried out in the moderate moisture / moderate temperature environment, as the genetic variance accounted for 22-32% of the total variance. In the unfavourable environment, genetic variance reduced to 8%.

Extreme temperatures were responsible for the elimination of a major thesis study being carried out in the Wanneroo glasshouse (Chapter 4.3). A glasshouse temperature of 47°C was recorded on the thermohygrograph. The majority of the *Pinus radiata* seedlings were discoloured (Plate 5.2), and some seedlings died in both the uninoculated pots and in pots that had been inoculated with *Phytophthora cinnamomi* thirty days previously. Following this extreme dry heat, seedlings continued to die in low numbers, in both the control and the inoculated pots. Shepherd and Pratt (1974) reported variation in response of isolates to high temperatures, but in general found little survival after exposure to 44oC for 7 hours.

The optimum glasshouse regime is to provide the minimum of stress for the pine seedlings, and the pathogen. This was achieved by maintaining pots in a moist condition, applying approximately 1000 ml of water twice weekly and keeping glasshouse temperatures below 30°C. Evaporative coolers were set for automatic operation at a temperature of 25°C.

All other environmental variables remain essentially the same as reported in Butcher *et al.* (1984). Equal parts of sand and peatmoss were mixed, fumigated with methyl bromide and filled into 8 litre capacity plant pots (height 20 cm), or seedling trays. Pine seed was stratified by cold-soaking in water for one day, followed by twenty days cold treatment at approximately 3°C, before sowing in seedling trays. Seedlings were grown to a height of approximately 8 cm, before they were individually labelled and transplanted into their experimental positions in the plant pots (Figure 5). Pots were mulched with pine needle litter, containing the mycorrhizal fungi *Rhizopogon luteolus* and *Thelephora terrestris,* and the seedlings were grown with regular watering and periodic liquid fertilizer application. Pots were moved into the glasshouse in May/June, where they were placed in individual water holding containers and positioned according to the experiment design. A later improvement was to place pots in holding tanks, twenty four pots per tank, where greater control of pot moisture level could be achieved.

The preparation of the pine branch-plug inoculum remained as it was reported in Butcher *et al.* (1984). Live *Pinus radiata* branches were cut into plugs 1-2 cm in diameter and approximately 2 cm long, after the bark was removed. The plugs were soaked overnight in distilled water, rinsed, and placed in conical flasks. Sufficient distilled water was added to cover the bottom of the flasks, which were plugged with non-absorbent cotton wool and autoclaved for 30 minutes at 103 kPa, then cooled to room temperature. *Phytophthora cinnamomi* isolates were incubated at 24-26°C for seven days in 10 ml lots of 10% V8 broth. Mycelial mats were aseptically lifted from the V8 broths and dropped onto the plugs, which were shaken gently, and then incubated at 24-26°C. After two to three days, when the fungus had started to colonise the plugs, they were again shaken to disperse the inoculum and were then left to incubate for a further two to three weeks.

Tests commenced with soil inoculation of pots with *P. cinnamomi,* usually in October when soil temperature and moisture conditions are most favourable for the growth and proliferation of the pathogen (Shea, 1975). Each pot was inoculated with four to eight infected plugs, equally spaced and buried 8 cm beneath the soil surface (Plate 1). The soil was saturated with water, and maintained in this condition for at least one day before being drained and normal watering was resumed.

PLATE 1
Inoculation of experimental pots with *Phytophthora cinnamomi*, grown on pine branch-plugs.

3.5 Experiment design and procedures

The exploratory test of Butcher *et al.* (1984) included a control (nil *P. cinnamomi* inoculation) as well as watering, as major treatments in the design. The design was a split-split-plot to prevent contamination of the controls and to allow for easier maintenance of the water treatment levels. A nil *P. cinnamomi* inoculation treatment was also included in their second glasshouse test. Butcher *et al.* (1984) reported only 2 seedling deaths from a total of 1,968 seedlings in non- *P. cinnamomi* inoculated pots. Sampling of root and collars of these 2 dead seedlings proved negative for *P. cinnamomi*.

In the *P. cinnamomi* inoculated pots, 1,140 seedlings died out of a total of 2,807, and *P. cinnamomi* was recovered from every dead seedling sampled.

The early tests have shown that it was not necessary to include an uninoculated control in the screening tests. This has simplified the experiment to a randomised block design. Tests have ranged from 5 to 8 seedlings per pot; 7 treatment seedlings per pot have been shown to be an optimum number. Combined with the other refinements of multi-family, multiple-inoculum pots, the most efficient design for the screening test is the 7 x 7 balanced lattice ($t = 49$, $k = 7$, $r = 8$; Cochran and Cox, 1957), using the plant pot as the incomplete block unit.

The number of seedlings inoculated for each family has varied in experiments from 6 to 64. The minimum number to use will depend on the form of the relationship of the family seedlings. Seedlings with the same ancestors will share more common genes and hence behave more similarly to each other than seedlings with dissimilar ancestries. By comparing the degrees of variation between related and unrelated trees, the genetic differences can be seen to have a strong effect if the data cluster in family groups, or a weak effect if family clusters are diffuse. Family seedlings used in our *P. cinnamomi* screening tests all have a half-sib relationship; the mother cone tree is the same in each family seedling while the pollen parent is assumed to be different.

Sample size was tested in a multi-family / multiple-inoculum pot trial conducted in an ideal glasshouse environment. The maximum number of seedlings per family inoculated with *P. cinnamomi* was 40. Complete blocks of $n = 24$, $n = 16$ and $n = 6$ single seedling replications were subsampled, and separate analyses of variance were carried out on the seedling disease codes (Chapter 3.8). Genetic variances were very high (Table 8), and similar over the 6 to 40 seedling sample range. The ranking of the 35 families for each of the sample sizes is given in Table 9. Kendall's coefficient of concordance (Kendall and Stuart, 1961) was calculated to be 0.85; this near perfect concordance is to be expected since it is obvious from a visual inspection of the Table 9.

TABLE 8

Partitioning of variances of seedling disease for a range of seedling sample sizes, from a trial of 35 half-sib *Pinus radiata* families inoculated with *Phytophthora cinnamomi*.

variance	Number of seedlings / family in the Variance Analysis			
	n = 40	n = 24	n = 16	n = 6
$ó^2_f$	31%	34%	29%	41%
$ó^2_b$	4	4	1	1
$ó^2_w$	65	62	70	59
$ó^2_t$	100	100	100	100

TABLE 9

Ranking of 35 *Pinus radiata* families on the character of seedling disease tolerance to *Phytophthora cinnamomi* for n = 6, to n = 40 observations / family.

Family code	Sample size - ranking n 40	n 24	n 16	n 6	Family code	Sample size - ranking n 40	n 24	n 16	n 6
1	34	34	33	33	**19**	4	9	1	1
2	21	21	21	20	**20**	7	6	11	13
3	32	32	31	32	**21**	30	32	25	20
4	22	19	9	30	**22**	5	14	18	11
5	14	17	10	13	**23**	17	19	3	16
6	10	11	6	6	**24**	19	16	19	23
7	9	6	9	5	**25**	13	11	17	20
8	24	30	22	16	**26**	11	9	15	20
9	26	25	26	26	**27**	18	14	19	9
10	29	24	32	30	**28**	27	26	26	29
11	6	8	5	16	**29**	3	3	4	4
12	20	23	16	9	**30**	28	27	28	23
13	31	31	29	26	**31**	1	1	2	3
14	5	4	6	8	**32**	33	27	35	35
15	8	5	14	6	**33**	23	22	23	25
16	2	2	2	2	**34**	12	13	8	16
17	34	35	33	33	**35**	15	18	11	11
18	25	29	24	28					

Sample size	n = 40	n = 24	n = 16	n = 6
Spearmans rank corr. coeff.	1	0.97	0.96	0.87

Spearmans coefficient of rank correlation (Siegal, 1956) was calculated by serial analysis for each of the sample sizes (Table 9). The ranking of each has near perfect correlation ($P < 0.001$). Even for a small sample size of 6 inoculated seedlings per family, Spearmans coefficient was 0.9.

Data from the examples listed in Tables 8 and 9 are representative of each of our *Pinus radiata* / *Phytophthora cinnamomi* tests. There is an unusually high consistency of results considering that only half-sib seedling stock was being tested. Data is strongly clustered in family groups indicating very high genetic variances. Small numbers of seedlings are adequate to measure the genetic variation. The optimum number appears to be 16, which allows for a duplication of the complete lattice design.

The length of the test period is another important parameter. Data was collected over a period of 250 days in the tests of Butcher *et al.* (1984), although family seedling deaths plateaued at about 130 days after inoculation. This plateau, occurring before the end of February, has been constant over most tests. Disease development curves, for the test average, are plotted for seven of our screening tests in Figure 7.

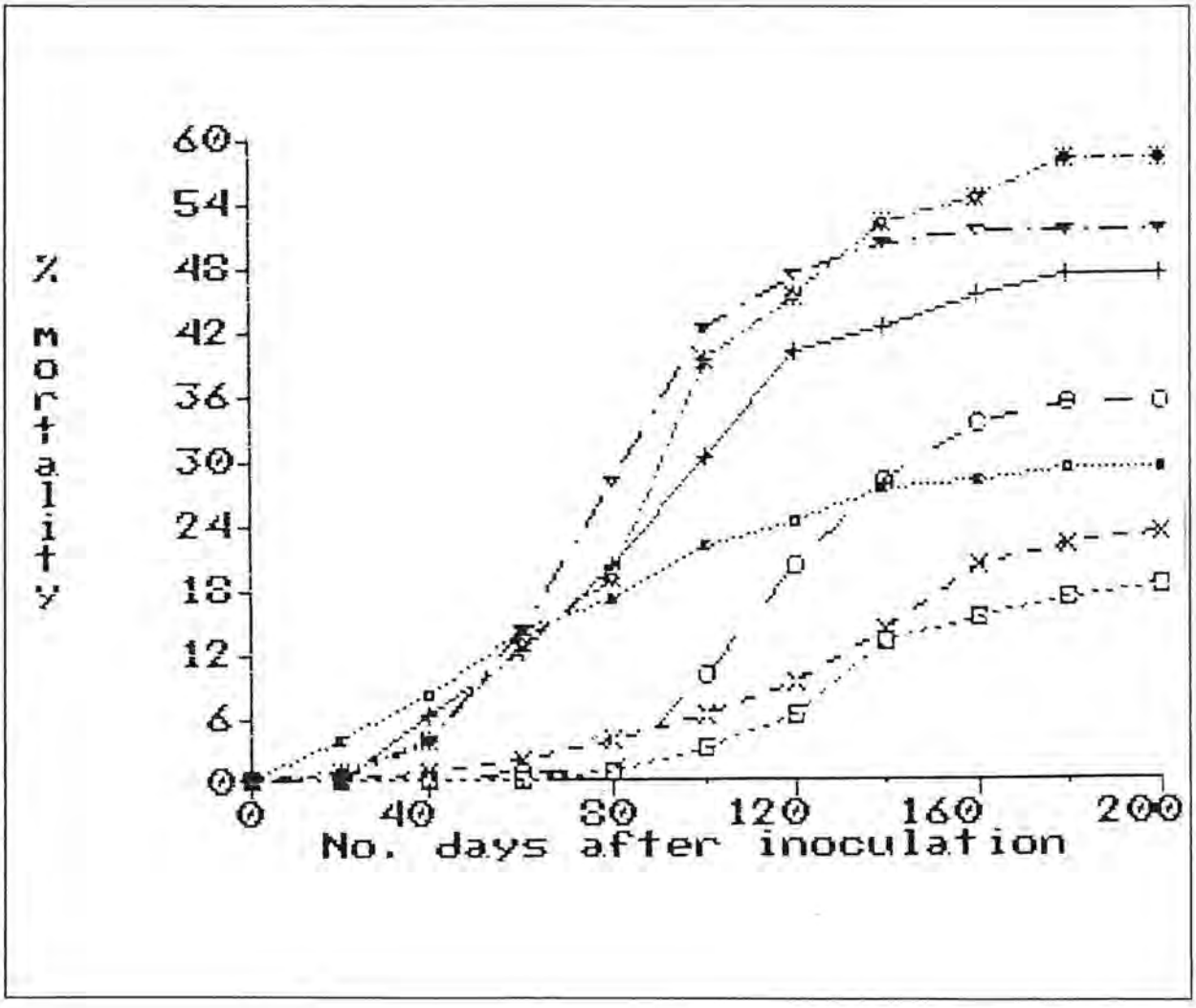

FIGURE 7
Progressive seedling mortality after inoculation with *Phytophthora cinnamomi*, in seven family screening tests of *Pinus radiata*.

Providing a moderate moisture / moderate temperature environment improves the efficiency of the test, in segregation of the genetic effects. Tests are now conducted in the optimum defined glasshouse environment, and end at 160 days after inoculation. Tops of the most vigorous seedlings are then removed, set as cuttings, rooted, and later established in genetic resource stands.

Repeatability of the family disease classification was demonstrated by Butcher *et al.* (1984) for the glasshouse and the field. This was corroborated with the second field test (Chapter 4.4). Glasshouse tests have been standardised by the inclusion of a common set of families in each test. These are susceptible families 60017, 20011 and 60001, and tolerant families 80007, 60027 and 60022 (Butcher and Stukely, 1986). Disease development curves in Figure 8 show a similar performance of these genotypes across tests. Even with the low moisture / high temperature environment regime, the performance of the standard families was consistent.

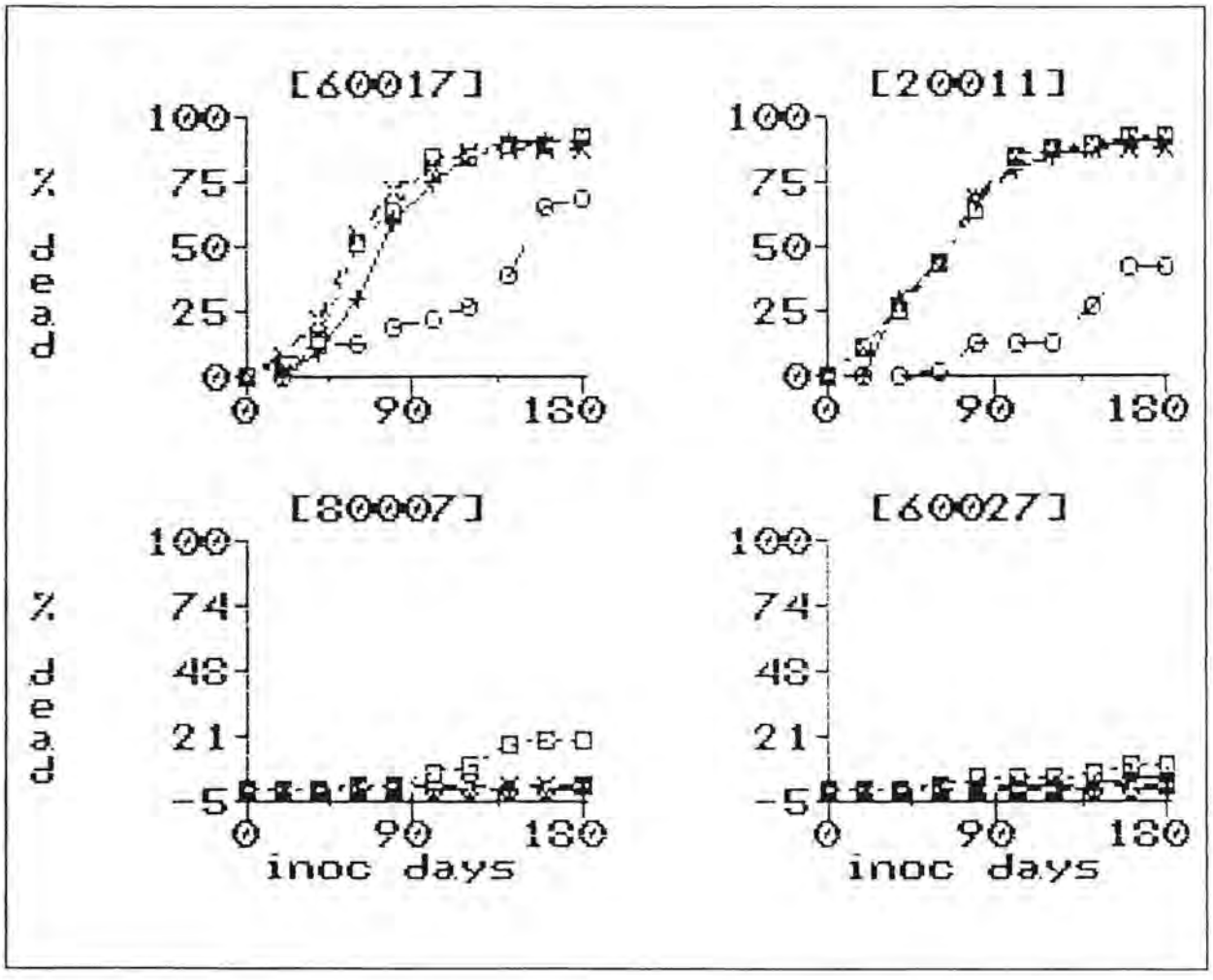

FIGURE 8

Repeatability of *Phytophthora cinnamomi* disease development in four families of *Pinus radiata*, in four screening tests.

(susceptible response : 60017 and 20011), (tolerant response : 80007 and 60027)

Another adjustment to the design, to improve the efficiency of the glasshouse test, has been the inclusion of a single susceptible family seedling near the centre in each inoculated pot. The susceptible seedling acts as a bait to the applied *P. cinnamomi* inoculum, rapidly multiplying the disease potential within a pot. Most of these seedlings die within 100 days (Table 10). Plating has shown massive infections throughout the root system, the collar and lower stem of the seedling (Chapter 3.6). Using the bait seedling has considerably reduced the amount of sampling and plating for *P. cinnamomi* at the end of a test, in pots in which no seedling had died.

TABLE 10

Seedling survival in *Phytophthora cinnamomi* susceptible families, planted as single seedling baits in each pot of the *Pinus radiata* tests.

Test	*Pinus radiata* susceptible family	seedling mortality 100 days	180 days
NP1	60001	63%	69%
DP1	60017	82	89
DP2	D655	74	84

3.6 Re-isolation of Phytophthora

At intervals during the experiments, seedlings and pots were sampled to detect the presence of *P. cinnamomi.* Dead seedlings, healthy seedlings or inoculum plugs were sampled. These were removed with sterile implements, adhering soil washed away and the sample surface-sterilized by immersion in 70% ethyl alcohol for 30 seconds, followed by four rinses in distilled water. The lower stem and collar of seedlings were cut into 8 mm segments and serially plated onto an agar medium selective for *Phytophthora* (Tsao and Guy, 1977). Randomly selected 1 cm root pieces were also plated. Inoculum plugs were first cut in half, lengthways before plating. After incubation for at least 72 hours at 24-26°C, the plates were examined and infections recorded.

The recovery of *P. cinnamomi* from dead seedling roots and collars has been consistently high, averaging 66% for individual sample pieces and 100% for the seedlings. In study A of Table 11, *P. cinnamomi* was recovered from half of the seedlings sampled at a height of 3 cm above the soil surface and from 91% of the seedlings, 2 cm above the soil. Infections were extensive in each case. There was no apparent difference between seedling genotypes and the degree of infection.

TABLE 11

Isolation of *Phytophthora cinnamomi* from dead seedlings of *Pinus radiata*

Sample		Seedlings n	infected	Segments n	+ve Recovery *P.c.*	%
A.	root pieces	44	100%	742	545	73%
	stem (3cm)	44		44	22	50 %
	stem (2cm)	44		44	40	91%
	stem collar	44		44	40	91 %
B.	root	75	100%	1500	930	62 %
	collar	75		1483	968	65 %
C.	root @ collar	172	99%			

At the end of an experiment all pots with no dead seedlings were sampled to check that *P. cinnamomi* was present and active. This was achieved by the plating of inoculum plugs, and healthy seedling root and collar segments. In 244 pots containing 903 plugs, *P. cinnamomi* was isolated from 819 plugs 180 days after the pot inoculation, showing that *P. cinnamomi* was present in each of the healthy seedling pots. The extent of infections in the healthy seedlings were reflected by the seedling genotype, with greater disease in roots and collars of the more susceptible families (Table 12).

The variable degree of root-rot infection of apparently healthy seedlings has been used to create a scale of disease intensity (Chapter 3.8). There is a direct relationship between root infection and root mass or bulk.

TABLE 12

Recovery of *Phytophthora cinnamomi* from healthy seedlings of tolerant, average and susceptible families of *Pinus radiata*, one year after inoculation with two isolates of *Phytophthora cinnamomi.*

	Family disease classification		
	Tolerant	Average	Susceptible
a) individual root pieces			
number of root pieces	2157	1253	1595
number with *P.c.* infection	113	152	378
% *P.c.* infection	5%	12%	24%
b) seedlings			
number of seedlings	31	20	21
% *P.c.* infection - root	68%	95%	100%
- collar	13%	50%	62%

3.7 Assessment

Disease offers three parameters for measurement, incidence, intensity and losses in production. Incidence infers the presence or absence of disease, i.e. the number of seedlings affected. The quantity of disease or intensity is more difficult to measure. This can be achieved by counting the number of lesions on roots, the number of roots with lesions, or by measuring lesion lengths etc. Disease intensity requires an estimate of the proportion of the seedling that is diseased. This is also directly related to the potential yield reduction.

Butcher *et al.* (1984) expressed disease as the percentage of dead seedlings for each *Pinus radiata* family and used height growth to support this data. No attempt was made to describe disease intensity on an individual seedling basis. Their data was adequate to show there was genetic variation in disease incidence, and to calculate family heritabilities to show what progress could be made from progeny test selection for this trait. However, the data was inadequate to calculate the genetical aspects of resistance.

Information on the disease intensity of each individual seedling is required for the genetical analysis. Dead seedlings were simply subdivided into classes on the time taken to kill the seedling (quantity of disease). Various methods of assessment were examined to develop the other end of the index. These included normalised height increment (yield reduction), subjective root bulk classification (quantity of disease) and xylem water potentials (quantity of disease) of the surviving seedlings.

Height growth of seedlings, in all families of *P. radiata* was depressed following inoculation with *Phytophthora cinnamomi* (Butcher *et al.*, 1984). This loss in growth varied according to the disease susceptibility of the family and could be used to form an index of disease intensity (Plate 4). However, as there was genetic variation in the height growth of seedlings in the absence of *P. cinnamomi*, any index based on seedling height growth must be adjusted for these genetic effects. Population parameters for each uninoculated family need to be calculated to adjust the increment of each inoculated seedling. Unfortunately it was not possible to recalculate the data of Butcher *et al.* (1984) because seedling positions in pots were not fixed.

Uninoculated treatments have been excluded from most of the screening experiments, to increase the efficiency of limited amounts of pedigree seed, and glasshouse space. Standardisation of height increments on uninoculated controls was not possible but adjustments were made using the general population parameters.

Surviving seedlings at the end of tests show a large variation in the mass or bulk of root systems, and in the degree of root-rotting. Trained observers have experienced little difficulty in similarly sorting seedlings into arbitrarily defined root disease classes. Four broad disease classification classes have been defined (Table 13).

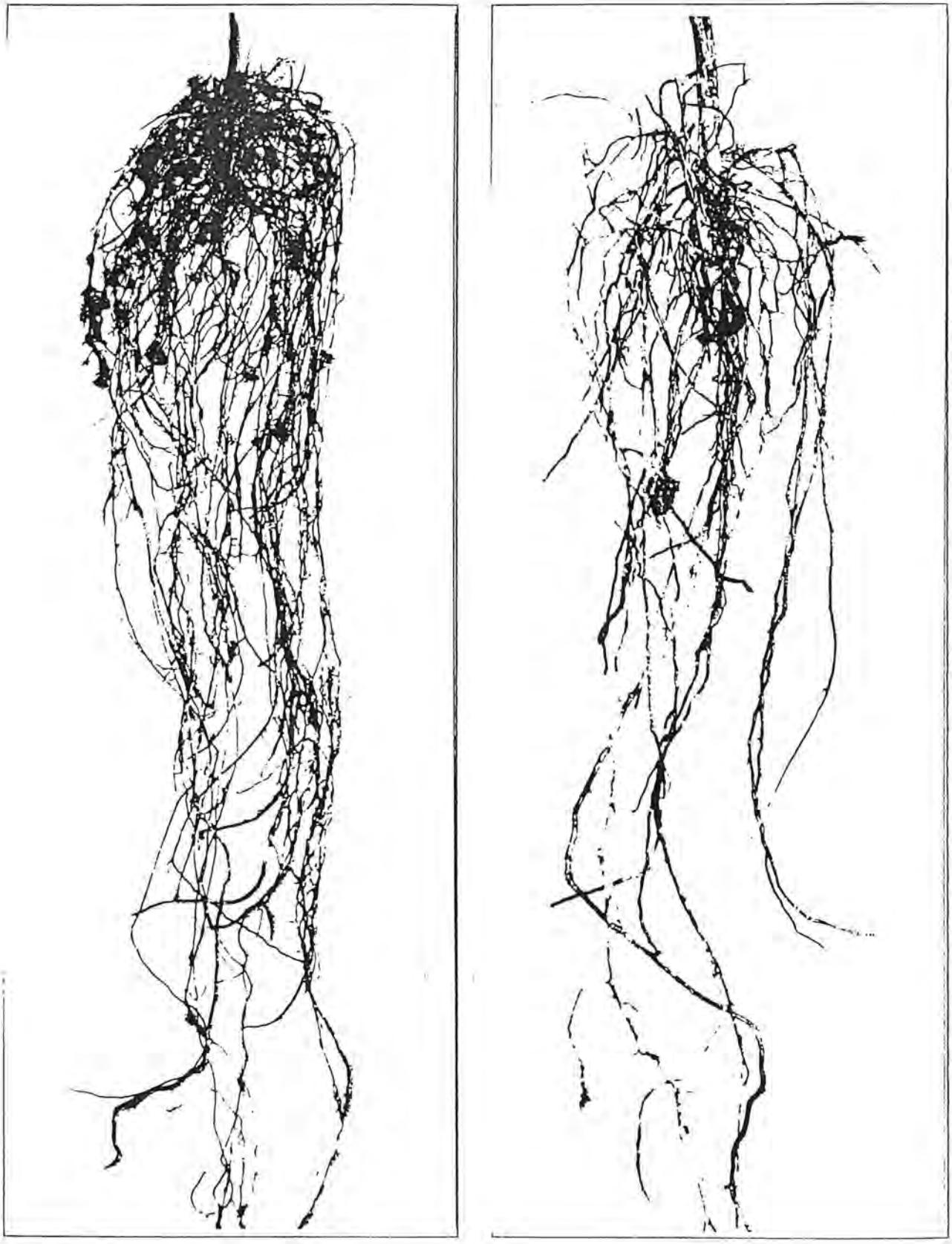

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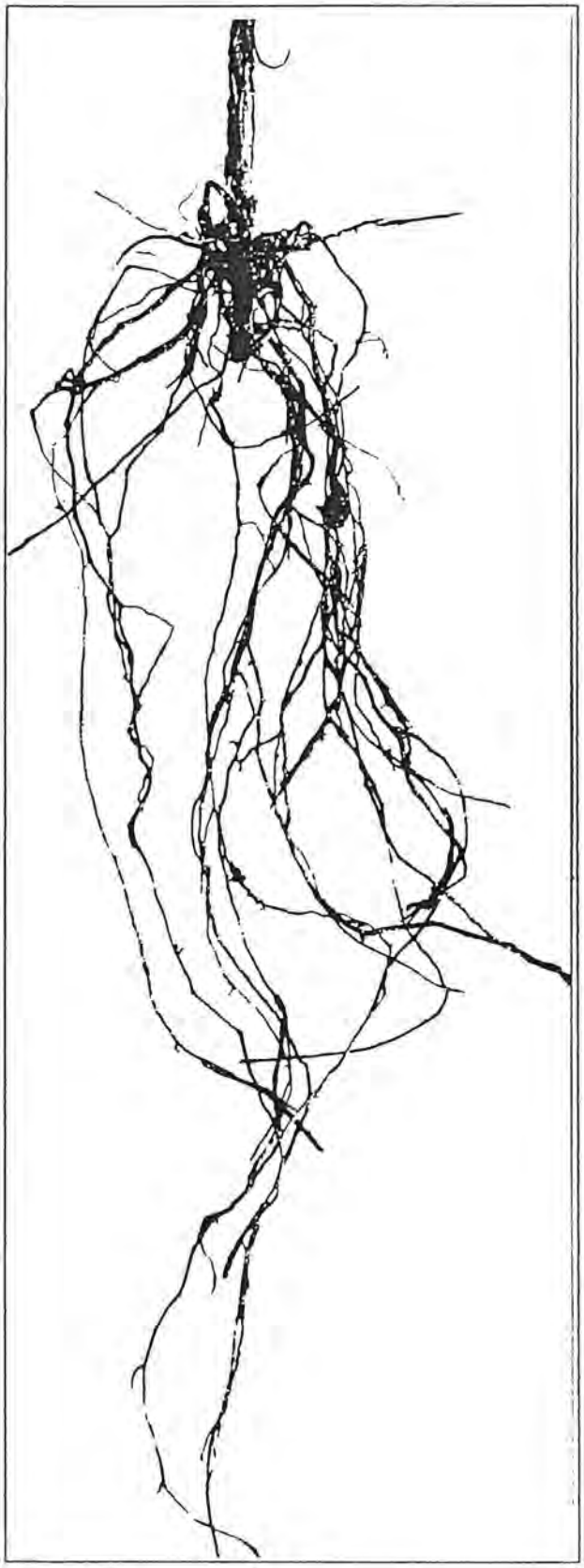
PLATE 2

Assessment and root system classification of surviving seedlings of *Pinus radiata*, 200 days after inoculation with *Phytophthora cinnamomi*.



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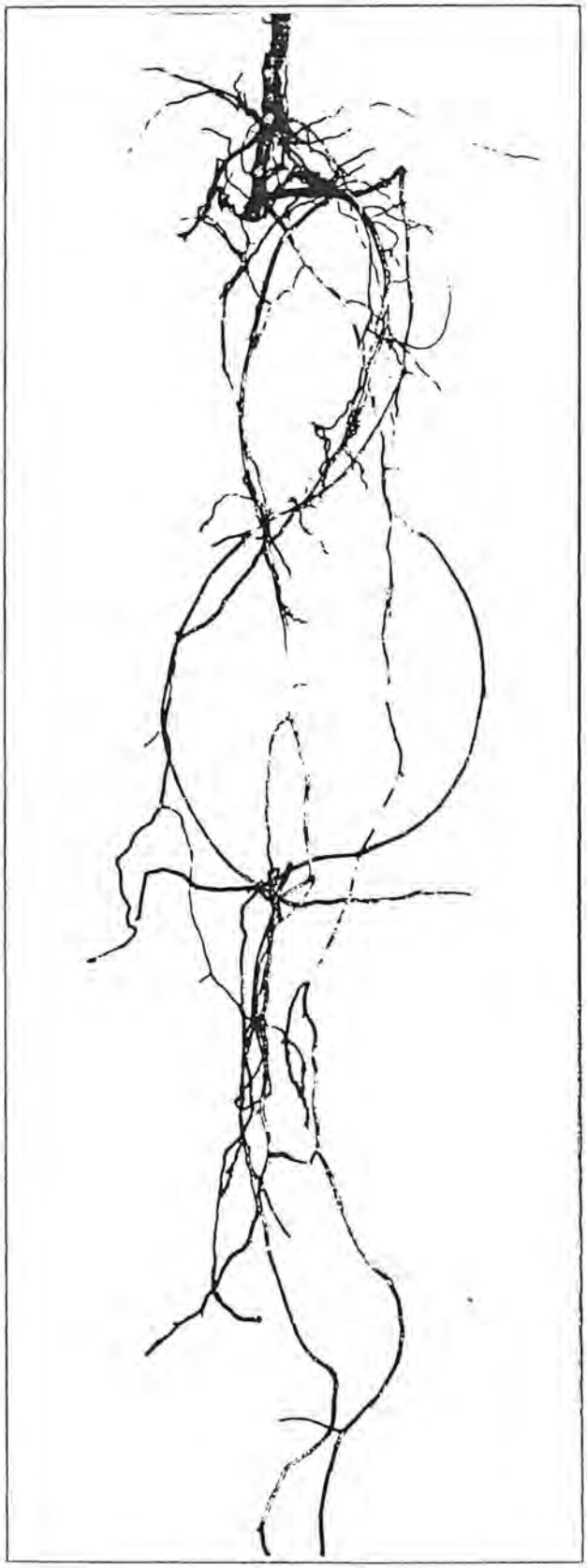

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PLATE 2 (cont)

Assessment and root system classification of surviving seedlings of *Pinus radiata*, 200 days after inoculation with *Phytophthora cinnamomi*.

Class 1 : Healthy, massive root system, well formed and branched with many active growing tips (Plate 2.1).

Class 2 : Healthy, well formed and branched root system, has less bulk than the first category (Plate 2.2).

Class 3 : Unhealthy, sparse root system. Roots are shorter in length and forked above rotted root ends (Plate 2.3), root bulk considerably reduced.

Class 4 : Unhealthy, rotted root system with few obvious living roots, generally unbranched as rotted roots have sheared away (Plate 2.4).

The method of assessment is to first remove seedling tops, approximately 10 cm above the collar (to eliminate bias with seedling height), empty the pot and physically separate seedling root systems. Initially, considerable care was taken to avoid root breakage but it was found that with quick separation, healthy roots remained intact and only rotted roots fell away. As the root classification was not affected, the quick separation has been adopted as the standard. Root systems are visually assessed and assigned to root classes by reference to representative samples.

There is some flexibility within each class to allow for variable competition within treatment pots that may be due to unequal numbers of survivors; for example, in a potwith six survivors, the root systems are ranked by degrees of bulk and then related to standards, while in pots containing only one or two survivors, the classification is absolute. Following the classification, seedlings are grouped by the root disease classes and at completion, each disease class is randomly sampled by taking 30 seedlings; stems are then cut at 1 cm above the collar, and roots are oven dried at 50°C for 32 hours to define the limits of each class.

TABLE 13

Definition of *Phytophthora cinnamomi* root disease classes from the assessment of surviving seedlings of *Pinus radiata*.

Root assessment	Root weight : mean and standard deviation			
	Test A	Test B	Test C	Test D
class 1	3.3 + 1.1g	2.9 + 0.8 g	4.1 + 1.8g	4.7 + 1.7g
class 2	2.2 + 1.0	1.5 + 0.5	2.1 + 0.5	2.9 + 0.8
class 3	0.7 + 0.3	0.9 + 0.4	1.0 + 0.4	1.2 + 0.4
class 4	0.2 + 0.1	0.5 + 0.3	0.5 + 0.3	0.4 + 0.3

Root mass for disease classes in three separate studies is presented in Table 13. Healthy (class 1 and 2) root systems were clearly distinctive from unhealthy (class 3 and 4) root systems, as demonstrated by the class standard deviations. Frequency distributions (Chapter 3.8) show a normal distribution of the data.

The root assessment has been found to be a reliable and repeatable index of disease.Applying both root assessment and normalised height increment to define the amount of disease in surviving seedlings has given identical results from the analysis of variance. For example, genetical variances were 22% from either the analysis of the root, or the height increment scale in one test.

Infection by root diseases was reported by Ayres (1978) to cause progressive water stress in the aerial parts of the plant by increasing stem and root resistances to the conduction of water. Death of seedlings resulted from the decline in water supply to the leaves. In one-year-old seedlings of *Pinus ponderosa*, infected with root stain disease (*Verticladiella wageneri*), Helms *et al.* (1971) reported leaf water potentials of -18 bars at four weeks increasing to -30 bars at six weeks after infection; most of the infected seedlings died soon afterward. Healthy seedlings had leaf water potentials from between -12 and -15 bars.

A small pot study was carried out in conjunction with the main thesis study at Wanneroo, to determine the effectiveness of the xylem water potential as a measure of *Phytophthora cinnamomi* disease severity in *Pinus radiata* seedlings. Each pot contained four susceptible and two tolerant *P. radiata* families, and two *P. pinaster* (resistant species) family seedlings. Half the number of pots were inoculated with eight isolates of *Phytophthora cinnamomi.* Xylem water potentials of excised seedling tops were assessed at 15 day intervals, using the Scholander *et al.* (1965) pressure bomb technique.

TABLE 14

Xylem water potentials of *Pinus radiata* and *P. pinaster* family seedlings, 20 days after inoculation with *Phytophthora cinnamomi.*

	xylem water potentials	
	P.c. inoculated	control
susceptible *Pinus radiata* family (n = 10)	360 kpa	270 kpa
tolerant *Pinus radiata* family (n = 5)	430	435
resistant *Pinus pinaster* family (n = 5)	210	210

The first result, 20 days after inoculation, appeared promising (Table 14). A difference of 90 kilopascals was recorded between inoculated and control seedlings of the susceptible *Pinus radiata* families, whereas xylem water potentials of the inoculated and control seedlings of the tolerant families and resistant *P. pinaster* family seedlings were

the same. However, the same pattern was not evident at the subsequent sampling dates, probably because the test was severely affected by the extreme temperatures experienced in the glasshouse that caused the elimination of the first domestic population study (Chapter 4.3).

The use of xylem water potentials as a measure of disease has not been pursued. The method requires uninoculated seedlings of each family and it could only be realistically applied at the end of a test to divide the surviving seedlings into disease severity classes. The simple visual classification appears to be adequate for this. In addition, the sampling is destructive and this would prevent the taking and setting of inoculated seedlings tops, as cuttings for genetic resources stands.

Assessment procedures have been defined as : Initial heights of seedlings are measured at the time of inoculation. Assessments are then carried out at 20 day intervals recording the number of dead seedlings and their heights. Heights of all surviving seedlings are measured at the end of the 160 day test period. Root systems are then inspected and given a subjective disease classification.

3.8 Scale

Analysis can be performed on all-or-none traits, for example dead or alive seedlings, to calculate genetical variances at the individual level (Robertson and Lerner, 1949; Bingham *et al.*, 1969). By definition, all-or-none traits are binomially distributed with variances dependent on means and this violates a fundamental requirement of the variance analysis. Data in the preceding section of this paper has shown that there was considerable variation in the amount of disease in alive seedlings. Similarly, Figure 7 shows a range in disease intensity of dead seedlings. From these, a disease scale representing continuous or quasi-continuous variation can be developed. Obviously a scale will be more efficient in describing and analysing the genetic variation.

The choice of an appropriate scale is the first step in the analysis of polygenic variation (Mather and Jinks, 1982). The scale must cover the intensity of disease, ranging from minimal effect on seedling health through to rapid seedling death. No complete resistance or immunity to *Phytophthora cinnamomi* disease was observed in healthy seedlings of *Pinus radiata* sampled. Either height growth has been suppressed, relative to uninoculated seedlings, or roots have been infected by the disease. *Phytophthora cinnamomi* has been readily isolated from healthy inoculated seedlings sampled.

Surviving seedlings were apportioned to four health classes, either on the basis of the subjective root assessment or on the objective height measurement (Chapter 3.7). Similarly, disease classes were developed from the time taken for the seedling to die following inoculation. This is demonstrated in Figure 9. Data from four trials, involving 4,564 seedlings of *Pinus radiata* inoculated with multiple isolates of *Phytophthora cinnamomi*, in an environment conducive to disease development, were collated to

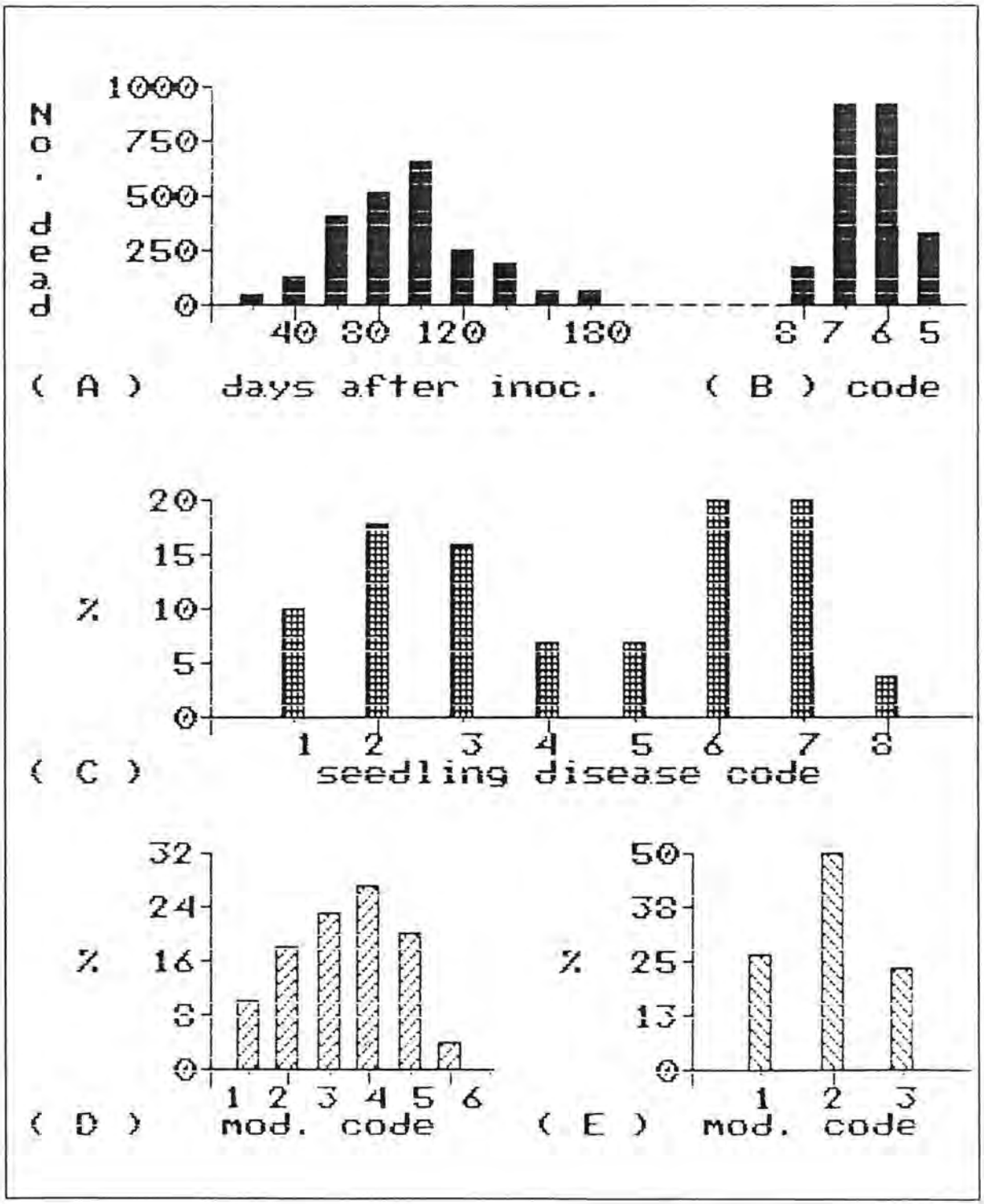

FIGURE 9
Development of a seedling disease scale.

FIGURE 9 (cont)
Development of a seedling disease scale.

A. frequency distribution of seedling deaths.

B. disease codes based on 40 day interval of deaths.

C. combining (B),with root assessment classes of surviving seedlings, to create the disease index.

D. modified scale was formed by combining diseased seedling codes 3 ,4 in (C) to make mod.code 3 and dead seedling codes 5 ,6 in (C) to make mod.code 4.

E. scale was further modified into :
healthy (1 = 1 + 2 in (D)),
diseased (2 = 3 + 4 in (D)),
and dead (3 = 5 + 6 in (D)).

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illustrate the frequency distribution of seedling deaths (Figure 9A). In these trials, seedling death averaged 50%. As the initial classification was based on two classes, it was then logical to have an equal subdivision within the dead and alive major classes. Four classes could be easily defined from the 20 day record to have a 40 day interval with an approximate normal distribution (Figure 9B).

Combining the four classes of alive seedlings (50%) and four classes of dead seedlings (50%) from this data creates the disease intensity scale with eight classes (Figure 9C). This empirically developed scale represents the continuous variation in disease expression of individual seedlings.

Seedling disease intensity scale :

Code 1 - healthy seedling, massive root system
2 - healthy seedling, good root system
3 - unhealthy seedling, sparse root system
4 - unhealthy seedling, rotted root system
5 - dead seedling, 180 days after inoculation
6 - dead seedling, 120 days after inoculation
7 - dead seedling, 80 days after inoculation
8 - dead seedling, 40 days after inoculation

A fundamental assumption of the analysis of variance is that there is a normal distribution of data for the individuals sampled (Sokal and Rohlf, 1981). This was achieved in Figure 9(D), by combining the codes 5 and 6 to create mod.code 4 and 3 and 4 to make mod.code 3. Seedlings in these classes were similar, either dying near the end of the experiment, or having a completely rotted root system and were on the point of death. Other conditions of the analysis of variance were met. Seedlings were randomly sampled and variances of families were homoscedastic. Analysis of variance on both the assigned codes (1 to 8) and modified codes (1 to 6) gave an identical partitioning of variance.

The frequency distribution of classes in Figure 9(C) shows a clear separation of data into two populations, with an equal mixture of two normal distributions. Models are illustrated in Allard (1960) that depict this. However, by changing the character scale, different interpretations can be made. For instance, Figure 9(D) could represent the polygenic model showing high heritability and no dominance. By further compressing the central distribution of the scale (Figure 9C) so that codes 3, 4, 5 and 6 are unhealthy seedlings, and the extremes, codes 1 and 2 are healthy, and codes 7 and 8 are dead, a ratio of 1 : 2 : 1 is achieved (Figure 9E). This is the typical segregation ratio of Mendel (Strickberger, 1968).

The choice of the empirical scale should remove environmental and other effects from the genetical analysis. The scale that is appropriate for the representation of variation in disease of one population under one set of glasshouse conditions may not be appropriate for a different population, given the same or different environmental conditions. One scale may be impossible, given our lack of understanding of gene action for this disease character.

3.9 Heritability

Genetic sources of variance are complex and difficult to measure and use. Many genes may effect a single response and many physiological conditions may interact to produce the composite trait of disease resistance. Genes cannot often be measured and are generally known only by their action on the trait being measured. The trait can be manipulated in a designed experiment to separate out the variation caused by genetic effects, from other unidentified sources.

For genetics experiments, the family groups are the experimental sources of variance that can be controlled and analysed. The degree of relationship and the strength of the genetic effects determine the physical distinctiveness of the family groupings. By knowing or controlling the degree of relationship, gene effects can be studied by measuring the similarity of family members. If variation is largely the result of gene effects then close relatives like parent-offspring or sib-sib will be very similar, as compared with unrelated pairs. If both parents are common between sibs (full-sibs), the covariance is higher than if only one parent (half-sib) is the same. In turn, the half-sib covariance is higher than for more distantly related pairs. The genetic variation is reflected in the variation

between family groups, which increases as the covariances of relatives increase within groups. Genetic variances can then be estimated from the known genetic covariances of the constructed family group.

Selfing in mature stands of *P. radiata* is most likely to be insignificant (Moran *et al.*, 1980). Bannister (1965) has shown that many trees have an equal chance of contributing pollen; as the density of the stand decreases, the proportion of pollen provided by neighbour trees increases. Bannister concluded that if a species is self-fertile, the breeding system may range from strong outcrossing in dense stands, through various proportions of outcrossing and selfing, to complete selfing in isolated individual trees. While open-pollinated seed is inexpensive, and is an easy and rapid progeny test medium, an element of uncertainty is introduced in the genetical analysis because of the possible variable rates of sib comparisons.

Heritability is an index which reflects the amount of genetic variation in the population relative to the total (genetic plus environmental) variation. To the tree breeder, it serves as a partial indication of the genetic gain possible in a certain trait. Hanson (1963) considers heritability important in quantifying whether progress from selection is relatively easy, or difficult to make in a breeding programme. Some knowledge of heritabilities is basic to the efficient operation of breeding programmes, particularly in the choice of breeding methods to use (Wright, 1962).

Heritability can be estimated in either of two ways. The most direct estimate is derived from parent / offspring regressions and correlations. This is not applicable to our situation as the selected *Pinus radiata* parent trees were not growing in a *Phytophthora cinnamomi* environment. The other method is to establish progeny tests with standard statistical designs, and to compute the genetic variances from an analysis of the experimental variances.

The glasshouse experiments we used had a randomised complete block design, or a slight modification of this. Components of variance and expected mean squares have been computed from the analysis of variance at the individual tree level and for family plots (Table 15). Most of the trials had no missing values. In trials with missing data, k coefficients for unequal subclass numbers were computed by the method of Harvey (1960). Standard errors of individual heritabilities were calculated according to Swiger *et al.* (1964).

Phenotypic variance and narrow sense heritability of *P. cinnamomi* disease resistance in *Pinus radiata* were calculated as:

$\acute{o}^2_P$ = phenotypic variance

$= \acute{o}^2_w + \acute{o}^2_{fb} + \acute{o}^2_f$

$\acute{o}^2_A$ = additive genetic variance

$= 4\,\acute{o}^2_f$

The variance component $\acute{o}^2_f$ is due to family groups, $\acute{o}^2_{fb}$ is due to the interaction of replications and families, and $\acute{o}^2_w$ is due to differences among trees within plots. The

size of $\acute{o}^2_f$ is due to differences between the family groups. The female parent (cone tree) is wind pollinated by a large number of male parents (pollen trees) so that the family groups are half-sib relatives. Therefore the variance component is equivalent to the covariance among half-sibs (Becker, 1975). True half-sibs have a coefficient of relationship (r) of $^1/_4$ (Falconer, 1960). The variance component $\acute{o}^2_f$ estimates one quarter of the additive genetic variance, while the $\acute{o}^2_w$ estimates the remainder of the genetic variance, plus all of the environmental variance (Becker, 1975).

$\mathbf{h^2_I}$ = heritability, narrow sense, individual.

$$= \frac{\text{additive genetic variance}}{\text{variance of individual tree phenotypic values}}$$

$$= \frac{\acute{o}^2_A}{\acute{o}^2_P}$$

$$= \frac{4\,\acute{o}^2_f}{\acute{o}^2_w + \acute{o}^2_{fb} + \acute{o}^2_f}$$

$\mathbf{h^2_F}$ = heritability, narrow sense, family mean

$$= \frac{\text{fraction of additive variance}}{\text{variance of family means}}$$

$$= \frac{\acute{o}^2_f}{\acute{o}^2_f + \acute{o}^2_{fb}/r + \acute{o}^2_w/nr}$$

(Falconer, 1960; Namkoong *et al.*, 1966; Kung, 1972)

Individual heritabilities are used to determine expected response from individual or mass selection; outstanding trees are selected according to their own individual phenotypic performance. Family heritabilities are used to determine expected responses from progeny test selection; superior trees are selected according to the performance of their progeny.

TABLE 15

Expected mean squares for the analysis of variance of populations in individual tests.

1. multi-family pots

Source of variation	d.f.	Expected mean squares
between families	f-1	$ó^2_w + bó^2_f$
individuals in families	f(b-1)	$ó^2_w$

2. single-family pots

Source of variation	d.f.	Expected mean squares
between families	f-1	$ó^2_w + nó^2_{fb} + bnó^2_f$
between blocks	b-1	$ó^2_w + nó^2_{fb} + fnó^2_b$
families in blocks	(f-1)(b-1)	$ó^2_w + nó^2_{fb}$
trees in plots	bf(n-1)	$ó^2_w$

3. host x pathogen studies

Source of variation	d.f.	Expected mean squares
between families	f-1	$ó^2_w + bó^2_{fi} + ió^2_{fb} + biό^2_f$
between inoculates	i-1	$ó^2_w + bó^2_{fi} + fbó^2_i$
between blocks	b-1	$ó^2_w + ió^2_{fb} + fió^2_b$
family * inoculate	(f-1)(i-1)	$ó^2_w + bó^2_{fi}$
families in blocks	(f-1)(b-1)	$ó^2_w + ió^2_{fb}$
seedlings in plots	residual	$ó^2_w$

Heritabilities were calculated from the hierarchal analysis of variance model for each intermating population in the natural and domestic population tests. A common intermating population was used for the field and host / pathogen studies.

The mating designs employed can be readily analysed by standard statistical procedures and interpreted into the components of variance of the design. To interpret these components genetically, Cockerham (1963) listed five assumptions which must be met :

1. diploid and normal Mendelian inheritance
2. lack of maternal effects
3. no linkage of genes
4. random sampling of candidates in populations
5. sampling from non-inbred populations

Regarding the validity of assumption 5, Moran *et al.* (1980)reported that the overall rate of outcrossing in a thirty clone *Pinus radiata* seed orchard was at least 90%. The outcrossing rate is expected to be higher for the thesis studies of the South African and South Australian domestic populations, as seed was collected from plus trees (ortets) in plantations. However, the outcrossing rate may be lower in the natural stands of California, in particular in the small isolated population of Guadalupe Island. This will affect the coefficient of relationship (r) used to calculate the genetic variance components, leading to inflated estimates of heritability. Bannister (1969) arbitrarily chose an (r) of $^{1}/_{3.5}$ to counteract the bias when the full requirements of the half-sib model could not be met. Some of Bannister's parent trees of *P. radiata* were isolated, or in small clumps, and these could be expected to be more homogeneous within themselves than families comprising half-sibs only. A similar (r) of $^{1}/_{3.6}$ for open-pollinated seed was also used by Squillace (1974).

Assumption 4 is wholly valid as there has been no selection for resistance to *Phytophthora cinnamomi* in the *Pinus radiata* parent trees. Parents in the domestic population studies were intensively selected for bole straightness, growth rate and small, flat-angled branching. In the natural populations, parent trees were selected at random (Eldridge, 1978a).

For the other assumptions, *P. radiata* is a diploid tree (Pederick, 1967), and there is no evidence of linkage of genes associated with resistance. However, maternal effects are uncertain. Indications are that there is a maternal effect because of the surprising reliability of the open-pollinated families used in the studies. The maternal contribution is being examined in current studies.

Heritability has been defined in two senses (Kempthorne, 1957). In the broad sense, heritability considers the total genetic variability (additive, dominance and epistasis) in relation to the phenotypic variability. It can be used by clonal propagation. In the narrow sense, heritability considers only the additive portion of the genetic variability in relation to the phenotypic variability. As such, it expresses the reliability of the phenotype as a guide to the breeding value. The concepts of additive and non-additive variance are based

on ideas about gene effects in a general sense. If genes at a certain locus always have the same effects regardless of what individual they are part of, they are said to act additively, or dependably. By contrast, if the effects of genes at a locus depend on what other genes are present in the same individual, then they are said to act in a non-additive manner and the effects are not dependable.

The concept of heritability is simple, but there are many published reports where its calculation is unclear and it has been incorrectly applied (Namkoong *et al.*, 1966). The particular estimate is a function of the experiment and it may be very different in a slightly different test. Therefore, when quoting heritability, it is necessary to include the pertinent details of experimental design and calculation procedures. The use of the estimate becomes ambiguous unless the unit of measurement, such as the individual, the average at a location or the plot mean, is stated precisely (Morley, 1963).

CHAPTER 4

GENOTYPIC VARIATION IN RESISTANCE OF PINUS RADIATA TO PHYTOPHTHORA CINNAMOMI

4.1 INTRODUCTION

Earlier glasshouse studies of genotypic variation in the response of *Pinus radiata* to *Phytophthora cinnamomi* have shown that this is large, highly heritable and repeatable in the field (Butcher *et al.*, 1984). From their tests of 49 families in the Western Australian breeding population, 9 were rated as tolerant and a further 13 families as moderately tolerant. These genotypes could be considered for deployment in seed orchards to produce seed having tolerance to *P. cinnamomi* as a major character. However, many more genotypes tolerant to *P. cinnamomi* are required for selection of a seed orchard population that is genetically superior in growth rate, stem and branching form, and wood quality, in addition to tolerance to *Phytophthora* root-rot.

The aim of the work described in this chapter is to examine the genetic variation in (a) the native Californian mainland and island populations, and (b) the major world domesticated populations of *Pinus radiata*, in response to inoculation with *Phytophthora cinnamomi*. The glasshouse test and methods of assessment were improved considerably (Chapter 3) over the earlier reported studies. The genotype response is now tested against a number of isolates and there is now a greater precision in the estimation of genetic variances.

The response test was conducted in the glasshouse. This has been shown to be repeatable in the field. A second, comprehensive field test is described. Production losses through death of planted seedlings and the effects of sub-lethal infections on increment are examined, and implications to management are discussed.

4.2 NATURAL POPULATIONS OF PINUS RADIATA

SEEDLING RESPONSE TO INOCULATION
WITH *PHYTOPHTHORA CINNAMOMI* IN GLASSHOUSE TESTS.

4.2.1 Introduction

Pinus radiata, of unknown origin, was first introduced into Australia around 1860 and the first forest plantings were in the 1870s. Since then, more than one half million hectares have been planted in plantations around Australia.

It is possible that all of the Australian plantations are derived from a very few original exports from California. Provenance testing of the Monterey pine was never given any emphasis because the very restricted coastal distribution suggested that provenance variation would be unimportant. Eldridge (1978b) set out to redress this oversight by making large seed collections in all natural stands of the species. His concern was to introduce the best selections from the natural range of the species and to increase the variability of the genetic resource for future domestic breeding programmes of the world's most important pine tree.

A principal objective of the Eldridge seed collection was "... to provide a long-term and very variable stock of genetic variation in many traits, particularly in possible resistance to pests and diseases" (Eldridge, 1979). This valuable provenance seed has been used to study variation in salinity tolerance (Cromer *et al.*, 1982) and variation in tolerance to western gall rust (Old *et al.*, 1986). Individual family seedlots were provided by the CSIRO, Division of Forest Research for this thesis study on the variability of the natural populations of *P. radiata* in response to inoculation with *Phytophthora cinnamomi.*

4.2.2 Materials and Methods

Each of the five native populations of *Pinus radiata* were sampled with 20 open-pollinated families. Sub-populations of the major Monterey population were also included at the suggestion of Dr. Eldridge, because of the variation found in the salt tolerance study (Cromer *et al.*, 1982). The coastal and the inland Jack's Peak sub-populations, representing the major differences in physiographic conditions in Monterey, were selected for the study. Trees chosen for the mainland populations were more than 100 metres apart, and where there was a choice, were larger and of better form than their

neighbours (Eldridge, 1978b), while the two island collections were mostly from randomly selected trees (Libby, 1978).

The 120 provenance/family seedlots, 50 seeds of each, except for Cedros Island families where there were 100 seeds, were stratified and sown in seedling trays in March 1983. Initial germination was variable (Figure 10), ranging from 6% for the Guadalupe Island seed to 34% for the Cambria and Ano Nuevo seed. With time, germination of all provenances improved to average 40%, but there was considerable variation within the provenance groups (Figure 10). Because of the poor and variable germination, the proposed experimental design had to be substantially modified, from a single test to a combination of two tests. The Guadalupe Island provenance could not be included in the first test because of its protracted germination and lack of seedlings of comparable size to the others. Many of the seed did not germinate until 200 days after sowing.

Natural Population Test (NP1) :

Population	Sub-population		Number of Families	Number of Seedlings
Ano Nuevo	01-1	coast	10	16
Monterey	02-1	coastal dunes	10	16
Monterey	02-4	inland, Jack's Peak	9	16
Cambria	03-2	town	10	16
Cedros Island	T	ridge tops	10	16

Experimental design

7 x 7 balanced lattice, duplicated ($t = 49, k = 7, r = 8$) x 2.

multi-family pot containing seven seedlings plus one susceptible family seedling, 30004 or 60001.

multiple-inoculum *P. cinnamomi*, using isolates (1), (2), (4), (6), (7), (9), (10) and (11), (Table 32).

16 families common with NP2.

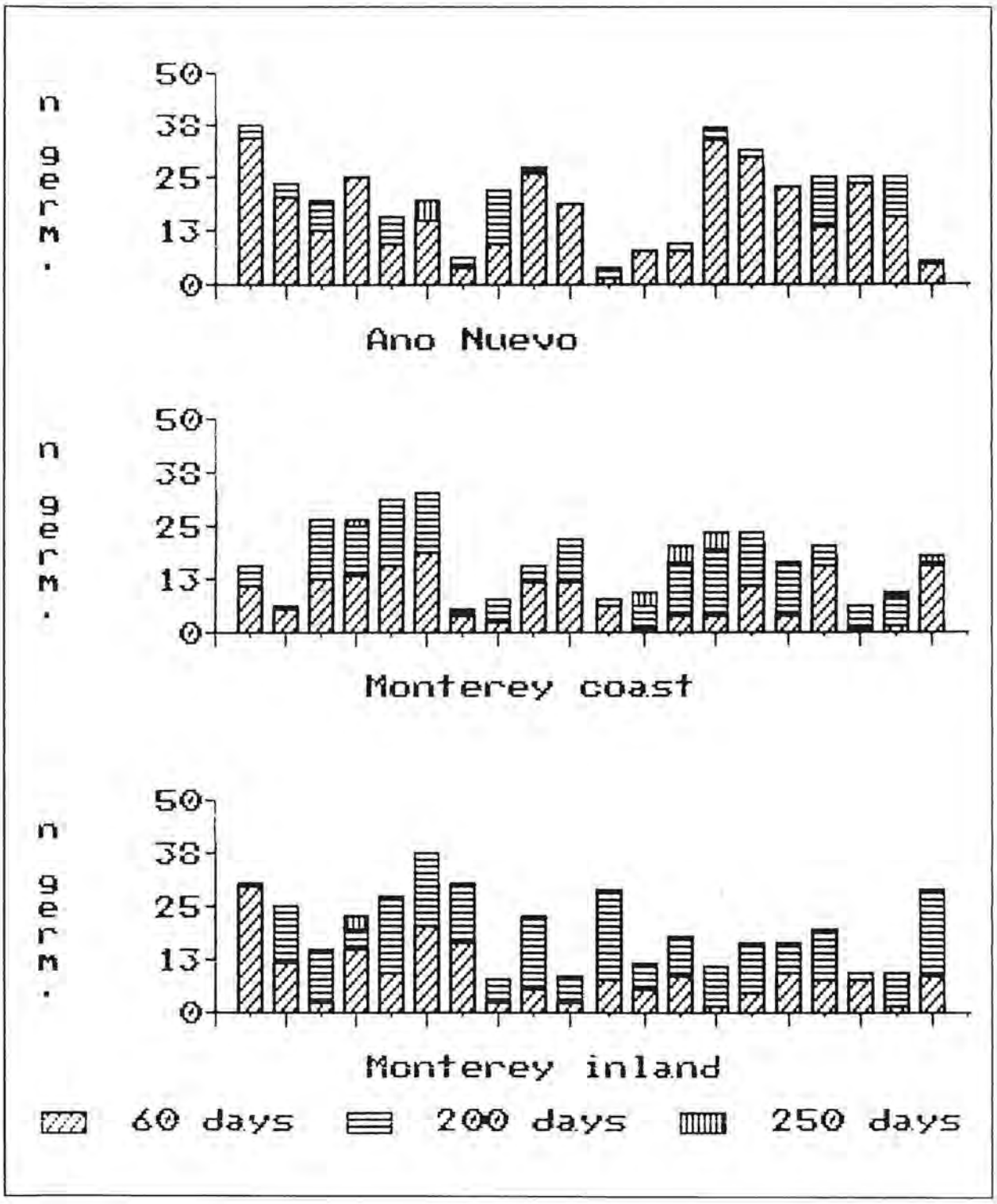


FIGURE 10

Germination of seedlings in natural populations of *Pinus radiata*, and numbers available for experiments.

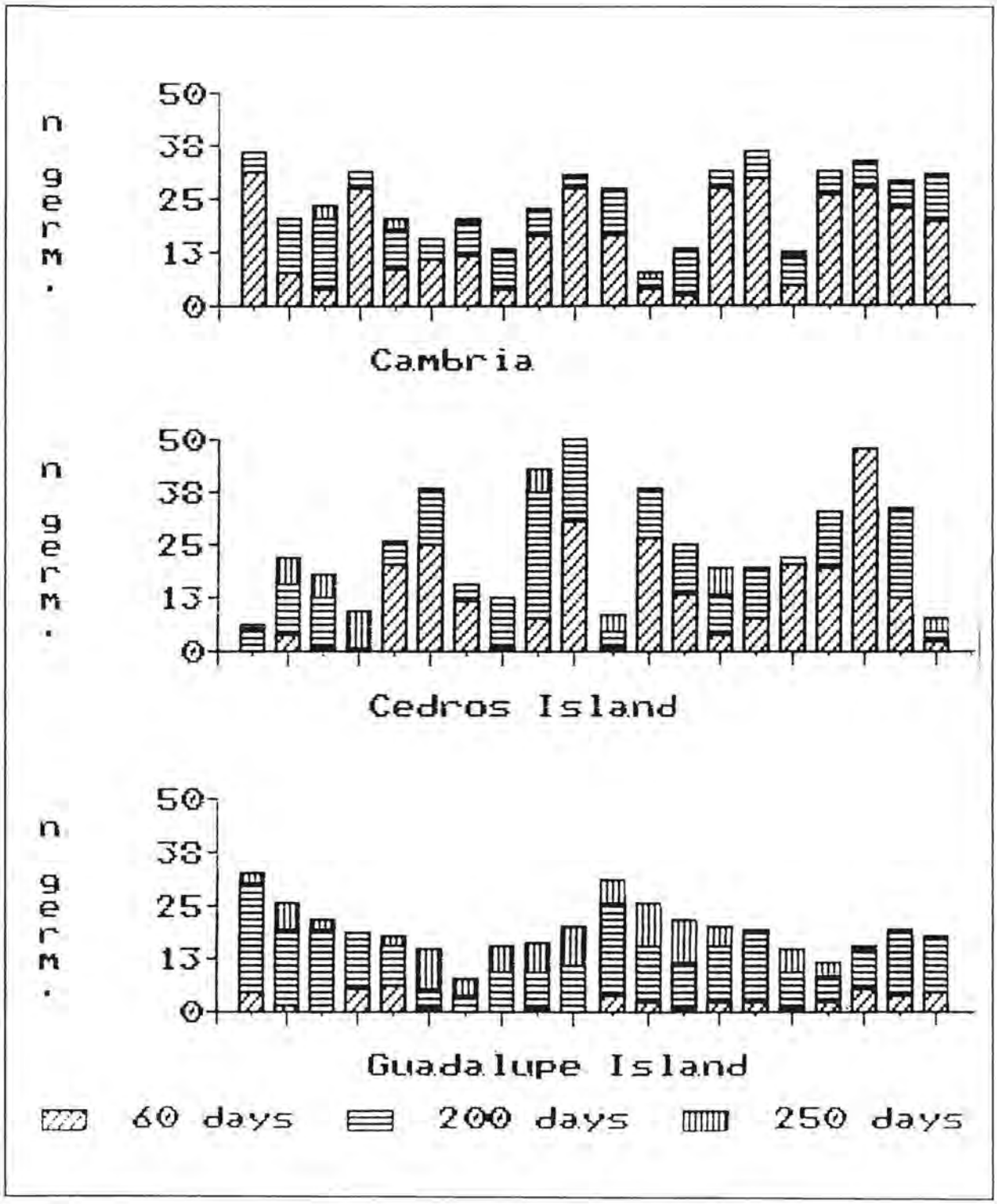


FIGURE 10 (cont)

Germination of seedlings in natural populations of *Pinus radiata*, and numbers available for experiments.

Natural Population Test (NP2) :

Population	Sub-population		Number of Families	Number of Seedlings
Ano Nuevo	01-1	coast	12	6
Monterey	02-1	coastal dunes	12	6
Monterey	02-4	inland, Jack's Peak	12	6
Cambria	03-2	town	12	6
Cedros Island	T	ridge tops	12	6
Guadalupe Island	L	ridge	12	6

Experimental design

complete randomised block of 72 families in 6 replications, ($t = 72, r = 6$).

multi-family pot containing 8 seedlings.

multiple-inoculum *P. cinnamomi*, using isolates (1), (2), (4), (6), (7), (9), (10) and (11), (Table 32).

Natural Population Test (NP3) :

Population	Sub-population		Number of Seedlings
Ano Nuevo	01-1	coast	72
Monterey	02-1	coastal dunes	72
Monterey	02-4	inland, Jack's Peak	72
Cambria	03-2	town	72
Cedros Island	T	ridge tops	72
Guadalupe Island	L	ridge	72

Experimental design

complete randomised block of 6 provenances in 9 replications, ($t = 6, r = 9$).

single provenance pot containing eight seedlings.

multiple-inoculum *P. cinnamomi,* using isolates (1), (2), (4), (6), (7), (9), (10) and (11), (Table 32).

Note for NP3 :

Late germinations of the Guadalupe Island seed enabled the implementation of the NP3 test. This test was necessary because the Guadalupe Island provenance was not well represented in the other tests. Seedlings were transplanted into the experimental pots in November, each pot containing one provenance, although the

family identity of positions were maintained. Families not used in the NP1 and NP2 tests, because of a lack of seedling numbers, were included in this test. The NP3 pots were inoculated with eight isolates of *P. cinnamomi* on the 11th January 1984, one month after the other tests were inoculated.

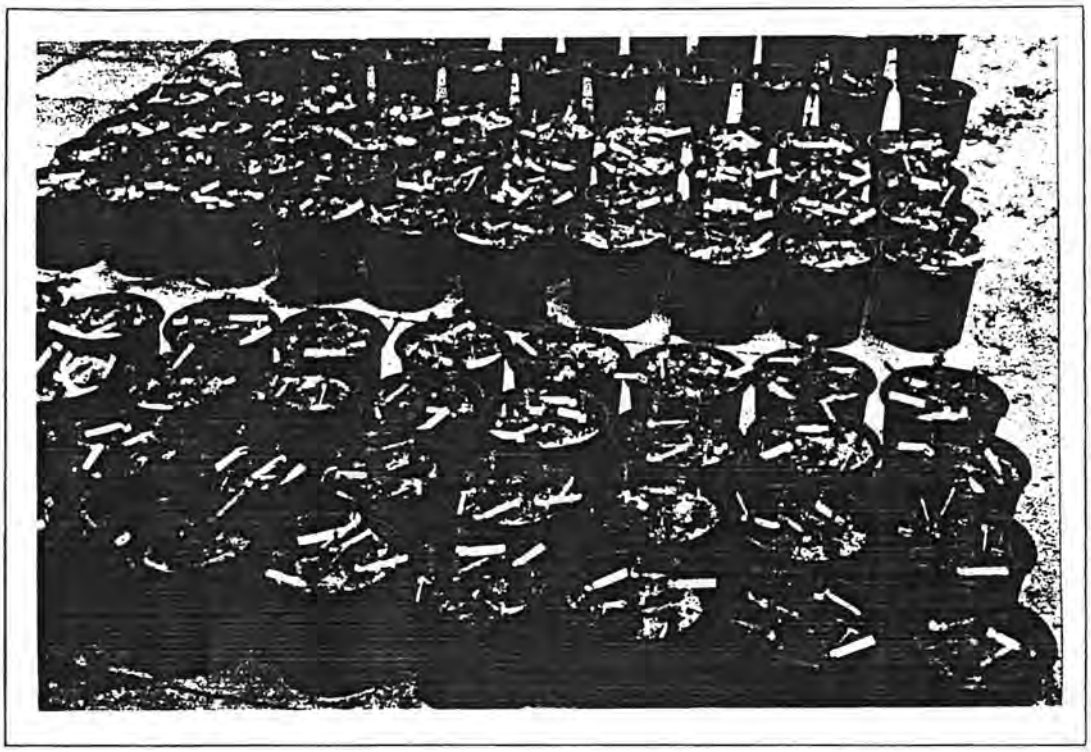


PLATE 3

Transplanting of natural population seedlings of *Pinus radiata* into experimental pot positions.

Labelled seedlings were transplanted into the NP1 and NP2 experimental pot designs in August 1983 (Plate 3). Seedlings were regularly fertilized with Aquasol liquid fertilizer, and pots were transferred to the Como glasshouse in October. Pine needle duff was collected from the Joondalup pine seed orchard in early November, was finely chopped, and spread over the surface of the pots for mycorrhizal inoculation.

Pine plug inoculum was prepared for eight different isolates of *P. cinnamomi* A2, according to the procedure outlined in Chapter 3(4). These were the same isolates used in the host x pathogen interaction study reported in Chapter 5. Pots were flooded for two days prior to the inoculation on the 7th December 1983. An inoculum plug for each *P. cinnamomi* isolate was buried in eight holes in each treatment pot (Figure 5). Pots were drained to field capacity two days later and the normal watering treatment was resumed.

The glasshouse environment for the NP1, NP2 and NP3 tests was described in Chapter 3(4) as the low moisture / high temperature regime. About 500 ml of water were added to each pot twice weekly, although the volume added was never strictly controlled. Temperature was maintained below about 35°C by the use of evaporative coolers.

Seedling heights were measured at the time of the inoculation, and then at 15 day intervals through to the end of experiments, 240 days after inoculation. Seedling deaths were also recorded. Surviving seedlings at the end of the experiments were each assessed for the degree of root-rotting (Chapter 3.7).

4.2.3 Results

Seedling heights varied considerably at the time of inoculation, both within and between populations (Table 16, Figure 11). Genetic variance accounted for 57% of the total variance in NP1 and 59% in NP2. Variance due to blocking was also very large in NP1 at 18%. This was explained by the planting of the tallest seedlings first in consecutive blocks so as to have similar size seedlings in the same pot. Because of their small size, this was not possible for the Cedros Island population. The heights of the Ano Nuevo, Monterey coast and Monterey inland seedlings were similar in NP1 and were significantly taller than the Cambria seedlings ($P < 0.001$). Cedros Island seedlings were 100 mm smaller than the Cambria population ($P < 0.001$). In the second test, NP2, the Ano Nuevo seedlings were significantly taller than other mainland seedlings ($P < 0.01$). Seedlings from Monterey were smaller because of the protracted germination, while the delayed germination of the Guadalupe Island seedlings also contributed to their relatively small size. Cedros Island seedlings were very much smaller than any other population.

The first seedling death occurred 21 days after the *Phytophthora cinnamomi* inoculation treatment, in the susceptible family 30004 bait seedling. Disease developed rapidly in all treated pots, reaching its maximum expression about 100 days after the inoculation. The mortality rate in the Ano Nuevo population was very high and exceeded the rate of the susceptible family bait seedling (Figure 12). The pattern of disease development was similar for each population, although there were large and significant differences in the incidence of disease between populations. Disease incidence was analysed by grouping family seedlings into noncontiguous family plots of 4 seedlings in NP1, and 2 seedlings in NP2. Family plot percentage of mortalities were calculated and transformed into arcsin values for the analysis of variance (Table 17). The incidence of disease was low in the Cambria and both Monterey populations, while it was more than doubled in the Ano Nuevo population. The Guadalupe Island population was only represented in the NP2 test and showed a similar high incidence of dead seedlings. This observation was supported from data in NP3, showing similar high mortality of the Ano Nuevo and Guadalupe Island seedlings. The Cedros Island population was ranked with the Cambria and Monterey populations as having low mortality in each of the three tests, even though most of the Cedros Island seedlings had an unhealthy appearance.

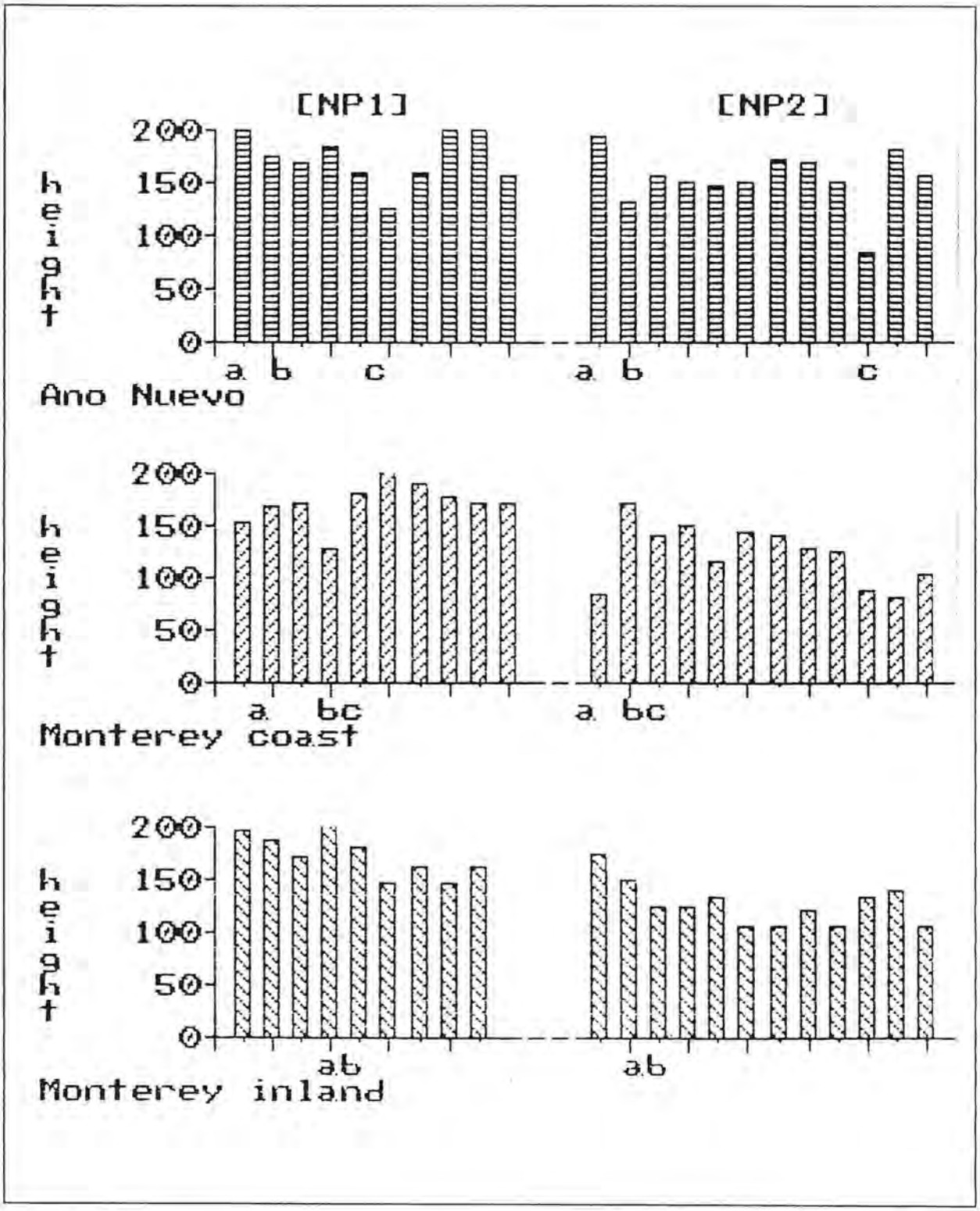


FIGURE 11

Height (mm) of seedlings in natural populations of *Pinus radiata*, before *Phytophthora cinnamomi* was applied.
(a, etc . - families common to both NP1 and NP2 tests)

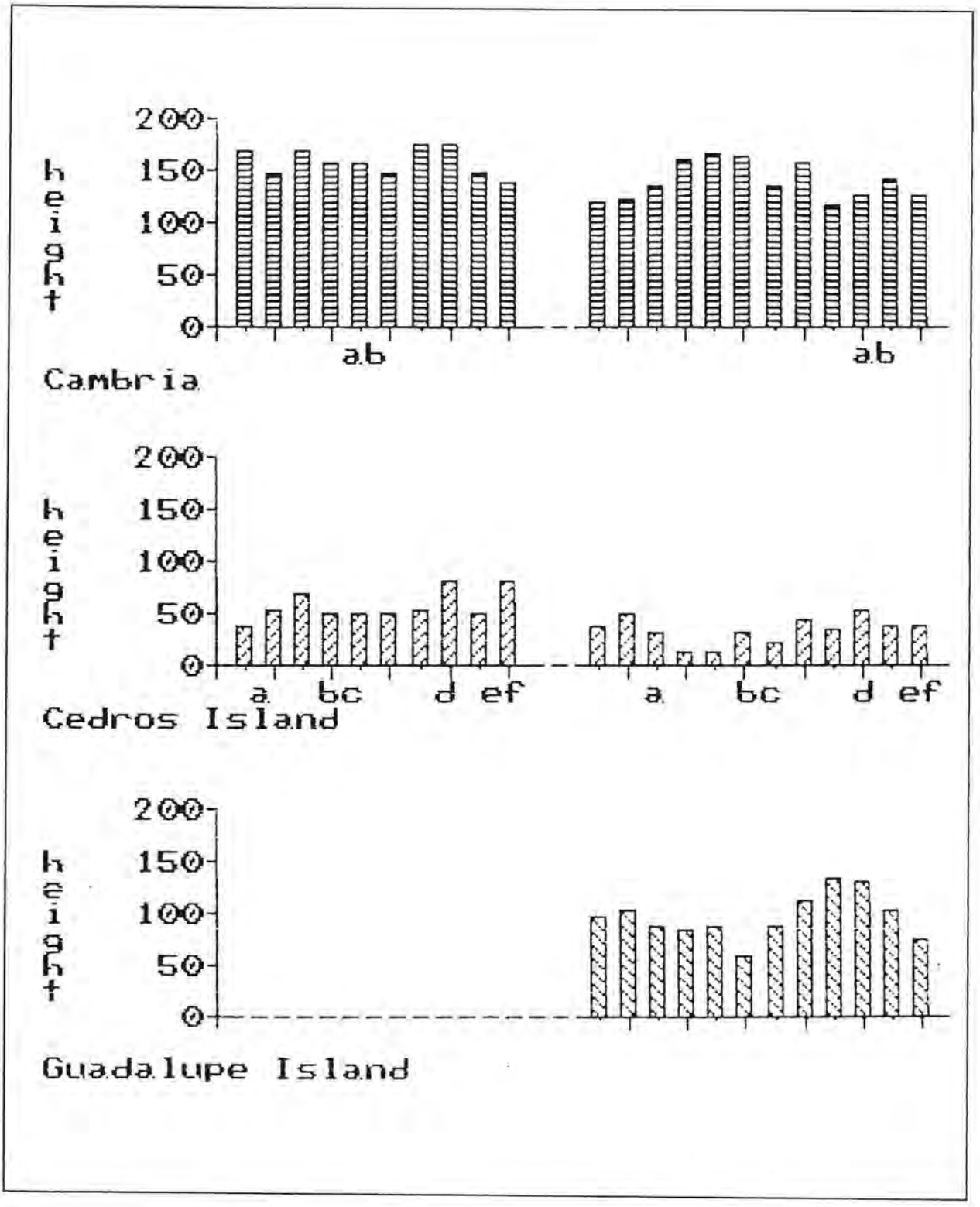


FIGURE 11(cont)

Height (mm) of seedlings in natural populations of *Pinus radiata*, before *Phytophthora cinnamomi* was applied.
(a, etc.- families common to both NP1 and NP2 tests)

TABLE 16

Analysis of variance and summary for seedling height growth of natural populations of *Pinus radiata*, prior to the inoculation with *Phytophthora cinnamomi*.

Source	NP1 d.f.	NP1 variance	NP1 sig.	NP2 d.f.	NP2 variance	NP2 sig.
within population						
Ano Nuevo	9	11474	***	11	4511	***
Monterey coast	9	6590	***	11	4927	***
Monterey inland	8	7225	***	11	2693	*
Cambria	9	3011	**	11	2014	
Cedros Island	9	3368	**	11	1026	
Guadalupe Island	-	-		11	2770	*
between populations	4	409116	***	5	132051	***
within species	48	39880	***	71	12079	***
replication	15	38802	***	5	2499	
residual	720	1222		355	1244	
Total	783	4312		431	3043	

Summary

Population	Initial mean height mm NP1		NP2	
Ano Nuevo	177	a	155	
			***	
Monterey inland	174	a	129	b
Monterey coast	173	a	124	b
	***		**	
Cambria	159		140	
			***	
Guadalupe Island			98	
	***		***	
Cedros Island	59		35	

* significant difference at 95% probability level, ** $P < 0.01$, *** $P < 0.001$
values followed by the same letter are not significantly different.

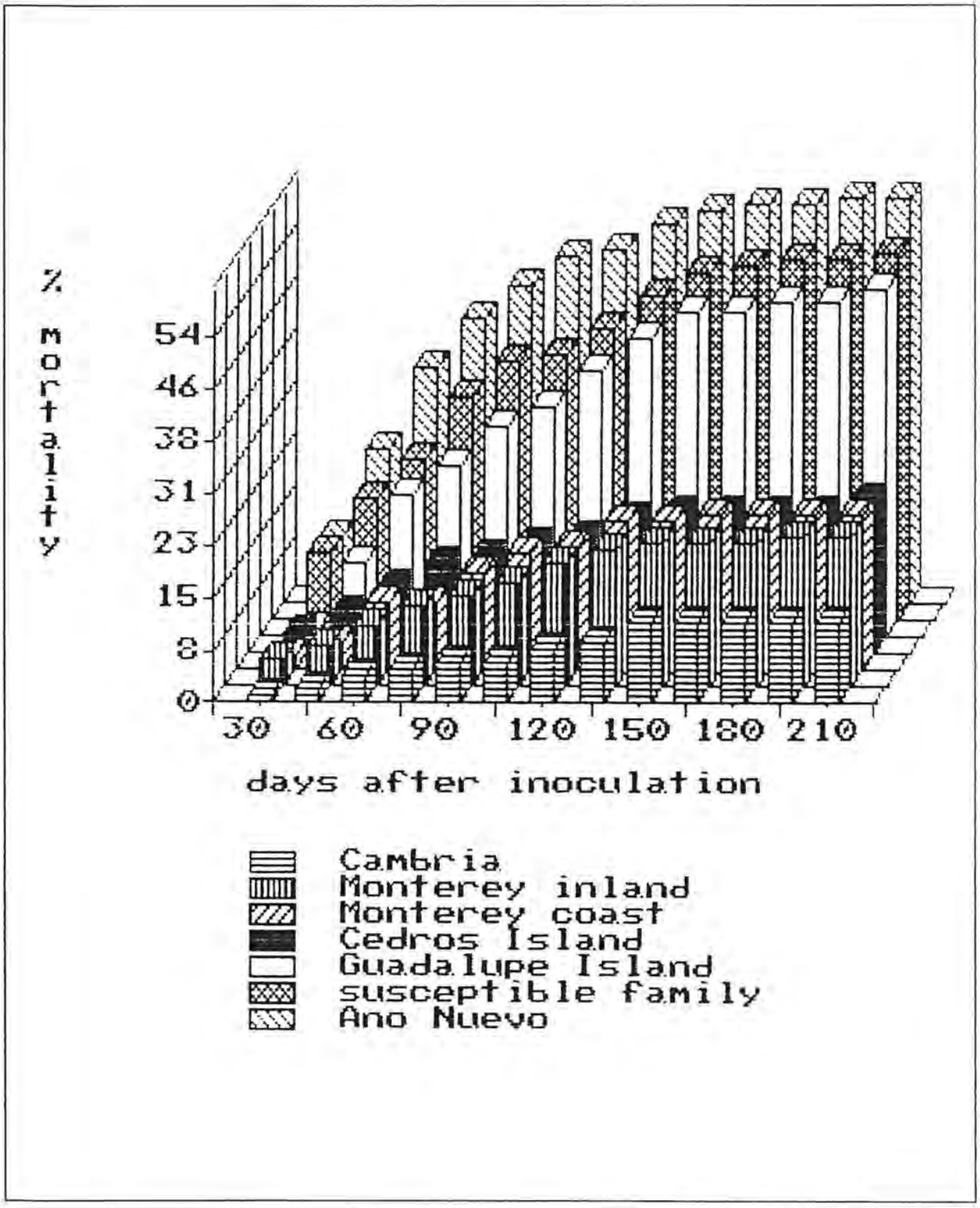


FIGURE 12

Progressive seedling mortality in *Pinus radiata* natural populations, after inoculation with *Phytophthora cinnamomi.*

TABLE 17

Analysis of variance, family heritabilities and natural population summary for percent mortality of *Pinus radiata* seedlings (transformed into arcsin square root), 240 days after *Phytophthora cinnamomi* was applied.

Source	NP1 d.f.	NP1 variance	NP1 sig.	NP2 d.f.	NP2 variance	NP2 sig.
within population						
Ano Nuevo	9	573		11	2511	**
Monterey coast	9	576		11	1473	*
Monterey inland	8	503		11	1284	*
Cambria	9	323		11	593	
Cedros Island	9	513		11	900	
Guadalupe Island	-	-		11	1841	**
between populations	4	9594	***	5	8228	***
within species	48	1255	***	71	1912	***
replication	3	664		2	1809	
residual	144	322		142	688	
Total	195	557		215	1102	

Summary

Population	NP1 mortality	NP1 h^2F	NP2 mortality	NP2 h^2F
Cambria	14 a	0.18	13 b	0.18
Monterey inland	20 a	0.13	24 bc	0.35
Monterey coast	20 a	0.56	30 c	0.53
Cedros Island	30 *	0.50	25 c	0.27
Guadalupe Island	-		45 d	0.71
Ano Nuevo	53 **	0.35	54 d	0.70

* significant difference at 95% probability level, ** $P < 0.01$, *** $P < 0.001$. values followed by the same letter are not significantly different

About two thirds of the inoculated seedlings survived the infection with P. cinnamomi, although it was obvious judging on the height growth (Plate 4) that some seedlings were more diseased than others. The root system of each surviving seedling was subjectively assessed for the amount of disease and classified into one of four disease intensity classes. Class parameters were defined from a random sample of 30 seedlings per class:

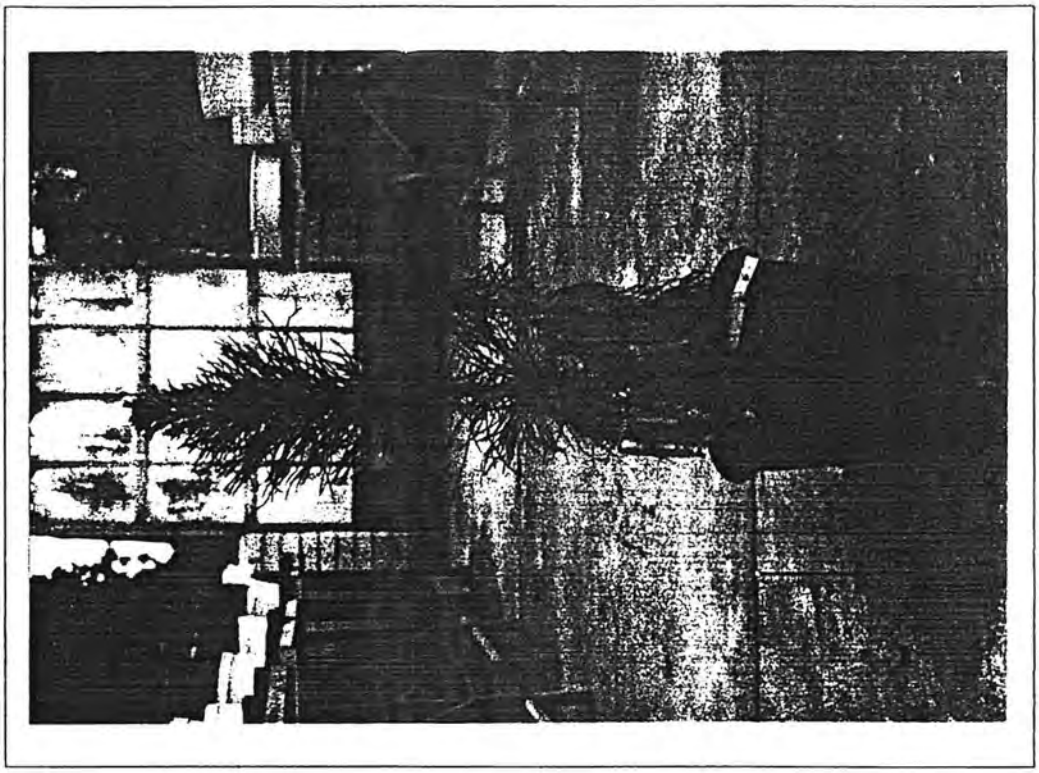
Disease intensity scale :

1	healthy seedling	root mass	3.3 + 1.1g
2	healthy seedling,	root mass	22. + 1.0g
3	unhealthy seedling,	root mass	0.7 + 0.3g
4	unhealthy seedling,	root mass	0.2 + 0.1g

Healthy seedlings were easily distinguishable from unhealthy seedlings. Alive seedlings, two thirds of all seedlings inoculated, were divided into a healthy class (codes 1, 2) and an unhealthy, or diseased class, (codes 3, 4) to give a better illustration of the amount of disease. Variation between the natural populations is illustrated in Figure 13, while within population variation is shown in Figure 14. The simple pie pictures (Figure 13) show an almost identical representation of disease for each population in both tests, even though different families constituted the population.

The Cambria and Monterey populations were superior with more than 60% healthy seedlings, compared with 30% healthy seedlings in the Ano Nuevo and Guadalupe Island populations. Cedros Island population scored only 6% healthy seedlings. This contrasts with the earlier analysis (Table 17) on seedling mortality, where it ranked with the Cambria and Monterey populations on response to *Phytophthora cinnamomi.* The difference was in the very large number of survivors being classified as unhealthy. The majority of the Cedros Island seedlings had a severely rotted root system. Sketches, drawn by Lousia de Braganca, illustrate this for three Cedros Island seedlings in Figure 15.

The non-parametric chi-square analysis was deployed as an independent assessment of the disease in natural populations, as a check to the analysis of variance, if the normality of data was questioned. Very large chi-square values were calculated for the NP1 and NP2 tests (Table 18), concluding that there were highly significant ($P < 0.001$) differences among the natural populations in the amount of *Phytophthora cinnamomi* disease. The parametric analysis of variance test (Table 17) also indicated highly significant differences ($P < 0.001$) among natural populations.



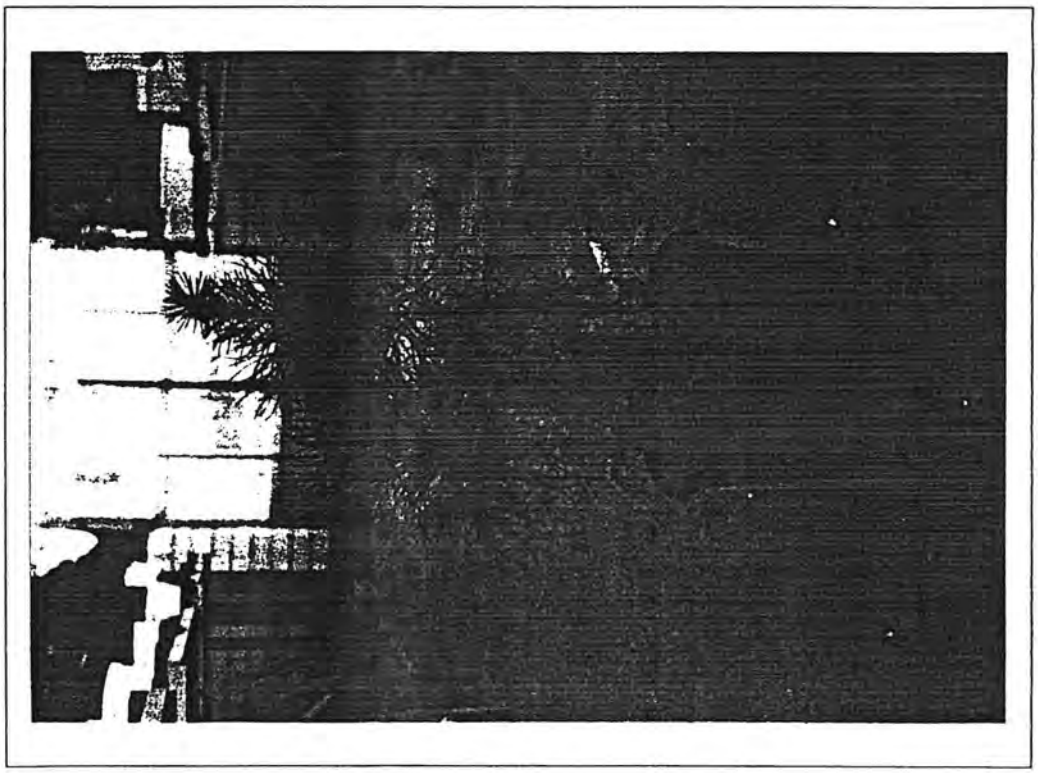
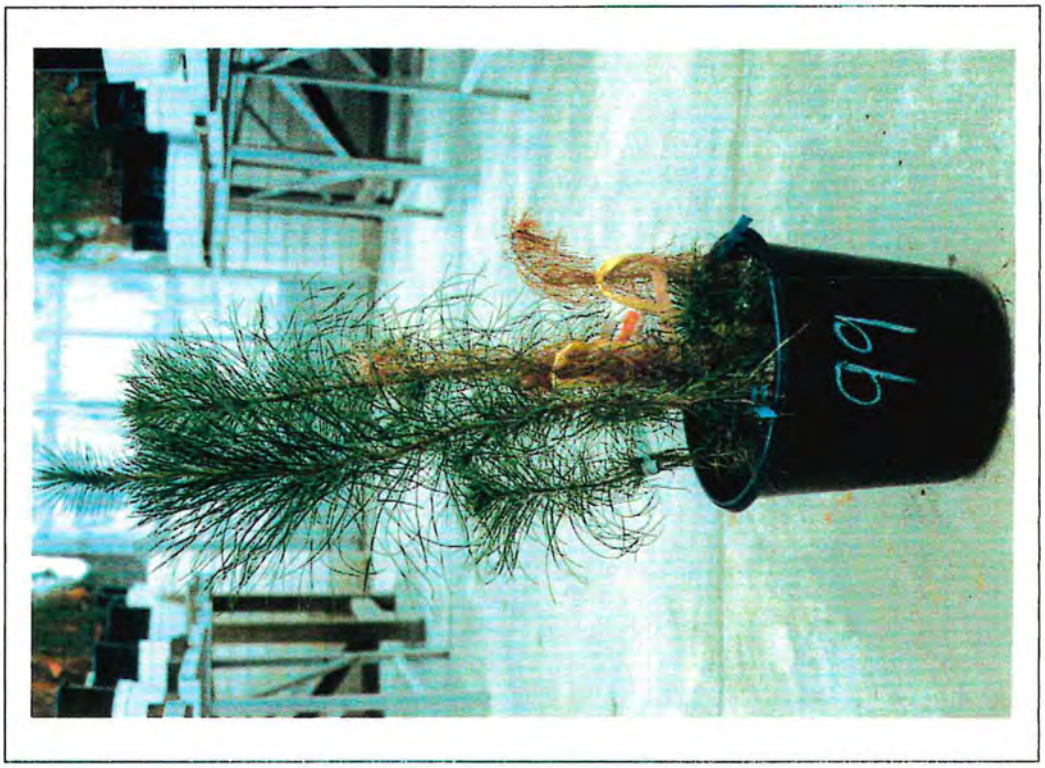


PLATE 4

Disease response of seedlings from the natural populations of *Pinus radiata*, 240 days after inoculation with *Phytophthora cinnamomi*.



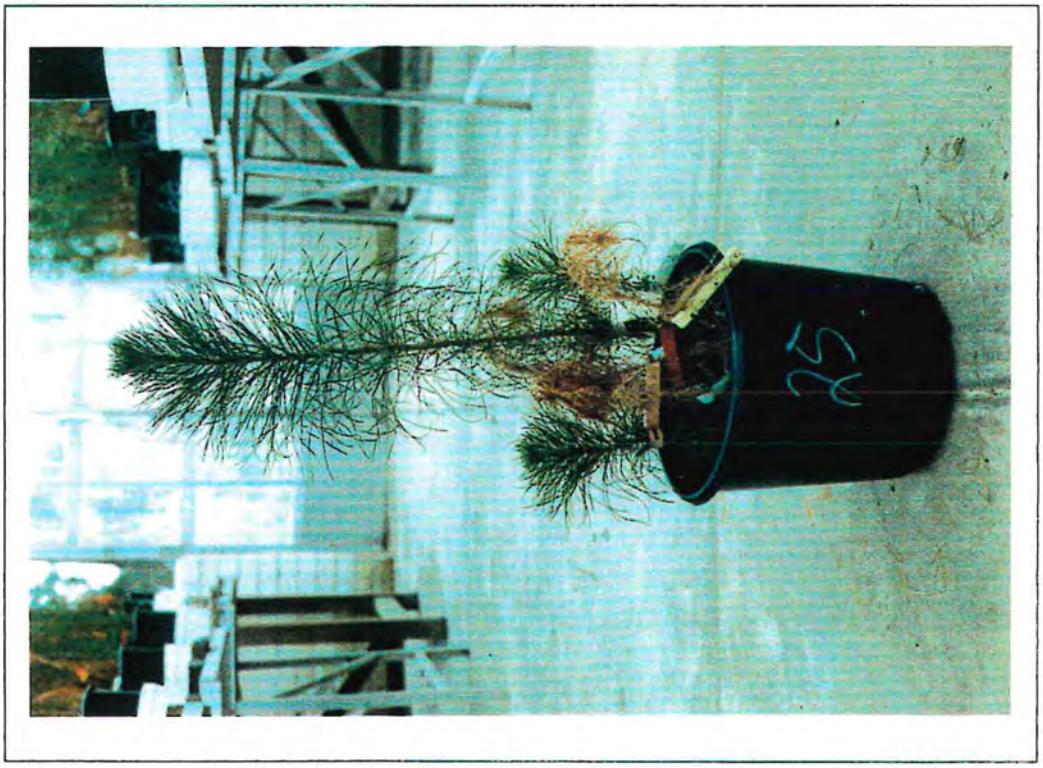


PLATE 4 (cont)

Disease response of seedlings from the natural populations of *Pinus radiata*, 240 days after inoculation with *Phytophthora cinnamomi*.

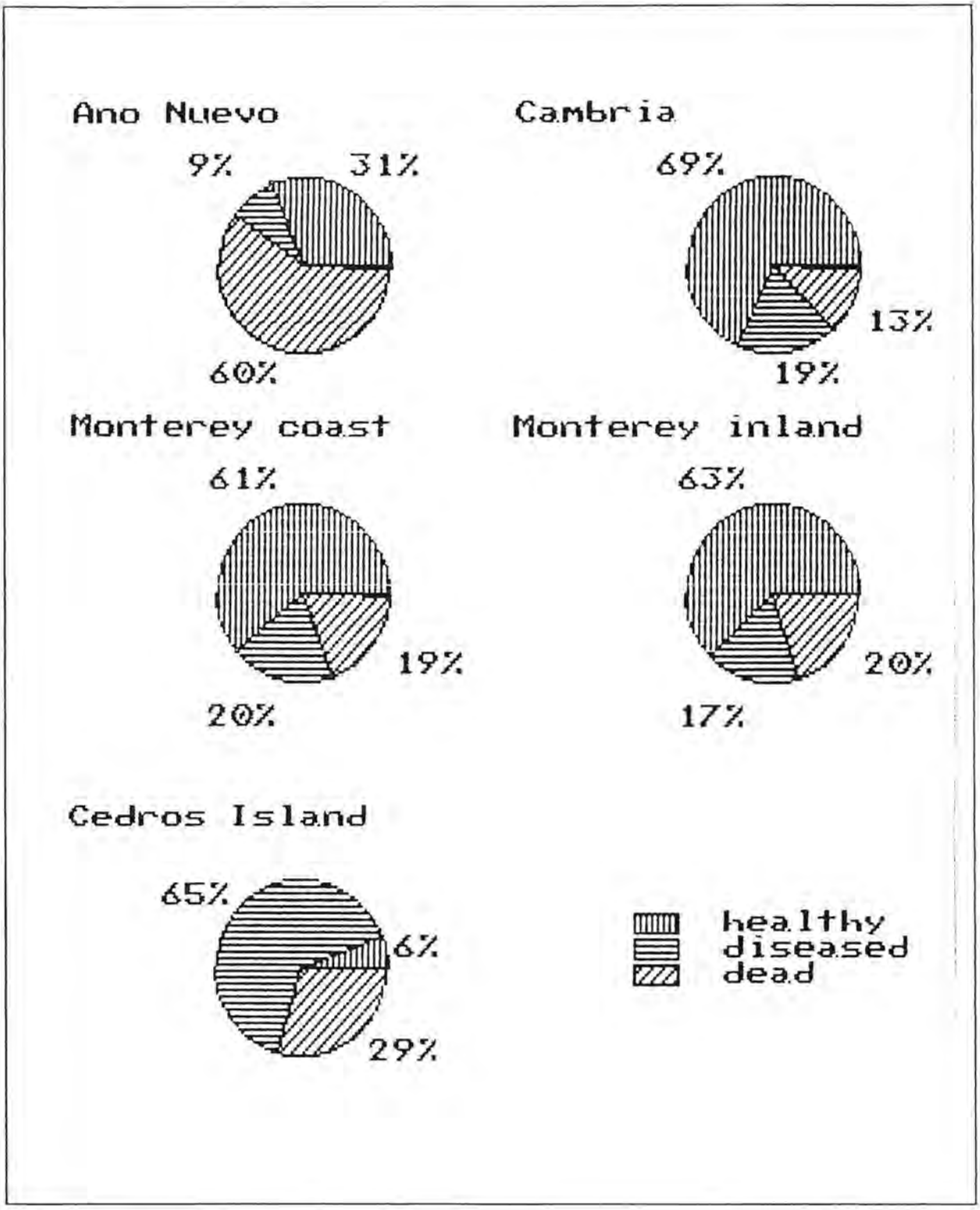


FIGURE 13 **NPI.**

The amount of disease in natural populations of *Pinus radiata*, 240 days after inoculation with *Phytophthora cinnamomi*.

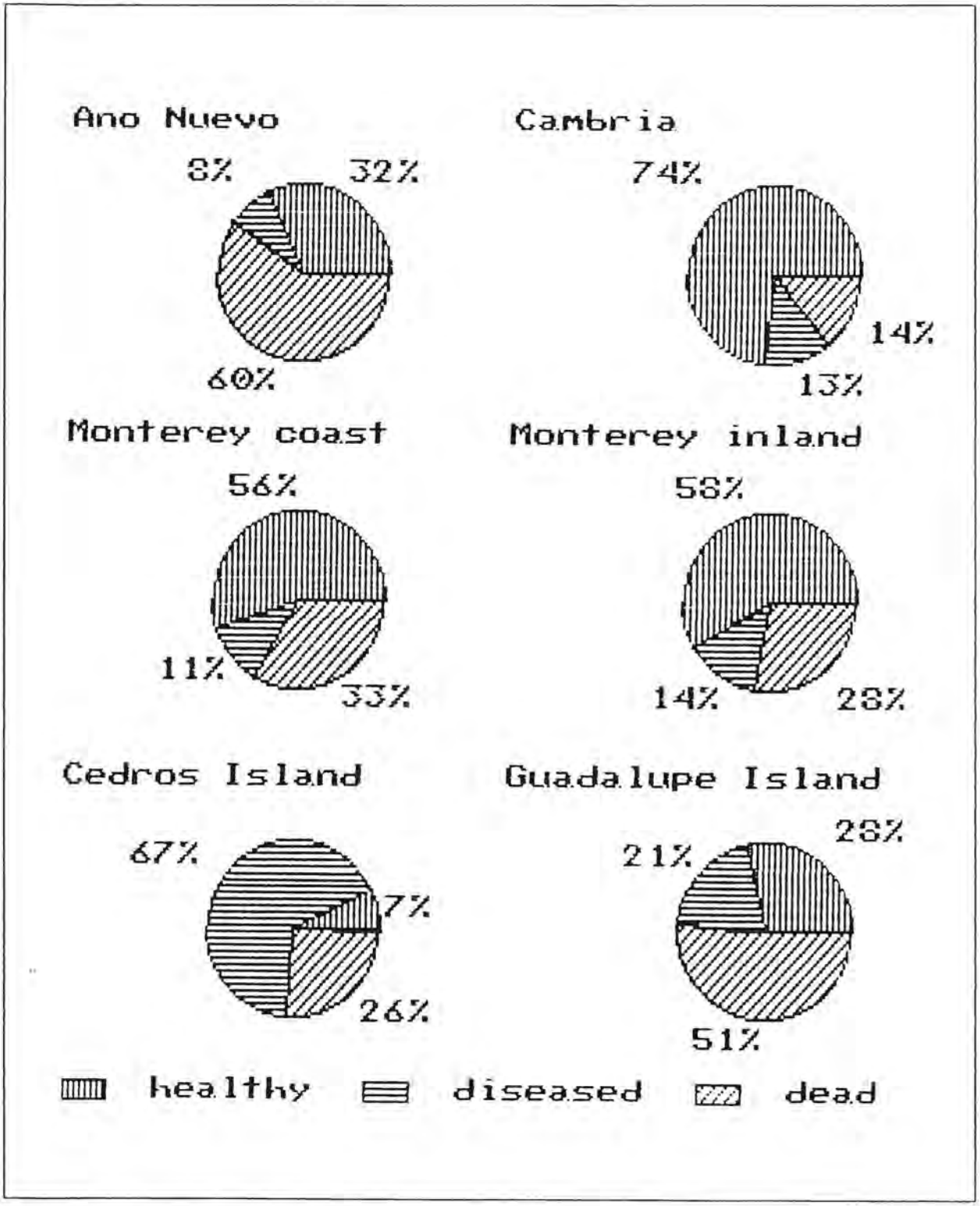


FIGURE 13 **NP2.**

The amount of disease in natural populations of *Pinus radiata*, 240 days after inoculation with *Phytophthora cinnamomi*.

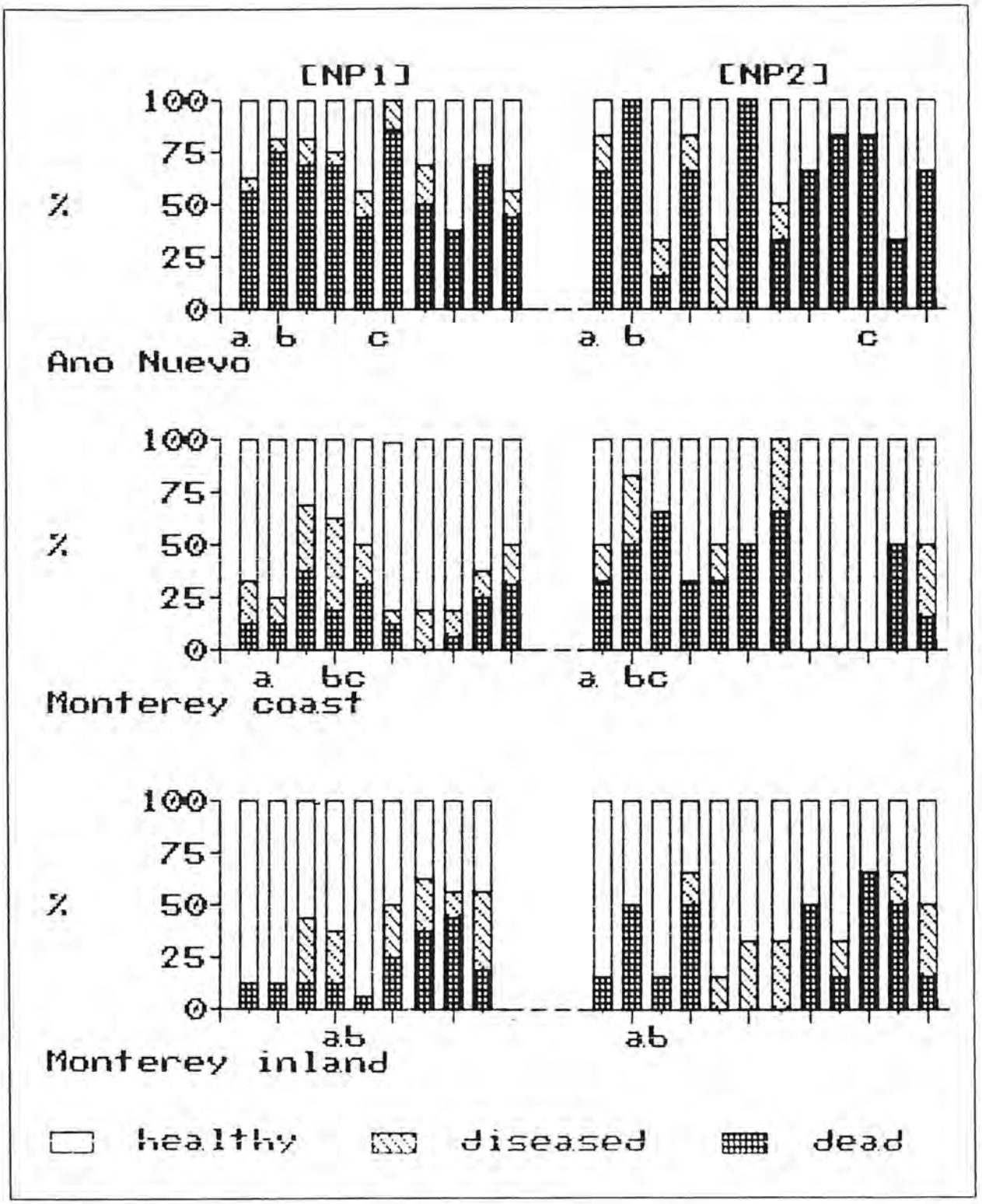


FIGURE 14

Variation in the amount of *Phytophthora cinnamomi* disease, within and between natural populations of *Pinus radiata*.
(a, etc . - families common to both NP1 and NP2 tests)

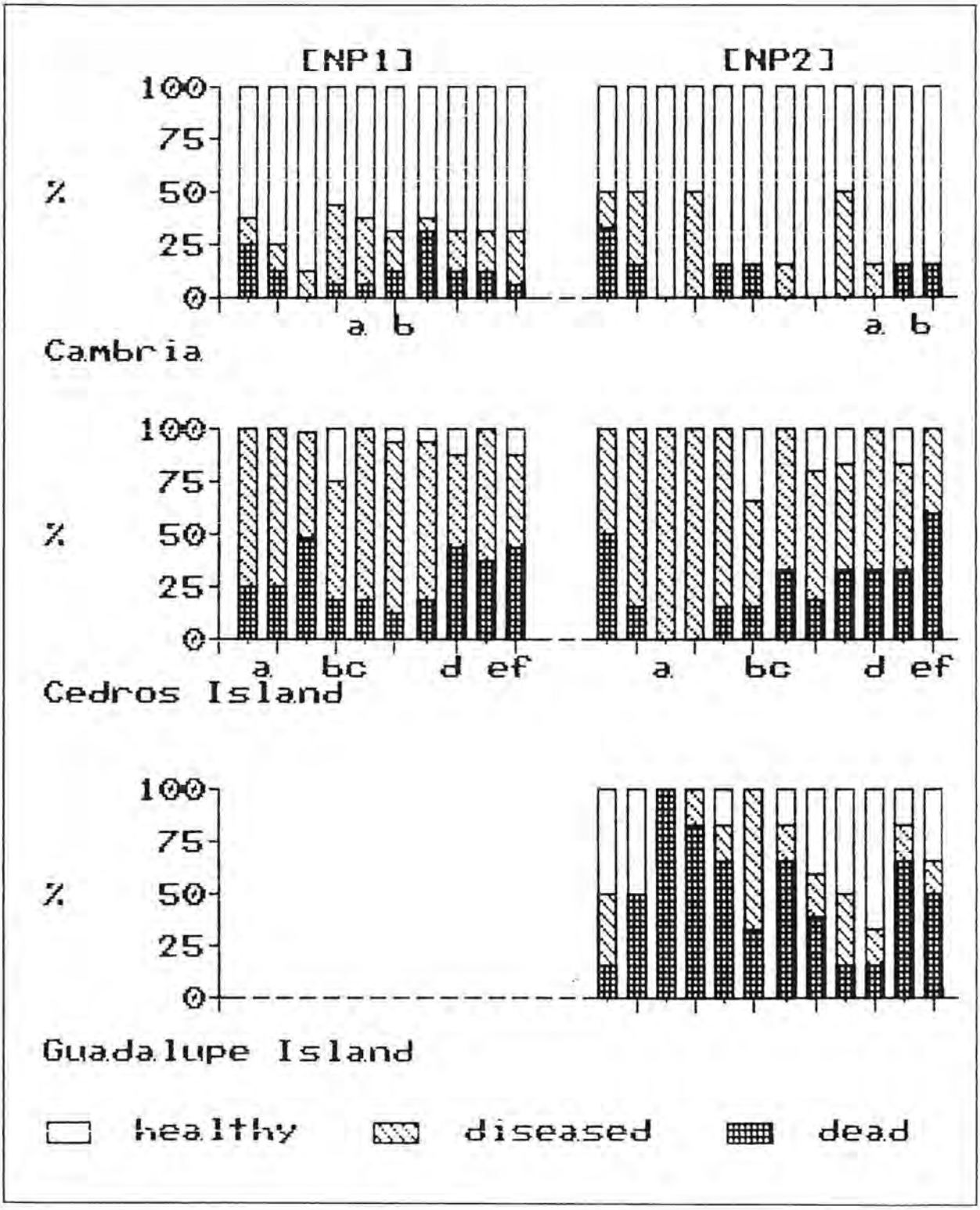


FIGURE 14 (cont)

Variation in the amount of *Phytophthora cinnamomi* disease, within and between natural populations of *Pinus radiata.*
(a, etc . - families common to both NP1 and NP2 tests)

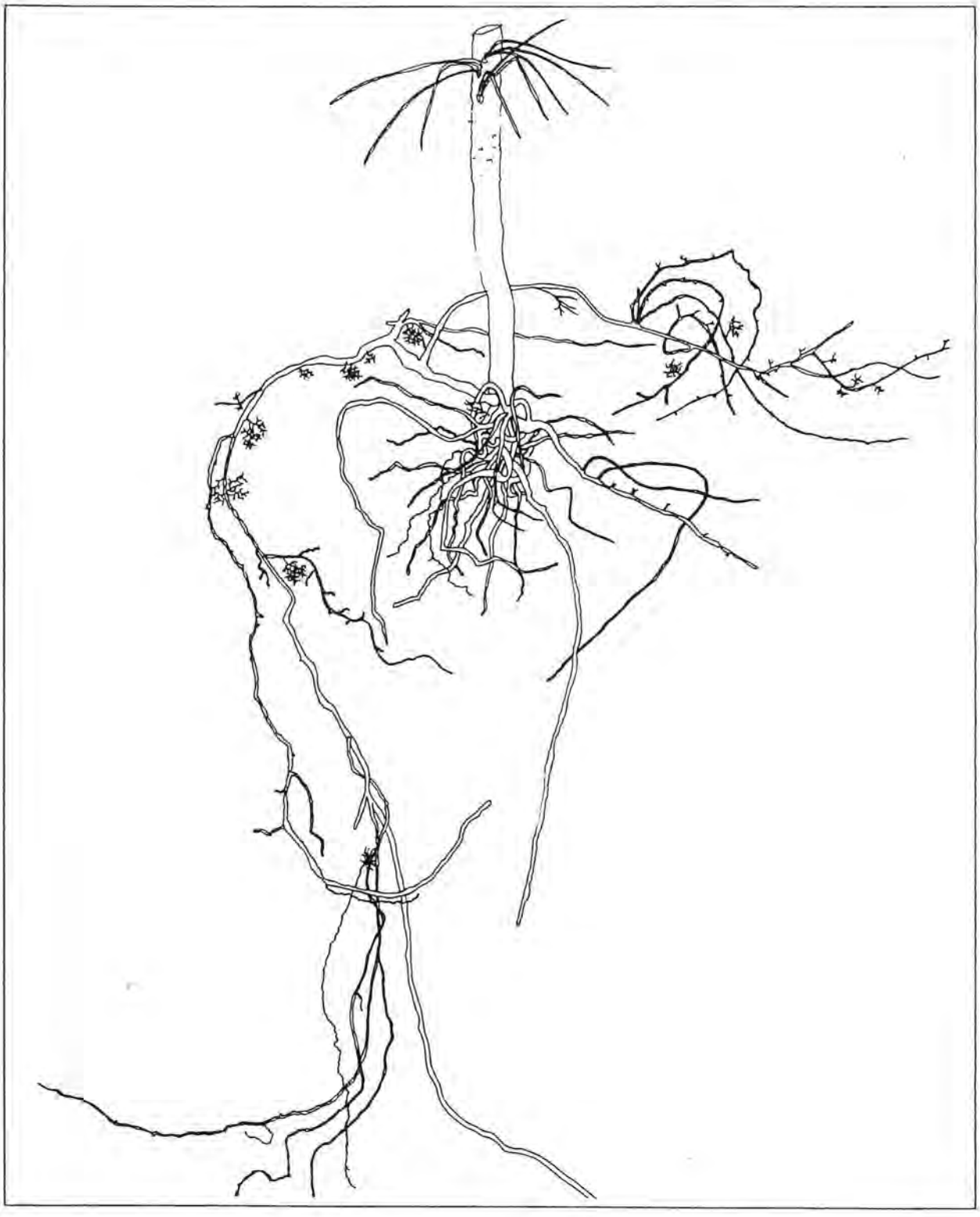


FIGURE 15

Illustrations of the root systems of diseased seedlings of *Pinus radiata*, from the Cedros Island population.

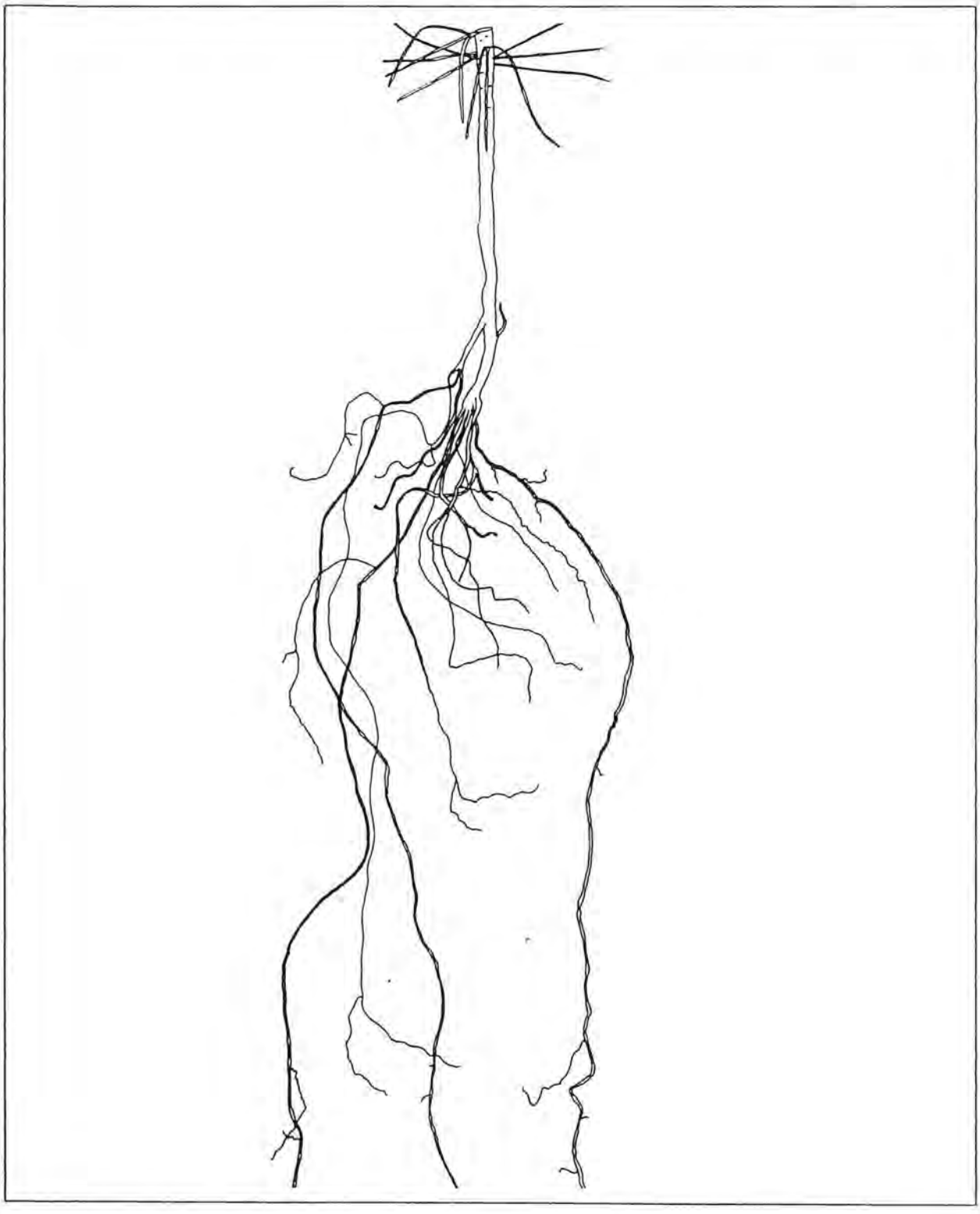


FIGURE 15 (cont)
Illustrations of the root systems of diseased seedlings of *Pinus radiata*, from the Cedros Island population.

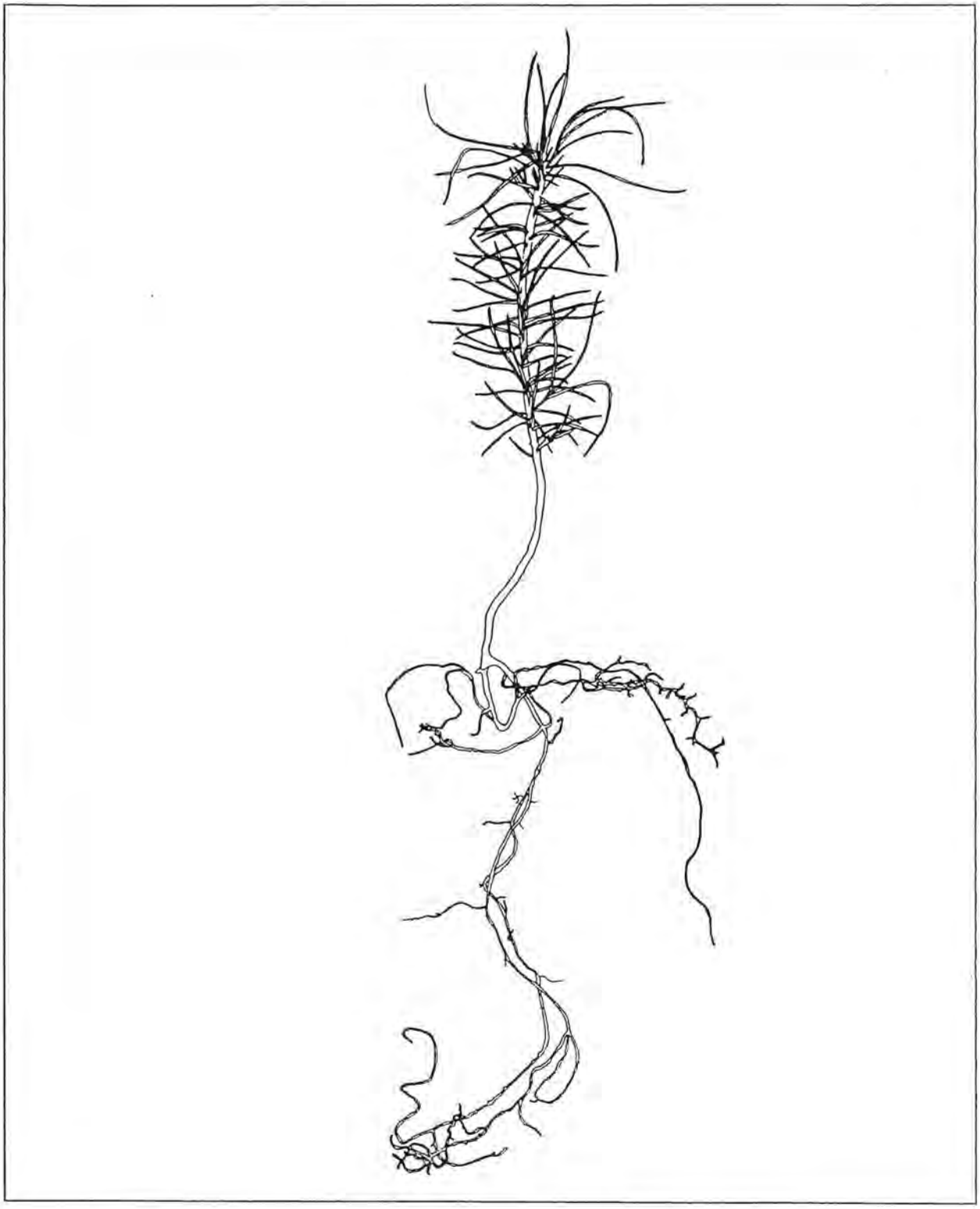


FIGURE 15 (cont)
Illustrations of the root systems of diseased seedlings of *Pinus radiata*, from the Cedros Island population.

TABLE 18

Chi-square test for the frequency of healthy, diseased and dead seedlings in six natural populations of *Pinus radiata,* 240 days after inoculation with *Phytophthora cinnamomi.*

Population	number of seedlings					
	NP1			NP2		
	healthy	disease	dead	healthy	disease	dead
Ano Nuevo	50	14	95	23	6	43
Monterey coast	97	31	30	40	8	24
Monterey inland	90	25	29	42	10	20
Cambria	109	30	20	53	9	10
Cedros Island	10	102	46	5	47	18
Guadalupe Island	-	-	-	20	15	36
P. radiata spp.	356	202	220	183	95	151

calculated Chi-square : NP1 = 291.6 NP2 = 162.1

tabular Chi-square = 26.1 (d.f. 8, $P < 0.001$). Fisher and Yates, 1963.

Frequency distributions of healthy, diseased and dead seedlings for the open-pollinated families in each population, in tests NP1 and NP2, are presented in Figure 14. Families that were common to both tests are indicated.

The remainder of the disease intensity scale was developed from the number of days taken for a seedling to die. Class intervals were derived from the disease development curves shown in Figure 12.

Disease intensity scale:

5 Dead at 240 days, after inoculation with *P. cinnamomi*

6 Dead at 120 days, after inoculation with *P. cinnamomi*

7 Dead at 60 days, after inoculation with *P. cinnamomi*

8 Dead at 30 days, after inoculation with *P. cinnamomi*

Table 19 lists the mean squares for the analysis of variance of the disease scale (1 to 8) and the natural population means, while Figure 16 shows the average disease values for each family. There were highly significant and consistent differences ($P < 0.001$) in *P. cinnamomi* disease intensity between the natural *Pinus radiata* populations in tests NP1 and NP2. The Cambria population had the least disease development and its expression was uniform over the 20 families sampled in the NP1 and NP2 tests. There was no difference between the coastal and inland populations of Monterey and they were only marginally less tolerant than the Cambria population. Both tests showed significant variation within the Monterey populations.

The island populations had significantly more disease ($P < 0.01$), while the most susceptible was the Ano Nuevo population of the northern mainland. Significant within population variation to *Phytophthora cinnamomi* was found in both of the important Ano Nuevo and Guadalupe Island provenances. No differences were found within the Cedros Island population.

Figure 17 shows that the disease scores were not normally distributed but skewed to the right. Normal distribution of data is a prerequisite to the analysis of variance. In a modified disease scale, codes 3 and 4 were given the same value as were the codes 5 and 6, resulting in a new scale ranging from 1 to 6 (Figure 17). This right skewed data was then transformed using square roots so that a normal distribution was achieved. Variance analysis of the transformed modified-disease codes has demonstrated significant differences ($P < 0.001$) within the Ano Nuevo, Monterey coast and inland, and Guadalupe Island populations, as well as between populations. Individual heritabilities were identical with the results of the analysis of the original disease codes (Table 19).

The ranking of families for two variables, seedling height before inoculation and disease code, was tested for independence by the Spearman's coefficient of rank correlation. The r_s for test NP1 (49 families) was 0.30, approaching significance at the 95% probability level, and for test NP2 (72 families) was 0.17, not significant. This leads to the conclusion that ranking of families on disease intensity, following inoculation with *P. cinnamomi,* was independent of their initial seedling heights.

Individual heritabilities for the *P. cinnamomi* disease intensity in natural populations ranged from 0 to 1.35 (Table 19). Population heritabilities were dependent on the number of parent trees in the sample and the variation in their response to inoculation with *P. cinnamomi.* Lower values, generally, were recorded in the ten parent tree populations of NP1, compared with the twelve parent tree populations of NP2. Heritabilities were low in populations where there was little difference in family response. When variances were computed from the model for the *Pinus radiata* within species variation, individual heritabilities for the *P. cinnamomi* disease intensity were near unity, being $0.97 + 0.21$ in NP1 and $1.08 + 0.25$ in NP2. However, these extremely high values need to be regarded with some scepticism as they involve separate and different intermating populations, thereby violating one of the assumptions of the genetical analysis (Cockerham, 1963).

TABLE 19

Analysis of variance, heritability and *Pinus radiata* natural population summary for disease intensity (scale 1 to 8), 240 days after inoculation with *Phytophthora cinnamomi*.

Source	NP1 d.f.	NP1 variance	NP1 sig.	NP2 d.f.	NP2 variance	NP2 sig.
within population						
Ano Nuevo	9	12.6	**	11	17.4	***
Monterey coast	9	7.1	*	11	10.5	**
Monterey inland	8	12.2	**	11	7.3	*
Cambria	9	1.3		11	5.0	
Cedros Island	9	3.5		11	1.0	
Guadalupe Island	-	-		11	9.4	**
between populations	4	162.8	***	5	52.3	***
within species	48	20.2	***	71	11.5	***
replication	15	7.4	**	5	9.1	
residual	720	3.3		355	3.6	
Total	783	4.4		431	5.0	

Summary

Population	NP1 disease code	NP1 h^2I	NP1 h^2F	NP2 disease code	NP2 h^2I	NP2 h^2F
Cambria	2.5 a	0	0	2.3 **	0.57 + 0.45	0.50
Monterey inland	2.7 a	0.56 + 0.34	0.72	3.2 b	0.38 + 0.41	0.39
Monterey coast	2.7 a ***	0.28 + 0.23	0.55	3.3 b **	0.84 + 0.50	0.61
Cedros Island	4.1	0.17 + 0.19	0.41	4.1 c	0	0
Guadalupe Island	- **			4.2 c	0.93 + 0.51	0.65
Ano Nuevo	4.8	0.30 + 0.24	0.57	4.6 c	1.35 + 0.55	0.75

* significant difference at 95% probability level, ** $P < 0.01$, *** $P < 0.001$. values followed by the same letter are not significantly different.

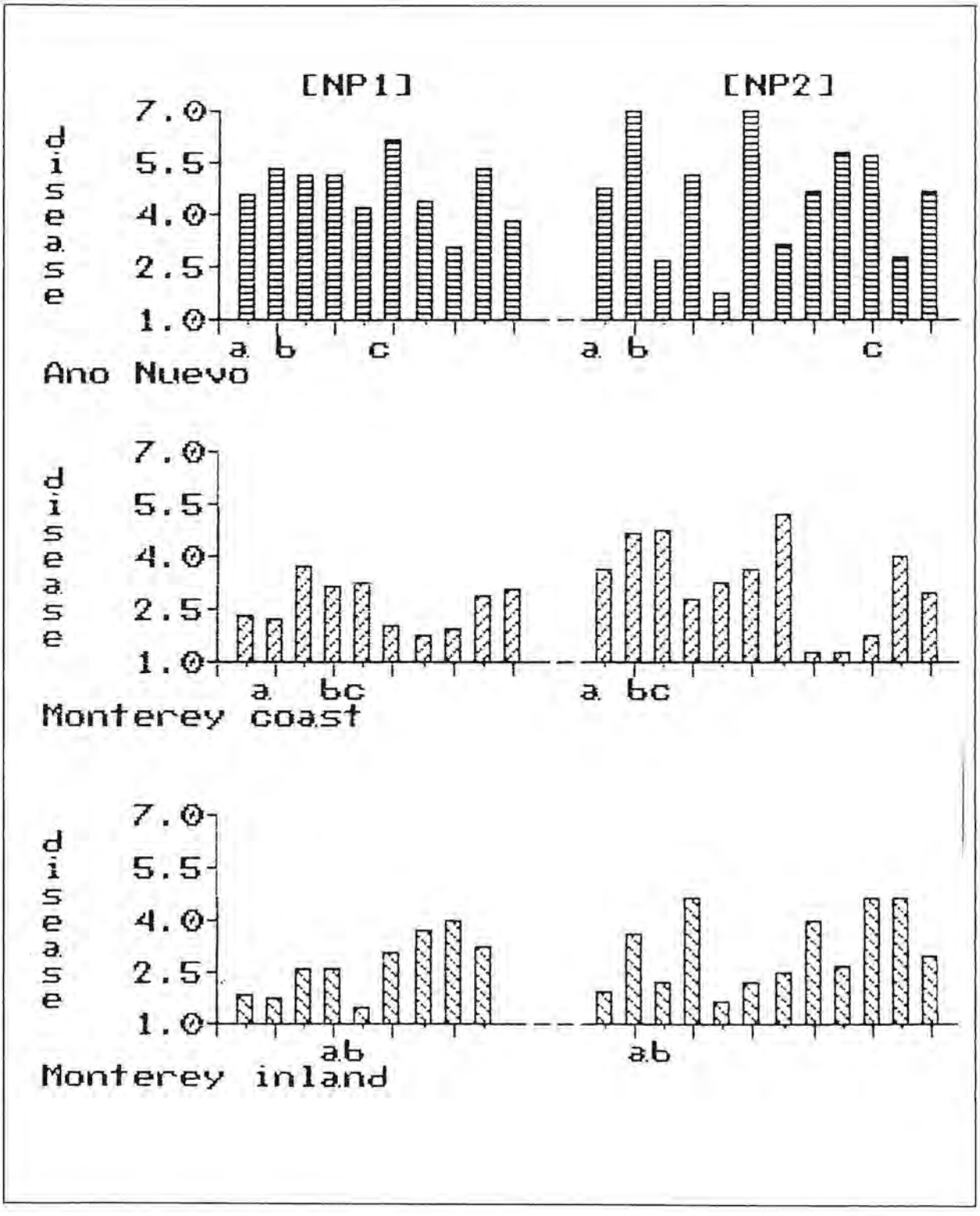


FIGURE 16

Disease intensity for each family in the natural populations of *Pinus radiata*, 240 days after inoculation with *Phytophthora cinnamomi*.
(a , etc. - families common to both NP1 and NP2 tests)

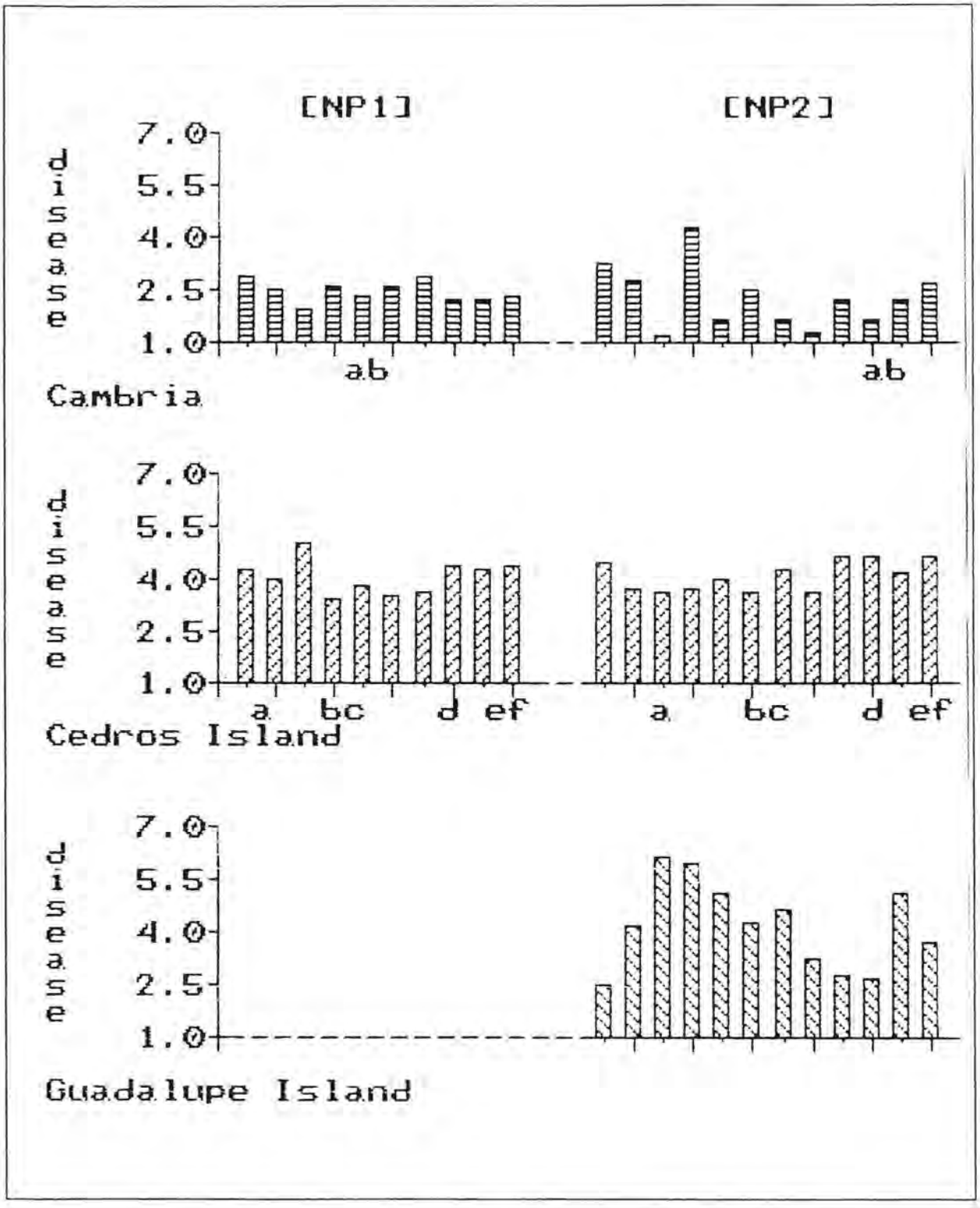


FIGURE 16 (cont)

Disease intensity for each family in the natural populations of *Pinus radiata*, 240 days after inoculation with *Phytophthora cinnamomi*.
(a , etc. - families common to both NP1 and NP2 tests)

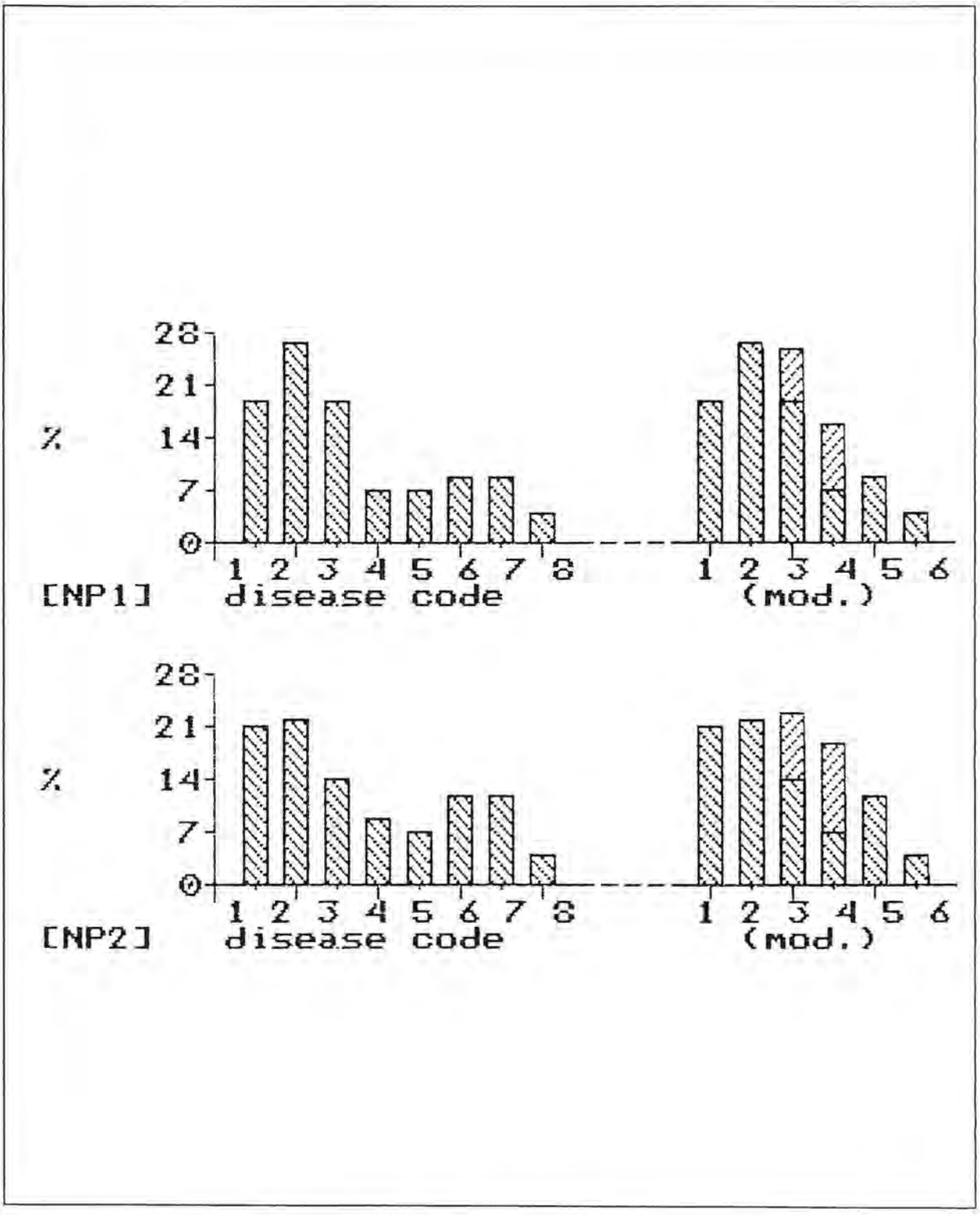


FIGURE 17

Frequency distribution of disease codes, for seedlings of the natural populations of *Pinus radiata*, 240 days after inoculation with *Phytophthora cinnamomi*.

4.2.4 Discussion

Data from the three glasshouse tests have shown that there is considerable intra-specific variation in the disease expression of one-year-old seedlings of *Pinus radiata* to infection with *Phytophthora cinnamomi.* Seedlings from the Cambrian population consistently had less disease development than any of the other populations. Response of each of the 20 families, constituting the population, was similar. The two sub-populations from Monterey gave a similar response and were only slightly more susceptible than the Cambrian provenance to the *P. cinnamomi* disease. However, in both of the Monterey populations, the intra-population variation was considerable.

Populations from Ano Nuevo, Guadalupe Island and Cedros Island had a poor resistance to *P. cinnamomi.* Intra-population variation was large in the important Ano Nuevo and Guadalupe Island populations. Two families from Ano Nuevo, and from Guadalupe Island, were ranked in the top quarter for disease resistance, for all of the families in the natural population studies.

Seedlings from the Cedros Island provenance nearly always had a severely rotted root system, although the number that died was similar to the tolerant Monterey populations. Few seedlings died because their shoots were considerably smaller than the other provenance seedlings and the transpiration requirement was reduced. Close examination of several surviving seedlings from the Cedros Island population at the end of the trial showed every root to be rotted, as well as the shoot collar and lower stem being discoloured by the infection with *P. cinnamomi.* The seedling tops were small, and the insignificant water demand did not provide the physiological stress to kill the seedling.

Shelbourne *et al.* (1979) found that Cedros Island trees grew much more slowly than the other provenances at all sites in New Zealand. In the nursery, Burdon and Bannister (1973) reported that Cedros Island and Guadalupe Island seedlings developed a strong taproot growth at the expense of a fibrous root development, presumably as an adaptation to the low rainfall and exposure on the islands. Soils were developed from volcanic rock at Guadalupe Island, whereas the Cedros Island soils were mostly derived from sedimentary and metamorphic rocks (Libby *et al.,* 1968). Eldridge (1978a) described the soils on Cedros Island as shallow and skeletal, and on Guadalupe Island as a well drained loam between the basaltic boulders. Rainfall is unreliable although fogs are prevalent in all seasons. *Phytophthora cinnamomi* was not recovered by Zentmyer (1977) from any native trees in undisturbed sites in California, or from undisturbed soils in southern California or Mexican avocado areas, but it was readily recovered from roots of trees brought into cultivation and affected with root-rot. He postulated that it was unlikely that *P. cinnamomi* was an indigenous fungus in this area.

Guadalupe and Cedros are small isolated islands with precipitous coastlines. Guadalupe Island is uninhabited, and habitation on Cedros Island is limited to small fishing villages. From Zentmyer's data, and the nature of the islands, it is extremely unlikely that *P. cinnamomi* would be present. The most likely explanation for the high susceptibility of the island populations to the disease is that (a) tree populations have developed in

the absence of *P. cinnamomi*, (b) development of an unbranched rooting system in response to the low rainfall, and (c) inherently low vigour of the Cedros Island population developed on infertile, shallow and dry soils. Differences between the populations in disease expression is largely due to the lower vigour of the Cedros Island seedlings. The low root regeneration potential of the Cedros Island population has resulted in a uniform response to *P. cinnamomi* infection.

A uniform, tolerant response to *P. cinnamomi* inoculation was achieved for the Cambrian population. This could indicate a balance in the indigenous plant and pathogen populations (Segal *et al.*, 1982). However, Zentmyer was certain that *P. cinnamomi* was not a native pathogen of southern California, and there have been no reports in the literature of any recoveries of the pathogen from the small Cambrian forest. The uniform response must be related to some other allied character. During seed collection early in the spring of 1978, Eldridge reported that many parts of the Cambria area were waterlogged after an unusually wet winter and some trees were windthrown. The gentle slopes and almost flat terrain at Cambria allows more opportunity for the accumulation of water than on steep, well-drained slopes (Eldridge, 1978a). This factor was argued by Cromer *et al.* (1982) for greater salt accumulation in the soil profile and adaptation of the Cambria population to be more salt tolerant than the other populations. Adaptation to waterlogged conditions also infers a greater tolerance to flooding and a higher root regeneration potential. In the absence of evolutional selection to an indigenous *P. cinnamomi* pathogen, the most likely explanation of the increased and uniform disease tolerance, is the adaptation of the Cambria population to waterlogging conditions and an improved root regeneration potential.

The tolerant response to *P. cinnamomi* infection was similar for both Monterey populations, even though the soil types were quite different. Forde (1966) considered that the Monterey and Cambria forests were ecologically similar, although the latter was reported in Cromer *et al.* (1982) to be subject to waterlogging. Chemically, the Monterey and Cambria trees have a similar turpentine composition, while the Ano Nuevo trees have a lower average -pinene content (Bannister *et al.*, (1962). Variation within the Monterey populations was large, suggesting that the populations have developed in the absence of *Phytophthora.*

Generally, the Ano Nuevo seedlings had a susceptible reaction to *P. cinnamomi* inoculation, although there was considerable intra-population variation. Ano Nuevo has the most rainfall, steep terrain and slightly heavier-textured soils, although soil depth rather than soil type is more important in determining the distribution of the pine tree (Forde, 1966). Evidence suggests that the Ano Nuevo population has developed in the absence of *P. cinnamomi.*

Genetic variation within *Pinus radiata* has been considered as a hierarchy of three levels for this study; between populations, between individual trees in populations and between seedlings within parent trees. In these experiments, populations have been represented by less than 20 families, and families have been represented by 16 seedlings in NP1 and only 6 seedlings in NP2. Families common to both tests had a similar disease ranking. Data in Chapter 3(5) indicated that the same ranking would be achieved from a sample of 6, 16 or 40 seedlings. As population trees were sampled at random for this character (*P. cinnamomi* is not indigenous in the natural stands), the large and significant differences calculated in both the parametric and non-parametric tests, indicate that there are real provenance differences in resistance to *P. cinnamomi*.

Although the individual heritability for disease intensity in the *Pinus radiata* species was close to unity in both tests, its validity is questionable as the estimates of variances for the parent trees, were for trees sampled in different intermating populations. The large and consistent differences between populations and between parent trees, and the large sample size, were responsible for this large species heritability estimate. Heritability estimates for each intermating natural population varied considerably, due to the small sample size and the different response of the populations. The heritability was very high for the Guadalupe Island, Ano Nuevo and Monterey populations, where there was large intra-population variation, and was low for the consistent response Cambria and Cedros Island populations.

Genetic variation in response to inoculation with *Phytophthora cinnamomi* has been shown to be large between natural populations of *Pinus radiata*. Large responses between populations have also been reported for the *Dothistroma pini* disease (Cobb and Libby, 1968), western gall rust (Old *et al.*, 1986), wood density (Nicholls and Eldridge, 1980) and salt tolerance (Cromer *et al.*, 1982). In each case there was considerable within population variation that can be exploited.

4.3 DOMESTIC POPULATIONS OF PINUS RADIATA

SEEDLING RESPONSE TO INOCULATION WITH
PHYTOPHTHORA CINNAMOMI IN GLASSHOUSE TESTS.

4.3.1 Introduction

Tree breeding programmes for *Pinus radiata* have been in progress in Australia, New Zealand and South Africa since the early 1950s. In each case, programmes were based on "plus trees" (outstanding phenotypes) selected from *P. radiata* planted in each particular country. Some of the New Zealand "850" selections were included in the Australian population, but neither the New Zealand nor the South African population contains any extraneous selections. No recourse was taken of the natural radiata stands in California, unlike the *P. pinaster* situation in Western Australia where plus tree selections in the native Portugal stands played such an integral role in the pinaster breeding programme (Perry and Hopkins, 1967). The large provenance variation in growth and form of *P. pinaster* (Hopkins, 1960), compared with *P. radiata,* could partly explain this.

Major objectives of the Australian, New Zealand and South African tree breeding programmes are the same; i.e., increased growth rate, straight stems, small diameter branches and improved wood quality. Considerable progress in the improvement of these characters has been reported from each programme, for example, Pederick and Eldridge (1983), and Shelbourne *et al.* (1986).

For the Western Australian programme, tolerance to the *Phytophthora cinnamomi* root-rot disease has been cited as a major objective (Butcher, 1986a). Excellent support has been given by the Research Working Group No.1 (Forest Genetics) of the Australian Forestry Council, in providing seedlots from the Australian population for screening. Results of the screening tests for populations from New South Wales, Victoria, South Australia, Tasmania, CSIRO-DFR, and APM forests in Victoria were given by Butcher and Stukely (1986). No single Australian population appeared to be different from the others in response to inoculation with *P. cinnamomi*; most of the variation was found within populations. The various Australian sub-populations could be considered as the same, which is not surprising as many clones are common to each programme and most of the nation's plantations have originated from seed collections in the southeast of South Australia.

The aim of this study is to examine the level of genetic variation within the major world land-races of *Pinus radiata* in their response to infection with *Phytophthora cinnamomi*. These races have been improved for a number of characters and they are an excellent source of genetic material for the Western Australian breeding programme.

4.3.2 Materials and Methods

Domestic populations from Western Australia, South Australia, New Zealand and South Africa formed the study.

The Western Australia parents were selected for their healthy appearance, superior growth rate and tree form from the Mundaring and Grimwade plantations. These selections were cloned and planted in the West Manjimup seed orchard. Seed for the study was collected from the individual mother trees, open pollinated in the 94 clone seed orchard. Standard test families (susceptible 60017, 20011, 60001 and tolerant 80007, 60027, 60022) were open pollinated in this orchard.

The South Australian population was represented by the "Super-80" series plus trees selected in the southeast region of the State (Boomsma *et al.*, 1983). This was a recent extensive search to broaden the genetic base for future breeding activities. Selection was based on tree health, vigour and form, at an intensity of 1:1000. Seed was collected from the open pollinated plus trees.

Seedlots of the New Zealand population comprised both the "850" series and "268" series parents (Shelbourne *et al.*, 1986). These open pollinated families were collected at the Kaingaroa and Waimihia seed orchards.

Seed orchard pollinated clones, and wind pollinated plus tree seedlots were sent from South Africa for this study. Notes on these selections were given by Lange (1979).

The study was designed as a nested analysis of variance (Sokal and Rohlf, 1981), where each major population (4) was subdivided into randomly chosen parent tree subgroups (21) and each family was replicated (20 inoculated + 10 control) using single tree plots. The test medium was a multi-family pot containing 7 different family seedlings and 1 susceptible family 60017 bait seedling near the centre of each pot. One replication consisted of 12 plant pots; the inoculated test consisted of 240 pots and the control, 120 pots. Family seedlots were stratified, sown into nursery trays, grown, transplanted into the experimental plant pots and moved into the Wanneroo glasshouse. The inoculated series pots were each inoculated with the same eight isolates of *Phytophthora cinnamomi* used in the natural population studies, in December 1983 (Plate 1). Control pots were treated identically, except that the infected plugs were first autoclaved.

Thirty days after the inoculation, 8 (of 240) seedlings of the bait family 60017 and 7 (of 1680) seedlings of the population families had died. There were no deaths in the control pots. Immediately after this assessment, a number of days of extreme high temperature and low humidity were recorded in the Wanneroo glasshouse, culminating with a thermohygrograph temperature recording of 47°C and humidity of 12% on the 10th January. Seedlings in both control and inoculated pots were wilted, with browning of tops and collapse of tissue (Plate 5.2). An assessment of seedling health was made by me on my return from leave, 10 days after the disaster. In the inoculated pots, 15% of the seedlings were burnt and 6% were dead, while in the control pots 14% were unhealthy and 3% were dead. The test was continued, with assessments at 15 day intervals, but

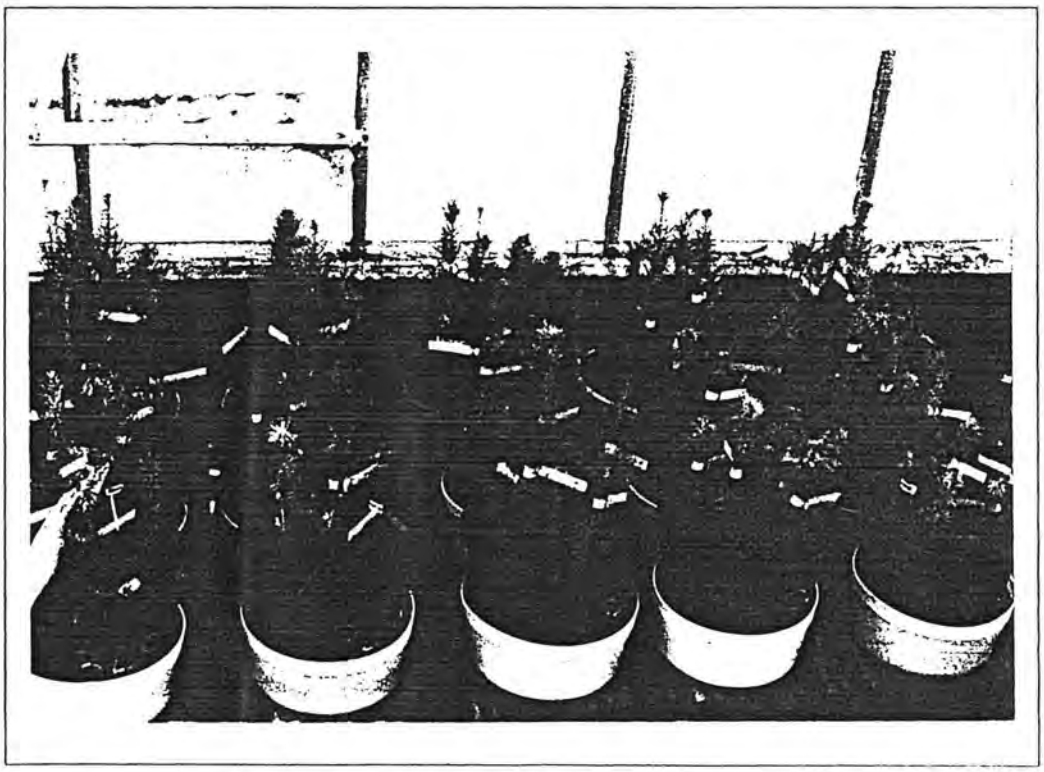


PLATE 5

Extreme temperature effects on the health of *Pinus radiata* seedlings in the failed domestic population test.

1. before the event.
2. ten days after the extreme temperature event.

detailed analysis of seedling height increments and deaths failed to give any consistent segregation of data. Families from the W.A. population that had previously been evaluated for tolerance to *P. cinnamomi,* were inconsistent in this test. There were also many deaths in the control uninoculated pots. *Phytophthora cinnamomi* had most likely been killed in the majority of pots. Theron *et al.* (1982) successfully used a hot water dip treatment of *Pinus radiata* seedlings to eradicate *Phytophthora cinnamomi;* similar conditions were inadvertently applied to our glasshouse test. Eventually, it was realised that all data from this test had to be ignored.

Failure of this main study required that it had to be repeated. However, because of seedlot availability this was not possible. Experiment designs were modified and two tests were conducted in the following three years to acquire data on the genetic variability of the major domesticated populations. These test designs were :

Domestic Population Test (DP1) :

Number of Seedlings Population	Families	(A)	(B)
South Africa	22	32	28
South Australia	18	32	28
New Zealand	5	32	28
WA Standards	4	32	28

Experimental design:

(A) : 7 x 7 balanced lattice, repeated x 4 (t = 49, k = 7, r = 8).

Multi-family pot containing seven different family seedlings plus one seedling of susceptible 60017.

(B) : 7 x 7 quadruple lattice, (t = 49, k = 7, r = 4).

Single family pot containing seven family seedlings plus one seedling of susceptible family 60017.

(A) and (B) : Application of four isolates of *P. cinnamomi* per pot: (1), (7), (9) and (10) (Table 32).

Domestic Population Test (DP2) :

Population	(A) Families	(A) Seedlings	(B) Families	(B) Seedlings
South Africa	12	16	10	40
South Australia	12	16	6	40
New Zealand	20	16	1	40
WA Standards	5	16	4	40

Experimental design:

(A) : 7 x 7 balanced lattice, repeated x 2 ($t = 49, k = 7, r = 8$).

(B) : 7 x 5 completely randomised block ($t = 35, r = 40$).

(A) and (B) : Multi-family pots with seven different family seedlings and one seedling of D655 per pot.

Application of four isolates of *P. cinnamomi* per pot: (1), (7), (9) and (10) (Table 32).

Seedlings were grown as previously described. Plant pots were positioned in large holding tanks (24 pots per tank), instead of individual pot containers, in the Como glasshouse to facilitate uniform moisture control between pots. Three days prior to inoculation, holding tanks were plugged, and filled with water to a depth of 8 cm at the centre of the tank. This depth saturated the soil volume of the treatment pots. Inoculum grown on pine branch-plugs, of four of the most virulent isolates of *P. cinnamomi* used in the host x pathogen tests (Chapter 5), were then buried to a depth of 8 cm in recorded positions in each pot. Pots were watered from the top after inoculation. The holding tanks were drained six days after inoculation and then normal watering was resumed.

Pots in test DP1 were inoculated on the 30th November 1984, when seedlings were about 10 months old and 165 mm tall. Pots in the DP2 test were inoculated on the 6th November 1986; pine seedlings were one-year-old and 280 mm tall.

The glasshouse regime for these tests was the moderate moisture / temperature regime described in Chapter 3(4). Pots were watered with about 1000 mls at least twice weekly, and glasshouse temperatures were maintained below 30°C. Evaporative coolers were set to automatic operation at a temperature of 25°C. Thermohygrograph records were maintained for the duration of both tests.

In both tests, seedling deaths were recorded at 20 day intervals through to the end of the experiments, 180 days after the inoculation. Heights of dead seedlings were measured at these periodic assessments and surviving seedling heights were measured at the end. The amount of disease was scored for the root systems of alive seedlings in the multi-family pots; this was not done in any single-family pot because of the possible observer bias. Any pot with no dead seedlings was tested for the presence of *P. cinnamomi*, either by the plating of inoculum plugs or seedling roots/collar.

4.3.3 Results

Before *P. cinnamomi* was introduced into the plant pots, there was significant variation in the heights of the one-year-old seedlings ($P < 0.001$). From the nesting of the analysis of variance, this family variation was partitioned into within population and among population effects (Table 20); both were shown to be highly significant ($P < 0.001$). Mixtures of seedling sizes with different competitive abilities in the multi-family pots could be expected to affect seedling response to infection with *P. cinnamomi*; i.e., small seedlings to be susceptible and large seedlings to be more able to withstand attacks on a larger root system. This association of seedling size and response to *P. cinnamomi* inoculation was tested by the Spearman coefficient of rank correlation. A negligible correlation of $r_s = 0.02$ was calculated for the DP1 test, indicating a complete independence of the rankings of family disease and initial seedling heights. This information was later used in the construction of the disease intensity scale for the single-family pots in the DP1(b) test.

The moderate moisture / temperature glasshouse regime used for the DP1 and DP2 tests was conducive to maximum disease development. The progression of *P. cinnamomi* disease, as the percentage of dead seedlings, is illustrated in Figures 18 and 19 for a range of *Pinus radiata* families. Note the comparable performance of the susceptible families 20011 and 60017 in both tests, and contrast this with the pattern of the tolerant standards 60027 and 80007. In test DP2, no seedlings of New Zealand family 268/323 died. Figures 18 and 19 show that most of the susceptible seedlings die within 100 days of the inoculation. The rate of seedling death was used in the construction of the disease scale.

Tables 21 and 22 show the incidence of disease, as the number of dead seedlings, 180 days after inoculation with *Phytophthora cinnamomi*. Data from the multi-family and single-family pot tests of DP1 have been combined for the multi-factor analysis in Table 21. Four replications of noncontiguous family plots of 8 seedlings were formed to calculate plot mean mortality for the multi-family test. Mean mortality in the single-family test was based on 7 family seedlings in the same pot. Percentages were transformed to the arcsin square root of the number of dead seedlings for the analysis of variance. Variation in all factors was large (Table 21). The difference in contiguous versus noncontiguous family seedling plots was highly significant ($P < 0.001$), 53% death compared with 40%. The interaction of family and plot design was also significant ($P < 0.05$). Both results reflect the increased build-up of *P. cinnamomi* inoculum associated with susceptible families of *Pinus radiata*. The South African population had significantly less dead seedlings than the others ($P < 0.001$). The number of dead

TABLE 20

Analysis of variance and summary for seedling height growth of domestic populations of *Pinus radiata*, prior to the inoculation with *Phytophthora cinnamomi*.

Source	DP1. d.f.	DP1. variance	DP1. sig.	DP2. d.f.	DP2. variance	DP2. sig.
within population						
South Africa	21	8563	***	11	62688	***
South Australia	17	8229	***	11	38372	***
New Zealand	4	3705	*	19	27728	***
W.A. standards	3	8266	***	4	24486	***
between populations	3	23506	***	3	24915	***
within species	48	8955	***	48	38227	***
replication	31	2051	*	15	11198	**
residual	1488	1331		720	4426	
Total	1567	1579		783	6627	

Summary

Population	Initial mean height mm DP1		DP2	
South Australia	173	a	271	b
W.A. standards	169	a	279	b
South Africa	168	a	297	
New Zealand	149		270	b

* significant difference at 95% probability level, ** $P < 0.01$, *** $P < 0.001$.

values followed by the same letter are not significantly different.

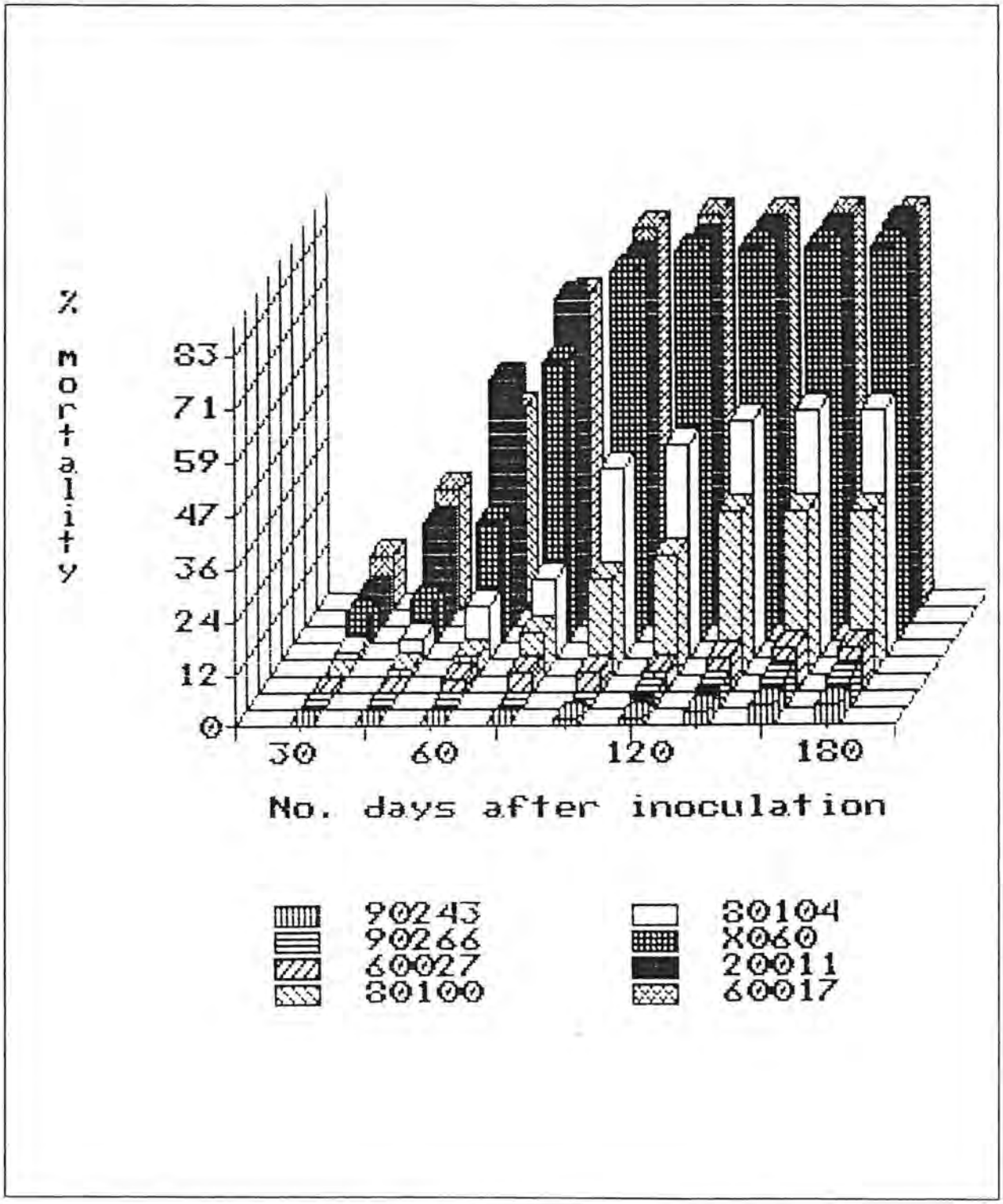


FIGURE 18

Progressive seedling mortality in eight families of *Pinus radiata*, following inoculation with *Phytophthora cinnamomi* in test DP1.

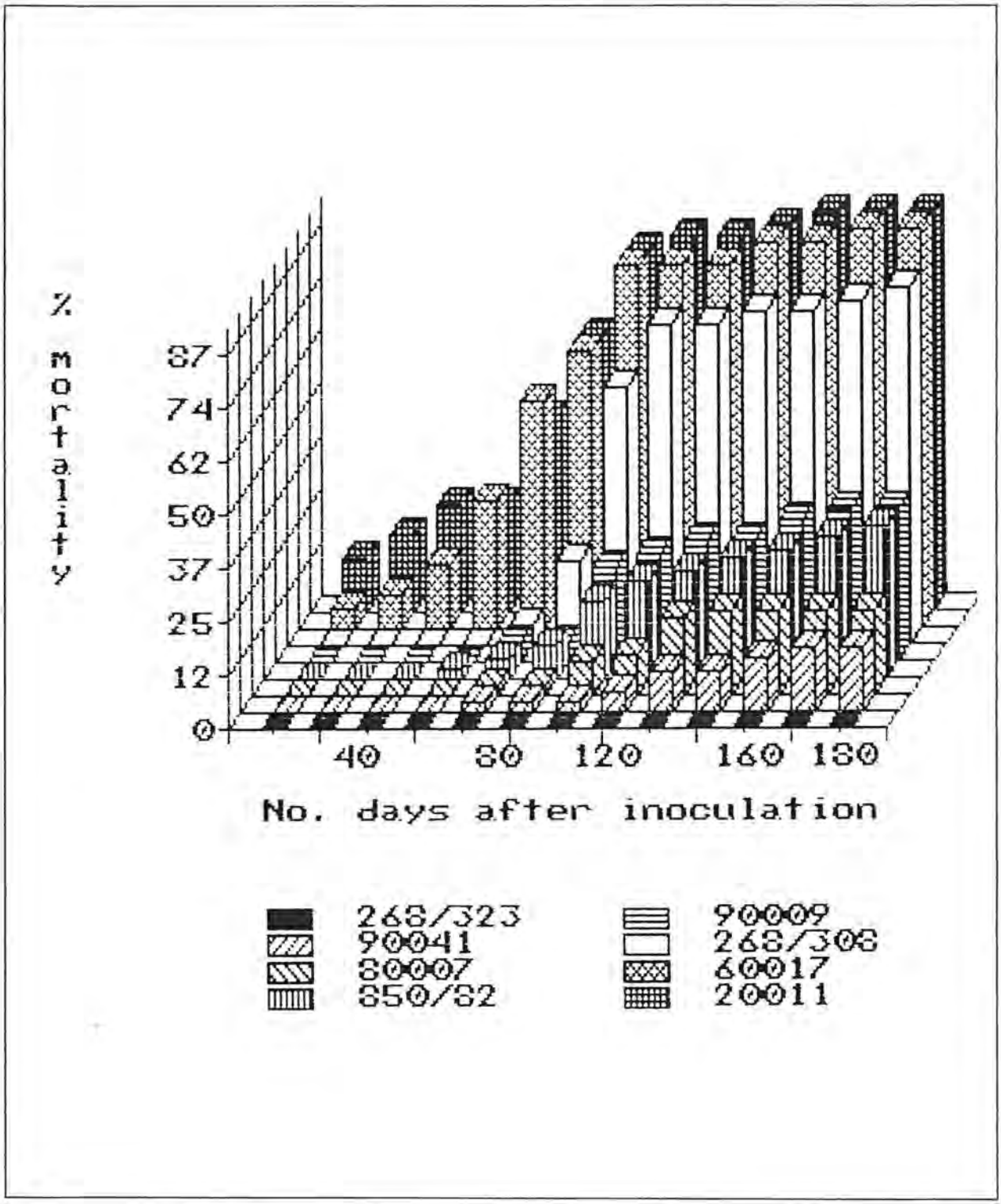


FIGURE 19

Progressive seedling mortality in eight families of *Pinus radiata*, following inoculation with *Phytophthora cinnamomi* in test DP2.

TABLE 21

Hierarchal and split-plot analysis of variance, family heritabilities and domestic population summary for percent mortality (arcsin square root transformed) of *Pinus radiata* seedlings, 180 days after inoculation with *Phytophthora cinnamomi* in DP1.

Source	d.f.	Variance	Significance
within population			
South Africa	21	2044	***
South Australia	17	838	***
New Zealand	4	984	**
W.A. standards	3	6511	***
between populations	3	19264	***
Main plots			
Design-pots (D)	1	15931	**
Replication (R)	3	844	
error	3	339	
Sub plots			
Family (F)	48	2884	***
FxD	48	355	*
F*R	144	267	
error	144	232	
Total	391	631	

Summary

DP1 Population	Mortality	h^2_F
South Africa	33 ***	0.87
New Zealand	54 a	-
W.A. standards	55 a	-
South Australia	59 a	0.58

* significant at $P < 0.05$, ** significant at $P < 0.01$, *** significant at $P < 0.001$.

values followed by the same letter are not significantly different.

TABLE 22

Hierarchal analysis of variance, family heritabilities and domestic population summary for percent mortality (transformed into arcsin square root) of *Pinus radiata* seedlings, 180 days after inoculation with *Phytophthora cinnamomi* in DP2.

Source	d.f.	Variance	Significance
within population			
South Africa	9	783	***
South Australia	5	610	**
New Zealand	14	775	***
W.A. standards	3	3670	***
between populations	3	5619	***
within species	34	1464	***
replication	4	386	*
residual	136	145	
Total	174	408	

Summary

DP2 Population	Mortality	h^2_F
South Africa	35 ***	0.82
New Zealand	50 a	0.84
W.A. standards	56 a *	-
South Australia	65	0.64

* significant at $P < 0.05$, ** significant at $P < 0.01$, *** significant at $P < 0.001$.

values followed by the same letter are not significantly different.

seedlings in the South Australian, New Zealand and W.A. standards populations were similar. There was significant variation between families within each of these populations. Variation between the twenty two South African families ranged from 8 to 60% (average 33 arcsin mortality), 39 to 76% (average 59) for eighteen South Australian families, and 37 to 65% (average 54) for five New Zealand "850" families. The calculated family mean heritability of 0.87 for South Africa and 0.58 for South Australia were very high, and similar to the test values of the Western Australian breeding population reported in Butcher *et al.* (1984). Heritabilities were not calculated for the New Zealand population as it was inadequately sampled, or for the W.A. standards as these parents were selected for their response to *P. cinnamomi*.

In the second test DP2(b), the 40 single seedling family plots were combined to form 5 noncontiguous family plots of 8 seedlings, to calculate family mean mortality for the analysis of variance. Genetic variation was again very large (Table 22). The South African population had significantly fewer dead seedlings ($P < 0.001$) than the New Zealand population, which had less dead seedlings than the South Australian population ($P < 0.05$). Family variation within each population was highly significant ($P < 0.001$), ranging from 20 to 54% (average 35 arcsin mortality) for the ten South African families, 31 to 69% (average 50) for the fifteen New Zealand families, and 44 to 73% (average 65) for the six South Australian families. Family mean heritabilities were calculated as 0.84 for New Zealand and 0.82 for South Africa. Note that different families constituted each population, apart from the W.A. standards, in the DP1 and DP2 tests.

TABLE 23

Root weights of *Pinus radiata* seedlings from the subjective assessment and classing of root systems in DP1 and DP2 tests.

Disease code	root weight (mean and standard deviation) DP1	DP2
1	2.9 + 0.8 g	4.4 + 1.8 g
2	1.5 + 0.5	2.5 + 0.8
3	0.9 + 0.4	1.1 + 0.4
4	0.5 + 0.3	0.5 + 0.2
healthy	2.2 + 1.0 g	3.5 + 1.7 g
diseased	0.7 + 0.4	0.8 + 0.7

Seedling root weights, calculated from a random sample of 30 seedlings for the four subjective assessment classes in DP1 and DP2, are shown in Table 23. Classes 1 and 2 had a large root mass and were clearly separated from the small weights of the diseased seedling roots of classes 3 and 4. These combinations were used to represent healthy and diseased seedlings in Figure 20.

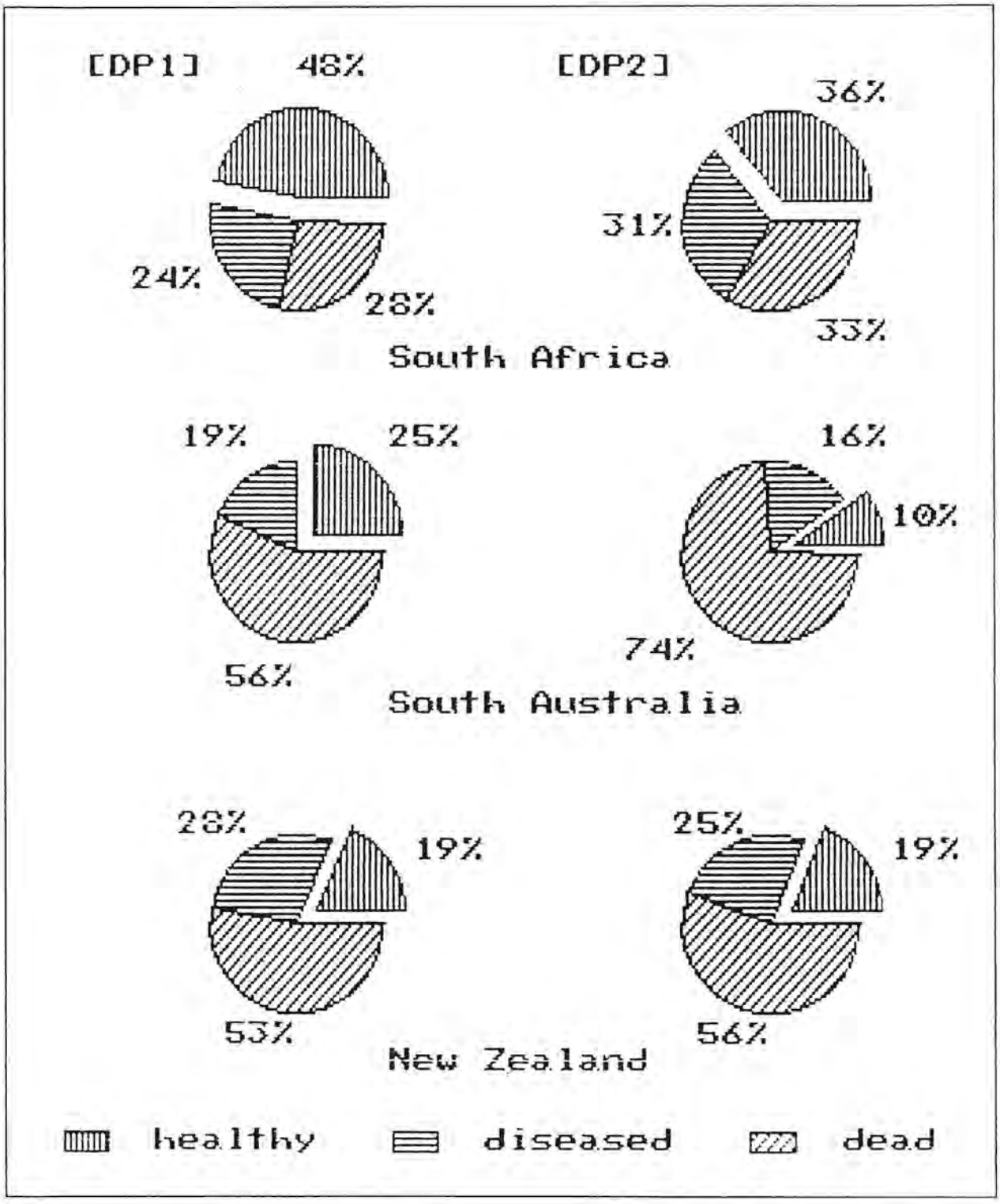


FIGURE 20

The amount of disease in domestic populations of *Pinus radiata*, 180 days after inoculation with *Phytophthora cinnamomi*.

TABLE 24

Chi-square test for the frequency of healthy, diseased and dead seedlings in three domestic breeding populations of *Pinus radiata*, 180 days after inoculation with *Phytophthora cinnamomi*.

Population	number of seedlings					
	DP1.			**DP2.**		
	healthy	disease	dead	healthy	disease	dead
South Africa	336	169	199	155	134	143
South Australia	144	107	325	34	53	249
New Zealand	30	45	45	127	170	372
P. radiata spp.	510	321	321	316	357	764

calculated Chi-square DP1 = 132.1 DP2 = 141.0

tabular Chi-square = 18.5 (d.f. 4, $P < 0.001$). Fisher and Yates, (1963).

The South African population consisted of 704 seedlings in test DP1, and 432 different open-pollinated family seedlings in DP2; 48% and 36% respectively were assessed as healthy (Figure 20). This contrasted with the 25% and 10% healthy assessment for the South Australian population of 276 seedlings in test DP1, and 336 different open-pollinated family seedlings in DP2. There were 19% healthy seedlings in both tests of the New Zealand population, from a total of 160 seedlings inoculated in DP1, and 669 different open-pollinated family seedlings in DP2. Chi-square test of this data (Table 24) was highly significant ($P < 0.001$), showing conclusively that there were differences between the domestic populations in their response to inoculation with *P. cinnamomi*.

Family differences within each population for the percentage of healthy, diseased and dead seedlings are illustrated in Figures 21 and 22.

Scales were developed to represent the continuous variation in disease of individual seedlings. One end of the scale was fixed by the four root assessment classes of the alive seedlings, and the other end was derived from the seedling death assessments carried out at 20 day intervals during the experiments.

The frequency distribution of the 56% alive seedlings and 44% dead seedlings in DP1 is shown in Figure 23. These had an approximately normal distribution, but there were too

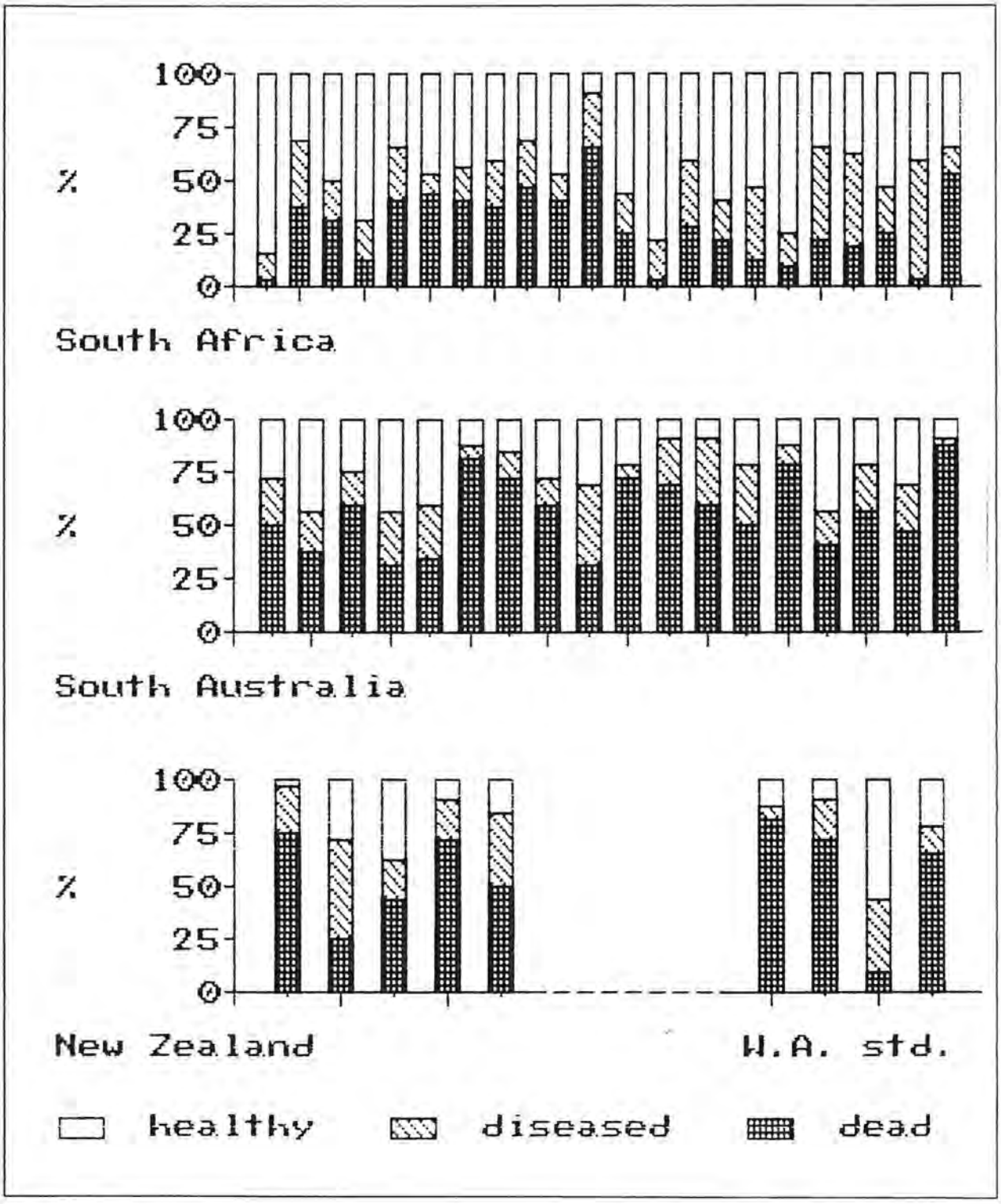


FIGURE 21

Variation in the amount of *Phytophthora cinnamomi* disease, within and between domestic populations of *Pinus radiata* in test DP1.

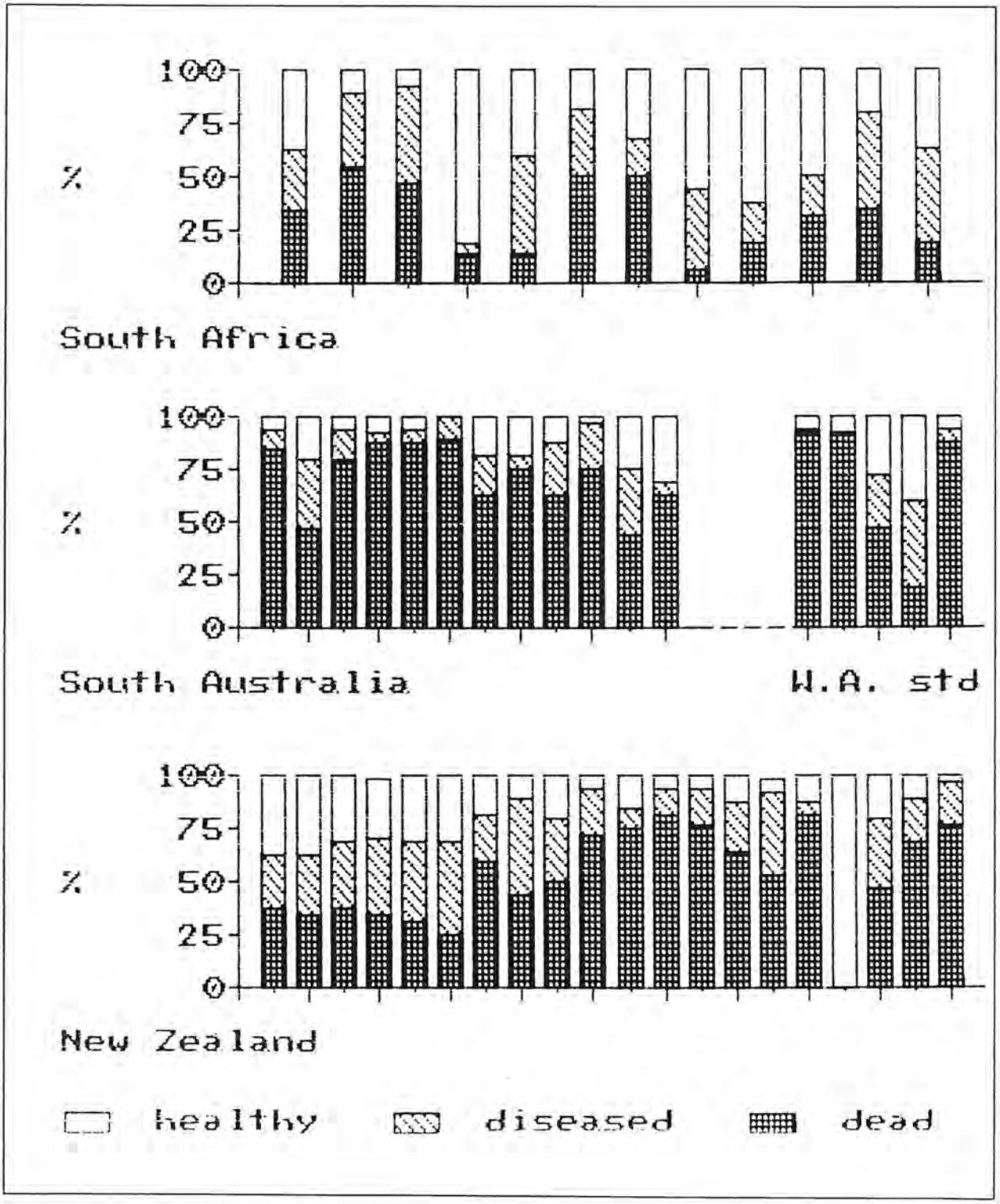


FIGURE 22

Variation in the amount of *Phytophthora cinnamomi* disease, within and between domestic populations of *Pinus radiata* in test DP2.

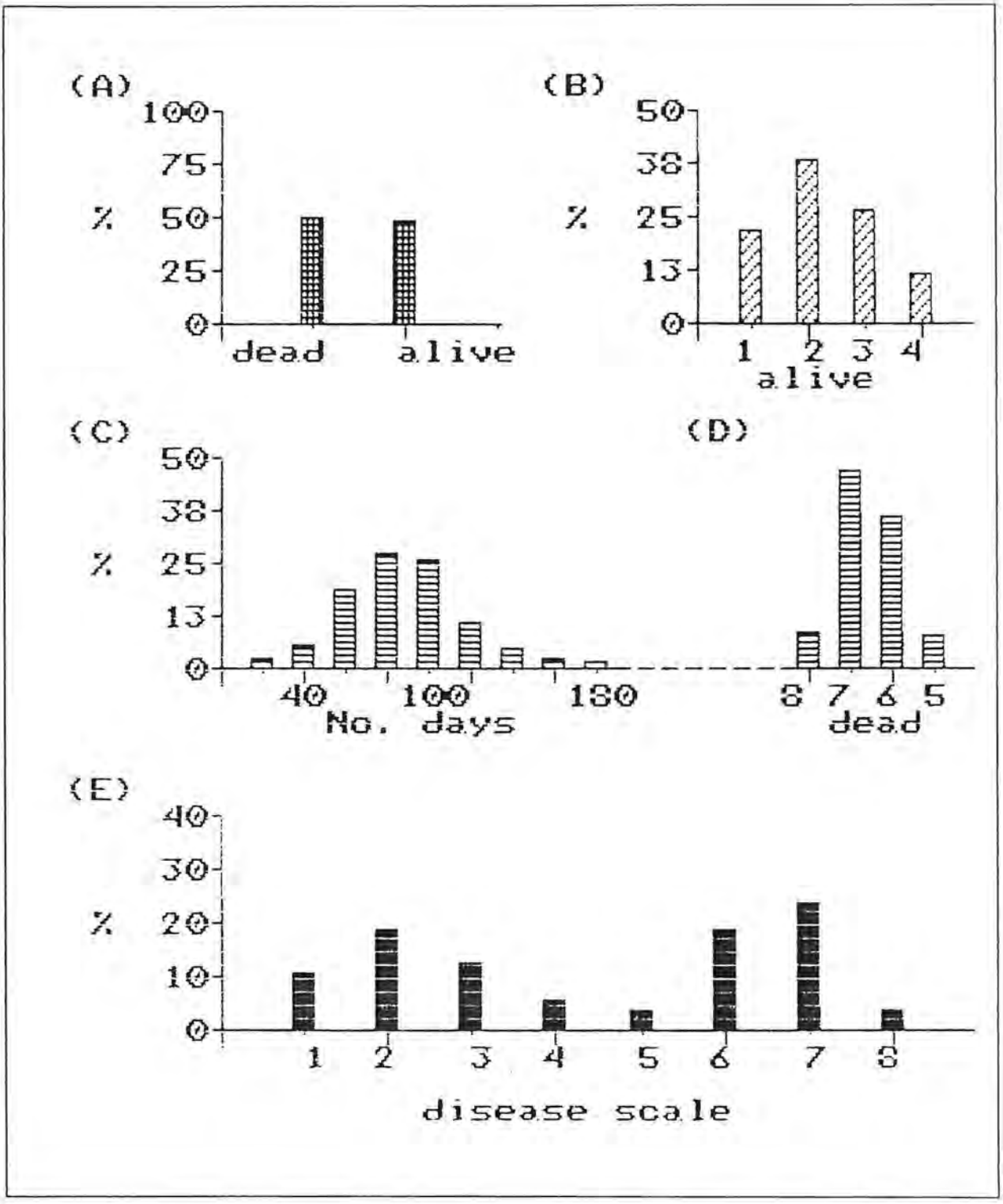


FIGURE 23

Frequency distribution and development of the disease intensity index for test DP1.

FIGURE 23 (cont)
Frequency distribution and development of the disease intensity index for test DP1.

A. distribution of alive and dead seedlings.

B. root assessment classes of the alive seedlings.

C. frequency distribution of dead seedlings.

D. disease codes for dead seedlings, based on 40 day intervals.

E. combination of the alive and dead classes to create the disease intensity index.

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many class intervals in the dead seedlings (C) for the analysis scale. Class intervals were condensed to deaths in 40 day periods in Figure 23(D). The disease scale, 1 to 8, is a combination of the alive (B) and dead (D) seedling scales (Figure 23 E). Variances of family means using this scale were found to be homogeneous by Bartlett's test (Sokal and Rohlf, 1981). Table 25 lists the results of the analysis of variance for the disease scale. Consistently large significant differences ($P < 0.001$) were calculated, within and between populations. The South African seedlings had significantly less disease ($P < 0.001$), while disease levels in the South Australian, New Zealand and WA standards were similar. Family variation in disease intensity is graphed in Figure 24.

The disease scale for the single-family pots in DP1 could not be constructed from the assessment of root systems because of the potential error introduced through assessor bias. The alternative scale, based on seedling height increment after inoculation with *P. cinnamomi* was used. Analysis of initial height (Table 20) indicated that there was considerable variation between families. Family variances were homoscedastic and disease classes were based on these variance intervals. Because the South African population had the least disease in the multi-family pot analysis, this population was used as the benchmark of the scale. Seedling disease was assessed using the following height increment classes:

Disease intensity scale (height) :

Code 1	Alive at 180 days	mean + 1 std. dev.	> 295 mm
Code 2	Alive at 180 days	mean to + 1 std. dev.	200 to 290 mm
Code 3	Alive at 180 days	mean to -1 std. dev.	105 to 195 mm
Code 4	Alive at 180 days	mean -1 std. dev.	< 100 mm

The remainder of the disease scale (5 to 8) was allocated as for the dead seedlings in the multi-family pots. Variance analysis of this height-disease scale in test DP1(b) is shown in Table 25. Differences between and within populations were large. Blocks in replication, or seedling positions in pot, were also significant ($P < 0.001$), with more

TABLE 25

Hierarchal and split-plot analysis of variance, heritability and domestic population summary for disease codes of *Pinus radiata* seedlings, 180 days after inoculation with *Phytophthora cinnamomi* in test DP1.

Source	DP1a (root code) d.f.	variance	sig.	DP1b (height code) d.f.	variance	sig.
within population						
South Africa	21	24.9	***	21	36.2	***
South Australia	17	19.7	***	17	15.3	*
New Zealand	4	28.7	***	4	6.7	
W.A. standards	3	81.3	***	3	117.3	***
between populations	3	246.9	***	3	285.7	***
main plots						
within species	48	40.8	***	48	47.0	***
replication	31	7.8	**	3	17.3	
error	1488	4.0		144	6.8	
sub-plots						
seedlings in pots	-	-		6	14.1	***
error	-	-		1170	2.9	
Total	1567	5.1		1371	4.9	

Summary

Population	DP1a disease code	h^2I	h^2F	DP1b disease code	h^2I	h^2F
South Africa	3.4 ***	0.58 + 0.18	0.85	4.1 ***	0.99 + 0.26	0.87
New Zealand	4.6 a	-		5.1 b	-	-
W.A. standards	4.8 a	-	-	5.4 b	-	-
South Australia	4.8 a	0.39 + 0.16	0.78	5.8	0.33 + 0.12	0.55

* significant difference at 95% probability level, ** $P < 0.01$, *** $P < 0.001$.

values followed by the same letter are not significantly different.

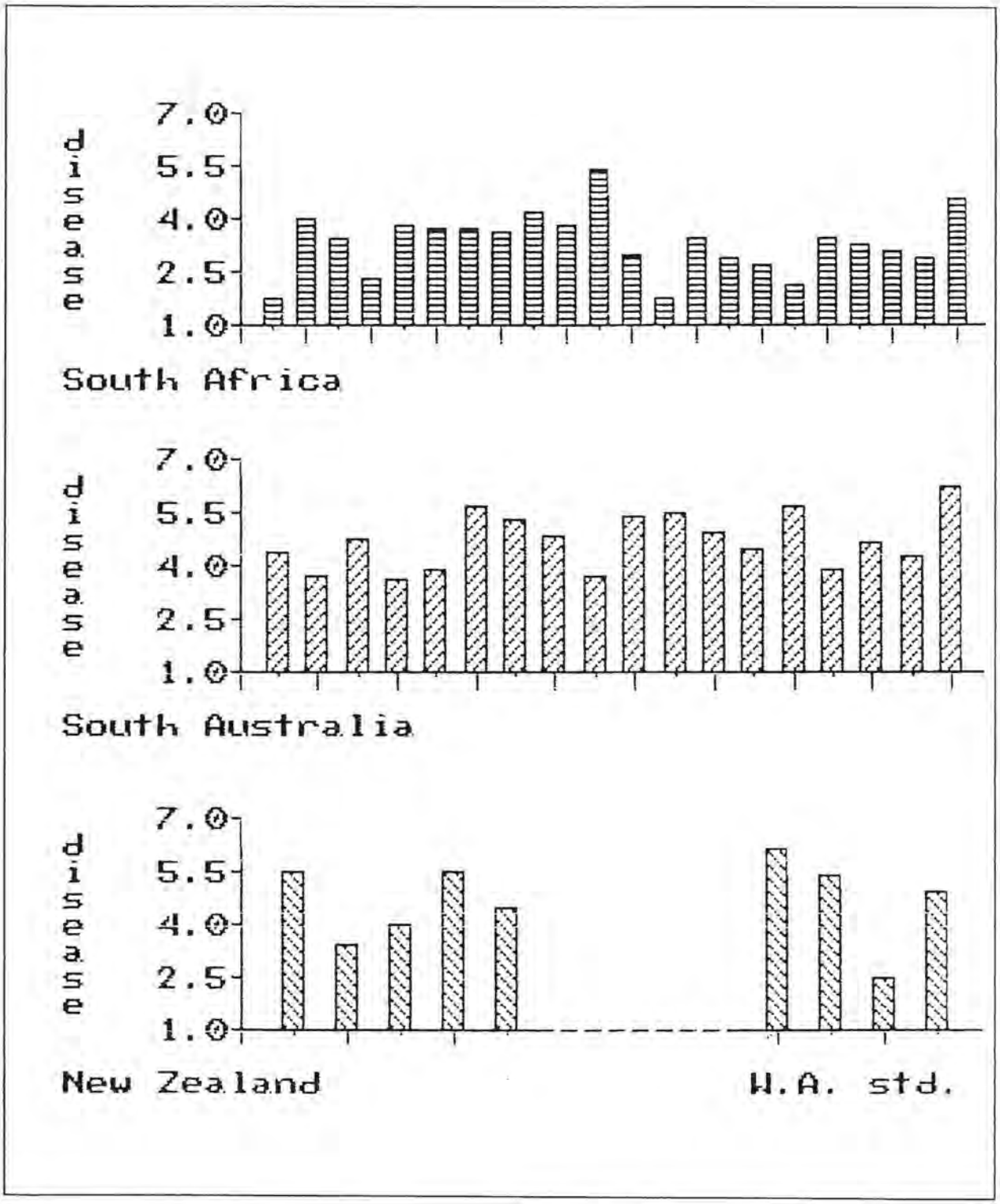

FIGURE 24

Seedling disease intensity for families in the major domestic populations of *Pinus radiata,* 180 days after inoculation with *Phytophthora cinnamomi* in test DP1.

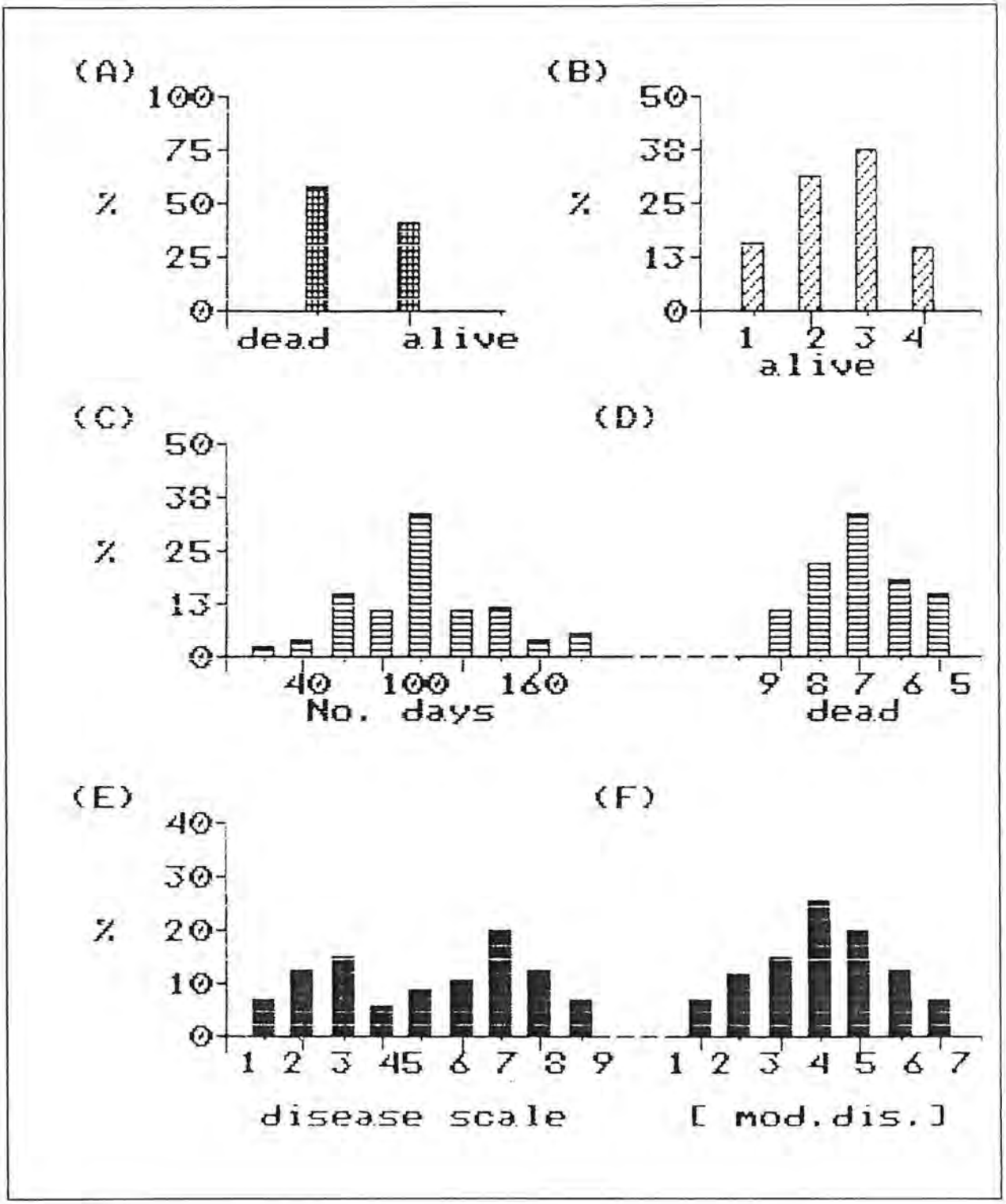

FIGURE 25

Frequency distribution and development of the disease intensity index for test DP2.

FIGURE 25 (cont)

Frequency distribution and development of the disease intensity index for test DP2.

A. distribution of alive and dead seedlings.

B. root assessment classes of the alive seedlings.

C. frequency distribution of dead seedlings.

D. disease codes for dead seedlings, based on 20 - 40 day intervals.

E. combination of the alive and dead classes to create the disease intensity index.

F. modified disease scale was formed by combining disease codes 4, 5, 6 in (E) to make the new code 4; this scale has a normal distribution.

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disease in seedling positions adjacent to the bait seedling. Variation within the South African population was increased as there was greater separation between the tolerant and susceptible families. Conversely, the variation within the South Australian population was diminished because of the greater susceptibility of these families. Ranking of the families was essentially the same using either disease code; Spearman's rank correlation coefficient was calculated as 0.99 for the 49 families.

There were more dead seedlings (55%) than alive seedlings in the DP2 tests. Frequency distributions are shown for the root assessment (Table 23) of surviving seedlings and for the seedling death assessment, in Figure 25. Data for dead seedlings (C) were compressed into 5 classes, forming a normal distribution (Figure 25 D); the additional class was included for the dead seedling scale because of the greater incidence of disease in this trial. The composite disease scale from 1 to 9 is shown in Figure 25(E); this was formed by combining (B) and (D).

Variance analysis for the disease intensity scale is shown in Table 26 for both experiments forming the DP2 test. Genetic variances were very large. In both experiments, the South African population had significantly less disease than the New Zealand population ($P < 0.001$), which in turn was significantly less diseased than the South Australian population ($P < 0.001$). The family variation in disease within each population was considerable ($P < 0.001$). This is illustrated in Figure 26.

The disease scale (Figure 25 E) was modified to create a scale with a normal distribution of disease (Figure 25 F). Disease code 4, the most diseased class of the surviving seedling scale (B), and codes 5 and 6, seedlings that died near the end of the test (D), were combined to form the single code 4 in the modified disease scale shown as Figure 25(F). Analysis of variance for this scale gave the same results as for the analysis of the seedling disease scale (Figure 25 E). Genetic variances were both 31% of the total variance and differences between and within populations were all highly significant ($P < 0.001$).

TABLE 26

Hierarchal analysis of variance, heritability and domestic population summary for disease codes of *Pinus radiata* seedlings, 180 days after inoculation with *Phytophthora cinnamomi* in test DP2.

Source	DP2a d.f.	DP2a variance	DP2a sig.	DP2b d.f.	DP2b variance	DP2b sig.
within population						
South Africa	11	15.8	***	9	23.0	***
South Australia	11	14.6	***	5	21.1	***
New Zealand	19	25.9	***	14	27.5	***
W.A. standards	4	49.5	***	3	128.9	***
between populations	3	193.7	***	3	203.9	***
within species	48	33.4	***	34	49.9	***
replication	15	7.0	***	23	12.3	***
residual	720	4.3		782	3.6	
Total	783	6.1		839	5.7	

Summary

Population	DP2a disease code	DP2a h^2_I	DP2a h^2_F	DP2b disease code	DP2b h^2_I	DP2b h^2_F
South Africa	3.3 ***	0.75 + 0.33	0.79	4.0 ***	0.82 + 0.36	0.85
New Zealand	4.5 ***	0.92 + 0.29	0.83	5.3 *	0.75 + 0.28	0.85
W.A. standards	5.3 a	-	-	5.8 **	-	-
South Australia	5.6 a	0.43 + 0.25	0.66	6.5	0.69 + 0.44	0.83

* significant difference at 95% probability level, ** $P < 0.01$, *** $P < 0.001$.

values followed by the same letter are not significantly different.

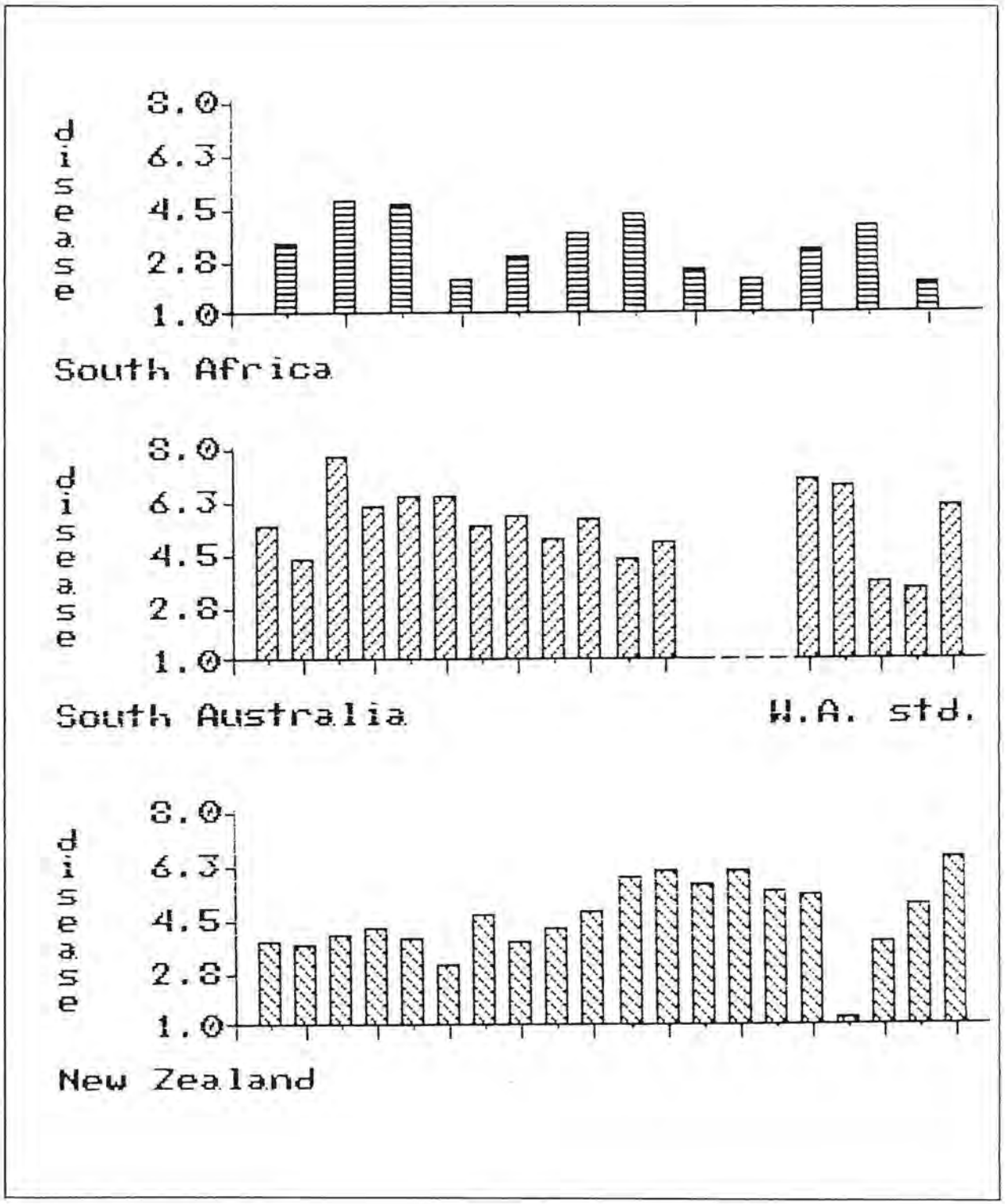


FIGURE 26

Seedling disease intensity for families in the major domestic populations of *Pinus radiata*, 180 days after inoculation with *Phytophthora cinnamomi in* test DP2.

The South African population has provided significantly more "tolerant" trees than the others to develop a breed of radiata pine that has resistance to the *P. cinnamomi* disease (Table 27). Priority should be given to screening of the South African population to rapidly increase the number of tolerant selections for the new breed.

TABLE 27

Chi-square test for the number of *Pinus radiata* families in *Phytophthora cinnamomi* disease response classes, for the major domestic breeding populations.

Population	number of families					
	DP1			**DP2**		
	tolerant	average	susceptible	tolerant	average	susceptible
South Africa	12	9	1	8	4	0
South Australia	1	5	12	0	5	7
New Zealand	1	2	2	7	6	8
P. radiata spp.	14	16	15	15	15	15

calculated Chi-square DP1 = 20.0 DP2 = 14.8

tabular Chi-square = 18.5 (d.f. 4, $P < 0.001$), 13.3 ($P < 0.01$). Fisher and Yates, 1963.

The individual narrow sense heritabilities, calculated for seedling disease intensity, were very large and consistent. They were calculated as 0.58, 0.99, 0.75 and 0.82 for the South African population, 0.39, 0.33 and 0.43 for the South Australian population, and 0.92 and 0.75 for the New Zealand population (Tables 25 and 26). These large and apparently anomalous values were due to the use of the half-sib covariance ($^1/_4\ \delta^2_A$) in the numerator of the heritability equation. Obviously, from Figures 27 and 28, the expression of disease in the half-sib families was too predictable for seedling groups where only the mother tree was common. This was further supported (Chapter 3.5) by a small family sample of 6 seedlings giving the same result as a family sample of 40 seedlings. The pollinator appears to be of little consequence.

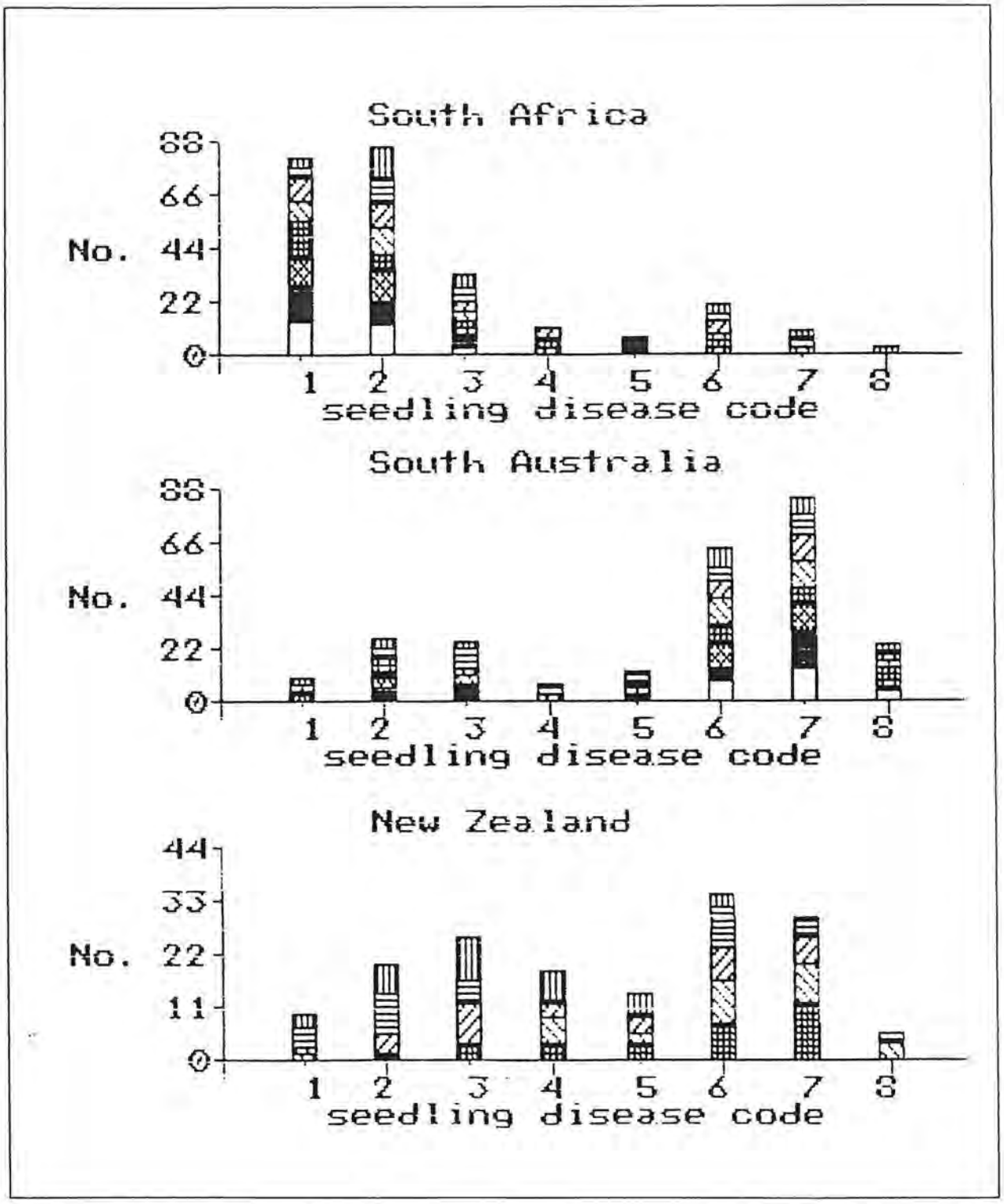


FIGURE 27

Distribution of seedling disease codes within families of the major domestic populations of *Pinus radiata*, 180 days after inoculation with *Phytophthora cinnamomi* in test DP1. Patterns are for the number of seedlings from the same family.

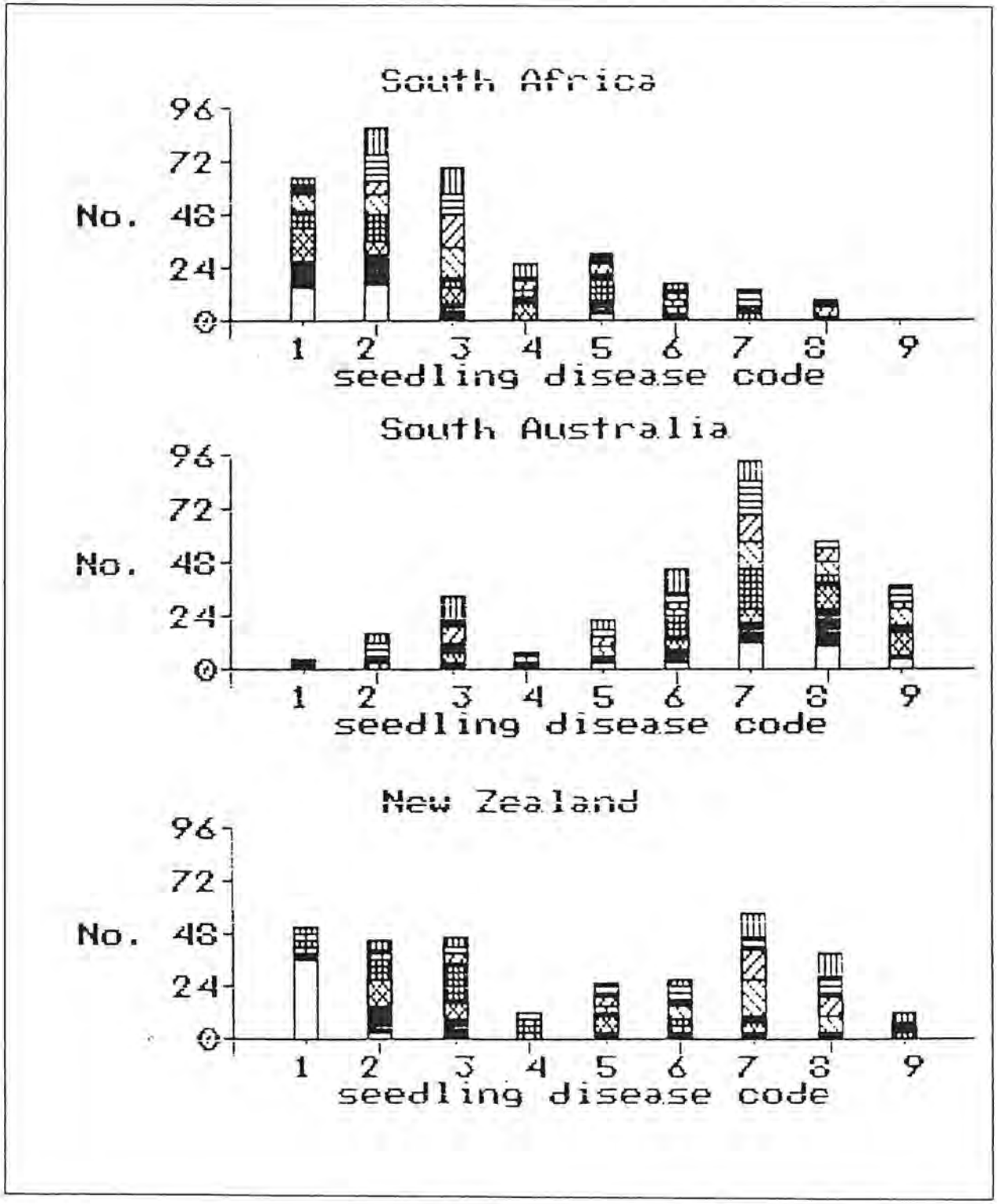


FIGURE 28

Distribution of seedling disease codes within families of the major domestic populations of *Pinus radiata*, 180 days after inoculation with *Phytophthora cinnamomi* in test DP2. Patterns are for the number of seedlings from the same family.

4.3.4 Discussion

Results have shown that there is tremendous genetic variation in resistance to *Phytophthora cinnamomi*, in the principal world domesticated populations of *Pinus radiata* and that it is very highly heritable. Consistency of results for the glasshouse screening method has been demonstrated by the similar performance of the genetic standards, in these and in all other tests.

Seedlings from the South African population have consistently shown a greater degree of resistance to *Phytophthora cinnamomi*, than have seedlings from New Zealand and South Australia. Variation within the South African population, and indeed for the others, was also very large. The South Australian population had double the number of dead seedlings compared with the South African population. This result was repeated in the second test using a completely different set of families to constitute the populations. Again, variation within the populations was very large.

The resistant response versus the susceptible response of these two populations could be indicative of a population change brought about by selection for the character. However, in neither case was selection of plus trees made in diseased forest areas. *Phytophthora cinnamomi* is not considered to be a problem in plantations in either country. From South Africa, Van der Sijde (personal communication, 1983) has said "... although *P. cinnamomi* is present in most of our forest areas, it seldom causes problems in our plantations albeit severe damage does occasionally occur in poorly managed tree nurseries". There may have been indirect selection for this character as plus trees had to be healthy crowned and vigorous. Von Broembsen (1984 a and b; 1987) has shown that *P. cinnamomi* is indigenous to the southwestern Cape Province in South Africa and is in *Pinus radiata* plantations where plus trees were selected.

Contrary to this, Davison and Bumbieris (1973) were confident that *Phytophthora cinnamomi* was not present in the Southeast plantations of South Australia, the source of the South Australian plus trees in this study. The difference in response to *P. cinnamomi*, by South African and South Australian seedlings could reflect an indirect selection for this disease character. The large variation within the South African population suggests that this indirect selection was not complete.

Another explanation for these observed population differences could be the selection of plus trees for health and vigour on marginal sites. The South Australian plus tree selection was extensive, and was concentrated on the highest site quality stands developed on deep yellow sands. Selection intensity was 1 in 1000, or the best tree per hectare (Boomsma *et al.*, 1983). A more intensive selection was practised in South Africa and a tree had to be outstanding in vigour and tree form in comparison to its neighbours to be included in the programme; there was also interest in trees which were adapted to marginal sites (Lange, 1979). This supposition is supported by Western Australian data, where a higher frequency of plus trees selected on marginal plantation sites at Grimwade were rated as tolerant to *P. cinnamomi* in glasshouse tests, compared with selections from more fertile sites. In addition, many more families from the intensive New Zealand "850"

selections were classed as tolerant, compared with the low intensity "268" selections conducted in the fertile volcanic soils of the Kaingaroa forest.

A final explanation infers the origin of *Pinus radiata* for the domesticated populations. Data from the natural population tests have shown large variations between the major provenances in their response to *Phytophthora cinnamomi* inoculation. The Cambrian provenance was the most tolerant and also the least variable. The Monterey provenance had a similar tolerance, but there was also considerable variation between the individual families within the population. The Ano Nuevo provenance was susceptible, with more than double the number of dead seedlings compared with the Monterey and Cambrian provenances. Variation within the susceptible Ano Nuevo population was very large. Because of their isolation, the Islands can be ignored as a gene source of the domestic populations. The origin of *Pinus radiata* in South Africa is uncertain, but Poynton (1977) reasoned that the Monterey provenance predominated. Moran and Bell (1987), using isozyme techniques to identify the source of Australian *P. radiata*, concluded that the Monterey and Ano Nuevo populations were probably the major source of the original introductions. There is no evidence that the Cambrian population formed any part of the original introductions to Australia, New Zealand or South Africa.

Greatest rewards were achieved from the South African population. Sixty percent of the selections tested were found to have an acceptable degree of resistance to *Phytophthora cinnamomi*. This compared with 30% for the New Zealand population, and only 3% for the South Australian "Super 80" series population. The most resistant family (268/323) was found in the generally susceptible New Zealand "268" series population. This suggests that no population should be ignored in the search for genotypes to develop a wide genetic base population that has resistance to *P. cinnamomi* as a major trait.

Genetic variation in resistance to *P. cinnamomi* was independent of the vigour of the seedling. Spearman's tests have shown that there was no correlation between the ranking of families on their height before inoculation, and disease response. Family heights of seedlings in the glasshouse have been correlated with the four to five-year growth of young trees on disease-free sites. In the absence of any genetic correlation between disease resistance and vigour, the implication is that breeding for disease resistance will have no effect on the vigour of the population used on disease-free sites.

Cotterill and Zed (1980) have shown that height and diameter were strongly and positively correlated, and both were similarly correlated with stem straightness. Breeding for disease resistance should have no deleterious effects on the stem straightness of the population. On a diseased site, there is a strong positive correlation between disease resistance and height and diameter growth. A positive correlation with stem straightness is also expected as trees will be more stable associated with a less rotted root system; trees with a rotted root system are unstable and are subject to butt and stem sweep.

Root regeneration capacity has been suggested earlier in this manuscript as a possible mechanism for disease resistance. Studies by Nambiar *et al.* (1982) have shown that *Pinus radiata* rooting characters were highly heritable. They have found that family 50048, which is one of the fastest growing first generation selections in Australian breeding programmes, was able to initiate more new roots than other families in their study, which included families 80055, 12038 and 10956. Families 50048 and 80055 have been classified as moderately susceptible to *Phytophthora cinnamomi*, and 12038 and 10956 were classified as tolerant (Butcher and Stukely, 1986). At a soil temperature of 14°C, root

initiation was similar for families 50048 and 12038 (Nambiar *et al.*, 1982) and was significantly higher than for families 80055 and 10956. The ability to produce more roots is not a simple explanation for differential disease resistance.

The moderate soil moisture / temperature regime for the glasshouse environment gave maximum expression of the disease and variability of response for the different *Pinus radiata* families. Disease development in this environment was significantly greater than in an environment inducing stress. Vigorous healthy growth of the pine seedlings, and soil moisture and temperature conditions optimum for the growth of *Phytophthora cinnamomi*, have been defined as the ideal conditions for the glasshouse screening test.

Phytophthora cinnamomi disease, expressed as the number of dead seedlings per family, was perfectly correlated with the disease intensity scores of individual seedlings. Family heritabilities for seedling mortality were 0.87 and 0.82 for South Africa, 0.58 and 0.68 for South Australia, and 0.84 for New Zealand, calculated from plot means. Family heritabilities for disease intensity, calculated from individual seedling scores, were 0.85, 0.87, 0.79 and 0.85 for South Africa, 0.78 and 0.55 for South Australia, and 0.83 and 0.85 for New Zealand.

The individual narrow sense heritabilities for seedling disease intensity were calculated as 0.58, 0.99, 0.75 and 0.82 for the South African population. 0.39, 0.33 and 0.43 for the South Australian trees, and 0.92 and 0.75 for the New Zealand population, in these studies. The values are extraordinarily high and consistent for different family sets and environmental test conditions. The very high and very consistent individual and family heritabilities of these open-pollinated families, in which the male pollen parent was not controlled, has led to the supposition that the female cone parent contribution may be more important than that of the male. They were estimated for each intermating population on the assumption that progeny raised from open-pollinated seed orchard parents and plantation ortets were true half-sibs and therefore have a coefficient of relationship of $^1/_4$ (Falconer, 1960). This assumption is correct for the plantation ortets and is realistic for the seed orchard parents (Moran *et al.*, 1980). Apparently the mitochondrial and chloroplast DNA has a greater proportional effect than nuclear DNA on the *P. cinnamomi* disease resistance of *Pinus radiata*.

Cytoplasmic inheritance of this disease resistance can be confirmed by progeny testing reciprocal crosses of parents that are tolerant and susceptible for this trait. None of the tests reported in this manuscript were of this nature. Our first cytoplasmic inheritance study was inoculated with *Phytophthora cinnamomi* in December 1987. Control pollinations for a six parent complete diallel were commenced in July 1986 for a major cytoplasmic inheritance study.

Data obtained by Langner (1952) indicated the occurrence of a cytoplasmic background of susceptibility to needlecaste in hybrid crossings of *Larix decidua* and *L. leptolepis*. Cytoplasmically inherited characteristics have not been utilised in tree breeding but are commonly used in hybrid corn breeding (Manion, 1981).

4.4 GENOTYPIC VARIATION OF PINUS RADIATA IN THE FIELD

YOUNG TREE RESPONSE TO *PHYTOPHTHORA CINNAMOMI.*

4.4.1 INTRODUCTION

Glasshouse inoculation tests with *Phytophthora cinnamomi* have been very effective in identifying families of *Pinus radiata* with high tolerance to this disease. The advantage of the glasshouse test is the control that can be exerted on the environment, the pathogen, the seedling and any other factor that can cause variation in response. However, where possible, screening for resistance to fungal diseases should be carried out in the field. Russell (1978) lists two main advantages of field tests over glasshouse tests; much larger populations can be dealt with in the field, and plants are tested for resistance under natural conditions.

The field test is important in the study of the host pathogen interaction in the natural environment, and its development with time and age of the tree. Intensive management practices, such as inter-row cultivation, clover pasture, fertilisation, pruning and thinning can all interact with the disease to affect the susceptibility of the host; these require field study.

The two-year results of the first field test, reported in Butcher *et al.* (1984), showed a very high correlation of the field test family seedling mortalities with the glasshouse test. An additional field test, with a greater number of families and a control site, was established to provide the essential backup to the glasshouse test. This section reports on the six-year results of the two field tests.

4.4.2 Materials and Methods

Areas showing severe jarrah dieback symptoms in the native forest were chosen in the Donnybrook Sunkland for the field inoculated trial sites. Soils were described by McCutcheon (1978) as grey coloured, sandy at the surface, often becoming heavier in texture at depth, poorly drained and waterlogged for much of the year. One replicate of the FP1 test was on a brownish-yellow, silty-clay loam soil of the valley floor. Chevis (1984) reported that deaths amongst jarrah and *Pinus radiata* were highest on these soils.

The control site for the FP2 test was chosen on a yellow sand soil, high in the landscape. Its vegetation consisted of a healthy *Banksia grandis* and *Eucalyptus marginata* overstory, and a healthy understory containing *Dasypogon bromeliaefolius* and *Pultenaea reticulata*.

Test sites were windrow cleared, burnt and mound ploughed prior to pegging of the field design. Plots were located within the windrow bays.

Stratified seed of open-pollinated families of *Pinus radiata* was sown in open nursery beds at Wanneroo in September. Seedlings were labelled and lifted for transplanting to the field site in May/June.

PLATE 6

Field test site FP1 in the Donnybrook Sunkland, ten weeks after planting.

Field Population Test (FP1) :

Experimental design

Split-plot, (I = 2, F = 26, R = 21).

2 *Phytophthora cinnamomi* inoculum levels (natural inoculum, natural plus applied inoculum).

26 *Pinus radiata* families (6 tolerant, 6 mod-tolerant, 4 average, 6 mod-susceptible, 4 susceptible).

21 single-tree-plot replications.

Seedlings completely randomised in the principal factor blocks.

Hand planted in May 1980, at the normal plantation spacing of 3.5 m x 2.5 m. After planting, each seedling received 100 g of an N-P fertiliser, and was later treated with a foliar spray containing Zn, Cu and Mn trace elements.

Phytophthora cinnamomi A2 isolate (318) was applied in September 1980, 17 weeks after the seedlings were transplanted, by burying 4 inoculum plugs adjacent to the treatment seedling (Plate 7)

In the FP1 test, treatment numbers were increased by the inclusion of seedlings from *Pinus radiata, P. pinaster* and *P. taeda* orchard sources. *Pinus radiata* from the Manjimup seed orchard is the routine seed source for planting, and the resistant *P. pinaster* and *P. taeda,* are the principal alternative species.

Field Population Test (FP2) :

Experimental design

Split-plot, (I = 3, F = 36, R = 25).

3 *Phytophthora cinnamomi* inoculum levels (natural inoculum, natural plus applied inoculum, and *P. cinnamomi* disease-free site).

36 *Pinus radiata* families (5 tolerant, 8 mod-tolerant, 11 average, 7 mod-susceptible and 5 susceptible, including the hybrid *P. attenuradiata*).

5 single-tree-plot replications.

Seedlings completely randomised in the principal factor blocks.

Hand planted in June 1981, at spacing of 3m x 3m. After planting, each seedling received 100 g of an N-P fertiliser, and was foliar sprayed with Zn, Cu and Mn trace elements in October 1981.

Phytophthora cinnamomi A2 isolates (318) and (1) were applied by burying two inoculum plugs of each isolate adjacent to the treatment seedlings, in September 1981, 9 weeks after the transplanting.

Complete rows of single families were planted adjacent to the FP2 test, for demonstration of the disease response (Plate 10).

Seedling health was assessed immediately prior to the inoculation in September. Death checks were made throughout the first summer, at two monthly intervals, commencing in November. After this, assessments were made in early January, and in late May of each year, and have continued through to the present. At every assessment, dying or dead trees have had their roots and lower stem collars sampled. Adjacent soil and inoculum plugs (if any) were also collected and were later plated for detection of Phytophthora species.

Tree heights were measured at the age of four years in the FP1 test, and five years in the FP2 test.

PLATE 7

Inoculation of the FP1 field test seedlings with four branch-plugs, inoculum of *Phytophthora cinnamomi*. (branch-plugs in position before burying)

4.4.3 RESULTS

In FP1, the seedlings were inspected in August 1980, about ten weeks after transplanting, and all appeared healthy (Plate 6). When the inoculum was applied seven weeks later, most of the seedlings were healthy and growing vigorously (Plate 7), although some of the *Pinus pinaster* and *P. taeda* seedlings were still small. No post-planting losses were observed.

The first seedling deaths in the FP1 test were in late November, about 60 days after inoculation. By the end of January, 75 seedlings in the test had died and *Phytophthora cinnamomi* was recovered from the roots and/or collar segments of 70 seedlings, while *P. citricola* was recovered from the roots of two dead *Pinus radiata* seedlings on the natural inoculum site. There was a high recovery of *Phytophthora cinnamomi* from the infected seedlings and also from the buried inoculum plugs, indicating survival within plugs in the summer soil conditions. Results of the first year sampling of all dead seedlings, and the recovery of *Phytophthora* species were tabled in Butcher *et al.* (1984).

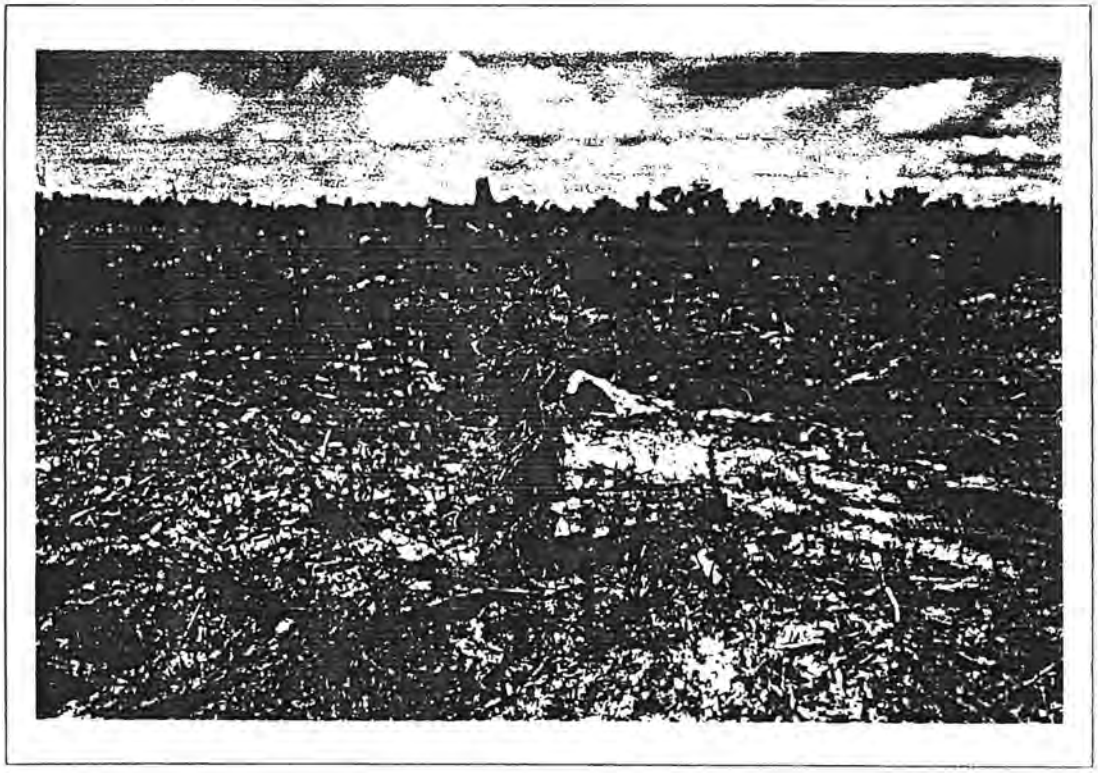


PLATE 8

Seedling death caused by *Phytophthora cinnamomi*, in January 1981, fifteen weeks after inoculation, in *Pinus radiata* susceptible family 60017.

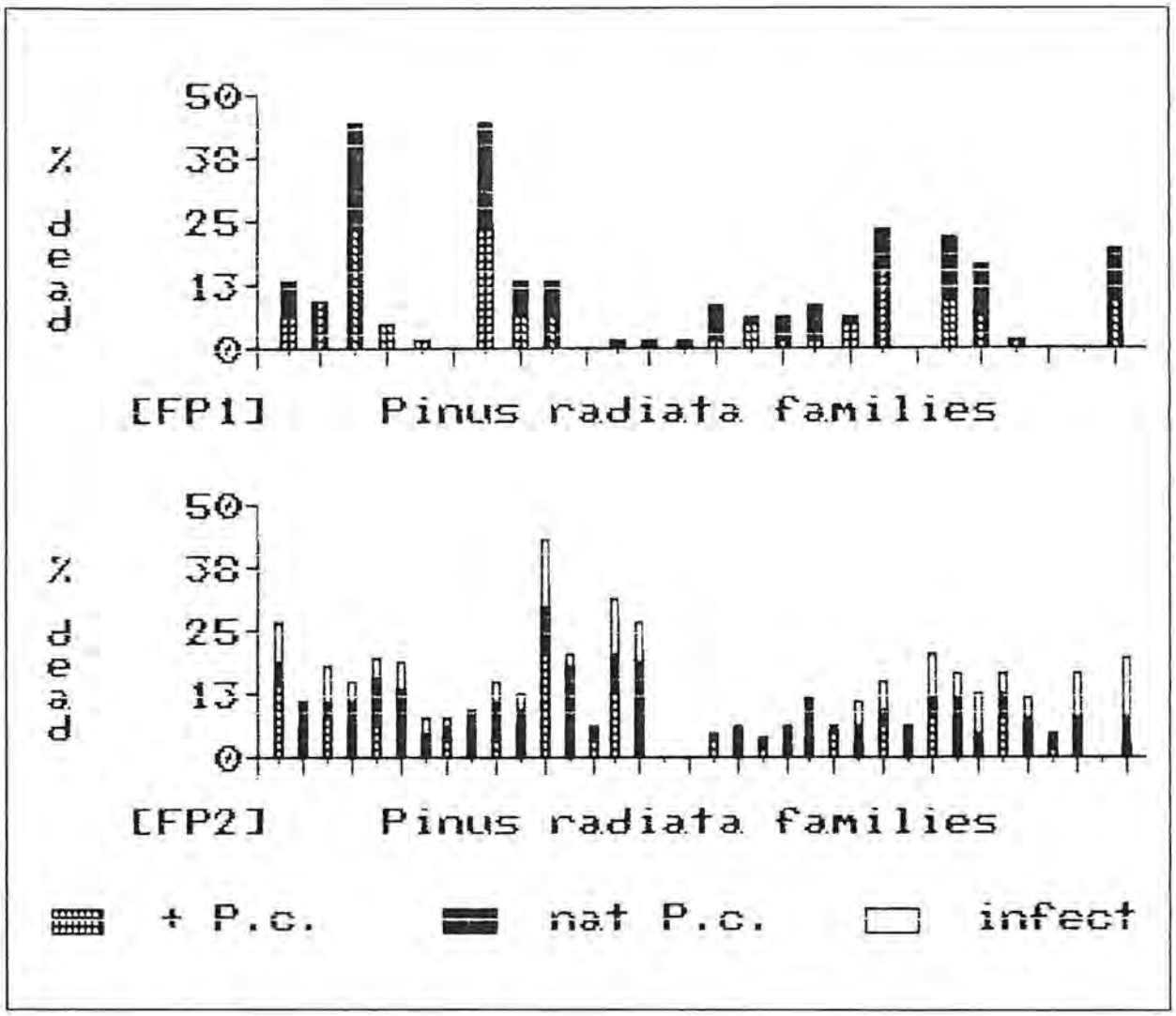


FIGURE 29

Percentage of seedlings killed by *Phytophthora cinnamomi*, for *Pinus radiata* families planted in the field tests.

The majority of seedlings that died were initially vigorous and healthy (Plate 8). If *Phytophthora* was isolated from the sampled roots and collar, then it was considered to be the causal agent of the seedling death. Figure 29 shows the percentage of *Phytophthora*-killed seedlings and young trees, for families of *Pinus radiata* planted on each site in both field tests.

Tree mortality after two years was calculated for noncontiguous family plots of seven trees for the analysis of variance (Table 28). The variation between families in their response to *Phytophthora cinnamomi* was very large and consistent. Differences in mortality ranged from 0% in tolerant families 12038, 60022, 60027, 60038 and 80007, through to maximum values of 42% and 48% for susceptible families 60024 and 60017. Between family variance accounted for 55% of the total test variance. Heritability of the family means was calculated as 0.87.

TABLE 28

Hierarchal and split-plot analysis of variance, and heritability for the two-year percent mortality (transformed into arcsin square root) of *Pinus radiata* seedlings killed by *Phytophthora cinnamomi*, in field tests FP1 and FP2.

Source	FP1. d.f.	FP1. variance	FP1. sig.	FP2. d.f.	FP2. variance	FP2. sig.
within disease groups						
tolerant	5	22		4	307	
mod-tolerant	5	86		7	148	
average	3	7		10	451	*
mod-susceptible	5	105		6	167	
susceptible	3	583	*	4	714	*
between disease groups	4	4851	***	4	1207	***
main plots						
inoculum (I)	1	28		2	2631	**
replication (R)	2	275		4	1311	*
error	2	21		8	772	
sub-plots						
family (F)	25	890	***	35	1207	***
F*I	25	113		70	282	
F*R	50	115		140	217	
error	50	109		280	218	
Total	155	238		539	316	

* significant difference at 95% probability level, ** $P < 0.01$, *** $P < 0.001$.

Heritability (family) h^2_F FP1 = 0.87 FP2 = 0.82

Inoculation of the FP1 sites with the test isolate of *P. cinnamomi* did not increase mortality. The same culture of this isolate (318) was very aggressive in the second glasshouse study of Butcher *et al.* (1984). There was an interesting effect of block or site. Similar numbers of dead seedlings were observed in the two blocks on type 4c soil (McCutcheon, 1978) and these were significantly higher than on the type 7 soil ($P < 0.05$). The whole area was severely affected by jarrah dieback disease, and a uniform distribution of the pathogen was expected over the sites. The heavier textured type 7 soil generally has a better soil moisture status and perhaps these trees were less stressed.

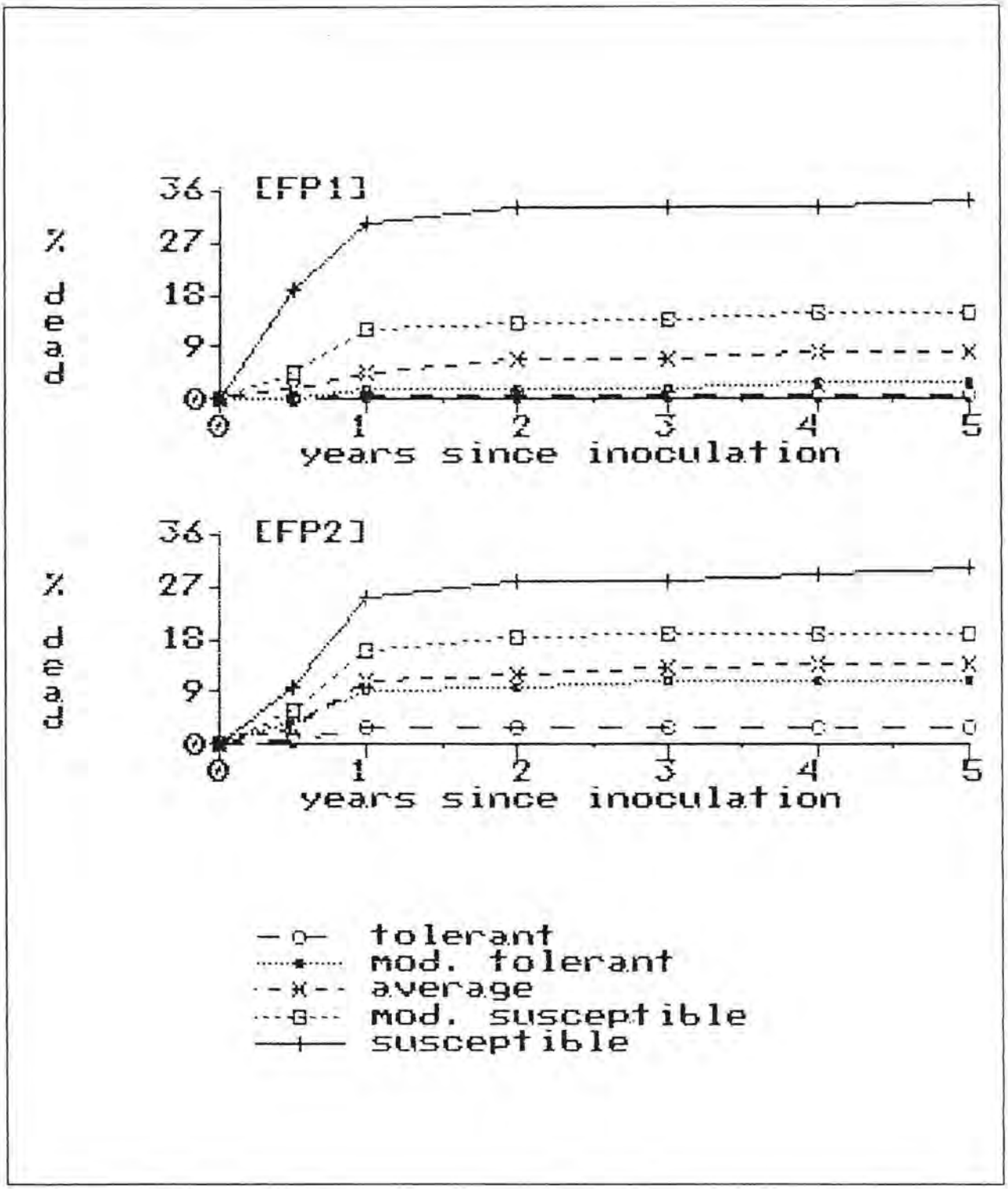


FIGURE 30

Progressive mortality of young trees of *Pinus radiata* up to five years after inoculation with *Phytophthora cinnamomi*, illustrated for the disease response classes.

In the second field test, FP2, 32 seedlings (4%) on the control site were recorded as being severely affected by cutworm (*Noctuidae, Agrotis* spp.) at the time of the inoculation, and they subsequently died. One percent of the seedlings on the inoculated site also died. These seedling deaths were excluded from any of the subsequent analyses.

Most of the seedlings highly vulnerable to *P. cinnamomi* died during the first summer, and these were mainly in families classified as susceptible to the pathogen (Figure 30). Each dead seedling was sampled and plated for *P. cinnamomi* detection, and only dead seedlings associated with the recovery of *P. cinnamomi*, were used in the analysis. *Phytophthora cinnamomi* was recovered from 343 of the 411 dead seedlings sampled. Family mortalities for the analysis of variance were calculated from noncontiguous plots of five trees, for data two years after the inoculation (Table 28).

Variation between families in test FP2, for *P. cinnamomi* death of seedlings, was very large and consistent between the sites (Table 28, $P < 0.001$). ranging from no death in tolerant families 30026, 30028 and 80007, to 40% death in susceptible family 60017. Heritability of the family means was calculated as 0.82. Family mortality at each site is illustrated in Figure 29.

The difference in seedling mortality between inoculated sites was highly significant ($P < 0.01$). Most deaths were associated with the applied inoculum site (18%) and these were significantly more than the natural inoculum site (13%). The control site, which was supposedly free of *P. cinnamomi*, had 11% of the planted seedlings killed by *P. cinnamomi*. Clearing of the healthy *Banksia* vegetation and site preparation has obviously spread *Phytophthora* to this "clean" site.

The abnormal summer rainfall in January 1982 (47 mm compared with the average 10 mm) was highly conducive to *Phytophthora* disease development. Soil moisture and temperatures were ideal for the colonisation of pine seedling roots from point infections of the pathogen. This may explain the different results at the natural inoculum site; inoculum plugs as the point infections increased the disease. At the control site, disease spots were the infection point.

The incidence of disease in families of *Pinus radiata* was very highly correlated between the glasshouse and the field tests. Spearman's correlation of the ranks of families was 0.80 for the test FP1, and 0.87 for the test FP2. Both were highly significant ($P < 0.001$), which indicates the same ordinal scale for mortality in the glasshouse and the field tests. This correlation is illustrated in Figure 31, using rank scores (Cotterill *et al.*, 1983).

In the glasshouse tests, disease intensity of individual seedlings was expressed on an ordinal scale, ranging from rapid death through to a minimal effect on root mass or height growth. A similar scale can be developed for the field inoculated seedlings. Figure 30 shows that most of the susceptible seedlings die during the first summer in the field. Death of the young trees continues at a reduced rate and is usually in "susceptible" families. Plate 9.1 shows the *Phytophthora cinnamomi*-caused death of a two-year-old tree of susceptible family 60017, and Plate 9.2, a recent death of a six-year-old tree of susceptible family 20083.

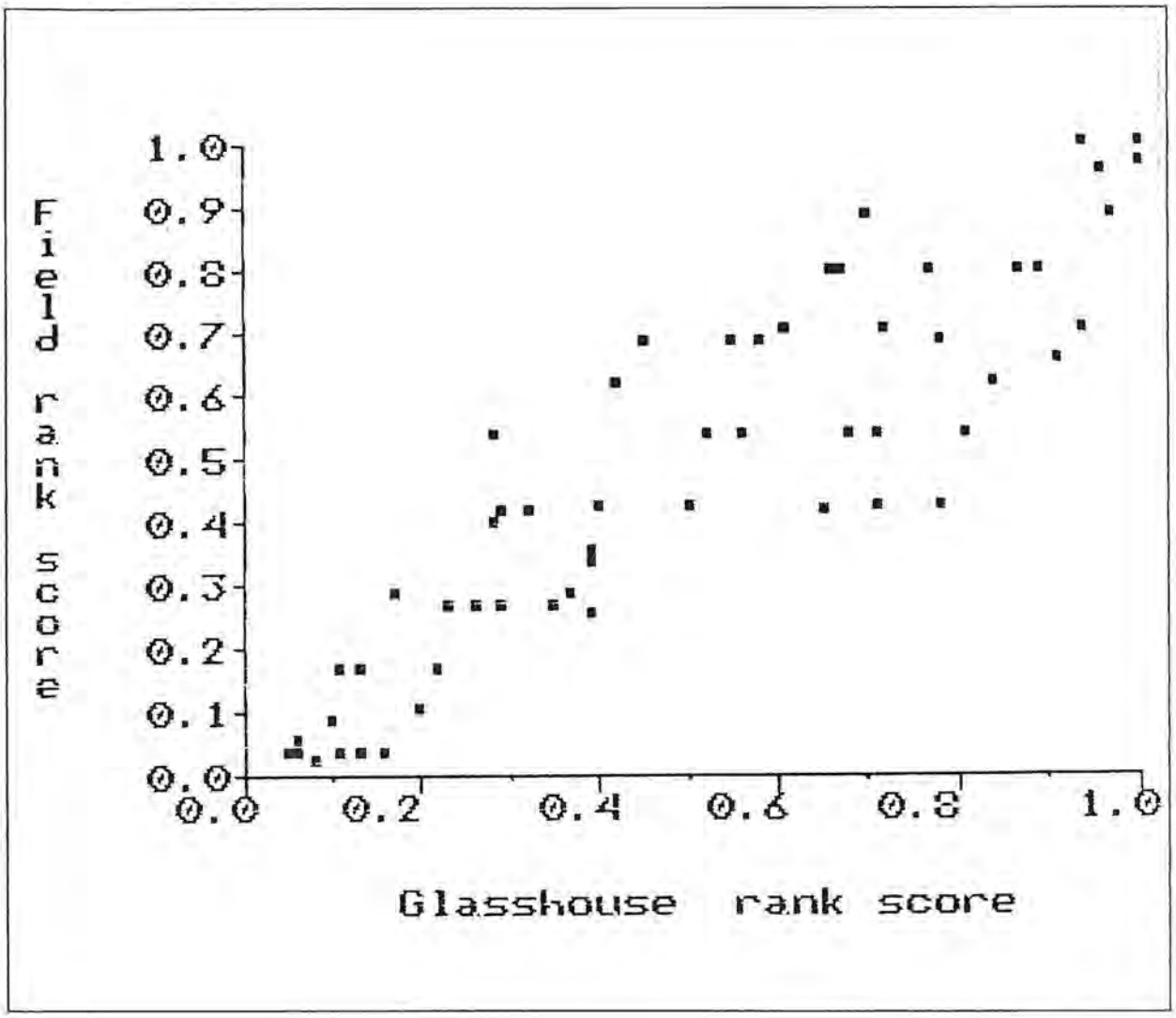
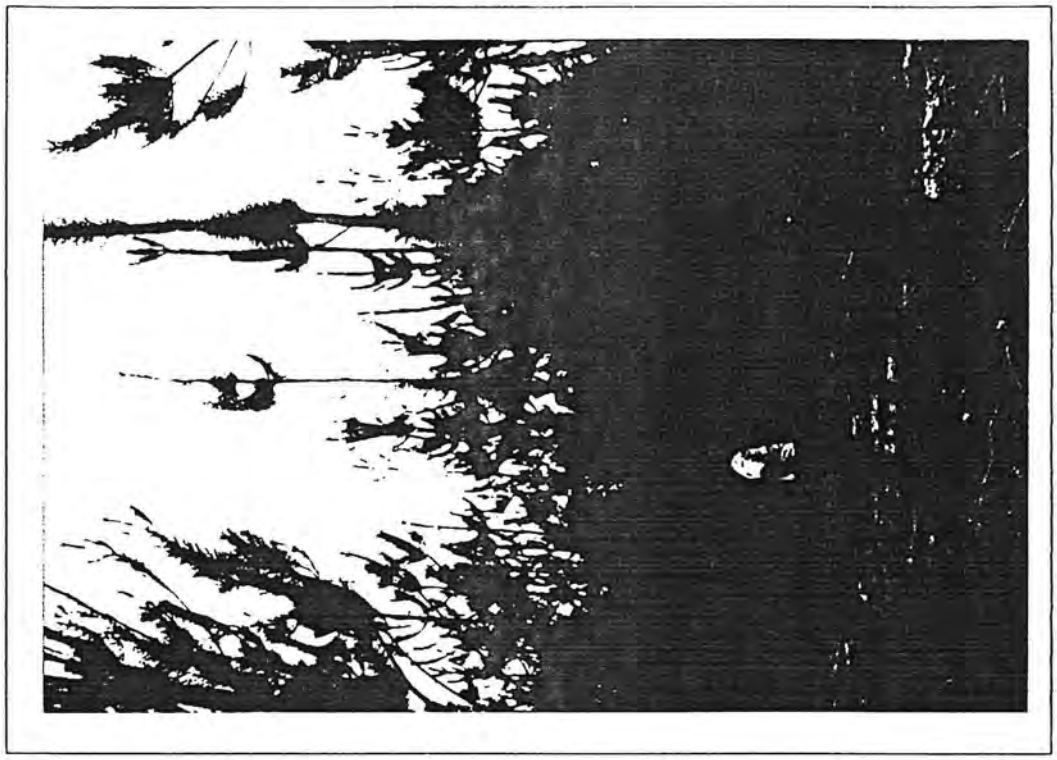


FIGURE 31

Comparison on ranking of seedling and young tree mortality, associated with *Phytophthora cinnamomi,* for *Pinus radiata* families common to the field and glasshouse tests.

The sub-lethal effects of *P. cinnamomi* on *Pinus radiata* may not be as obvious but are of equal significance (Tables 29 and 30). Tree heights were measured at the age of four years in test FP1, and five years in test FP2. The analysis of variance of the noncontiguous plot means is shown in Table 29 for both tests. Variation between families in height growth was highly significant ($P < 0.001$). Standard progeny tests of the *P. radiata* breeding population on *Phytophthora cinnamomi* disease-free sites, have indicated that individual heritabilities for tree height were low. Cotterill and Zed (1980) reported intermediate heritability values for height. The high family heritabilities calculated in FP1 ($h^2 = 0.82$) and FP2 ($h^2 = 0.83$) point to other factors having a strong effect on height growth, on



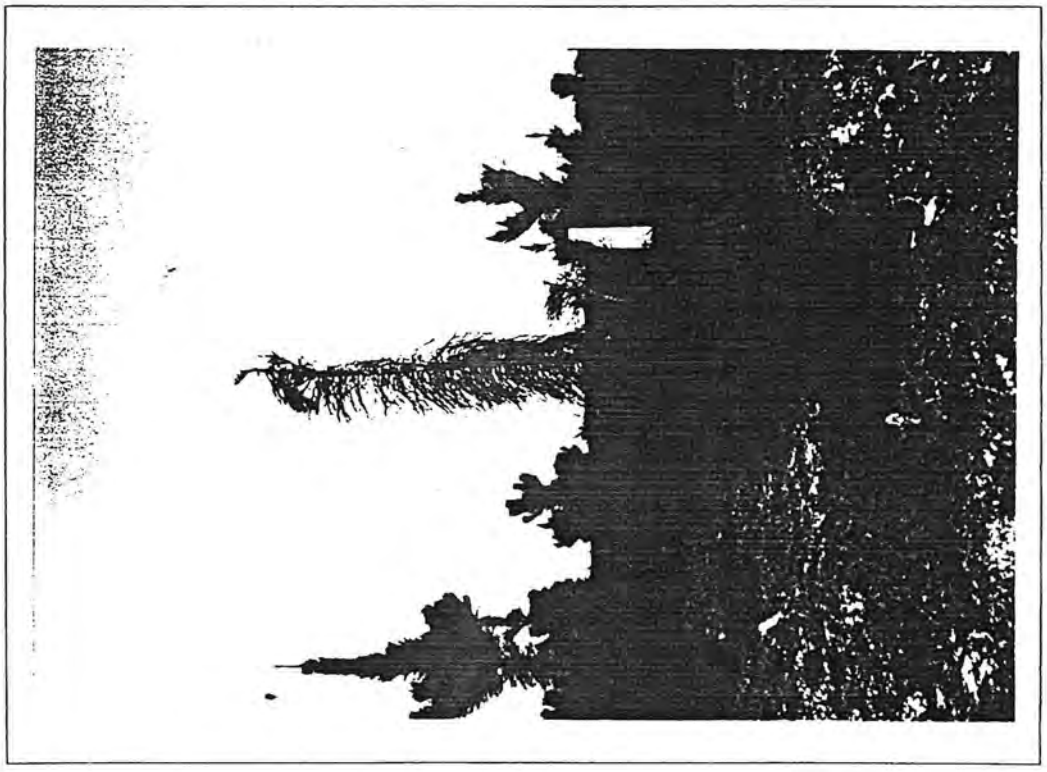


PLATE 9

Death of two-year-old *Pinus radiata* tree of susceptible family 60017, and six-year-old tree of susceptible family 20083 in the FP2 test. Both trees had extensive collar and root infections of *Phytophthora cinnamomi*.
1. two-year-old 60017. 2.six-year-old 20083.

P. cinnamomi infested sites. These factors were revealed in the hierarchal analysis of families placed in the disease groups of Butcher and Stukely (1986). Family variance in height growth was conditioned by their disease classification (Table 29); most of the variation was between these groups. Height growth has been significantly reduced by *P. cinnamomi* infection. *Phytophthora cinnamomi* effects on the incidence and intensity of disease, for the *Pinus radiata* disease response groups, are highlighted in Table 30.

TABLE 29

Hierarchal and split-plot analysis of variance, and heritability for the height of four-year-old *Pinus radiata* trees in field test FP1, and five-year-old trees in test FP2.

	FP1.			FP2.		
Source	d.f.	variance	sig.	d.f.	variance	sig.
within disease groups						
tolerant	5	0.46	*	4	0.4	
mod-tolerant	5	0.55	*	7	1.3	*
average	3	0.24		10	0.7	
mod-susceptible	5	0.94	**	6	0.9	
susceptible	3	0.90	**	4	3.6	***
between disease groups	4	2.55	***	4	7.0	***
main plots						
inoculum (I)	1	0.01		1	7.6	
replication (R)	2	1.09		4	39.2	***
error	2	0.09		4	1.4	
sub-plots						
family (F)	25	0.94	***	35	2.0	***
F*I	25	0.14		35	0.5	
F*R	50	0.18		140	0.6	
error	50	0.17		140	0.4	
Total	155	0.30		359	1.0	

* significant difference at 95% probability level, ** $P < 0.01$. *** $P < 0.001$.

Heritability (individual) h^2_I FP1 = 0.47 + 0.14

Heritability (family) h^2_F FP1 = 0.82 FP2 = 0.83

TABLE 30

Summary of *Pinus radiata / Phytophthora cinnamomi* disease response classes for two year mortality, and height growth of four-year-old and five-year-old trees in field tests FP1 and FP2.

	FP1			FP2.		
family response	n	mortality (arcsin%)	tree height m	n	mortality (arcsin%)	tree height m
tolerant	6	1 a	4.0 b	5	3 **	7.8 **
mod-tolerant	6	5 a *	4.3 b	8	9 *	7.3 d
average	4	12 **	4.0 b *	11	13 **	7.1 d
mod-susceptible	6	20 ***	3.5 c	7	19 ***	7.2 d **
susceptible	4	34	3.7 c	5	29	6.6
modified class						
tolerant	12	3 **	4.1 b	13	7 ***	7.5 ***
average	4	12 ***	4.0 b ***	11	13 ***	7.1 d
susceptible	10	26	3.6	12	23	6.9 d

* significant difference at 95% probability level, ** $P < 0.01$, *** $P < 0.001$.

values followed by the same letter are not significantly different.

Contamination of the FP2 control site prevented the direct comparison of families growing in the presence/absence of *Phytophthora cinnamomi*, and the quantification of the sub-lethal infection effect. This was examined by comparing the 3.5 year height growth of sixteen families in a standard progeny test at Collie on a *P. cinnamomi* disease-free site, with families common to the FP2 test. Variance analysis and summary

TABLE 31

Hierarchal analysis of variance and summary of *Pinus radiata* height growth, for families common to the *Phytophthora cinnamomi* inoculated field test FP2 and to the disease-free progeny test at Collie.

Source	FP2 d.f.	FP2 variance	FP2 sig.	Collie d.f.	Collie variance	Collie sig.
within disease classes						
tolerant families	5	1.91	**	5	0.35	
average families	5	1.07	*	5	0.22	
susceptible families	3	3.62	***	3	0.74	*
between disease classes	2	2.62	**	2	0.05	
within species	15	2.07	***	15	0.34	
replication	7	5.72	***	5	14.91	***
residual	105	0.45		75	0.20	
Total	127	0.94		95	1.00	

Summary

P. c. disease / response class	n	FP2 (*P. c.* site) height at 5 yr.		n	Collie (control site) height at 3.5 yr.	
tolerant families	(272)	7.4m **		(263)	4.8m	b
average families	(265)	7.0	a	(265)	4.8	b
susceptible families	(162)	6.9	a	(184)	4.9	b

n (272) number of living trees measured.

* significant difference at 95% probability level, ** $P < 0.01$, *** $P < 0.001$.

values followed by the same letter are not significantly different.

are given in Table 31. At the disease-free site, seedling death and height growth were similar for each disease group classification. Conversely, the differences between the same family groups on the *P. cinnamomi* inoculated site were highly significant ($P < 0.001$) for height growth and mortality. Tolerant family trees were 0.5 m taller than susceptible family trees.

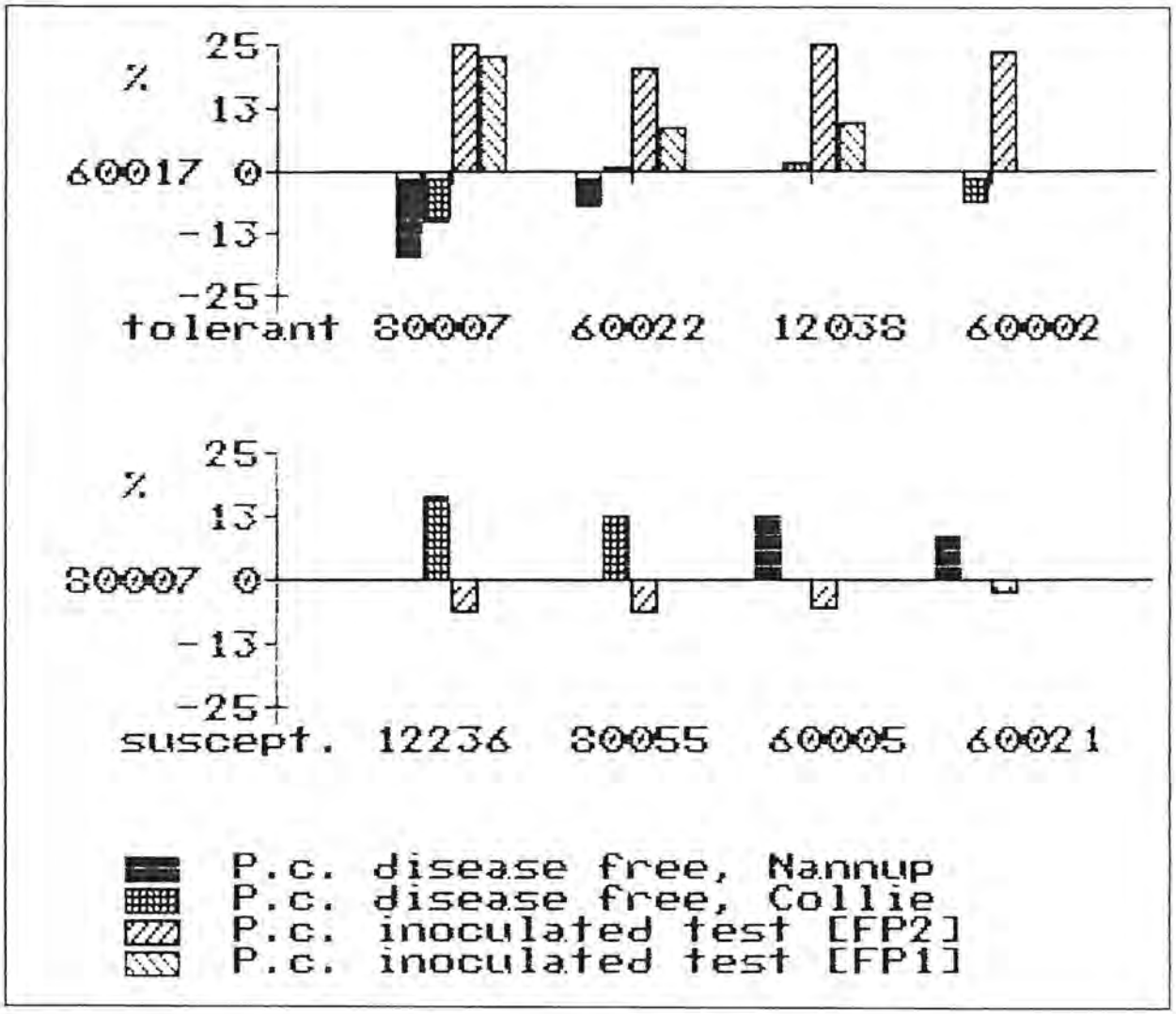


FIGURE 32

Comparison of height growth for four to five-year-old *Pinus radiata,* tolerant and susceptible families, growing on sites inoculated with *Phytophthora cinnamomi,* and on disease-free sites.

Individual family comparisons for four to five-year height growth are illustrated in Figure 32, for tolerant families 80007, 60022, 60002 and 12038, relative to the height growth of susceptible family 60017, and for susceptible families 12236, 80055, 60005 and

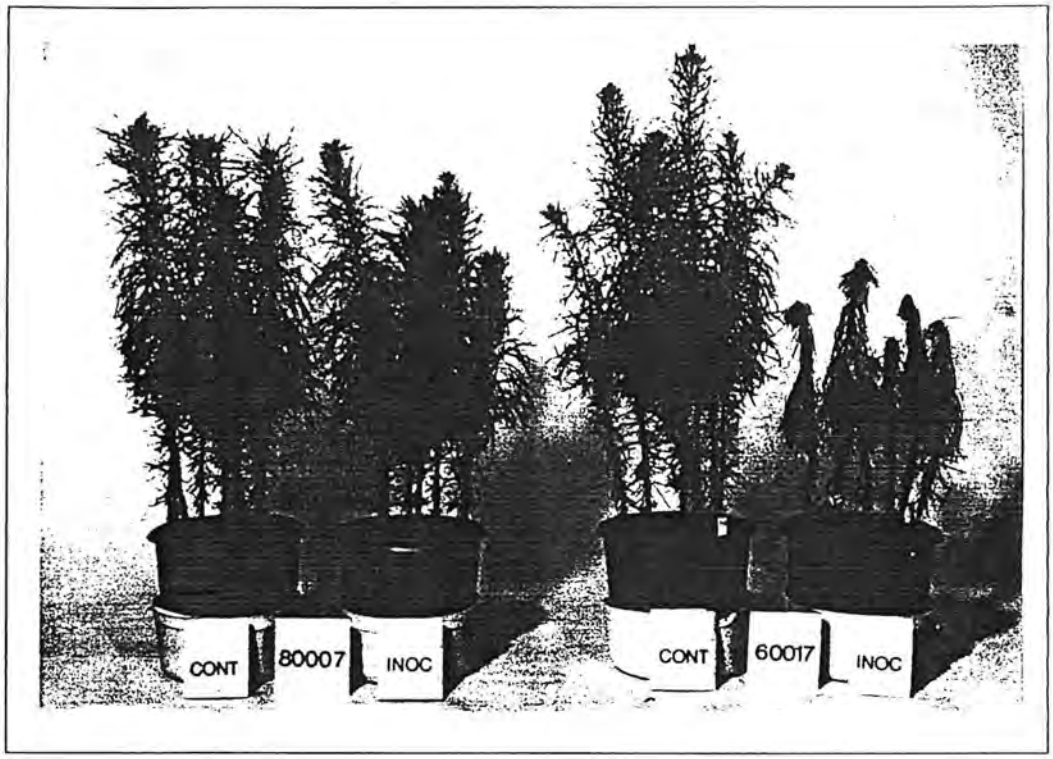


PLATE 10 Comparison of tolerant family 80007 and susceptible family 60017 following inoculation with *Phytophthora cinnamomi*, as seedlings in the glasshouse test, and as four-year-old trees in the FP2 test.

1. glasshouse test, uninoculated pots on the left (cont) *P. cinnamomi* inoculated pots on the right.
2. field test, *P. cinnamomi* applied to site; left tree row - tolerant 80007, right tree row - susceptible 60017

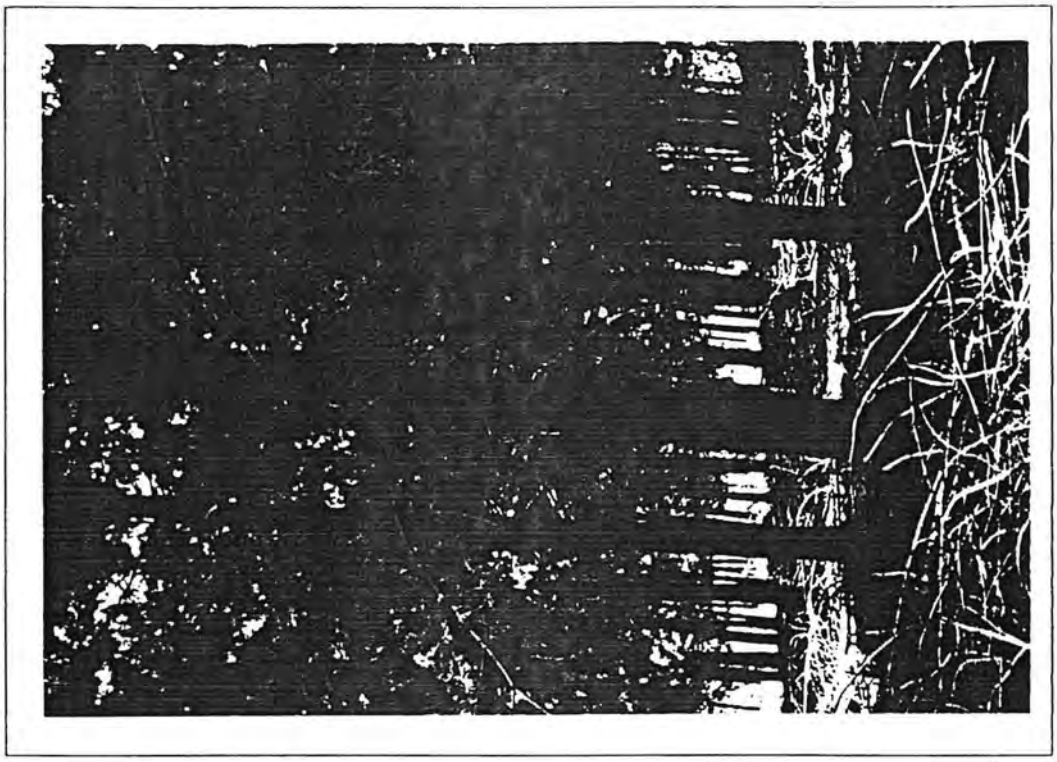


PLATE 11 Comparison of tolerant family 80007 and susceptible family 60017, as fourteen-year-old trees, growing on dieback disease-free sites at Grimwade and Nannup. (trees with yellow band are family 80007, trees with green band are family 60017).

1. disease-free Grimwade site. 2. disease-free Nannup site.

60021, relative to the height growth of tolerant family 80007. The datum line or the zero x-axis line is the height of the susceptible family 60017, or tolerant family 80007 in these two graphs. The trees were grown in the FP1 and FP2 tests, and in disease-free progeny tests at Collie and Nannup. Interpretation of this figure is illustrated for the growth of tolerant family 80007 relative to the susceptible family 60017. Tolerant family 80007 height on the disease-free site at Nannup was 5.5 m, which was 1.1 m (-17%) smaller than the height of susceptible family 60017. Similarly, on the disease-free site at Collie, height of tolerant family trees were 4.5 m, which was 0.5 m (-10%) smaller than trees of the susceptible family 60017. Conversely, on the *P. cinnamomi* inoculated test sites, tolerant family 80007 trees were 4.3 m tall, or 0.8 m taller (+ 23%) than trees of susceptible family 60017 in test FP1, and were 7.5 m tall, or 1.5 m taller (+ 25%) in test FP2.

The ultimate demonstration of the potential impact of *P. cinnamomi* on productivity of *Pinus radiata* plantations is depicted in Plate 10. In the glasshouse, there were no seedling deaths of tolerant family 80007, and seedling heights were depressed by 5% due to *Phytophthora cinnamomi* infections, while 91% of the seedlings of susceptible family 60017 died and height growth of survivors was depressed by 33% (Plate 10.1). Plate 10.2 shows the paired rows of 40 trees of 80007 and 60017 families, planted alongside the FP2 test for demonstration purposes. No trees in the tolerant family 80007 row had died, compared with the death of 24 (60%) seedling/trees in the susceptible family 60017 row. The large difference between the height growth of the surviving three-year-old trees is obvious. Finally, Plate 11 shows these same families as fourteen-year-old trees on disease-free sites at Nannup and Grimwade.

4.4.4 Discussion

The incidence of disease in *Pinus radiata* families was very highly correlated between the field and the glasshouse. These results are confirmation of the glasshouse test as a reliable selection method for resistance to disease.

Susceptible seedlings died within the first year of planting. Seedling mortality ranged from 30% for susceptible families to about 3% for tolerant families. Young tree mortality declined significantly after two years, although trees are continuing to die, mainly in susceptible families. Newhook (1959) also reported that Monterey pine was highly susceptible as seedlings and in the juvenile stage to four to five years.

The clearing and site preparation of the disease-free control site in test FP2 resulted in its infestation with *Phytophthora cinnamomi*. Unless very rigid hygiene practices are enforced and strictly controlled, there is the potential to infest any area that is being prepared for the planting of *Pinus radiata*. If the site conditions are conducive to *Phytophthora cinnamomi,* then disease will develop and the plantation productivity will be lessened.

The abnormal summer rainfall in January 1982 provided the optimum soil moisture and temperature conditions for an epidemic reaction by *P. cinnamomi* on susceptible hosts. This coincided with the first summer of the FP2 test and the second summer of the FP1 test. The moist soil and relatively high soil temperature conditions were responsible for the significantly higher seedling deaths on the artificially inoculated site, and for the proliferation of the disease at the control site. Intensification of the disease by these optimal conditions was mainly in the susceptible families of radiata pine. There was no evidence of any breakdown of the host/pathogen system, given these epidemic environmental conditions. In the second test, the pattern of disease was unchanged. Sunkland progeny tests of *Pinus radiata,* ranging in age from two years to eight years, were also seemingly unaffected by this high summer rainfall event.

Families were ranked similarly for survival and height growth on the natural inoculum site and the artificially applied *Phytophthora cinnamomi* site, for both tests. This shows that resistance is effective against a broad spectrum of the natural population of the pathogen. In addition, although *P. cinnamomi* was the most commonly isolated *Phytophthora* spp. from the tests, *P. citricola* was recovered from the roots and collar of a dead seedling of susceptible family 60004 and from an orchard stock seedling in the FP1 test, and an as yet unidentified *Phytophthora* spp. was isolated from a dead seedling of the susceptible family 60017 in the FP2 test. The development of a durable disease resistant population must have field resistance to other pathogens, as well as general resistance to different isolates of the principal pathogen. These early results are promising.

The most obvious effect of *P. cinnamomi* infection was seedling death in the first year. This averaged 10% for all of the families in test FP1, the same as the Manjimup seed orchard lot, and 13% in the test FP2. The simple and often stated solution, to then plant 10% more seedlings is similarly unintelligible. Tree death has continued in these tests (age six years), and in Agro-forestry trial plots after ten years (Moore, personal communication). There has also been a resurgence of tree deaths following silvicultural pruning or thinning operations, associated with the improved soil moisture and temperature status of these sites.

The insidious sub-lethal infections of *P. cinnamomi* on *Pinus radiata* root systems have been shown to be severely limiting the potential growth of susceptible family trees. The example was given showing a 40% reduction in the five-year height growth of susceptible family 60017 trees, and a 20% reduction in the height growth of moderately-susceptible family 80055 trees. Using all of the height data from the two field tests, FP1 and FP2, the average height of the tolerant / moderately-tolerant disease response group (twenty five families, 1150 trees) was 6.0 m, while the average height of the susceptible / moderately-susceptible group (twenty two families, 900 trees) was 5.5 m. This difference of 0.5 m, or 9% was attributed to the greater disease in the susceptible family trees. When the same analysis was applied to these disease response groups for height growth on a disease-free site, the heights of the trees between the disease response groups were found to be similar.

CHAPTER 5

PINUS RADIATA
PHYTOPHTHORA CINNAMOMI
HOST x PATHOGEN INTERACTION

5.1 Introduction

Just as there is genetic variation in the *Pinus radiata* host population, there is genetic variation in the *Phytophthora cinnamomi* pathogen population. Whether they act independently or in concert affects the planning of the disease control strategy. To be effective, disease resistance must act against all potential strains of the pathogen population. If resistance is race-specific, then there is the potential for selection pressure by the genotypes of the host, on the constitution of the races in the population of the pathogen (Scott *et al.*, 1978), and stability is bound to decline in long rotations of plantation trees. Genetic systems concerned in non-specific resistance can be relied upon against all of the tested races of the pathogen, and have been proposed as those most likely to result in permanent resistance (Watson, 1970).

Durable resistance to a disease is resistance that remains effective during its prolonged and widespread use in an environment favourable to the disease (Johnson, 1984). Thus, environment is a factor of disease, and is interactive with the host and pathogen, forming the "disease-triangle". To these can be added a fourth factor, time or tree age, forming the "disease-pyramid" (Browning *et al.*, 1977). Resolution of these factors is desirable for a better understanding of the epidemiology of the *Pinus radiata* / *Phytophthora cinnamomi* plant pathosystem.

The major components of the disease pyramid, host and pathogen, were studied in two glasshouse experiments using one-year-old seedlings. Inferences on the other factors, firstly, the environment, were drawn from tests conducted in different glasshouse environment regimes, and secondly, tree age, from the development of the disease from seedlings to young trees in two field tests.

5.2 Materials and Methods

Pinus radiata families used in these experiments had been previously tested for response to *Phytophthora cinnamomi* and were classified in Butcher and Stukely (1986). Families were chosen to represent the extremes in response, from susceptible to tolerant. Families were open-pollinated seedlots collected from mother trees in the Manjimup seed orchard.

The two glasshouse studies reported in Butcher *et al.* (1984) used a single isolate of *P. cinnamomi* (mating type A2), originally isolated from the roots of a dead *Pinus radiata* tree in the Donnybrook Sunkland. This isolate, *P. cinnamomi* A2 (318), was included in both field tests, FP1 and FP2, and also in the third and fourth glasshouse tests with the isolate *P. cinnamomi* A2 (1). The isolate, *P. cinnamomi* A2 (1), had the greater virulence (Table 6) and was included in the host / pathogen studies. Other isolates were chosen to sample a broad spectrum of the pathogen from the types immediately available. *Phytophthora cinnamomi* was extracted from pine trees of tolerant and susceptible families that had died at Baudin in the Donnybrook Sunkland, and from jarrah trees, banksias and sheoaks, ranging from Wanneroo in the North, to Augusta in the South (Table 32). An isolate of the A1 mating type, from South Australia, was included in the second test.

The same procedures, reported in Chapter 3(5), were used for the germination, growing and preparation of experimental pots. Each experimental pot in the HP1 test contained a single seedling of the five treatment families, and the HP2 test contained single seedlings of the eight treatment families, arranged as shown in Figure 5. Inoculum for each of the *P. cinnamomi* isolates was prepared as described in Chapter 3(5). Inoculum for the control plugs was killed by autoclaving.

Host Pathogen Test (HP1) :

Experimental design

Split-plot (I = 8, F = 5, R = 10).

8 *Phytophthora cinnamomi* isolates (Table 32, isolates 1, 2, 3, 4, 5, 6, 7 and 8).

5 *Pinus radiata* families (tolerant 80007 and 60027; average 50015; susceptible 20011 and 60001).

10 replications.

Multi-family pots of five seedlings, and four inoculum plugs of the same isolate buried in each pot.

Inoculation date, 1st November 1982.

TABLE 32

Description of the *Phytophthora cinnamomi* isolates used in the experiments.

Isolate	*Phytophthora cinnamomi* original host
Test HP1	
P. c. A2 (1)	*Pinus radiata*, 5-year-old tree, Jarrahwood
P. c. A2 (2)	*Pinus radiata*, 1-year-old susceptible 60017, Baudin
P. c. A2 (3)	*Pinus radiata*, 1-year-old susceptible 60021, Baudin
P. c. A2 (4)	*Banksia attenuata*, gas pipeline, Wanneroo
P. c. A2 (5)	*Eucalyptus marginata*, CSIRO SC-90, Kirup
P. c. A2 (6)	*Pinus radiata*, 1-year-old tolerant 12038, Baudin
P. c. A2 (7)	*Casuarina fraseriana*, CSIRO SC-381, Jarrahdale
P. c. A2 (8)	*Pinus radiata*, 1-year-old tolerant 60002, Baudin
Test HP2	
P. c. A2 (1)	*Pinus radiata*, 5-year-old tree, Jarrahwood
P. c. A2 (2)	*Pinus radiata*, 1-year-old susceptible 60017, Baudin
P. c. A2 (4)	*Banksia attenuata*, gas pipeline, Wanneroo
P. c. A2 (6)	*Pinus radiata*, 1-year-old tolerant 12038, Baudin
P. c. A2 (7)	*Casuarina fraseriana*, CSIRO SC-381, Jarrahdale
P. c. A2 (9)	*Banksia* species, Molloy Island, Augusta
P. c. A2 (10)	*Eucalyptus marginata*, Churchman block, Jarrahdale
P. c. A2 (11)	*Eucalyptus marginata*, Gordon block, Jarrahdale
P. c. A1 (12)	A1 mating type, DCE-21, Waite, South Australia
control (c)	autoclaved *P. c.* branch-plugs

Host Pathogen Test (HP2) :

Experimental design

Split-plot (I = 10, F = 8, R = 8).

10 *Phytophthora cinnamomi* isolates (Table 32, isolates 1, 2, 4, 6, 7, 9, 10, 11, 12 and control).

8 *Pinus radiata* families (tolerant 20058, 30043 and 60022; susceptible 12112, 20011, 50048, 60000 and 60001).

8 replications.

Multi-family pots of eight seedlings, and six inoculum plugs of the same isolate buried in each pot.

Inoculation date, 7th November 1983.

Experimental pots were flooded for two days prior to the inoculation and this level was maintained for seven days before the pots were drained. Inoculum plugs in HP1 test were buried in four holes, 8 cm deep, and equally spaced about 3 cm in from the pot edge. In the HP2 test the six inoculum plugs were buried in 8 cm deep holes, positioned at the centre of the triangles formed by the seedlings.

The glasshouse environment for the HP1 test was described as the low moisture / high temperature regime in Chapter 3(4). Evaporative coolers in the glasshouse were only turned on when temperatures exceeded 35°C in the glasshouse. Pots were watered twice weekly, with about 500 ml, although this was not controlled. In some instances plants were wilting when water was re-applied.

A similar glasshouse environment regime was prescribed for the second HP2 test, although watering was increased to about 1000 ml at each application. Glasshouse evaporative coolers were set for automatic operation at a temperature of about 30°C on weekends only. Otherwise, they were operated manually when glasshouse temperatures reached 35°C. The HP2 test was conducted adjacent to the NP1 and NP2 tests, in the same glasshouse.

Seedling heights were measured at inoculation and then at 20 day intervals, through to the end of the experiments, 280 and 200 days later. Seedling deaths were recorded at each assessment. Dead seedlings were periodically sampled and plated for detection of *Phytophthora cinnamomi*. Inoculum plugs were sampled at the end of the experiments and plated for all inoculated pots containing no dead seedlings.

5.3 Results

Mortality in test HP1 was only 13% of the 400 seedlings inoculated. This ranged from 25% for the susceptible families 20011 and 60001, 14% for the average family 50015, and 2% for the tolerant families 80007 and 60027. Very similar values were recorded in these families in an adjacent screening test using *P. cinnamomi* isolates (1) and (2). There was also considerable variation in the numbers of dead seedlings in susceptible families, depending on the isolate of *P. cinnamomi* applied to the pot. For example, in susceptible family 20011, 50% of seedlings died with isolate (1), 40% with isolates (2) and (6), 20%

with isolates (7) and (8), and no seedlings died with isolates (4) and (5). In fact, there were no dead seedlings in any of the plant pots inoculated with *P. cinnamomi* isolates (4) and (5) in the HP1 test.

TABLE 33

Sampling of collars and roots of dead *Pinus radiata* seedlings in test HP2 and recovery of *Phytophthora* cinnamomi.

Phytophthora cinnamomi isolate	seedling collar sample n	*P.c.*	%	roots *P.c.* recovery
P. c. A2 (1)	197	131	66 %	65 %
P. c. A2 (2)	123	79	64	91
P. c. A2 (4)	88	52	59	44
P. c. A2 (6)	83 #	1	1	1
P. c. A2 (7)	123	56	46	54
P. c. A2 (9)	281	197	7 0	72
P. c. A2 (10)	188	148	79	46
P. c. A2 (11)	193	92	49	54
P. c. A1 (12)	290	213	73	72

sample from healthy seedlings in the same pot (2 susceptible and 2 tolerant families), 280 days after inoculation.

In the second, HP2 test, 37% of the 576 inoculated seedlings died. The number of deaths was increased over the HP1 test because there were more susceptible families (5/8 compared with 2/5) and the glasshouse environment was not as severe. Maximum mortality was recorded in the susceptible standard family 20011 (61%), and was also high in the other susceptible families 12112 (47%), 60001 (42%), 50048 (40%) and 60000 (39%). Mortality in the tolerant families was significantly less; 20058 (19%), 30043 (20%) and 60022 (26%). Variability within the pathogen population was greater than the variation within the host, in terms of the number of seedlings killed. The variability between the *P. cinnamomi* isolates ranged from the maximum with A1 isolate (12) 59% and A2 isolate (10) 50%, down to 2% for the A2 isolate (6). By contrast, the A2 isolate (6) in test HP1 killed 26% of the seedlings inoculated (Figure 33) in an environment not as favourable to the development of the pathogen. This isolate was recovered from less

than 1% of the seedling roots and collars sampled in healthy seedlings of susceptible and tolerant families (Table 33). This was significantly less than the recoveries reported in Table 12. The apparent loss of virulence will be discussed later in this chapter. There were no seedling deaths in the control uninoculated pots.

There was no assessment of seedling root systems at the end of the study, in either test. An empirical disease scale was developed based on the number of days to death and the height increment of surviving seedlings. Because the analysis of the host x pathogen interaction is dependent on this arbitrary scale, a complete description to its development is given.

The first step in the development of the surviving seedling disease scale was to establish family population parameters, before *P. cinnamomi* was applied. As there was no control uninoculated treatment in the HP1 test, the *P. cinnamomi* A2 isolates (4) and (5) were selected, because they caused no seedling death, to form the index for the comparison of the other inoculate effects. The initial heights of the isolate (4) and (5) allocated seedlings, were similar to the heights of other seedlings in each family. Their height increments for the 280 day period following inoculation are given in Table 34. Standard deviations were calculated for each family using the coefficients of variation measured for the initial height of all family seedlings, and this was used for the disease class intervals (Table 34).

Seedling disease scale (HP1)

Code 1 (23%)	Alive at 280 days, Ht. inc. > mean + 1 std. dev.
Code 2 (20%)	Alive at 280 days, Ht. inc. mean to + 1 std. dev.
Code 3 (22%)	Alive at 280 days, Ht. inc. mean to -1 std. dev.
Code 4 (23%)	Alive at 280 days, Ht. inc. < mean -1 std. dev.
Code 5 (1%)	Dead at 280 days
Code 6 (4%)	Dead at 200 days
Code 7 (6%)	Dead at 140 days
Code 8 (2%)	Dead at 70 days

The distribution of the seedling disease codes is shown above, and is shown for the families in Figure 33. Although the data distribution was not normal, the other requirements of the analysis of variance were met. Sampling was at random as there were no differences for allocated inoculate treatments within families, for the initial height measurement and code variances were similar. The analysis of variance was performed on the non-transformed disease codes (Table 35). This analysis of variance table is a combination of two separate analyses, the split-plot analysis to partition variance into the major effects of *P. cinnamomi* isolates and *Pinus radiata* families, and the interactive effects. Data was then nested for family groups in the other part of the Table 35, to show the variation within a *P. radiata* family in response to the different *Phytophthora cinnamomi* isolates.

TABLE 34
Development of disease codes for the analysis of test HP1.

HP1		*Pinus radiata* family				
		80007	60027	50015	60001	20011
population parameters, seedling height before inoculation.						
	mean (n = 80)	241 mm	258	252	213	215
	Std. Dev. (SD.)	47 mm	43	44	33	35
	Coeff. Var.	20 %	17	18	15	16
Height increment, 280 days after inoculation, for *P. c.* A2 isolates (4) and (5).						
	mean (n = 20)	335	318	364	297	284
	calculated SD.	67	54	66	45	45
Disease scale for alive seedlings.						
1.	> mean + 1 SD.	> 402	> 372	> 430	> 342	> 329
2.	mean to +1 SD.	402-335	372-318	430-364	342-297	329-284
3.	mean to -1 SD.	334-268	317-264	363-298	266-252	283-239
4.	< mean - 1 SD.	< 268	< 264	< 298	< 252	< 239

In test HP1, there were significant differences between families ($P < 0.001$), isolates ($P < 0.01$) and the interaction ($P < 0.05$). As expected, tolerant families 80007 and 60027 were similar and had significantly less disease than the average family 50015, which had less disease than susceptible families 20011 and 60001 (Table 36). The most virulent *P. cinnamomi* were isolates from tolerant *Pinus radiata* families in the Donnybrook Sunkland, ranging to low pathogenic isolates from jarrah and banksia trees (Table 32). Although the variance analysis showed a significant effect of the host x pathogen interaction (Table 35), the hierarchal analysis showed this to be false. There were significant differences within the susceptible and average family groups for the various *P. cinnamomi* isolates, but there were no differences in disease expression within either of the tolerant families. The ratio of the interactive variance to the family variance was 0.10, which is insignificant. Variation between families and inoculates acted independently.

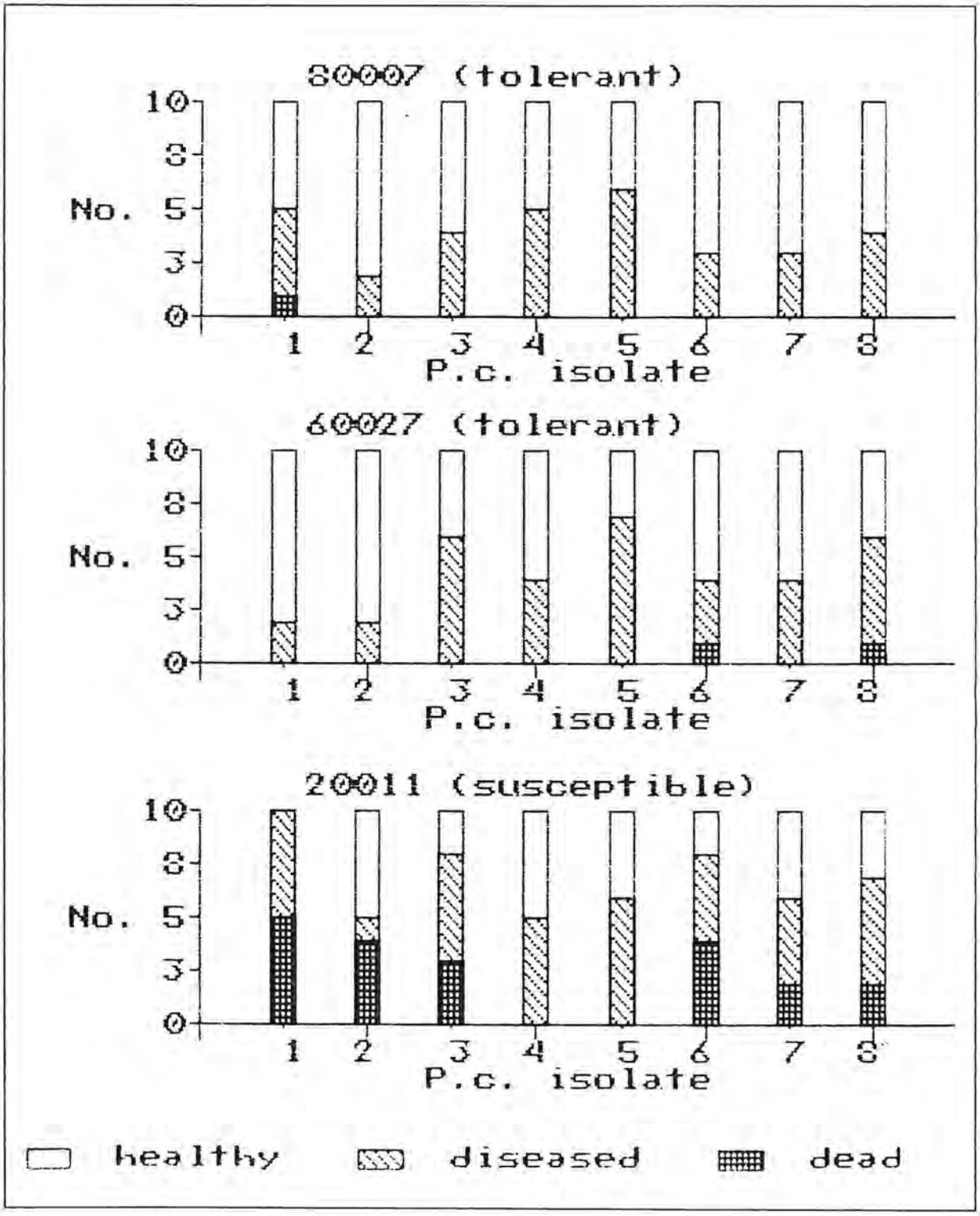


FIGURE 33

The amount of disease in *Pinus radiata* families for eight different isolates of *Phytophthora cinnamomi*, in test HP1.

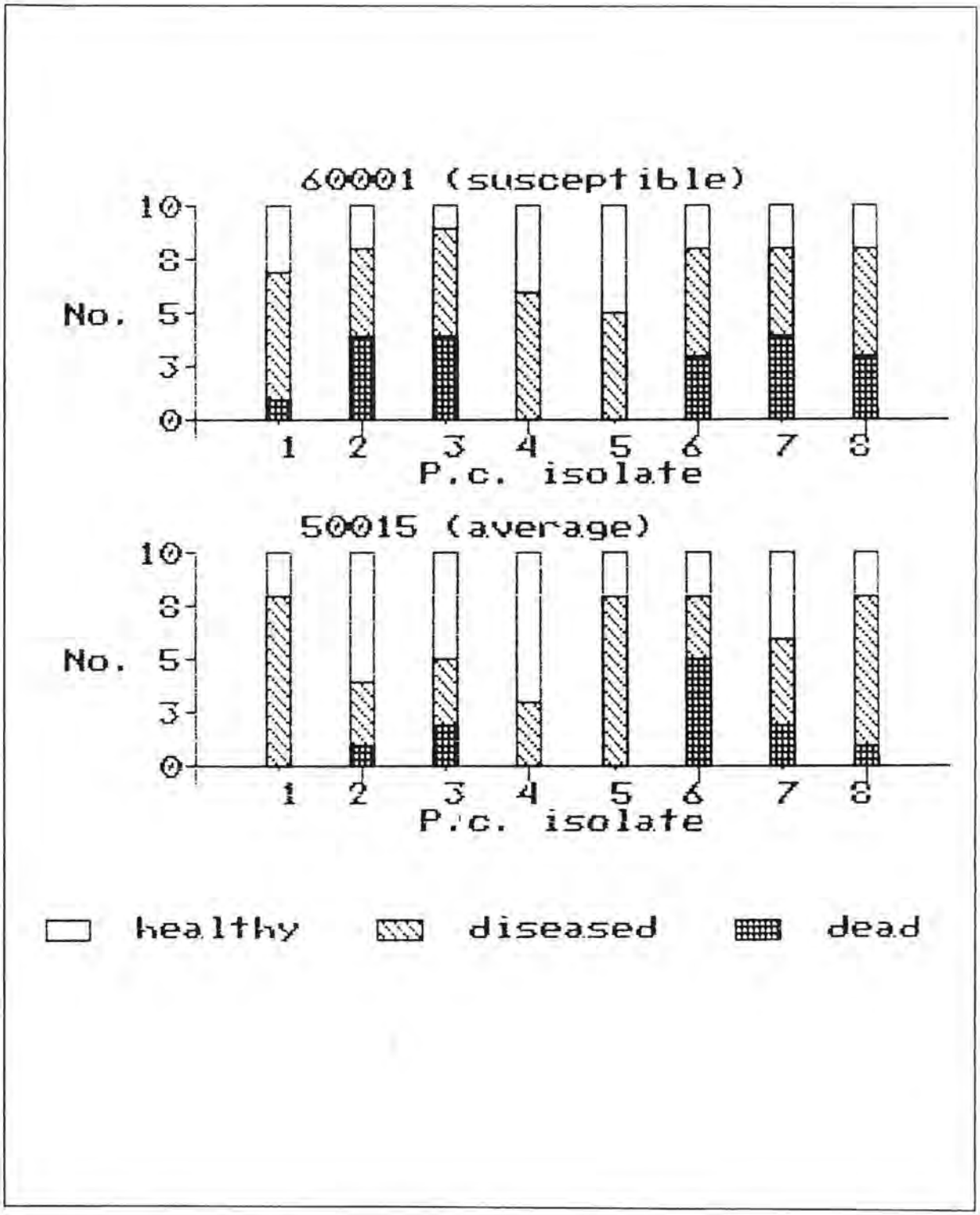


FIGURE 33 (cont)

The amount of disease in *Pinus radiata* families for eight different isolates of *Phytophthora cinnamomi*, in test HP1.

TABLE 35

Heritability and components of disease variance for the host *Pinus radiata*, pathogen *Phytophthora cinnamomi* and their interaction in test HP1.

Source	d.f.	Variance	Significance
within *Pinus radiata* family for 8 isolates of *Phytophthora cinnamomi*.			
80007 (tolerant)	7	1.7	
60027 (tolerant)	7	1.9	
50015 (average)	7	6.7	*
60001 (susceptible)	7	8.1	**
20011 (susceptible)	7	8.1	**
between families	4	41.3	***
Main plots			
Inoculum (I)	7	8.4	**
Replication (R)	9	2.1	
error	63	2.7	
Sub plots			
Family (F)	4	41.3	***
F*I	28	4.3	*
F*R	36	2.4	
error	252	2.7	
Total	399	3.2	

* significant at $P < 0.05$, ** significant at $P < 0.01$, *** significant at $P < 0.001$.

Heritability (individual) $h^2I = 0.58 + 0.39$

(family) $h^2F = 0.90$

TABLE 36

Virulence of *Phytophthora cinnamomi* isolates and *Pinus radiata* family response, 280 days after inoculation in test HP1.

Pinus radiata family		80007 (tolerant)		60027 (tolerant)		50015 (average)	
Phytophthora cinnamomi isolate	disease	*P. c.*	disease	*P. c.*	disease	*P. c.*	disease
P. c. A2 (4)	2.4 a	(2)	2.0 a	(1)	1.7 a	(4)	1.9 a
P. c. A2 (5)	2.6 ab	(6)	2.1 a	(2)	1.9 a	(2)	2.6 ab
P. c. A2 (2)	2.9 abc	(7)	2.1 a	(7)	2.2 a	(1)	3.1 ab
P. c. A2 (7)	3.1 bcd	(3)	2.2 a	(4)	2.3 a	(5)	3.2 ab
P. c. A2 (1)	3.2 bcd	(5)	2.3 a	(6)	2.3 a	(3)	3.3 ab
P. c. A2 (3)	3.4 cd	(8)	2.5 a	(3)	2.5 a	(7)	3.4 b
P. c. A2 (8)	3.4 cd	(4)	2.6 a	(5)	2.6 a	(8)	3.4 b
P. c. A2 (6)	3.6 d	(1)	2.7 a	(8)	3.1 a	(6)	4.8 c
Pinus radiata family			2.3 e		2.3 e		3.2 f

Table 36 (cont)

Pinus radiata family		60001 (susceptible)		20011 (susceptible)	
Phytophthora cinnamomi isolate	disease	*P. c.*	disease	*P. c.*	disease
P. c. A2 (4)	2.4 a	(5)	2.4 a	(4)	2,5 a
P. c. A2 (5)	2.6 ab	(4)	2.7 ab	(5)	2.5 a
P. c. A2 (2)	2.9 abc	(1)	3.3 abc	(7)	3.3 ab
P. c. A2 (7)	3.1 bcd	(8)	4.0 bcd	(2)	3.7 abc
P. c. A2 (1)	3.2 bcd	(6)	4.2 cd	(3)	3.9 abc
P. c. A2 (3)	3.4 cd	(7)	4.4 cd	(8)	4.0 bc
P. c. A2 (8)	3.4 cd	(2)	4.5 cd	(6)	4.6 bc
P. c. A2 (6)	3.6 d	(3)	4.9 d	(1)	5.0 c
Pinus radiata family			3.8 g		3.7 g

Disease scores with different letters are significantly different at the 95% probability level

The disease scale for the second test HP2 was also based on height increment after inoculation, but in this test the standard for comparison were uninoculated seedlings of the same family. The population parameters for each family were established from their initial heights (Table 37). This indicated that adjustments were necessary only for the 12112 and 60000 family controls, to be representative of the family populations. Disease class intervals were based on the height increment of uninoculated seedlings (control mean), 200 days after the inoculation treatment and the calculated population standard deviation (Table 37).

Seedling disease scale (HP2)

Code 1 (22%)	Alive at 200 days, Ht. inc. > control mean + 1 std. dev.
Code 2 (12%)	Alive at 200 days, Ht. inc. control mean to + 1 std. dev.
Code 3 (15%)	Alive at 200 days, Ht. inc. control mean to -1 std. dev.
Code 4 (17%)	Alive at 200 days, Ht. inc. < control mean -1 std. dev.
Code 5 (5%)	Dead at 200 days
Code 6 (16%)	Dead at 120 days
Code 7 (10%)	Dead at 60 days
Code 8 (2%)	Dead at 40 days

Distribution of seedling disease classes by family are shown in Figure 34, grouped for healthy (codes 1, 2), diseased (codes 3, 4) and dead seedlings (codes 5, 6, 7, 8). The data was not normally distributed but disease scale variances were homoscedastic. There were no significant differences within families in seedling heights before inoculum was added.

Table 38 is a composite variance analysis table detailing the split-plot analysis and the separate hierarchal analysis of the HP2 test. The uninoculated treatment was excluded from the split-plot analysis as these variances were used to calculate heritability. Differences between the *Pinus radiata* families and the *Phytophthora cinnamomi* isolates in disease intensity were highly significant ($P < 0.001$). The tolerant families were similar and had significantly less disease than the susceptible families (Table 39). Most disease, as in the HP1 test, was in the susceptible family 20011. The variation between the *P. cinnamomi* isolates was as large as the family variation. The single A1 isolate from South Australia was highly virulent as were the three A2 isolates from banksia, pine and jarrah trees (Table 39). *Phytophthora cinnamomi* isolates (1) - high, and (4) - low virulence, had similar pathogenicity in both tests for a different set of families. However, the response of the number (6) isolate was different in both tests, giving maximum disease expression in the HP1 test and the minimum in the HP2 test.

The interactive host x pathogen effect was also shown to be significant ($P < 0.05$) in the HP2 test (Table 38), although its ratio with the family variance, computed as 0.12, was very low. The hierarchal analysis in Table 38 shows highly significant ($P < 0.001$) variation in the pathogenicity of the nine isolates within each of the susceptible families. Within the tolerant families, there were no differences in disease expression among the isolates and disease was similar to the uninoculated seedlings.

TABLE 37

Development of disease codes for the analysis of test HP2.

HP2	*Pinus radiata* family			
	20058	30043	60022	60000
population parameters, seedling height before inoculation.				
mean (n = 80)	172 mm	184	187	212
Std. Dev. (SD.)	37 mm	35	57	57
control mean (n = 10)	174 mm	181	187	234
Height increment, 200 days after inoculation, for uninoculated control pots.				
mean (n = 10)	213	193	228	211
popn. mean adjusted	213	193	228	190
calculated SD.	47	37	68	51
Disease scale for alive seedlings.				
1. > mean + 1 SD.	> 260	> 230	> 296	> 241
2. mean to +1 SD.	260 - 213	230 - 193	296 - 228	241 - 190
3. mean to -1 SD.	213 - 166	193 - 156	228 - 160	190 - 139
4. < mean - 1 SD.	< 166	< 156	< 160	< 139

TABLE 37 (cont)

HP2	*Pinus radiata* family			
	60001	50048	12112	20011
population parameters, seedling height before inoculation.				
mean (n = 80)	242 mm	149	169	206
Std. Dev. (SD.)	54 mm	40	41	49
control mean (n = 10)	243 mm	143	194	202
Height increment, 200 days after inoculation, for uninoculated control pots.				
mean (n = 10)	282	214	146	184
popn. mean adjusted	282	214	128	184
calculated SD.	62	58	31	44
Disease scale for alive seedlings.				
1. > mean + 1 SD.	> 344	> 272	> 159	> 228
2. mean to +1 SD.	344 - 282	272 - 214	159 - 128	228 - 184
3. mean to -1 SD.	282 - 220	214 - 156	128 - 97	184 - 140
4. < mean - 1 SD.	< 220	< 156	< 97	< 140

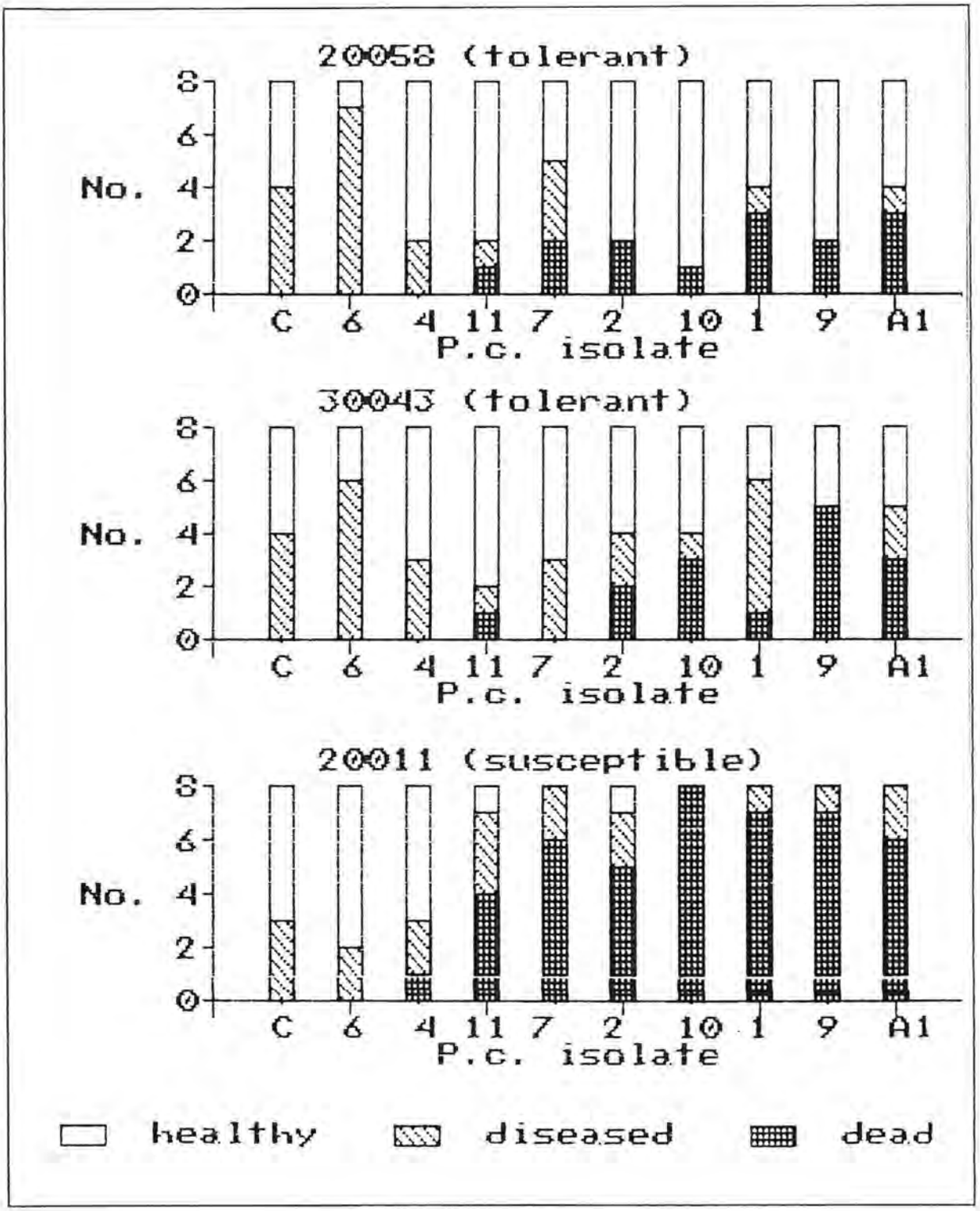


FIGURE 34

The amount of disease in resistant and susceptible response families of *Pinus radiata*, for nine different isolates of *Phytophthora cinnamomi*, in test HP2.

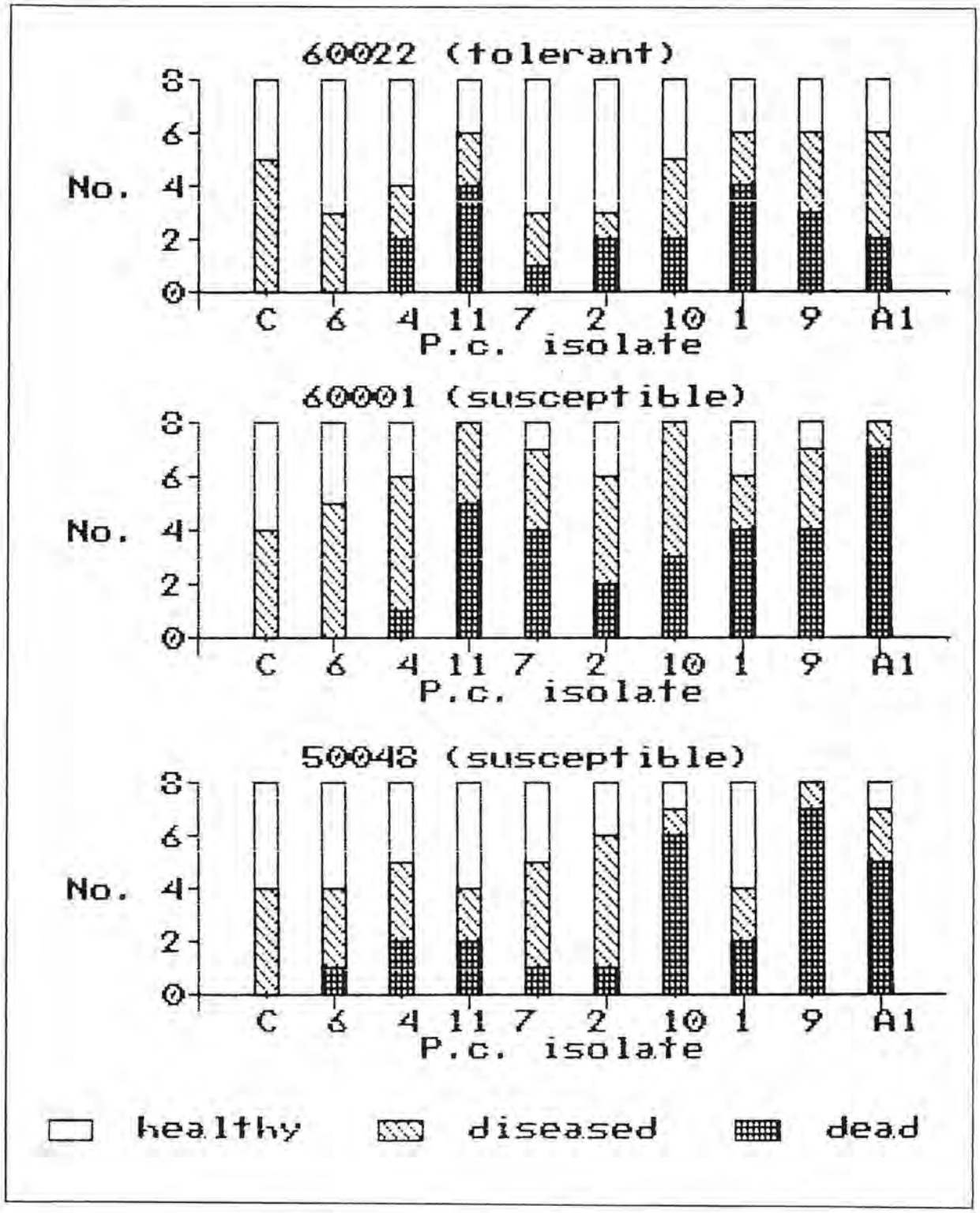


FIGURE 34 (cont)

The amount of disease in resistant and susceptible response families of *Pinus radiata*, for nine different isolates of *Phytophthora cinnamomi*, in test HP2.

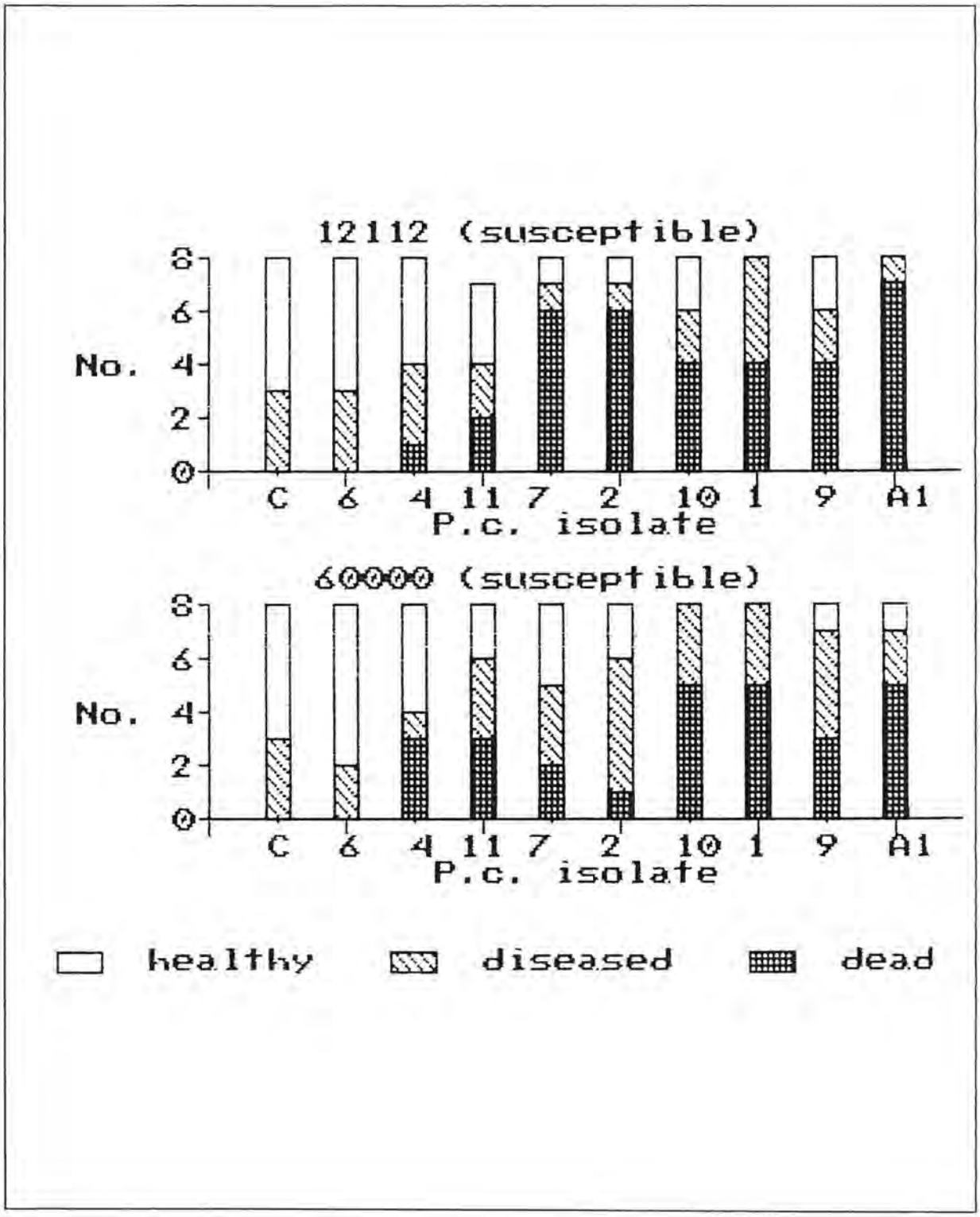


FIGURE 34 (cont)

The amount of disease in resistant and susceptible response families of *Pinus radiata*, for nine different isolates of *Phytophthora cinnamomi*, in test HP2.

TABLE 38

Heritability and components of disease variance for the host *Pinus radiata*, pathogen *Phytophthora cinnamomi* and their interaction in glasshouse test HP2.

Source	d.f.	Variance	Significance
within *Pinus radiata* family for nine isolates of *P. cinnamomi* and control.			
20058 (tolerant)	9	3.01	
30043 (tolerant)	9	4.91	
60022 (tolerant)	9	4.17	
60000 (susceptible)	9	12.47	***
60001 (susceptible)	9	9.68	***
50048 (susceptible)	9	12.17	***
12112 (susceptible)	9	14.14	***
20011 (susceptible)	9	28.22	***
between families	7	38.31	***
Main plots			
Inoculum (I)	8	40.75	***
Replication (R)	7	6.68	
error	56	5.65	
Sub plots			
Family (F)	7	44.40	***
F*I	56	5.34	*
F*R	49	3.53	
error	392	3.02	
Total	575	4.62	

* significant at $P < 0.05$, *** significant at $P < 0.001$.

Heritability (individual) $h^2_I = 0.58 + 0.31$

(family) $h^2_F = 0.89$

TABLE 39

Virulence of *Phytophthora cinnamomi* isolates and *Pinus radiata* family response, 200 days after inoculation in test HP2.

Pinus radiata family		20058 (tolerant)		30043 (tolerant)		60022 (tolerant)		60000 (susceptible)	
Phytophthora cinnamomi isolate	disease	*P. c.*	disease	*P. c.*	disease	P. c.	disease	P. c.	disease
control	(c) 2.4 a	(4)	1.8 a	(7)	1.9 a	(6)	2.3 a	(c)	2.0 a
P. c. A2	(6) 2.5 a	(10)	2.0 a	(4)	2.0 a	(7)	2.5 ab	(6)	2.0 a
P. c. A2	(4) 2.8 ab	(11)	2.3 a	(11)	2.0 a	(c)	2.6 ab	(2)	3.3 ab
P. c. A2	(11) 3.5 bc	(c)	2.6 a	(c)	2.5 ab	(2)	2.8 ab	(4)	3.5 ab
P. c. A2	(7) 3.7 cd	(9)	2.6 a	(1)	3.0 ab	(4)	3.0 ab	(7)	3.5 ab
P. c. A2	(2) 3.8 cd	(1)	3.1 a	(6)	3.1 ab	(12)	3.4 ab	(11)	3.8 bc
P. c. A2	(10) 4.5 de	(2)	3.1 a	(2)	3.4 ab	(9)	3.8 ab	(9)	4.1 bcd
P. c. A2	(1) 4.5 de	(6)	3.1 a	(10)	3.4 ab	(10)	3.9 ab	(12)	4.6 bcd
P. c. A2	(9) 4.5 de	(7)	3.5 a	(12)	3.5 ab	(1)	4.1 ab	(10)	5.4 cd
P. c. A1	(12) 4.7 e	(12)	3.5 a	(9)	4.3 b	(11)	4.3 b	(1)	5.8 d
Pinus radiata family			2.8 f		2.9 f		3.3 f		4.0 g

Pinus radiata family		60001 (susceptible)		50048 (susceptible)		12112 (susceptible)		20011 (susceptible)	
Phytophthora cinnamomi isolate	disease	*P. c.*	disease	*P. c.*	disease	P. c.	disease	P. c.	disease
control	(c) 2.4 a	(c)	2.3 a	(c)	2.5 a	(6)	2.0 a	(6)	2.0 a
P. c. A2	(6) 2.5 a	(6)	2.6 ab	(6)	2.9 a	(c)	2.1 a	(c)	2.3 a
P. c. A2	(4) 2.8 ab	(4)	3.6 abc	(11)	3.0 a	(4)	2.8 ab	(4)	2.8 ab
P. c. A2	(11) 3.5 bc	(2)	3.8 abcd	(7)	3.3 ab	(11)	3.3 abc	(11)	4.4 abc
P. c. A2	(7) 3.7 cd	(10)	4.3 bcde	(4)	3.4 abc	(9)	4.3 bcd	(2)	5.3 cd
P. c. A2	(2) 3.8 cd	(1)	4.4 cde	(2)	3.6 abc	(10)	4.6 cd	(7)	5.8 cd
P. c. A2	(10) 4.5 de	(7)	4.4 cde	(1)	4.8 bcd	(1)	4.9 cd	(1)	6.3 d
P. c. A2	(1) 4.5 de	(9)	4.8 cde	(12)	5.1 cd	(2)	5.0 cd	(12)	6.3 d
P. c. A2	(9) 4.5 de	(11)	5.4 de	(10)	5.5 d	(7)	5.0 cd	(9)	6.5 d
P. c. A1	(12) 4.7 e	(12)	5.8 e	(9)	6.0 d	(12)	5.8 d	(10)	7.0 d
Pinus radiata family			4.3 g		4.2 g		4.2 g		5.1 h

Disease scores with different letters are significantly different at the 95% probability level.

Family ranking in disease intensity for this range of *P. cinnamomi* isolates was unchanged in either test. In another test, adjacent to the HP1 test, of 35 families from the Victorian and New South Wales breeding populations, inoculated with *P. cinnamomi* A2 isolates (1) and (2), the interactive variance was shown to be insignificant and the Spearman's test affirmed the family ranking for both isolates to be very highly correlated ($P < 0.01$). Further support was gained from an earlier screening test of 100 families from the Tasmanian, CSIRO and APM breeding populations using *P. cinnamomi* isolates (318) and (1). The interactive variance was insignificant, while the non-parametric rank test of Spearman was very highly significant ($P < 0.001$); ranking of *Pinus radiata* families was similar for either isolate of *Phytophthora cinnamomi.*

5.4 Discussion

Families chosen for these host / pathogen studies were selected for their known response to *P. cinnamomi* inoculation. In both tests, the families responded to the range of applied isolates as per their formal classification. There were significant differences in the level of disease between these major classes.

Narrow sense heritabilities calculated for the individual seedling disease scores in each test were both calculated as 0.58. These were large, compared with heritabilities reported for other *Pinus radiata* traits (Cotterill and Zed, 1980), but were considerably below the constant ultra-high values recorded in the DP tests for the South African and New Zealand populations. The standard error of the estimates were also considerably larger, 0.3 to 0.4, and provides a clue to the difference. A small number of families were sampled, and treatment pots in the DP tests each received a mixture of four *Phytophthora cinnamomi* A2 isolates, compared with single isolates in the HP tests. Variability between the isolates was large, and disease scores were dependent upon population parameters calculated for each family. Because of this, and the selection of parents on their disease response, caution should be exercised in the use of these estimates.

The differences between *P. cinnamomi* A2 isolates in their pathogenicity to *Pinus radiata* family seedlings were quite large. The single A1 mating type isolate was found to be the most aggressive pathogen in the second test. Shepherd *et al.* (1974) reported that both mating types were similarly highly pathogenic, killing 75% of their inoculated *P. radiata* seedlings within 15 days. No differences were reported in the pathogenicity of their twenty A1 and eighteen A2 isolates, probably because they used 45 day-old germinants of *P. radiata* in their test. Both mating types were highly pathogenic to two-year-old plants of *Nothofagus cunninghamii* (Weste, 1975), and symptoms were more severe in plants inoculated with the A1 mating type of the *Phytophthora cinnamomi* compared with the A2 type. Zentmyer and Guillemet (1981) found that an A1 isolate from *Camellia japonica* caused a severe reaction on both avocado and camellia, whereas an A2 isolate of *P. cinnamomi* from avocado was highly pathogenic to avocado but not to camellia. This was significant as all but one isolation from avocados have been of the A2 mating type. There have been several reports indicating varying degrees of virulence of isolates on different hosts, but there is no evidence for physiologic races of *P. cinnamomi* (Erwin, 1983).

The loss of virulence of the *P. cinnamomi* A2 isolate (6) in the HP2 test is apparently not an uncommon phenomenon. Caten (1971) reported that continued culturing can result in a loss of virulence of the pathogen and that this type of variation should be recognised by plant breeders. Genetic tests to investigate responses to pathogens should include a number of isolates to compensate for variations of this nature, and for different degrees of aggressiveness.

Isolates of *P. cinnamomi* originating from tolerant families of *Pinus radiata* growing in the Baudin plantation were seen as the most aggressive pathogens in test HP1, compared with isolates from other species. This could indicate an increase in virulence associated with the resistant *P. radiata* and the potential for the frequency of virulent strains to increase when the pathogen encounters large numbers of resistant trees. However, the second test dispelled this theory. Isolates from jarrah and banksia trees, from widely dispersed locations, were just as virulent as isolates from pine trees.

Stable, horizontal, uniform or race-non-specific resistance can be discerned from unstable, vertical, differential or race-specific resistance by a designed test in which a number of host genotypes are assessed for their reaction to a number of pathogen isolates (Van der Plank, 1968). Vertical resistance is characterised by a significant variance component for the interaction of the host and pathogen. Van der Plank (1978) later cautioned on the interpretation of the variance model in cases where fictitious interactions appear in the analysis of variance, when no differential interaction in reality existed. Horizontal resistance (stable) by definition implies that the variation in the host and pathogen are not correlated.

Variability within the *P. radiata* host population and the *Phytophthora cinnamomi* pathogen population has been found to be considerable. In both designed tests, the variance was principally due to main effects, while the interactive variance was small (10% of family variance) and was just discernable from the error variance. The hierarchal analysis of variance has clearly shown the fallacy of a significant interaction. Variability in the virulence of the pathogen was only encountered in susceptible families and it was constant between susceptible families in a test. Levels of virulence were low and constant between tolerant families. In the second test, the inoculated seedlings of tolerant families had a similar health condition to the uninoculated seedlings. In these tests there was no evidence to support a positive host x pathogen interaction.

Resistance which functions against individual races of a pathogen is known as specific resistance, whereas resistance which functions more generally against all pathogenic races or biotypes is non-specific resistance (Williams, 1975). Specific resistance is recognised by hypersensitivity and a correspondence between fungal genes and host genes (Watson, 1970). The gene-for-gene relationship of Flor (1971) has matching genes conditioning resistance in the host with pathogenicity or non-pathogenicity of the pathogen. With breakdown of this match, parasitism occurs. Specific resistance is usually under monogenic or oligogenic control (Van der Plank, 1968). Non-specific resistance is normally considered to be under polygenic control, and although it is expressed widely against all races of a pathogen, it may vary in the degree to which it functions in restricting the development of individual races (Nelson, 1972). Differences in the aggressiveness of various races of a pathogen may be a reflection of the varying genetic composition of non-specific resistance in the host plant (Williams, 1975).

In the majority of cases, specific resistance, while providing a high level of protection, is only effective against some races of the pathogen, which has led to "breakdown" with the emergence of new races able to attack the host. Increased pathogenicity in pathogens is probably the commonest cause for the failure of resistance (Johnson, 1984), although changes in the environment have undoubtedly played a role in reducing the effectiveness of the resistance. Where vertical resistance has been lost in important agricultural crops, the generation time has been short enough to counteract this by a flow of new resistant varieties. Breakdown is not the inevitable result of the use of vertical resistance for crop protection, but the dangers are enough to justify its avoidance for forest crops (Russell, 1978). The extent and uniformity of tree plantations and their lengthy rotations make them particularly vulnerable.

More lasting protection has been found in horizontal or non-specific resistance. Rather than being expressed as a complete or hypersensitive form of resistance, non-specific resistance is shown as a reduction in numbers and the rate at which propagules of the pathogen are produced on the host. No great selection pressure is provoked on the pathogen. This rate-reducing resistance appears to be conditioned by several to many genes, each having small effects. The disease is not simply the qualitative expression of dead or alive but is a quantitative character continuously ranging from rapid seedling death through to minimal effects on seedling health. Such resistance is additively inherited and can be manipulated in the same way as other quantitative traits of importance, to achieve long term durability of resistance.

Constant ranking of host genotypes exposed to different races of the pathogen is regarded by many researchers, including Person *et al.* (1982), as a distinguishing characteristic of polygenically-controlled resistance. Their theoretical analysis concluded that such systems will be relatively more stable than those that are based exclusively on major genes and specific gene-for-gene interaction.

Not only were the family rankings constant in the *Pinus radiata* / *Phytophthora cinnamomi* tests reported in this chapter, but the same families had common rankings in other glasshouse tests, carried out in different environment regimes, and in the field tests. In addition, screening tests using two different isolates of *P. cinnamomi* gave similar ranking of the 100 and 35 families in the tests for both isolates.

Seedling death in the HP2 test was 37% of all seedlings inoculated, compared with 13% for the HP1 test. Factors contributing to this disparity were the different *Pinus radiata* families, five susceptible out of eight compared with two out of five, different and more virulent isolates of *Phytophthora cinnamomi* including the virulent A1 mating type, an eight seedling compared with a five seedling treatment pot, and a more moderate glasshouse test environment. Resistance is the expression of the interaction between the host, the pathogen and the environment. These tests have shown that the host reaction is stable. Russell (1978) has suggested testing for resistance using different sets of environmental conditions to identify types of disease based on major genes. If a hypersensitive reaction is involved, the same expression of disease will result for different environments, whereas resistance based on many genes will be influenced in its expression by the environment. Standard test families have maintained their rankings in the glasshouse and field tests (Figure 8), although the levels of disease among tests has fluctuated considerably. This strategy suggests that resistance in *Pinus radiata* to Phytophthora cinnamomi is based on many genes.

CHAPTER 6

GENERAL DISCUSSION

Phytophthora cinnamomi, although it is not considered to be indigenous, is widespread throughout the southwest and southern areas of Western Australia. It must be recognised that the intensive site preparation of land for pine plantations, their subsequent management and eventually the extraction and haulage of the timber produce, has the potential to infest any area developed for pine plantations. The extension and proliferation of the disease will then depend on the type of soil, particularly the soil texture and depth. As the majority of plantations will be sited on marginal land, it is reasonable to expect that these plantations will become infested with *P. cinnamomi*, and these infestations will reduce the productive potential of the *Pinus radiata* trees.

Various cultural methods could be applied for the control of disease but they are not expected to have a significant effect. Sites having the greatest potential for disease development could be avoided, an alternative species such as the resistant *Pinus pinaster* could be used, strict hygiene could be implemented to minimise the spread of the pathogen, plantations could be managed to maintain a pine canopy closure, and thinning and timber extraction could be carried out in the summer months only. The solution to the problem lies in breeding a radiata tree that has resistance to the disease while maintaining all of the other improved characters of the tree.

Libby *et al.* (1969) stated "... In a forest plantation, a genetically poor tree will continue to produce below the potential of the land and climate resource for as long as it occupies the site. ..... investment in genetically good trees will maintain above average growth on the land resource and will continue without further cost. ..... breeding for pest resistance is compatible both with more efficient management and with a higher harvest per hectare by avoiding loss to disease." Zobel and Talbert (1984) have stated "... Although the development of resistant strains is expensive and takes special skills and much time, it has the advantage that, once obtained, resistance is relatively permanent, and more resistance can be added ..... one can breed for economic characteristics such as straightness and wood quality while at the same time one can breed for broad adaptability to pests and diverse environments." Again, a quote from Zobel (1982a) "... breeding for disease resistance is economically profitable and a necessity for most tree improvement programmes." Umaerus (1982) concluded "... Clearly disease resistant plants are the

number one defence against plant diseases and pests ..... the use of plant resistance presents little hazard to the environment and presents to the growers a built-in plant protection, which without risks and extra costs or efforts is provided with the seed."

Breeding for resistance to *Phytophthora cinnamomi* has resulted in very large genetic gains which are potentially actual gains because of the very high heritability. The genetic gain of 60% in seedling survival has been calculated for using only genotypes that have a tolerant or moderately-tolerant response as compared with using a seed population that ignores this *Phytophthora* tolerance character. This estimate was derived from seven glasshouse tests and two field tests, and was consistent over this range of environments. Test average seedling mortality ranged from 10% to 52% and in each case the multiplication of the test average by the factor of 0.4 gave a close approximation to the mortality of the tolerant / moderately-tolerant group. Seedling losses in the field were about one third to one quarter of the deaths in the glasshouse. This data indicates that a considerable number of diseased seedlings survive in the field. These trees cannot achieve their genetic potential for growth and are subject to tree death at any stage during the plantation rotation, if the host / pathogen / environment balance alters to favour the pathogen.

The effect of sub-lethal infections of *P. cinnamomi* on the growth of plantation trees of *Pinus radiata* has been quantified. Examples were given showing a 20-40% reduction in potential height growth, of four to five-year-old surviving trees of susceptible families on a dieback site. The field tests, FP1 and FP2, have indicated that height growth was reduced by 9% when compared with the growth of a population that included tolerance to *Phytophthora cinnamomi* as a major character.

Tremendous variation exists within the *Pinus radiata* species in response to *Phytophthora cinnamomi* infection. Variability was large for all of the population hierarchal levels. At the provenance or major population level, seedlings from Cambria showed the greatest degree of resistance, Monterey was similar, while seedlings from Ano Nuevo and the two island populations of Guadalupe and Cedros generally had a susceptible reaction. With the exception of the uniform response of the resistant Cambria population and the susceptible Cedros Island population, variation within the population groups was very large. Individual families within the Monterey, Ano Nuevo and Guadalupe Island populations were identified that have a high level of resistance to infection.

The variation in disease response was equally as large between the world's major domestic populations as it was among the natural populations. *Pinus radiata* from South Africa was consistently less affected by *Phytophthora cinnamomi* infection than was *Pinus radiata* from Australia and New Zealand. Double the number of seedlings from Australia and New Zealand died as a result of *Phytophthora cinnamomi* infection, compared with the South African population. Variability within the South African, Australian and New Zealand populations was also very large. Many more families from South Africa were classified as resistant compared with the others, although the family recognised as the most resistant was located within the generally susceptible New Zealand "268" population. All *Pinus radiata* resources should be searched to locate resistant individuals to add to the developing *Phytophthora cinnamomi* tolerant population. Priority should be given to the generally tolerant South African population and to other populations where the genetic information on tree growth and form are already known.

Diversity is the key to the development and use of genetic disease resistance in forest crops. A broad genetic base will be maintained and deployed to provide functional diversity for the control of other diseases and pests that could eventually be introduced into Western Australia; for example, *Dothistroma pini*, *Endocronartium harknessii*, *Sirex noctilio*. Genetical resistance in *Pinus radiata* to *Dothistroma pini* is currently being investigated in East Africa (Ivory and Patterson, 1970), in New Zealand (Wilcox, 1982) and in New South Wales (Johnson, 1986), and resistance to *Endocronartium harknessii* is being researched in California (Old *et al.*, 1986).

The main *P. radiata* breeding population in Western Australia is being developed on the multiline concept (Browning and Frey, 1969). Special sublines have been created that have resistance to *Phytophthora cinnamomi* as a principal character, as well as being outstanding for vigour, stem and branch form (Butcher, 1986a). Crossings between the independent sublines will be effected in intensive management seed orchards (HAPSO) to ensure completely outbred offspring at any time in the future (Butcher, 1986b). Genetic material from other programmes can be introduced immediately, at any time, directly into the HAPSO as a pollen mix to provide resistance to any other introduced disease.

Heritability of the response of seedlings and trees of *Pinus radiata* to *Phytophthora cinnamomi* root-rot was very high and consistent, both at the family and individual level. The individual heritability for disease intensity in the native *Pinus radiata* species was close to unity in the natural population tests, but its validity is questionable, as the estimates of variances for the parent trees are for trees sampled in different intermating populations. Heritability estimates for each intermating natural population varied considerably, due to the small sample size and the different response of the populations. The heritability was very high for the Guadalupe Island, Ano Nuevo and Monterey populations, where there was large intra-population variation, and was low for the consistent response Cambria and Cedros Island populations.

Family heritability for incidence of disease in the domestic populations and field studies averaged 0.80 (range 0.58 to 0.87), and was 0.80 (range 0.55 to 0.87) for the intensity of disease. Narrow sense heritability for the intensity of disease of individuals was extremely high, averaging 0.79 (range 0.58 to 0.99) in the South African population, and 0.84 (range 0.75 to 0.92) for the New Zealand trees. The heritability for the South Australian population was constant at 0.38 (range 0.33 to 0.43). For the domestic population and field studies, the intermating populations were either plantations or clonal seed orchards, so that mating can be assumed to be at random and to involve a large number of pollinators. These families fit the half-sib model quite well, making the results all the more impressive. Identical individual and family heritabilities suggests that the exactness of the half-sib relationship is immaterial. This conclusion is important to the natural population studies as trees in the very small Guadalupe Island population are highly related and more homozygous within themselves, than the larger heterozygous mainland populations.

The additive genetic variance has accounted for most of the phenotypic variance for the disease resistance trait. These results are most unusual as there was no control on the pollen parentage in any of the reported tests, the complete spectrum of the *Pinus radiata* species was tested, and the tests were conducted in different glasshouse environments, and in the field. The female contribution to the character is completely dominant and the male apparently has little effect. Therefore, cytoplasmic inheritance is most likely to

be a major mode of resistance in the *Pinus radiata* / *Phytophthora cinnamomi* system. Confirmation of this is required, as it has a profound effect on the constitution and development of sublines within the Western Australian breeding population.

Resistance to *P. cinnamomi* disease in *Pinus radiata* is under strong genetic control. This has been shown to be constant in a diverse range of environments and for a wide range of pathogen isolates. The level of disease expression has varied according to the conditions of the environment and the virulence of the *Phytophthora cinnamomi* isolates used in the tests. However, ranking of families for their response to the pathogen has remained unchanged and particularly, the variation in disease expression in the host appears to be independent of the variation in the pathogen. Both of these forms of variability are rarely encountered with disease resistance that is controlled by major genes (Russell, 1978). Indications are that there are a number of genes in *Pinus radiata*, each having a small effect, acting additively to give general resistance to *Phytophthora cinnamomi*. General or non-specific resistance will be more difficult to define in genetic and biochemical terms than specific resistance (Williams, 1975) as complex interactions between numerous genes may be involved. In tree crops, data on the genetic base of resistance, comparable to agricultural crops, are not available, even for the much studied biotrophic leaf rusts of poplar (Heather and Chandrashekar, 1982) and white pine (Bingham, 1983). Our use of open-pollinated families and heterogeneous sources of inoculum is unlikely to provide precise identification of resistance genes, but it will result in a diversity of resistance genes selected in a broad *Pinus radiata* population. This should guarantee durable resistance to *Phytophthora cinnamomi* over the long thirty-year plantation rotation. Dinus (1982) made the important statement "... Knowing the how and why of resistance will facilitate continued progress but much work can be deferred until more important questions are answered. Hastening release of a first usable product, however preliminary, is more important."

The two field tests have shown that family response to *P. cinnamomi* is similar in the glasshouse and in the field. Family rankings for the incidence of disease were very highly correlated in both test environments. Intensity of disease in the field, as measured by the height growth of trees, also gave a similar ranking of families as for the glasshouse test. The glasshouse test can be used as a reliable and rapid method for determining the *Pinus radiata* family response to *Phytophthora cinnamomi*.

Techniques for the glasshouse test have been defined, and refined to give the maximum expression of the *P. cinnamomi* disease for each family. The most important of these were the use of a multi-family treatment pot so that all families were subjected to similar inoculum levels of the pathogen, the use of multiple isolates of the pathogen in each pot so that resistance is tested against a broad spectrum of the pathogen, and experiments are conducted in a moderate moisture / moderate temperature regime in the glasshouse. Other improvements were the development of seedling disease intensity scales for the quantitative analysis of the disease.

The development of the empirical scale to represent the continuous variation in response of *Pinus radiata* seedlings to infection with *Phytophthora cinnamomi*, was paramount to the statistical model for the partitioning of experimental variances and their interpretation. The root assessment of the surviving seedlings and sorting into disease expression classes is obviously better than the height increment following inoculation sorting, even with standardisation of the increment classes. The height increment scale is dependent on a prescribed mean performance and the total population variance, before the pathogen was applied. How representative the standard for comparison is can have

an effect on this arbitrary scale. The root assessment is independent of any family mean and is a realistic sample of the surviving seedling population for the intensity of disease. Disease at the other end of the scale, dead seedlings, has been divided into classes subject to the length of time taken for seedlings to die. Again, the class limits are fairly arbitrary but they are based on the experience of a large number of *Pinus radiata* / *Phytophthora cinnamomi* screening tests.

BIBLIOGRAPHY

Allard, R.W., 1960. Principals of Plant Breeding. John Wiley and Sons, New York, 485 pp.

Allen, J.D., 1973. Pests and diseases of radiata pine. N.Z. J. For., 18: 265-272.

Ashby, S.F., 1929. Strains and taxonomy of *Phytophthora palmivora.* Trans Br. Mycol. Soc., 14: 18-38.

Ayres, P.G., 1978. Water relations of diseased plants. In: T.T. Kozlowski (Editor), Water Deficits and Plant Growth. Volume 5, Water and Plant Disease. Academic Press, New York, pp. 1-59.

Bain, J., 1981. Forest monocultures - how safe are they? An entomologist's view. N.Z. J. For., 26: 37-42.

Baker, R., 1978. Inoculum potential. In: J.G. Horsfall and E.B. Cowling (Editors), Plant Disease, An Advanced Treatise. Volume 2, How disease develops in populations. Academic Press, New York, pp. 137-157.

Bannister, M.H., 1959. Artificial selection and *Pinus radiata.* N.Z. J. For., 8: 69-90.

Bannister, M.H., 1965. Variation in the breeding system of *Pinus radiata.* In: H.G. Baker and G. Ledyard Stebbins (Editors), The Genetics of Colonizing Species. Academic Press, New York, pp. 353-372.

Bannister, M.H., 1969. An early progeny trial in *Pinus radiata* 1. Juvenile characters. N.Z. J. Bot., 7: 316-335.

Bannister, M.H., Williams, A.L., McDonald, I.R.C. and Forde, M.B., 1962. Variation of turpentine composition in five population samples of *Pinus radiata.* N.Z. J. Sci., 5: 489-495.

Barnett, H.C., 1959. Plant disease resistance. Ann. Rev. Microbiol., 13: 191-209.

Barrett, R.L. and Mullin, L.J., 1968. A review of introductions of forest trees in Rhodesia. Salisbury : The Rhodesia Forestry Commission.

Bassett, C. and Will, G.M., 1964. Soil sterilisation trials in two forest nurseries. N.Z.J. For., 9: 50-58.

Batini, F.E., 1973. Jarrah dieback - a disease of the jarrah forest of Western Australia. Forests Department of Western Australia, Bulletin 84, 45 pp.

Batini, F.E., 1978. Screening of *Pinus pinaster* seedlings for resistance to *Phytophthora cinnamomi* in pot trials. Forests Department of Western Australia, Research Paper 39, 7 pp.

Batini, F.E. and Podger, F.D., 1968. Shelterbelt mortalities on the Swan coastal plain. Aust. For. Res., 3: 39-45.

Bazzigher, G., 1981. Selection of blight-resistant chestnut trees in Switzerland. Eur. J. For. Path., 11: 199-207.

Becker, W.A., 1975. Manual of Quantitative Genetics. Students Book Corp., Pullman, Washington, 170 pp.

Bega, R.V., 1962. Tree killing by *Fomes annosus* in a genetics arboretum. Plant Dis. Rept., 46: 107-110.

Bell, A.A., 1980. The time sequence of defence. In: J.G. Horsfall and E.W. Cowling (Editors), Plant Disease - An Advanced Treatise. Volume 5 - How plants defend themselves. Academic Press, New York, pp. 53-73.

Bingham, R.T., 1983. Blister rust resistant western white pine for the inland empire : the story of the first 25 years of the research program. U.S.D.A. For. Serv. Int. Forest and Range Exp. Stat., General Technical Report INT-146, 45 pp.

Bingham, R.T., Olsen, R.J., Becker, W.A. and Marsden, M.A., 1969. V. Estimates of heritability, combining ability and genetic advance based on tester matings. Silvae Genetica, 18: 28-38.

Boomsma, D.B., Kroemer, P. and Munn, R., 1983. Cooperative plus tree selection of radiata pine in the southeast of South Australia and southwestern Victoria. Proc. 8th Meeting Aust. For. Council, Research Working Group No.1, Perth, Western Australia, pp. 76.

Boughton, T.J., 1983. Results of a preliminary screening of clover cultivars as potential hosts for *Phytophthora cinnamomi* in the Donnybrook Sunkland. Forests Department of Western Australia, Research Paper 74, 7 pp.

Boughton, T.J. and Crane,C.E., 1984. Assessment of *Phytophthora* disease risk at the Nannup nursery. Forests Department of Western Australia, Research Paper 77, 8 pp.

Bowman, R.H. and Estrada, D.C., 1980. Soil survey of Santa Cruz County, California. U.S.D.A. Soil Conserv. Serv.

Boyce, J.S., 1954. Forest plantation protection against diseases and insect pests. F.A.O. Forestry Development Paper 3.

Browning, J.A. and Frey, K.J., 1969. Multiline cultivar as a means of disease control. Ann. Rev. Phytopath., 7: 355-381.

Browning, J.A., Simons, M.D. and Torres, E., 1977. Managing host genes : epidemiologic and genetic concepts. In: J.G. Horsfall and E.B. Cowling (Editors), Plant Disease - An Advanced Treatise. Volume 1 - How disease is managed. Academic Press, New York, pp. 191-212.

Bryan, W., 1954. *Pinus radiata* and associated species in the granite belt. Aust. For., 18: 15-20.

Bryan, W.C., 1965. Testing shortleaf pine seedlings for resistance to infection by *Phytophthora cinnamomi.* U.S.D.A. For. Serv. Res. Note SE-50, 4 pp.

Bryan, W.C., 1973. Height growth of shortleaf pine progenies from trees selected for resistance to littleleaf disease. U.S.D.A. For. Serv. Res. Note SE-185, 7 pp.

Bumbieris, M., 1976. The role of *Phytophthora cryptogea* and waterlogging in a decline of *Pinus radiata.* Aust. J. Bot., 24: 703-709.

Bumbieris, M. and Boardman, R., 1979. *Phytophthora cinnamomi* in a pine forest in South Australia. Aust. Plant Path., 8: 49.

Burdon, R.D., 1985. Introductions of *P. radiata* to New Zealand. I.U.F.R.O., Working Party S2-03-09, Breeding *Pinus radiata*. Newsletter No. 6, pp. 3-4.

Burdon, R.D. and Bannister, M.H., 1973. Provenances of *Pinus radiata* : their early performance and silvicultural potential. N.Z. J. For., 18: 217-232.

Burdon, R.D., Currie, D. and Chou C.K.S., 1982. Responses to inoculation with *Diplodia pinea* in progenies of apparently resistant trees of *Pinus radiata*. Aust. Plant Path., 4: 37-39.

Bureau of Agricultural Economics, 1984. Australian Forest Resources 1983. Bureau of Agricultural Economics, A.G.P.S., Canberra, 32 pp.

Butcher, T.B., 1977. Impact of moisture relationships on the management of *Pinus pinaster* plantations in Western Australia. For. Ecol. Manage., 1: 97-107.

Butcher, T.B., 1986 a. Concepts of a revised plan for the breeding of *Pinus radiata* in Western Australia. Proc. 9th Meeting Aust. For. Council, Research Working Group No.1 , Burnie, Tasmania, pp. 23-26.

Butcher, T.B., 1986 b. HAPSO - Hedged Artificially Pollinated Seed Orchard. Proc. 9th Meeting Aust. For. Council, Research Working Group No.1. Burnie, Tasmania, pp. 15-16.

Butcher, T.B. and Stukely, M.J.C., 1986. Update of *Pinus radiata* / *Phytophthora cinnamomi* studies in Western Australia. Proc. 9th Meeting Aust. For. Council, Research Working Group No.1, Burnie, Tasmania, pp. 36-39.

Butcher, T.B., Stukely, M.J.C. and Chester, G.W., 1984. Genetic variation in resistance of *Pinus radiata* to *Phytophthora cinnamomi*. For. Ecol. Manage., 8: 197-220.

Byrt, P.H. and Holland, A.A., 1978. Infection of axenic *Eucalyptus* seedlings with *Phytophthora cinnamomi* zoospores. Aust. J. Bot., 26: 169-176.

Caldwell, R.M., Schafer, J.F., Compton, L.E. and Patterson, F.L., 1958. Tolerance to cereal leaf rusts. Science, 128: 714-715.

Calvert, O.H., 1982. Coming to terms with terms. Plant Disease, 66: 760.

Campbell, W.A., and Copeland, O.L., 1954. Little leaf disease of shortleaf and loblolly pines. U.S.D.A. circular 940, 41 pp.

Carson, S.D., 1985. Development of *Dothistroma*-resistant breed of *Pinus radiata*. I.U.F.R.O., Working Party S2-03-09, Breeding *Pinus radiata*. Newsletter No.6, pp. 6-8.

Caten, C.E., 1971. Single zoospore variation in *Phytophthora infestans* and attenuation of strains in culture. Trans. Brit. Mycol. Soc., 56: 1-7.

Chee, K.H. and Newhook, F.J., 1965. Variability in *Phytophthora cinnamomi*. N.Z. J. Agric. Res., 8: 96-103.

Chevis, H.W., 1984. *Pinus radiata* deaths rates associated with *Phytophthora* spp. on different soil types in the Donnybrook Sunkland of Western Australia. Forests Department of Western Australia, Research Paper 79, 5 pp.

Chevis, H.W., and Stukely, M.J.C., 1982. Mortalities of young established radiata pine associated with *Phytophthora* spp. in the Donnybrook Sunkland plantations in Western Australia. Aust. For., 45: 193-200.

Chilean Forestry News, 1987. Growth of the national forest mass. Chilean Forestry News, No.109, pp. 6-7.

Chou, C.K.S., 1976. A shoot dieback in *Pinus radiata* caused by *Diplodia pinea*. 1. Symptoms, disease development and isolation of the pathogen. N.Z. J. For. Sci., 6: 72-79.

Chou, C.K.S., 1977. Effect of tree age on *Diplodia pinea* infection of *Pinus radiata*. Plant Dis. Rept., 61: 101-103.

Chou, C.K.S., 1981. Monocultures, species diversification and disease hazards in forestry. N.Z. J. For., 26: 20-36.

Cobb, F.W. and Libby, W.J., 1968. Susceptibility of Monterey,Guadalupe Island, Cedros Island and Bishop pines to *Scirrhia* (*Dothistroma*) *pini*, the cause of red band needle blight. Phytopathology, 58: 88-90.

Cobb, F.W. and Miller D.R., 1968. Hosts and geographic distribution of *Scirrhia pini* - the cause of red band needle blight in California. J. For., 66: 930-933.

Cochran, W.G. and Cox, G.M., 1957. Experimental Designs. John Wiley and Sons, New York, 611 pp.

Cockerham, C.C., 1963. Estimation of genetic variances. In: W.D. Hanson and H.F. Robinson (Editors), Statistical Genetics and Plant Breeding. NAS-NRC., Public. No. 982, Washington, pp. 53-93.

Conservation and Land Management, 1987. Timber production in Western Australia. A strategy to take W.A.'s Forests into the 21st Century. Department of Conservation and Land Management, Western Australia, 192 pp.

Cooke, R.C. and Rayner, A.D.M., 1984. Ecology of Saprophytic Fungi. Longman, London, 415 pp.

Cooke, T.D., 1978. Soil survey of Monterey County, California. U.S.D.A. Soil Conserv. Serv.

Cotterill, P.P. and Zed, P.G., 1980. Estimates of genetic parameters for growth and form traits in four *Pinus radiata* progeny tests in South Australia. Aust. For. Res., 10: 155-167.

Cotterill, P.P., Correll, R.L. and Boardman, R., 1983. Methods of estimating the average performance of families across incomplete open-pollinated progeny tests. Silvae Genetica, 32: 28-32.

Cowling, E.B., 1980. Agricultural and forest practices that favour epidemics. In: J.C. Horsfall and E.B. Cowling (Editors), Plant Disease - An Advanced Treatise. Volume 2 How disease develops in populations. Academic Press, New York, pp. 361-381.

Cromer, R.N., Eldridge, K.G., Tompkins, D. and Barr, N.J., 1982. Intra-specific variation in the response of *Pinus radiata* to saline and water waste. Aust. For. Res., 12: 203-215.

Currie, D. and Toes, E., 1978. Stem volume loss due to severe *Diplodia* infection in a young *Pinus radiata* stand. N.Z. J. For., 23: 143-148.

Davison, E.M. and Bumbieris, M., 1973. *Phytophthora* and *Pythium* spp. from pine plantations in South Australia. Aust. J. Biol. Sci., 26: 163-169.

Day, P.R., 1980. The genetic basis of epidemics. In: J.G. Horsfall and E.B. Cowling (Editors), Plant Disease - An Advanced Treatise. Volume 2 - How disease develops in populations. Academic Press, New York, pp. 263-285.

De Gryse, J.J., 1955. Forest pathology in New Zealand. N.Z. For. Serv., Bulletin No. 1.

Dinus, R.J., 1982. Integrating resistance breeding and tree improvement. In: H.M. Heybroek, B.R. Stephan and K. Von Weissenberg (Editors), Resistance to Diseases and Pests in Forest Trees. C.A.P.D., Wageningen, pp. 452-459.

Donald, D.G.M. and Von Broembsen, S.L., 1977. The control of *Phytophthora cinnamomi* in a South African forest nursery. S.Afric. For. J., 100: 50-55.

Edwards, D.W. and Walker, J., 1978. *Dothistroma* needle blight in Australia. Aust. For. Res., 8: 125-137.

Eldridge, K.G., 1978 a. Seed collections in California in 1978. C.S.I.R.O., Div. For. Res., Annual Report 1977 - 1978, pp. 8-17.

Eldridge, K.G., 1978 b. Refreshing the genetic resources of radiata pine plantations. C.S.I.R.O., Div. For. Res., Genetics Sect. Rep. No. 7 (unpubl.), 120 pp.

Eldridge, K.G., 1979. *Pinus radiata* and *P. muricata* seed collections, California 1978. Aust. For. Council, RWG No.1, Forest Genetics Newsletter No. 1979-1, pp. 3-6.

Eldridge, K.G., 1983. Genetic resources available. In: A.C. Matheson and A.G. Brown (Editors), Radiata Pine Breeding Manual. C.S.I.R.O., Div. For. Res., pp. 3.1-3.15.

Erwin, D.C., 1983. Variability within and among species of *Phytophthora*. In: D.C. Erwin, S. Bartnicki-Garcia and P.H. Tsao (Editors), *Phytophthora* : Its Biology, Taxonomy, Ecology and Pathology. Amer. Phytopathol. Soc., St. Paul, Minn., pp. 149-165.

Falconer, D.S., 1960. Introduction to Quantitative Genetics. Longman, New York, 340 pp.

Fenton, R., 1978. Risks, or the case for diversification of production plantation species and the role of Douglas Fir. In: R.N. James and E.H. Bunn (Editors), A review of Douglas Fir in New Zealand. N.Z. For. Serv., For. Res. Inst. Symp. No. 15.

Fielding, J.M., 1957. The introduction of *Pinus radiata* to Australia. Aust. For., 21: 15-16.

Fielding, J.M., 1961. The pines of Cedros Island, Mexico. Aust. For., 25: 62-65.

Fisher, R.A. and Yates, F., 1963. Statistical Tables for Biological, Agricultural and Medical Research. Sixth edition. Oliver and Boyd, Edinburg, 138 pp.

Flor, H.H., 1956. The complementary genic systems in flax and flax rust. Advan. Genet., 8: 29-54.

Flor, H.H., 1971. Current status of the gene-for-gene concept. Ann. Rev. Phytopathol., 9: 275-296.

Forde, M.B., 1966. *Pinus radiata* in California. N.Z. J. For., 11: 20-42.

Forests Department of Western Australia, 1969. 50 years of forestry in Western Australia. Forests Department of W.A., 90 pp.

Forests Department of Western Australia, 1975. Afforestation with pines in the Donnybrook Sunkland. Statement of Intent. Forests Department of W.A., 84 pp.

Gadgil, P.D., 1984. *Dothistroma* needle blight. Forest Pathology in New Zealand, No. 5., 8 pp.

Gallegly, M.E., 1970. Genetics of *Phytophthora.* Phytopathology, 60: 1135-1141.

Gerrettson-Cornell, L., Humphreys, F.R. and Townsend, S.R. 1976. Results of a preliminary investigation on the use of *Pinus radiata* bark against *Phytophthora cinnamomi*. Phyton, 34: 3-6.

Gibson, I.A.S., 1972. *Dothistroma* blight of *Pinus radiata.* Ann. Rev. Phytopathol., 10: 51-72.

Gibson, I.A.S., Christensen, P.S. and Munga, F.N., 1964. First observations in Kenya on a foliage disease of pines caused by *Dothistroma pini.* Common. For. Rev., 43: 31-48.

Gilmour, J.W., 1966. Pathology of forest trees in New Zealand. N.Z. For. Serv., Tech. Paper 48, 82 pp.

Gilmour, J.W., 1967. Distribution and significance of the needle blight of the pine caused by *Dothistroma pini* in New Zealand. Plant Dis. Rept., 51: 727-730.

Gilmour, J.W., Alma, P., Marks, G.C. and Neumann, F.G., 1975. The status of diseases and insects in New Zealand and Australia. 2nd. World Technical Consultation on Forest Diseases and Insects. F.A.O. and I.U.F.R.O., Delhi.

Gisi, U., 1983. Biophysical aspects of the development of *Phytophthora*. In: D.C. Erwin, S. Bartnicki-Garcia and P.H. Tsao (Editors), *Phytophthora* : Its Biology, Taxonomy, Ecology and Pathology. Amer. Phytopathol. Soc., St. Paul, Minn., pp. 109-119.

Haasis, F.A., Nelson, R.R. and Marx, D.H., 1964. Morphological and physiological characteristics of mating types of *Phytophthora cinnamomi.* Phytopathology, 54: 1146-1151.

Hanson, W.D., 1963. Heritability. In: W.D. Hanson and H.F. Robinson (Editors), Statistical Genetics and Plant Breeding. NAS-NRC., Public. No. 982, Washington, pp. 125-139.

Harlin, J.R., 1976. Diseases as a factor in plant evolution. Ann. Rev. Phytopathol., 14: 31-51.

Harris, M.K. and Frederiksen, R.A., 1984. Concepts and methods regarding host plant resistance to arthropods and pathogens. Ann. Rev. Phytopathol., 22: 247-272.

Hartigan, C., 1964. Some observations on the effect of *Phytophthora* in monterey pine. N.S.W. Forests Commission Forests and Timber, 2: 5-6.

Harvey, W.R., 1960. Least-squares analysis of data with unequal subclass numbers. U.S.D.A. Agric. Res. Serv. Public. No. ARS20-8, 157 pp.

Heather, W.A. and Chandrashekar, M., 1982. Evolutionary, epidemiological and ecological implications of forms of resistance in *Populus* spp. to *Melampsora* leaf rust. Aust. For. Res., 12: 231-244.

Heather, W.A. and Pratt, B.H., 1975. Association of *Phytophthora drechsleri* with death of *Pinus radiata* in southern New South Wales. Aust. J. Bot., 23: 285-288.

Helms, J.A., Cobb, F.W. and Whitney, H.S., 1971. Effect of infection by *Verticladiella wagenerii* on the physiology of *Pinus ponderosa.* Phytopathology, 61: 920-925.

Hepting, G.H., 1949. Managing pines in littleleaf areas. Forest Farmer, 8(11): 7-10.

Hepting, G.H., 1971. Disease of forest and shade trees of the United States. U.S.D.A. For. Serv. Agric. Handbook No. 386, pp. 338-343.

Hepting, G.H. and Newhook, F.J., 1962. A pine disease in New Zealand resembling littleleaf. Plant Dis. Rept. 46: 570-571.

Heybroek, H.M., 1982. Monoculture versus mixture: interactions between susceptible and resistant trees in a mixed stand. In: H.M. Heybroek, B.R. Stephan and K. Von Weissenberg (Editors), Resistance to Diseases and Pests in Forest Trees. C.A.P.D., Wageningen, pp. 326-341.

Hooker, A.L., 1974. Cytoplasmic susceptibility in plant disease. Ann. Rev. Phytopathol., 12: 167-179.

Hopkins, E.R., 1960. Variation in the growth rate and quality of *Pinus pinaster* in Western Australia. Forests Department of Western Australia, Bulletin 67, 32 pp.

Hosking, G.P., 1984. Forestry pest problems of the South Pacific - the price of progress. N.Z. J. For., 29: 185-192.

Ivory, M.H. and Patterson, D.N., 1970. Progress in breeding *Pinus radiata* resistant to *Dothistroma* needle blight in East Africa. Silvae Genetica, 19: 38-42.

Jackson, L.W.R., 1945. Root defects and fungi associated with he littleleaf disease of Southern pines. Phytopathology, 35: 91-105.

Jackson, L.W.R., 1951. Effect of littleleaf disease on growth of shortleaf pine. J. For., 49: 778-779.

Jehne, W., 1971. Soil conditions and the occurrence of *Phytophthora cinnamomi* in relation to deaths in young plantations of radiata pine near Jervis Bay. Aust. For. Res., 5(3): 39-46.

Johnson, I.G., 1986. *Dothistroma* resistance in clones of radiata pine. Proc. 9th Meeting Aust. For. Council, Research Working Group No.1. Burnie, Tasmania, pp. 35.

Johnson, R., 1984. A critical analysis of durable resistance. Ann. Rev. Phytopathol., 22: 309-330.

Keirle, R.M., 1979. *Phytophthora cinnamomi* in proposed softwood plantation areas on the central tablelands of New South Wales. Aust. Plant. Path., 8: 20-21.

Kempthorne, O., 1957. An Introduction to Genetical Statistics. Wiley, New York.

Kendall, M.G. and Stuart, A., 1961. The Advanced Theory of Statistics. Volume 2. Hafner, New York, 676 pp.

King, N.L., 1925. *Pinus insignis* (*Pinus radiata*) in South Africa. Union of South Africa, Forest Department Bulletin No. 15, 30 pp.

Kleinschmit, J., 1979. Limitations for restriction of the genetic variation. Silvae Genetica, 28: 61-67.

Kinloch, B.B., 1982. Mechanisms and inheritance of rust resistance in conifers. In: H.M. Heybroek, B.R. Stephan and K. Von Weissenberg (Editors), Resistance to Diseases and Pests in Forest Trees. C.A.P.D., Wageningen, pp. 119-129.

Kung, F.H., 1972. Experimental design and analysis for half-sib progeny test. In: J.F. Kraus (Editor), Proc. Meeting of the Working Party on Progeny Testing, I.U.F.R.O., Macon, Georgia Forest Research Council, pp. 82-102.

Lange, P.W., 1979. A note on *Pinus radiata* improvement in South Africa. Breeding *Pinus radiata*. I.U.F.R.O., Working Party S2-03-09, Newsletter No. 3, pp. 3-4.

Langer, W., 1952. Kreuzungsversuche mit *Larix europaea* und *L . leptolepis*. Z. Forstgenet., 1: 40-56.

Ledig, F.T., 1986. Conservation strategies for forest gene resources. For. Ecol. Manage., 14: 77-90.

Libby, W.J., 1978. The 1978 expedition to collect radiata seed from Cedros and Guadalupe Islands. Breeding *Pinus radiata*. I.U.F.R.O., Working Party S2-03-09, Newsletter No. 2, pp. 8-12.

Libby, W.J., 1983. Concluding synthesis and the way ahead. I.U.F.R.O., Working Party S2-03-09, Breeding *Pinus radiata*. Newsletter No. 5, pp. 26-32.

Libby, W.J., Bannister, M.H. and Linhart, Y.B., 1968. The pines of Cedros and Guadalupe Islands. J. For., 66: 846-852.

Libby, W.J. and Russell, J.H., 1985. Return of the expatriate radiata. I.U.F.R.O., Working Party S2-03-09, Breeding *Pinus radiata*. Newsletter No. 6, pp. 12-16.

Libby, W.J., Stettler, R.F. and Seitz, F.W., 1969. Forest genetics and forest tree breeding. Ann. Rev. Genet., 3: 469-494.

Lindsay, A.D., 1932. Monterey pine (*Pinus radiata*) in its native habitat. Comm. For. Bur., Australia, Bulletin No. 10, 57 pp.

Lippik, E.E., 1970. Gene centres of plants as sources of disease resistance. Ann. Rev. Phytopathol., 8: 323-344.

Luckhoff, H.A., 1964. Diseases of exotic plantation trees in the Republic of South Africa. F.A.O / I.U.F.R.O. Symposium, Meeting No. V1.

MacKenzie, M. and Shaw, C.G., 1977. Spatial relationships between *Armillaria* root-rot of *Pinus radiata* seedlings and the stumps of indigenous trees. N.Z. J. For. Sci., 7: 374-383.

Magnani, G., 1966. Malattic fungine del *Pinus radiata*. Rome. Centro Di Spermentazione Agricola E. Forestale Publicazione Vol. 9, pp. 23-30.

Manion, P.D., 1981. Tree Disease Concepts. Prentice-Hall, New Jersey, 399pp.

Marais, L.J. and Kotze, J.M., 1976. Ectomycorrhizae of *Pinus patula* as biological deterrents to *Phytophthora cinnamomi*. S. Afric. For. J., 99: 35-39.

Marks, G.C., 1979. Review of the host-parasite relations 0f *Eucalyptus* spp. infected by *Phytophthora cinnamomi*. In: K.M. Old (Editor), Phytophthora and Forest Management in Australia. C.S.I.R.O., Melbourne, pp. 28-42.

Mather, K.M. and Jinks, J.L., 1982. Biometrical Genetics. Third edition. Chapman and Hall, London, 396 pp.

Matheson, A.C., 1985. *Dothistroma* needle blight in Australia. I.U.F.R.O., Working Party S2-03-09, Breeding *Pinus radiata*. Newsletter No. 6, pp. 8-10.

McCutcheon, G.S., 1978. Broadscale forest site survey techniques used in the Donnybrook Sunkland. Forests Department of Western Australia, Research Paper 48, 10 pp.

McKinnell, F.H., 1981. Review of the dieback disease situation 1981. Forests Department of Western Australia, Technical Paper No. 2, 8 pp.

Mitchell, D.J. and Zentmyer, G.A., 1971. Effects of oxygen and carbon dioxide tensions on growth of several species of *Phytophthora*. Phytopathology 61: 787-791.

Moran, G.F. and Bell, J.C., 1983. The genetic resources of *Pinus radiata* in the natural populations and in Australian breeding programs. Biennial Report, C.S.I.R.O., Div. For. Res. 1981-1983, Canberra, pp. 25-26.

Moran, G.F. and Bell, J.C., 1987. The origin and genetic diversity of *Pinus radiata* in Australia. Theor. Appl. Genet., 73: 616-622.

Moran, G.F., Bell, J.C. and Matheson, A.C., 1980. The genetic structure and levels of inbreeding in a *Pinus radiata* seed orchard. Silvae Genetica, 29: 190-193.

Morley, F.H., 1963. Statistics and plant selection. In: W.D. Hanson and H.F. Robinson (Editors). Statistical Genetics and Plant Breeding. NAS-NRC., Public. No. 982, Washington, pp. 218-222.

Munnecke, D.E. and Bricker, J.L., 1976. *Phytophthora* root-rot of *Pinus radiata* Christmas tree plantings. Plant Dis. Rept., 60: 928-932.

Mussell, H., 1980. Tolerance to disease. In: J.G. Horsfall and E.B. Cowling (Editors), Plant Disease : An advanced Treatise. Vol. 5 - How plants defend themselves. Academic Press, New York, pp. 39-51.

Nambiar, E.K.S., Cotterill, P.P. and Bowen, G.D., 1982. Genetic differences in the root regeneration of radiata pine. J. Exp. Bot., 33: 170-177.

Namkoong, G., Snyder, E.B. and Stonecypher, R.W., 1966. Heritability and gain concepts for evaluating breeding systems such as seedling orchards. Silvae Genetica, 15: 76-84.

National Academy of Sciences, 1968. Plant - Disease Development and Control. Nat. Acad. Sci. Public. No. 1596, 205pp.

Nelson, R.R., 1972. Stabilising racial populations of plant pathogens by use of resistance genes. J. Environ. Quality, 1: 220-227.

Nelson, R.R., 1982. On genes for disease resistance in plants. In: H.M. Heybroek, B.R. Stephan and K. Von Weissenberg (Editors), Resistance to Diseases and Pests in Forest Trees. C.A.P.D., Wageningen, pp. 84-93.

Nesbitt, H.J., Malajczuk, N. and Glenn, A.R., 1979. Effect of soil moisture and temperature on the survival of *Phytophthora cinnamomi* in soil. Soil Biol. Biochem., 11: 137-140.

Newhook, F.J., 1959. The association of *Phytophthora* spp. with mortality of *Pinus radiata* and other conifers. 1 : Symptoms and epidemiology in shelter belts. N.Z. J. Agric. Res., 2: 808-843.

Newhook, F.J., 1960 a. Some comments on the relationship of climate to *Phytophthora* infection of *Pinus radiata*.N.Z. J. For., 8: 261-265.

Newhook, F.J., 1960 b. Climate and soil type in relation to *Phytophthora* attack on forest trees. Proc. N.Z. Ecol. Soc., 7: 14-15.

Newhook, F.J., 1964. Forest disease situation, Australasia. F.A.O. / I.U.F.R.O., Symposium on Internationally Dangerous forest diseases and insects. Oxford, 20-30 July 1964.

Newhook, F.J., 1970. *Phytophthora cinnamomi* in New Zealand. In: T.A. Toussoun, R.V. Bega and P.E. Nelson (Editors). Root Diseases and Soil-Borne Pathogens. University of California Press, pp. 173-176.

Newhook, F.J. and Podger, F.D., 1972. The role of *Phytophthora cinnamomi* in Australian and New Zealand forests. Ann. Rev. Phytopathol., 10: 299-326.

Nicholls, J.W.P. and Eldridge, K.G., 1980. Variation in some wood and bark characteristics in provenances of *Pinus* radiata. Aust. For. Res., 10: 321-335.

Offord, H.R., 1964. Diseases of Monterey pine in native stands of California and in plantations of western North America. U.S.D.A. For. Serv., Research Paper PSW.14, 37pp.

Ohmart, C.P., 1980. Insect pests of *Pinus radiata* plantations : present and possible future problems. Aust. For., 43: 226-232.

Ohmart, C.P., 1981. An annotated list of insects associated with *Pinus radiata* in California. C.S.I.R.O., Div. For. Res., Div. Rept. No. 8, 50 pp.

Ohmart, C.P., 1982. Destructive insects of native and planted *Pinus radiata* in California and their relevance to Australian forestry. Aust. For. Res., 12: 151-161.

Old, K.M., 1979. Pathology of radiata pine in California. C.S.I.R.O., Div. For. Res., Div. Path. and Microbiol., Sect. Rept. No. 1, 75 pp.

Old, K.M., 1981 a. Western gall rust, a serious disease of *Pinus radiata* in California. Aust. For., 44: 178-184.

Old, K.M., 1981 b. Solar heating of soil for the control of nursery pathogens of*Pinus radiata*. Aust. For. Res., 11: 141-147.

Old, K.M., Libby, W.T., Russell, J.H. and Eldridge, K.G., 1986. Genetic variability in susceptibility of *Pinus radiata* to western gall rust. Silvae Genetica, 35: 145-149

Parlevliet, J.E. and Zadoks, J.C., 1977. The integrated concept of disease resistance; a new view including horizontal and vertical resistance in plants. Euphytica, 26: 5-21.

Parmeter, J.R. and Newhook, F.J., 1967. New Zealand *Pinus* radiata is susceptible to western gall rust disease. N.Z. J. For., 12: 200-201.

Patton, R.F., 1961. The effect of age upon susceptibility of eastern white pine to infection by *Cronartium ribicola*. Phytopathology, 51: 429-434.

Peace, T.R., 1957. Approach and perspective in forest pathology. Forestry, 30: 47-56.

Pederick, L.A., 1967. The structure and identification of the chromosomes of *Pinus radiata*. Silvae Genetica, 16: 69-77.

Pederick, L.A. and Eldridge, K.G., 1983. Characteristics of future radiata pine achievable by breeding. Aust. For., 46: 287-293.

Perry, D.H. and Hopkins, E.R., 1967. Importation of breeding material of *Pinus pinaster* from Portugal. Forests Department of Western Australia, Bulletin No. 75, 66 pp.

Person, C., Fleming, R. and Cargeeg, L., 1982. Non-specific interaction based on polygenes. In: H.M. Heybroek, B.R. Stephan and K. Von Weissenberg (Editors), Resistance to Diseases and Pests in Forest Trees. C.A.P.D., Wageningen, pp. 318-325.

Podger, F.D., 1972. *Phytophthora cinnamomi*, a cause of lethal disease in indigenous plant communities in Western Australia. Phytopathology, 62: 972-981.

Podger, F.D., 1975. The role of *Phytophthora cinnamomi* in dieback diseases in Australian eucalypt forests. In: G.W. Bruehl (Editor), Biology and Control of Soil-Borne Pathogens. Amer. Phytopathol. Soc., St. Paul, Minn., pp. 27-36.

Podger, F.D. and Newhook, F.J., 1971. *Phytophthora cinnamomi* in indigenous plant communities in New Zealand. N.Z. J. Bot., 9: 625-638.

Podger, F.D., Doepel, R.F. and Zentmyer, G.A., 1965. Association of *Phytophthora cinnamomi* with a disease of *Eucalyptus marginata* forest in Western Australia. Plant Dis. Rept., 49: 943-947.

Politowski, K. and Browning, J.A., 1978. Tolerance and resistance to plant disease : an epidemiological study. Phytopathology, 68: 1177- 1185.

Popovich L., 1980. Monoculture, a bugaboo revisited. J. For., 78: 487-489.

Power, A.B. and Dodd, R.S., 1984. Early differential susceptibility of juvenile seedlings and more mature stecklings of *Pinus radiata* to *Dothistroma pini*. N.Z. J. For. Sci., 14: 223-228.

Poynton, R.J., 1957. Notes on exotic forest trees in South Africa. Union of South Africa, Forest Department Bulletin No. 38.

Poynton, R.J., 1977. Tree planting in South Africa. Volume 1., The Pines. Republic of South Africa, Forest Department, pp. 417-433

Pratt, R.G. and Mitchell, J.E., 1976. Interrelationships of seedling age, inoculum, soil moisture level, temperature and host and pathogen genotype in *Phytophthora* root-rot of alfalfa. Phytopathology, 66: 81-85.

Raabe, R.D., 1982. Variation in pathogenicity and virulence in *Fomes annosus* and *Armillaria mellea*. In: H.M. Heybroek, B.R. Stephan and K. Von Weissenberg (Editors), Resistance to Diseases and Pests in Forest Trees. C.A.P.D., Wageningen, pp. 251-259.

Rands, R.D., 1922. Streepkanker van kaneel, veroorzaakt door *Phytophthora cinnamomi* n.sp. Meded. Inst. Plantenziekt., 54: 41.

Rawlings, G.B., 1957. The pathology of *Pinus radiata* as an exotic. Forest Research Institute, New Zealand Forest Service, Technical Paper No. 20, 16pp.

Robertson, A. and Lerner, I.M., 1949. The heritability of all-or-none traits : viability of poultry. Genetics, 34: 395-411.

Robertson, G.I., 1970. Susceptibility of exotic and indigenous trees and shrubs to *Phytophthora cinnamomi*. N.Z. J. Gar. Res., 13: 297-307.

Ross, E.W. and Marx, D.H., 1972. Susceptibility of sand pine to *Phytophthora cinnamomi*. Phytopathology, 62: 1197-1200.

Roy, D.F., 1966. Silvical characteristics of Monterey pine (*Pinus radiata*). U.S.D.A. For. Ser., Res. Pap. PSW-31, 21pp.

Ruehle, J.L. and Campbell, W.A., 1971. Adaptability of geographic selections of shortleaf pine to littleleaf sites. U.S.D.A. For. Ser., Res. Pap. SE-87. 8pp.

Russell, G.E., 1978. Plant Breeding for Disease and Pest Resistance. Butterworths, London, 485pp.

Savage, E.J., Clayton, C.W., Hunter, J.H., Brenneman, J.A., Laviola, C. and Gallegly, M.E., 1968. Homothallism, heterothallism and interspecific hybridization in the genus *Phytophthora.* Phytopathology 58: 407-410.

Schafer, J.F., 1971. Tolerance to plant disease. Ann. Rev. Phytopathol., 9: 235-252.

Schmidt, R.A., 1980. Diseases in forest ecosystems : The importance of functional diversity. In: J.G. Horsfall and E.B. Cowling (Editors) Plant Disease - An Advanced Treatise, Volume 2 - How disease develops in populations.Academic Press, New York, pp. 287-315.

Schoeneweiss, D.F., 1975. Predisposition, stress, and plant disease. Ann. Rev. Phytopathol., 13: 193-211.

Scholander, P.F., Hammel, H.T., Bradstreet, E.D. and Hemmingsen, E.A., 1965. Sap pressure in vascular plants. Science, 148: 339-346.

Scott, C.W., 1960. *Pinus radiata.* F.A.O., Forestry and Forest Product Studies No. 14. F.A.O. of the United Nations, Rome, 328 pp.

Scott, P.R., Johnson, R., Wolfe, M.S., Lowe, H.J.B. and Bennett, F.G.A., 1978. Host-specificity in cereal parasites in relation to their control. Ann. Rept. Plant Breed. Instit., Cambridge, pp. 27-62.

Segal, A., Manisterski, J., Browning, J.A., Fischbeck, G. and Wahl, I., 1982. Balance in indigenous plant populations. In: H.M. Heybroek, B.R. Stephan, K. Von Weissenberg (Editors) : Resistance to Diseases and Pests in Forest Trees. C.A.P.D., Wageningen. pp. 361-370.

Shaw, C.G. and Calderon, S., 1977. Impact of *Armillaria* root rot in plantations of *Pinus radiata* established on sites converted from indigenous forest. N.Z. J. For. Sci., 7: 359-373.

Shaw, C.G. and Toes, E.H.A., 1977. Impact of *Dothistroma* needle blight and *Armillaria* root rot on diameter growth of MIPinus radiata. Phytopathology, 67: 1319-1323.

Shea, K.R., 1971. Disease and insect activity in relation to intensive culture of forests. In: Proceedings XV I.U.F.R.O. Congress, March 14-20 1971, University of Florida, Gainesville, Florida, U.S.A., pp. 109-118.

Shea, S.R., 1975. Environmental factors of the northern jarrah forest in relation to pathogenicity and survival of *Phytophthora cinnamomi.* Forests Department of Western Australia, Bulletin No. 85, 83 pp.

Shea, S.R., 1976. Jarrah forests could be destroyed. Aust. For. Indust. J., 42: 16-22.

Shea, S.R., Gillen, K.J. and Leppard, W.I., 1980. Seasonal variation in population levels of *Phytophthora cinnamomi* in soil in diseased, freely-drained *Eucalyptus marginata* sites in the northern jarrah forest of southwestern Australia. Prot. Ecol., 2: 135-156.

Shelbourne, C.J.A., Burdon, R.D., Bannister, M.H. and Thulin, I.J., 1979. Choosing the best provenance of radiata pine for different sites in New Zealand. N.Z. J. For., 24: 288-300.

Shelbourne, C.J.A., Burdon, R.D., Carson, S.D., Firth, A. and Vincent, T.G., 1986. Development plan for radiata pine breeding Forest Research Institute, Rotorua, New Zealand, 142 pp.

Shepherd, C.J., 1975. *Phytophthora cinnamomi,* an ancient immigrant to Australia. Search, 6: 484-490.

Shepherd, C.J. and Forrester, R.I., 1977. Influence of isolation method on growth rate characteristics of populations of *Phytophthora cinnamomi.* Aust. J. Bot., 25: 477-482.

Shepherd, C.J. and Pratt, B.H., 1974. Temperature-growth relations and genetic diversity of A2 mating-type isolates of *Phytophthora cinnamomi* in Australia. Aust. J. Bot., 22: 231-249.

Shepherd, C.J., Pratt, B.H. and Taylor, P.A., 1974. Comparative morphology and behaviour of A1 and A2 isolates of *Phytophthora cinnamomi.* Aust. J. Bot., 24: 461-470.

Siegal, S., 1956. Non-parametric Statistics for the Behavioural Sciences. International Student Edition, McGraw-Hill Book, Tokyo, 312 pp.

Snedecor, G.W. and Cochran, W.G., 1967. Statistical Methods. Sixth edition. Iowa State University Press. Ames, Iowa, U.S.A., 593 pp.

Sokal, R.R. and Rohlf, F.J., 1981. Biometry; The Principles and Practice of Statistics in Biological Research. Second edition. W.H. Freeman and Company, New York, 859 pp.

Squillace, A.E., 1974. Average genetic correlations among offspring from open-pollinated forest trees. Silvae Genetica, 23: 149-156.

Stahl, W., 1966. Needlecaste fungi on conifers in the Australian Capital Territory. Aust. For., 30: 20-32.

Stahl, W., 1968. *Diplodia pinea*: a preliminary report on some aspects of fungus and host relationships. Aust. For. Res., 3: 27-32.

Stakman, E.C. and Christensen, J.J., 1960. The problem of breeding resistant varieties. In: J.G. Horsfall and A.E. Dimond (Editors), Plant Pathology. Academic Press, New York, pp. 567-624.

Strickberger, M.W., 1968. Genetics. MacMillan Co., New York, 868 pp.

Sutherland, C.F., Newhook, F.J. and Levy, J., 1959. The association of *Phytophthora* spp. with mortality of *Pinus radiata* and other conifers. II: Influence of soil drainage on disease. N.Z. J. Agric. Res., 2: 844-858.

Swart, W.J., Knox-Davies, P.S. and Wingfield, M.J., 1985. *Sphaeropsis sapinea* with special reference to its occurrence on *Pinus* spp. in South Africa. S. Afric. For. J., 135: 1-8.

Swiger, L.A., Harvey, W.R., Everson, D.O. and Gregory, K.E., 1964. The variance of intraclass correlation involving groups with one observation. Biometrics, 20: 818-826.

Tarr, S.A.J., 1972. Principles of Plant Pathology. MacMillan Press, London.

Theron, J.M., Donald, D.G.M., Broembsen, S.L. and Van der Merwe, J.A., 1982. The effect of warm water treatment of *Pinus radiata* seedlings on mycorrhizae survival root growth capacity and *Phytophthora* eradication. S. Afric. For. J., 123: 31-35.

Toda, R., 1974. Notes on the Japanese State Government forest tree breeding project. In: R. Toda (Editor), Forest Tree Breeding in the World. Government Forest Experiment Station, Meguro, Tokyo, pp. 161-169.

Tsao, P.H. and Guy, S.O., 1977. Inhibition of *Mortierel la Pythium* in a *Phytophthora* -isolation medium containing hymexazol. Phytopathology, 67: 796-801.

Umaerus, V., 1982. New problems, trends and visions in agricultural resistance breeding. In: H.M. Heybroek, B.R. Stephan and K. Von Weissenberg (Editors), Resistance to Diseases and Pests in Forest Trees. C.A.P.D., Wageningen, pp. 382-386.

Umaerus, V., Umaerus, M., Erjefalt, L. and Nilsson, B.A., 1983. Control of *Phytophthora* by host resistance : problems and progress. In: D.C. Erwin, S. Bartnicki-Garcia and P.H . Tsao (Editors), Phytophthora : Its Biology, Taxonomy, Ecology and Pathology. Amer. Phytopathol. Soc., St. Paul, Minn., pp. 315-325.

Van der Pas, J.B., Hood, I.A. and MacKenzie, M., 1983. *Armillaria* root rot. Forest Pathology in New Zealand, No. 4, 8 pp.

Van der Pas, J.B., Slater-Hayes, J.D., Gadgil, P.D. and Bulman, ., 1984. *Cyclaneusma* (*Naemacycleus*) needlecaste of *Pinus radiata* in New Zealand. 2: Reduction in growth of the host and its economic implication. N.Z. J. For. Sci., 14: 197-209.

Van der Plank, J.E., 1968. Disease Resistance in Plants. Academic Press, New York .

Van der Plank, J.E., 1978. Genetic and molecular basis of plant pathogenesis. Berlin, Heidelburg, New York, Springer-Verlag.

Van der Plank, J.E., 1982. Host-Pathogen Interactions in Plant Diseases. Academic Press, New York, 207 pp.

Van Emden, H.F. and Williams, G.F., 1974. Insect stability and diversity in agro-ecosystems. Ann. Rev. Ent., 19: 455-475.

Von Broembsen, S.L., 1984 a. Occurrence of *Phytophthora cinnamomi* on indigenous and exotic hosts in South Africa with special reference to the South-Western Cape Province. Phytophylactica, 16: 221-225.

Von Broembsen, S.L., 1984 b. Distribution of *Phytophthora cinnamomi* in rivers of the South-Western Cape Province. Phytophylactica, 16: 227-229.

Von Broembsen, S.L., 1985. *Phytophthora cinnamomi* associated with mortality of native vegetation in South Africa. Plant Disease, 69: 715-717.

Von Broembsen, S.L., 1987. Decline of *Ocotea bullata*. South African F.R.I., Research Review 1985/86., pp. 54.

Von Broembsen, S.L., and Brits, G.J., 1985. *Phytophthora* root-rot of commercially cultivated Proteas in South Africa. Plant Disease, 69: 211-213.

Walker, J.C., 1965. Host resistance as it relates to root pathogens and soil micro-organisms. In: K.F. Baker and W.C. Snyder (Editors), Ecology of Soil-Borne Plant Pathogens - prelude to biological control. John Murray, London, pp. 314-320.

Walker, A.K. and Schmitthenner, A.F., 1984. Comparison of field and greenhouse evaluations for tolerance to *Phytophthora* rot in soybean. Crop Science, 24: 487-489.

Wager, V.A., 1942. *Phytophthora cinnamomi* and wet soil in relation to the dying-back of avocado trees. Hilgardia, 14: 519-532.

Watson, I.A., 1970. Changes in virulence and population shifts in plant pathogens. Ann. Rev. Phytopathol., 8: 209-230.

Weste, G., 1975. Pathogenicity of *Phytophthora cinnamomi* towards *Nothofagus cunninghamii*. Aust. J. Bot., 23: 277-283.

Weste,G., 1983. Population dynamics and survival of *Phytophthora* In: D.C. Erwin, S. Bartnicki-Garcia and P.H. Tsao (Editors), *Phytophthora* : Its Biology, Taxonomy, Ecology and Pathology. Amer. Phytopathol. Soc., St. Paul, Minn., pp. 237-257.

Weston, G.C., 1956. Fertilizer trials in unthrifty pine plantations at Riverhead Forest. N.Z. J. For., 7: 35-46.

Weston, G.C., 1957. Exotic forest trees in New Zealand. New Zealand Forest Service, Wellington, N.Z., 104 pp.

White, R.P., 1937. Rhododendron wilt and root rot. N. J. Agric. Exp. Stat., Bulletin No. 615, 32 pp.

Wilcox, M.D., 1982. Genetic variation and inheritance of resistance to *Dothistroma* needle blight in *Pinus radiata*. N.Z. J. For. Sci., 12: 14-35.

Wilcox, M.D., 1983 a. Reciprocal cross effects in *Pinus radiata*. N.Z. J. For. Sci., 13: 37-45.

Wilcox, M.D., 1983 b. Forestry. In: G.S. Wratt and H.C. Smith (Editors), Plant Breeding in New Zealand. Butterworths, N.Z., pp. 181-194.

Wilcox, M.D., Shelbourne, C.J.A. and Firth, A., 1975. General and specific combining ability in eight selected clones of radiata pine. N.Z. J. For. Sci., 5: 219-225.

Williams, P.H., 1975. Genetics of resistance in plants. Genetics, 79: 409-419.

Wingfield, M.J. and Knox-Davies, P.S., 1980. Observations on disease in pine and eucalyptus plantations in South Africa. Phytophylactica, 12: 57-63.

Woollons, R.C. and Hayward, W.J., 1984. Growth losses in *Pinus radiata* stands unsprayed for *Dothistroma pini*. N.Z. J. For. Sci., 14: 14-22.

Wriht, J.P. and Marks, G.C., 1970. Loss of merchantable wood in radiata pine associated with infection by *Diplodia pinea*. Aust. For., 34: 107-119.

Wright, J.W., 1962. Genetics of Forest Tree Improvement. F.A.O. Forestry and Forest Product Studies No. 16, 399 pp.

Zagory, D. and Libby, W.J., 1985. Maturation-related resistance of *Pinus radiata* to western gall rust. Phytopathology, 75: 1443-1447.

Zak, B., 1955. Inheritance of resistance to littleleaf in shortleaf pine. U.S.D.A. For. Serv. Res. Note SE-88, 2 pp.

Zentmyer, G.A., 1977. Origin of *Phytophthora cinnamomi* : Evidence that it is not an indigenous fungus in the Americas. Phytopathology, 67: 1373-1377.

Zentmyer, G.A., 1980. *Phytophthora cinnamomi* and the diseases it causes. Mono. No.10, Amer. Phytopathol. Soc., St. Paul, Minn., 96 pp.

Zentmyer, G.A. and Guillemet, F.B., 1981. Evidence for strains of *Phytophthora cinnamomi.* Plant Disease, 65: 475-477.

Zentmyer, G.A. and Munnecke, D.E., 1952. *Phytophthora* root rot of nursery stock. Plant Dis. Rept., 36: 211-212.

Zobel, B.J., 1978. Gene conservation - as viewed by a forest tree breeder. Forest Ecol. Manage., 1: 339-344.

Zobel, B.J., 1982 a. The world's need for pest - resistant trees. In: H.M. Heybroek, B.R. Stephan and K. Von Weissenberg (Editors), Resistance to Diseases and Pests in Forest Trees. C.A.P.D., Wageningen, pp. 1-8.

Zobel, B.J., 1982 b. Developing fusiform - resistant trees in the south eastern United States. In: H.M. Heybroek, .R. Stephan and K. Von Weissenberg (Editors), Resistance to Diseases and Pests in Forest Trees. C.A.P.D., Wageningen, pp. 417-42.

Zobel, B.J. and Talbert, J., 1984. Applied Forest Tree Improvement. John Wiley and Sons. New York, 505 pp.