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# SOME FACTORS INFLUENCING THE SUDDEN DEATH SYNDROME IN CUT FLOWER PLANTS

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## ABSTRACT

Soil/root mixes from plants with the Sudden Collapse Syndrome of cut flower plants were tested for *Phytophthora* infection using a lupin (*Lupinus angustifolius*) baiting technique. *Boronia heterophylla* and *Leucadendron* 'Wilsons Wonder' root samples both caused the lupin seedlings to exhibit symptoms of *Phytophthora* infection.

The efficacy of phosphorous acid (Foschek® 500 at 1000 ppm and 2000 ppm) and a combination of phosphorous acid and an additional product (Foschek® 500 and C408 at 1000/200 ppm and 2000/400 ppm) in controlling *Phytophthora cinnamomi* root infections of *L.* 'Wilsons Wonder', *B.heterophylla* and *B. megastigma* rooted cuttings was compared with fosetyl-Al (Aliette® 80 SP at 1000 ppm and 2000 ppm) under conditions of high disease pressure.

The fungicides were applied as a root drench 7 days prior to the roots being inoculated by a split wheat technique and the effect of the fungicides and their concentrations on the rate of plant mortality was measured.

The results were species dependent. The treatments delaying plant mortality most effectively were fosetyl-Al at 2000 ppm on *L.* 'Wilsons Wonder', phosphorous acid at 2000 ppm on *B. heterophylla* and both fosetyl-Al at 1000 or 2000 ppm and phosphorous acid at 2000 ppm on *B. megastigma*.

The allelopathic activity of the root bark of *Protea cynaroides*, L. 'Wilson's Wonder', *Macadamia* 'Beaumont' and *Knightia excelsa* was evaluated as a growth inhibitor for *Phytophthora cinnamomi*.

The results indicate that by day 4 the root bark of *M.* 'Beaumont' reduced the growth rate of *Phytophthora cinnamomi* by 76.8% while that of *Protea cynaroides* inhibited the growth totally. The root bark of L. 'Wilson's Wonder' had no effect on the growth rate but that of *K. excelsa* enhanced the growth rate by 128% by day 4.

The root bark of *Protea cynaroides* plants previously infected with an unnamed, indigenous *Phytophthora* species provided greater resistance to the growth rate of *Phytophthora cinnamomi* than the root bark of uninfected plants. On the corn meal agar, the leachate of the infected *Protea cynaroides* root bark exhibited a 'zone of inhibition' which prevented the growth of *Phytophthora cinnamomi*. Possible reasons for this are discussed.

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# **Chapter 1**

## **GENERAL INTRODUCTION**

## 1.0 INTRODUCTION

The sudden collapse and death of mature, fully producing cut flower and foliage plants is of major concern to commercial growers in the Bay of Plenty and in other regions of New Zealand. These losses can reduce potential earnings from an export crop by between 10 and 50% annually.

### 1.0.1 Rationale for the study

The plant deaths usually follow periods of wet weather, often in autumn or spring. The symptoms usually include a general decline of the plant, chlorosis, wilting of the terminals and death. Similar symptoms have been reported on cut flower plants in Australia and South Africa where the disease has been attributed to infection with the root rot fungus, *Phytophthora cinnamomi* Rands (hereafter to be referred to as *P. cinnamomi*). This disease is probably responsible for causing many plant deaths in the Bay of Plenty on a regular basis.

The distribution of *P. cinnamomi* is widespread throughout the world, although there is some doubt as to the extent of its centre of origin.

Chemical control of *P. cinnamomi* is seldom adequate, particularly in periods of high disease pressure when environmental conditions favour active growth of the pathogen. The disease pressure is at a maximum during the warm and wet conditions experienced in autumn and spring in the Bay of Plenty. Currently, the best chemical protection is afforded by products containing acylalanines or organic phosphonites.

In part of this study two chemical products are compared to the fosetyl-Al product, Aliette®, which has been used extensively by local growers attempting to control *Phytophthora* in their plants. It was hypothesised that one or both of the products would achieve greater control of *P. cinnamomi* on a range of plant species than Aliette®.

Many plants exhibit a range of tolerances and susceptibility to *P. cinnamomi*. The king protea, *Protea cynaroides* R. Br., is considered to be very tolerant to *P. cinnamomi* due to the reported presence of a compound in the root bark which confers a degree of disease resistance. Nevertheless, in the spring of 1995, *Protea cynaroides* plants in the Bay of Plenty appeared to be dying from a disease with similar symptoms to *P. cinnamomi*. It was hypothesised that the plants which died as a result of the disease had a lower concentration of the resistance-conferring compound in the roots than is found in healthy, uninfected plants.

This study provides the opportunity to investigate the cause of the problem and suggest possible solutions which could be of immense value to the cut flower and foliage industry. *P. cinnamomi* infects more than 400 species globally which suggests that any new method of control, whether chemical or an alternative method, could have world-wide economic importance.

There is increased competition from both within New Zealand and offshore in the cutflower and foliage industry. Growers will continue to experience further pressure to become more efficient and reduce plant loss if they are to remain economically viable and competitive in the future.

## **1.0.2 Objectives**

The principal purpose of this research project was to assess methods of control of *P. cinnamomi*, both natural and man-made, which may provide alternatives to currently accepted practices.

In particular, this study focuses on the effects of the root rot disease, *P. cinnamomi* on a range of ornamental plant species grown for commercial production of cut flowers and essential oil production.

This investigation concentrates on three areas.

### **1.0.2.1 Investigation of Cause of Ornamental Plant Deaths**

To determine whether the cause of the sudden collapse and death of plants from a range of species on local cut flower growing properties can be attributed to the root fungal pathogen, *P. cinnamomi*.

### **1.0.2.2 Chemical Control of *P. cinnamomi*.**

To assess a range of chemicals which have been developed to control *P. cinnamomi* in a range of Proteaceous and Rutaceous plant species.

### **1.0.2.3 Natural Defences and Resistance Against *Phytophthora cinnamomi*.**

To assess whether the bark of a range of Proteaceous species has fungistatic influences on the root-invading fungus, *P. cinnamomi*.

This part of the study was to determine the ability of the root bark in a range of plants in the Proteaceae to resist *P. cinnamomi*. Investigation was also carried out to determine the level of resistance to *P. cinnamomi* in both healthy and infected *Protea cynaroides* plants. This investigation may reveal alternative strategies for control of the pathogen.

# **Chapter 2**

## **REVIEW OF LITERATURE**

## 2.0 INTRODUCTION

In this chapter a number of issues will be reviewed.

The terminology of ornamental plants used for cut flowers and the origin and distribution of the major families involved are examined. The natural habitats of 'proteas' and the way in which they influence the growth of the species involved are discussed.

Concerns of root disease in ornamental plants used for cut flowers by growers in the Bay of Plenty region are described in this chapter with possible causal agents proposed.

The origin, morphology and distribution of the root rot fungus, *Phytophthora cinnamomi* and the resistance and susceptibility of ornamental plants to this pathogen are discussed.

## 2.1 CUT FLOWER FAMILIES AND GENERA

Cut flower species grown commercially in New Zealand encompass a range of flowering shrub genera which originate mainly from southern Africa and Australia. The name 'protea' is commonly used because they are predominantly, but not exclusively, from the Proteaceae family.

A number of genera involved are from families which are botanically distinct from the Proteaceae, eg, Rhamnaceae, Rutaceae. However, they are often grouped under this title in New Zealand for commercial convenience.

In this study cut flower and cut foliage genera which occur in South Africa (SA), Australia (Aus), and New Zealand (NZ) are investigated. The families and genera involved include Proteaceae eg *Protea* (SA), *Leucadendron* (SA), *Macadamia* (Aus), *Knightia* (NZ) and the Rutaceae eg *Boronia* (Aus)

### 2.1.1 The Proteaceae

The Proteaceae is a very ancient family of plants with ancestors of today's members being present in Gondwana well before it began to break up some 300 million years ago. It had already divided into its two sub-families Proteioideae and Grevilleioideae at that time. As the continents drifted apart the Proteioideae remained in Southern Africa (329 species). However, the Grevilleioideae became concentrated in Australia (more than 800 species), South America (approximately 90 species), the islands east of New Guinea (80), New Caledonia (45), and other areas of Asia (from India eastwards) to the islands of the Pacific Ocean. The two indigenous species in New Zealand are *Knightia excelsa* R. Br. and *Toronia toru* Cunn.

No genus is common to both South Africa and Australia although South America shares more than half its genera with Australia. This suggests that Africa was isolated before the links between the other parts of the supercontinent were broken (Vogts, 1989).

In total, the family Proteaceae contains over 1400 species in 60 genera. The majority of the species and cultivars commercially grown in New Zealand are to be found in a relatively small number of genera. These originate from South Africa, ie *Protea*, *Leucospermum*, *Leucodendron*, *Serruria* and *Mimetes* (with a few others currently being trialled) and Australia ie *Banksia*,

*Dryandra*, *Grevillea*, *Hakea*, *Isopogon*, and *Telopea* (with at least three others being currently trialled).

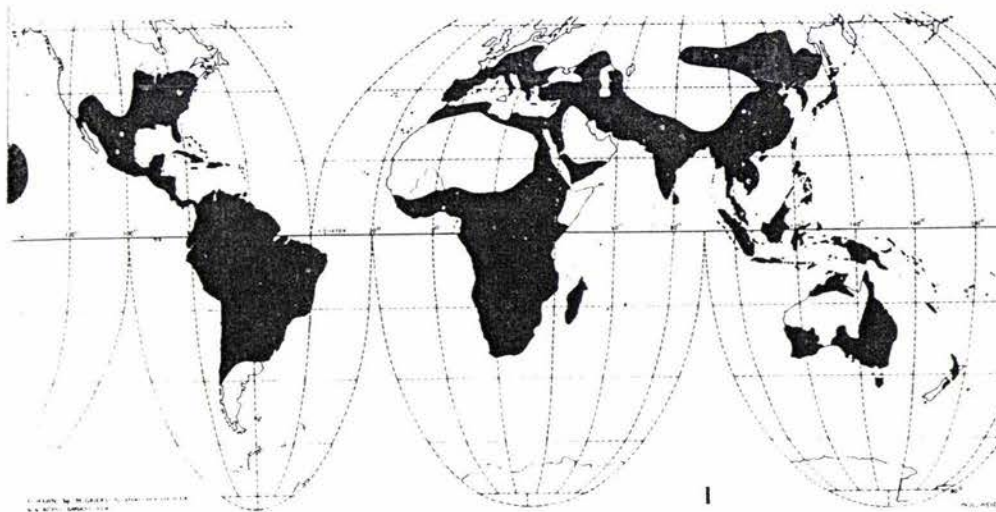
### 2.1.2 The Rutaceae

The Rutaceae is a large family of some 12 tribes consisting of 150 genera and 1600 species (Armstrong, 1978) with a world-wide distribution (see Map 1). It occurs from both the hottest to the temperate regions of the earth, eg the tribe *Zanthoxylae* is found extensively throughout the tropics and sub-tropics of all the major continents. Of the five tribes which have limited distribution, the *Boroniae* is predominantly Australian and contains a number of economically important genera for the ornamental plant and cut flower industries, eg *Boronia*, *Correa*, *Crowea*, *Eriostemon* and *Phebalium*. Only one species in this tribe, *Phebalium nudum* Hook., occurs in New Zealand. The Australian species in this tribe (at least 242) account for 72% of all the Rutaceous species in Australia and have a wide distribution throughout the sub-continent (see Map 2)

The genus *Boronia* is the largest genus in the tribe *Boroniae* with 94 species endemic to Australia. *Boronia heterophylla* FJ Muell. originates from the heathlands of Western Australia. It is widely grown in Australia and New Zealand as a cut flower producing red, bell-shaped flowers open in September/ October. These are well established in both the Japanese and North American markets where florists use it as an autumn filler in flower arrangements.

In contrast, *B. megastigma* Nees ex Bartl., which also originates from the Western Australian heathlands, is cultivated commercially for the highly

valued essential oil which is extracted from the flowers. Oil production is currently under evaluation in New Zealand.



Map 1: The distribution of the Family Rutaceae throughout the world (Armstrong, 1978)



Map 2: The distribution of the tribe Boroniae throughout Australia (Armstrong, 1978)

In order for other genera to be suitable for cultivation with 'proteas', they must be able to be grown in conditions which are similar to those preferred by members of the Proteaceae.

## **2.2 NATURAL HABITAT OF THE CUT FLOWER CROPS USED IN THIS STUDY.**

*Boronia* species have only reached economic significance in the last decade in Australia and New Zealand. This accounts for the limited amount of research undertaken on this genus. In contrast, research into the economic genera of the Proteaceae has been extensive in South Africa, Israel, Hawaii, Australia and New Zealand over the last 25 years.

The five regions of the world which are characterised by a Mediterranean type of climate (ie long, hot, dry summers and cool, moist winters) are the coastal regions of the Mediterranean Sea, California, Chile, south/south-west Australia and the south-west Cape of Africa. Heathlands occurring in these regions are known as macchia, chaparral, matoral, heath and fynbos respectively. Both the heathlands of Australia and the fynbos region of South Africa are occupied by a large number of species of the Proteaceae and the Rutaceae subtribe Boroniae (see Map 2). Although the majority of the Proteaceae are found in Mediterranean climates, a few species have adapted to the humid, tropical conditions of Queensland and New Guinea.

### 2.2.1 Rainfall

The annual rainfall in South Africa is classified into 4 main categories (Vogts, 1989):

In the first category there is a very pronounced winter peak with the occurrence of the main rainy season from May to September.

The second category has rainfall peaks twice a year, firstly in February-March and again in September-November.

The third category contains a single pronounced peak in midsummer with the rainy season extending from September to April.

In areas of the fourth category a single pronounced peak in March with the rainy season extending from November to the end of April.

Most of the South African proteas originate from the south-western region (SW) where the majority of the rainfall occurs in winter. However, the actual rainfall can vary from 300-500 mm on the flatlands to 3000 mm in some mountain peaks.

The other major region in terms of protea distribution is the southern coastal region (SC) which, as its name suggests, has a more even coastal climate in which the extremes are not so marked as they are in the SW region. The annual rainfall ranges from 400 mm to 1100 mm and frosts occur only on the higher mountain peaks. This is in spite of the average winter temperatures being lower than those in the SW region while the summer heat is reduced by the cool sea breezes.

### **2.2.2 Temperature**

Variation can also be observed in the temperature patterns of the region. The average daily maximum is about 28°C in summer and 17°C in winter while the average minimum is about 15°C in summer and 6°C in winter. Although these figures suggest a mild climate, they mask the range of temperatures experienced. The day temperatures can soar to 43°C in the hot, dry months and drop to -5°C in the long nights of June/July. Light to moderately severe frosts can be regularly encountered in the mountains during winter, but are usually rare in the coastal areas.

### **2.2.3 Wind**

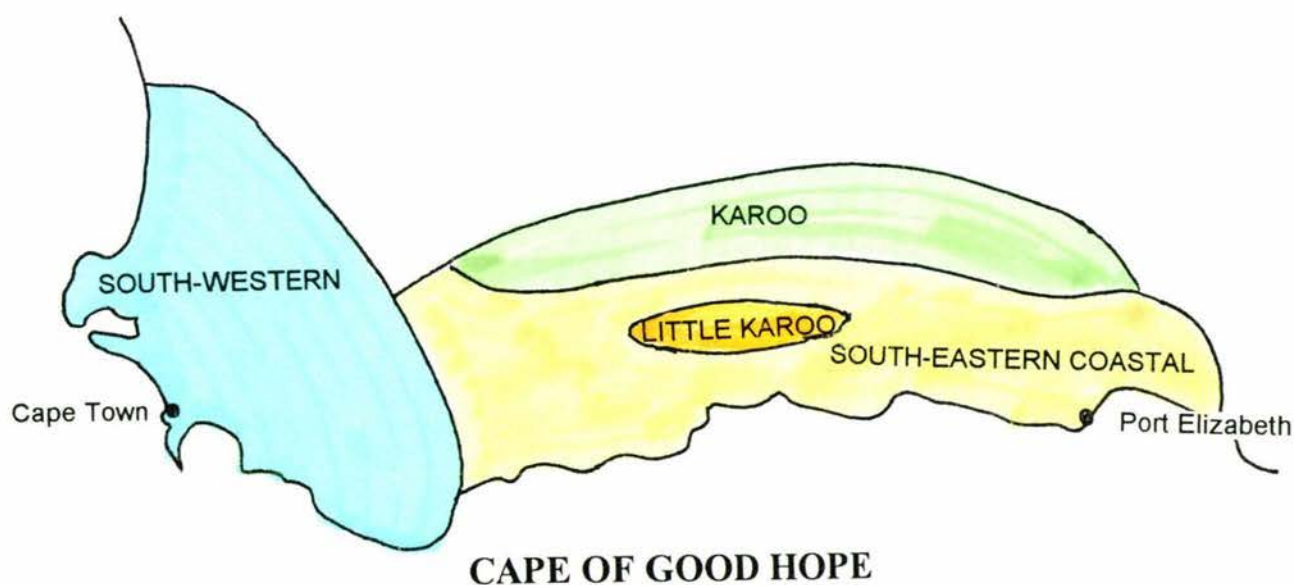
Wind is an important feature of the climate of the SW region in the summer. The south-easterly wind brings the cool, moisture-laden clouds which often blanket the mountains providing additional water for the plants and reducing the intensity of the light from the summer sun. At lower altitudes and along the coastal flats the south-easterly wind is usually very strong, causing a drying effect. Winter winds, however, are mainly north-westerly bringing rain and cool, humid days.

### **2.2.4 Variations in microclimate**

The climate of regions bordering the natural habitat indicate conditions detrimental to the plant and which must be avoided or manipulated in cultivation (Vogts, 1989).

This is clearly illustrated by the Karoo region (see Map 1). The Little Karoo is surrounded by, and the Great Karoo is adjacent to the SC region yet no protea grows in either area. This is because the climatic conditions do not satisfy the demands of the Proteaceae. Annual rainfall is less than 250 mm per year (and not supplemented by moist clouds or sea mists). Seasonal and diurnal temperature fluctuations with contrasts of up to 28°C within a 24 hr period are not unusual. Furthermore severe frosts can occur for at least 3 months in winter.

Growers should be aware that South African species of the Proteaceae will not tolerate adverse conditions (Vogts, 1989). Such conditions include less than 225 mm or more than 3000 mm annual rainfall, large diurnal fluctuations of air temperatures, more than 2 months of near constant frost or hot, humid, windless conditions which encourage fungal disease problems.



Map 3: Climatic Regions of the Cape of Good Hope, South Africa (Adapted from Vogts, 1989)

### 2.2.5 Soil type

The soil type of the habitats in which the Proteaceae and the Rutaceae subtribe, *Boroniae* naturally flourish plays an even more critical role in their distribution, isolation and environmental adaptations, than either climate or topography.

*Boronia heterophylla*, *B. megastigma* and most of the the 72 species of the Proteaceous genus *Banksia* are found in the woodlands of the Swan (Perth) Coastal Plain (kwongan) areas of South-West Australia (Dixon *et al*, 1984) and have developed on the heathlands. These are heavily weathered soils, often of great age and experience a harsh, dry climate in which fires are frequent. They are generally well-drained and impoverished with low nutrient availability. Deficiencies in phosphorous, nitrogen, potassium, calcium, copper, zinc, molybdenum, manganese, sulphur, and cobalt are common.

The soils of the South African fynbos are considered to be closely allied to those of the Australian heathlands with both being oligotrophic (strongly-leached). They have a pH of less than 6.0, total nitrogen less than 0.1%, total phosphorous less than 0.02% and total exchangeable cations less than 5 meq/100 g soil (Specht and Moll, 1983).

In South Africa such soils are usually developed from the quartzite rock from either the Table Mountain Group or the Witteberg Group. du Preez and Venter (1990) describe the open savanna community dominated by the 3m *Protea caffra* Meisn. as being restricted to the eastern and south-eastern slopes of the quartzite ridges and shale slopes with a shallow soil occurring amongst the rocks. The sandy soil is less than 0.1 m deep, has a pH of 4.8 and up to 90% of the surface is covered by stones and rocks,. The tree layer

has an average canopy of 22% with *Protea caffra* being the dominant species. This dominance is not unusual for members of the Proteaceae in these harsh conditions and it is related to their adaptive ability that has enabled them to be so successful.

As a result of that successful occupation of such marginal habitats, there is a widespread belief that proteas should not be fertilised, will not respond to fertilisation and in fact, will die if fertilisers are applied around their roots.

However, in both South African and Australian Proteaceae habitats there is the regular occurrence of fire. This renders available high quantities of available forms of nitrogen and other nutrients in ash for short periods to promote rapid re-growth (Rundel et al,1983). Nitrogen and phosphorous released during a fire in 21-year old mountain fynbos at Jonkershoek may be up to 15.8 and 0.64 g/m<sup>2</sup> respectively (van Wilgen and le Maitre,1981). A fire in a coastal fynbos ecosystem increased the nitrogen concentration by 6.6 g nitrogen/m<sup>2</sup> at the soil surface (Stock and Lewis, 1986a). Such increases in nutrient availability are generally short-lived and plant growth rates decline steadily as post-fire age of the vegetation increases (Rundel and Parsons,1979).

Lamont (1981) suggests that a plant's capacity to survive in conditions of low soil nutrients depends on an ability to utilise relatively inaccessible sources of nutrients.

Many members of the Proteaceae have developed specialised roots known as proteoid or cluster roots. These enable a plant, seemingly without microbial assistance, to enhance its uptake of key limiting nutrients such as phosphorus and nitrogen. Lamont (1972) suggested that proteoid roots are a

type of mycorrhizal symbiosis, but the evidence now shows that certain soil bacteria have an inductive role in the initiation of proteoid roots, but do not invade the roots, because such roots are not formed under sterile conditions (Lamont, 1986). Addition of organic matter can stimulate proteoid root formation by increasing the microbial activity around the plants as well as increasing the nutrients available.

Proteoid roots are dense clusters of rootlets which vary in length from a few millimetres to over 20 cm and may consist of hundreds or even thousands of hairy rootlets (Lamont, 1986). A *Protea* seedling often contains a substantial number of proteoid roots by the end of its first season of growth. By the second year they may account for up to 56% of the weight of the root system (Brits, 1983).

Proteoid roots are concentrated in the upper 2-5 cm of the soil near the base of the plant (Lamont, 1972) growing up into the decomposing leaf litter layer as it forms. However, they have been observed to a depth of 70 cm and up to 9 m away from a 1.5 m wide plant of several *Banksia* species (Lamont et al, 1985). Lamont (1973) also showed that the proteoid roots of *Hakea laurina* R. Br. are most efficient with regards to water and nutrient uptake when grown in the uppermost 12 cm of the soil profile.

New season's roots of the Proteaceae arise adventitiously from old roots, the root stock or the base of stems. This contrasts with many other plants, which form new roots as growth resumed from the tips of the previous season's growth (Lamont, 1986). Genera such as *Telopea* and *Hakea* produce proteoid roots at irregular intervals along the new roots with most of the root remaining non-proteoid. However, in *Banksia* and *Dryandra*, most of the new

root branches may be almost entirely proteoid, creating a dense mat of roots and rootlets near the soil surface (Lamont, 1986).

The ability of proteoid roots to increase the uptake of nutrients by a plant has been clearly demonstrated in studies by Lamont et al (1984). They found the surface area to weight ratio of proteoid roots to parent roots of *Leucadendron lauratum* (Lam) Four. to be almost 15 times greater. This large surface area was responsible for a 13 times greater uptake of P<sup>32</sup> under experimental conditions (Lamont, 1982).

Proteaceous plants have adapted to xeric habitats which are both hot and dry. Dodd, 1985 (reported in Bowen and Pate, 1992) reported that on soils in *Banksia* woodlands the volumetric content remained below 0.1 cm<sup>3</sup>/cm<sup>3</sup> throughout the unsaturated profile of soil above the water table. The gravimetric soil moisture content at 100-200 mm depth varied from less than 1 % in late summer to a maximum of around 5% in winter. Therefore there is virtually no water available from the top few metres of soil during the summer months.

In order to make optimum use of available water in these soils *Boronia heterophylla* and *Boronia megastigma* have developed extensive fibrous root systems. While the plants are usually found growing in full sun or partial shade their root systems are always established in the shade of a rock or other plants. As a result both species prefer a cool shaded root area when established commercially to reduce the potential stress of excessive heat causing root damage.

Many members of the Proteaceae and Rutaceae will grow in more amenable habitats. However they are simply unable to compete with the diverse range

of other genera which also successfully occupy such habitats. Consequently, the Proteaceae and many members of the Rutaceae have adapted to marginal habitats to survive.

Species must be able to thrive without the stresses of their natural habitats if they are to be a successful commercial species. They must also be able to adapt to alternative stresses which their new environments may place upon them.

### 2.3 ROOT DISEASE REPORTS

Several of the cut flower species grown by growers have been noted as being susceptible to a root disease. The visible symptoms have been reported to include yellowing of the leaves, wilting of the terminals and rapid collapse and death of the plants similar to those noted by Von Broembsen and Brits (1985) in South Africa. The plants are possibly infected by *Phytophthora cinnamomi*, a root and collar rot fungus which is of widespread occurrence in New Zealand soils (Newhook and Podger, 1972). It has the ability to attack a wide range of host species, primarily through the root system with some growers losing up to 10% of some species regularly each year (B Hooper, pers comm). However, some major outbreaks of the disease during periods of intense disease pressure can destroy far greater numbers of plants. Growers cannot prevent such occurrences due to a lack of available controlling chemicals.

*Phytophthora cinnamomi* is, arguably, the most common causal agent involved in the root rot, wilt or decline infections of a wide range of ornamental and cut flower species. It is distributed in soils throughout the

world (Dingley, 1969; Coffey and Joseph, 1985; Von Broembsen and Brits, 1985; Linderman and Zeitoun, 1989)

### **2.3.1 Symptoms of *Phytophthora cinnamomi***

Plant propagators around the world recognise *P. cinnamomi* as being one of the most serious of plant pathogens. In the United States it is aptly known as 'the plant killer'. because it has been recorded as being responsible for the death of plant species in at least 48 families (Newhook and Podger, 1972). In comparison, most plant pathogens such as Chestnut Blight, Dutch Elm disease or White Pine Blister Rust pathogens, usually only cause epidemics in one or two species.

One viewpoint held in the United States is that many plants leaving nurseries are already infected with *P. cinnamomi*. However, growing conditions in nurseries often favour the plant, ie cool temperatures, well-drained media and no environmental stress. The disease may not, therefore, progress sufficiently to manifest itself in the foliage or roots the sale of such plants (Linderman and Zeitoun, 1989).

Newhook (1970) notes that many nurseries throughout New Zealand regularly suffer heavy losses in a number of species, particularly in the Proteaceae.

Commercial cut flower plantings in South Africa established on sites with poor drainage have been the subject of the phenomenon known as 'patch death' (Von Broembson and Brits, 1985). The symptoms begin with dull coloured foliage and general unthriftiness, ie a plant may not be growing as quickly as

the surrounding plants in the row. This is followed by chlorosis, rapid wilting and death.

This situation has arisen on many cut flower properties in New Zealand (Brian Hooper pers comm) with similar symptoms being reported for dying plants. Plants from the properties of growers, C and M Bowyer (see Photo 1) and B and L Hooper (see Photo 2) regularly exhibit these symptoms.

The first above ground symptoms of *P. cinnamomi* invasion can be attributed to the initial death of the smallest feeding roots by the invading fungus. The plant begins to collapse as the larger roots and the lower stem become infected. Death almost always results from stem girdling. Turnbull (1992) observed stem girdling in dying *Leucadendron* 'Sylvan Red' plants, while retaining apparently healthy roots.

The symptoms exhibited by plants attacked by *P. cinnamomi* are numerous and varied. This is due to the wide range of species susceptible to the pathogen. However a range of general symptoms can be distinguished. The leaves of many species change colour, eg *Leucadendron* 'Wilsons Wonder', begin to yellow although those of *Boronia heterophylla* take on a characteristic red colour. A rapid collapse of the terminal shoots of the plant occurs with the sudden death of the leaves. A reduction in leaf size can be observed in plants which are being slowly debilitated by fungal attack. Stunted growth and epicormic shooting may occur. Dieback of branches is common. Cankers, lesions, stem girdling, bark cracking and bleeding are all to be observed on the stems of susceptible plants. At this stage the fine roots and root hairs are brown and rotten, although larger roots do not appear to be affected.



Plate 1: The sudden collapse and death of an 18-month old *Boronia heterophylla* plant in a commercial planting at the author's property at Ohauiti, Tauranga, 1995.

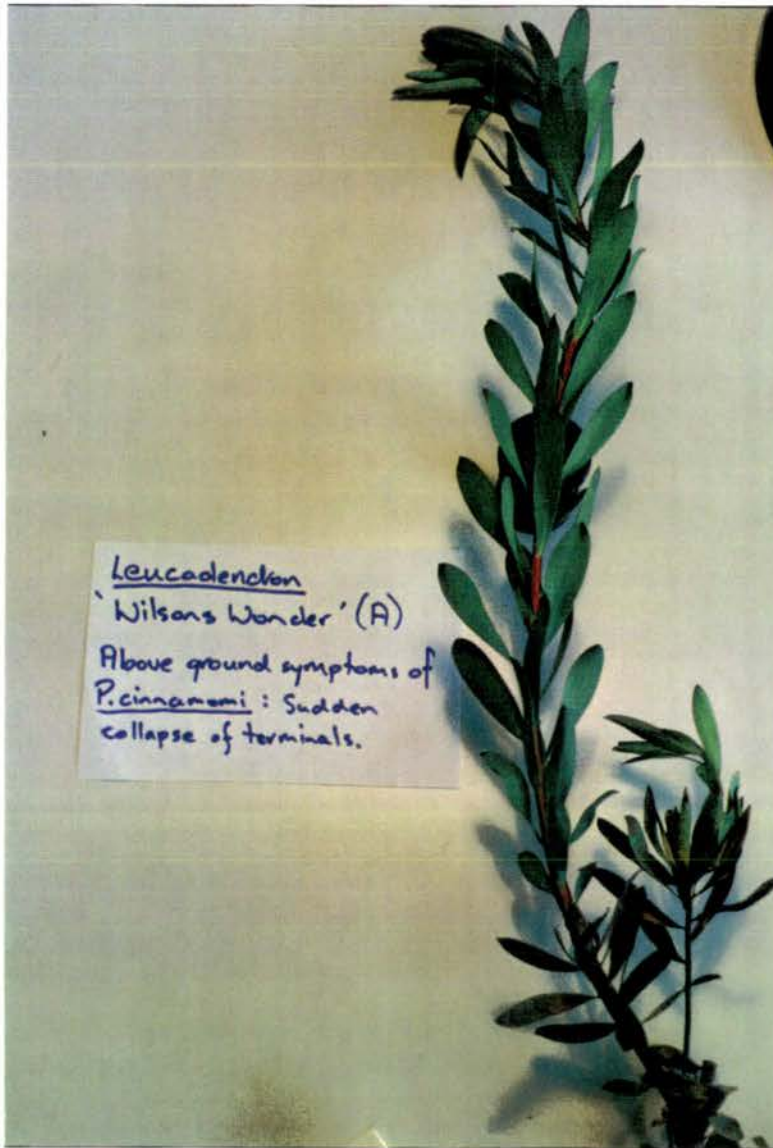


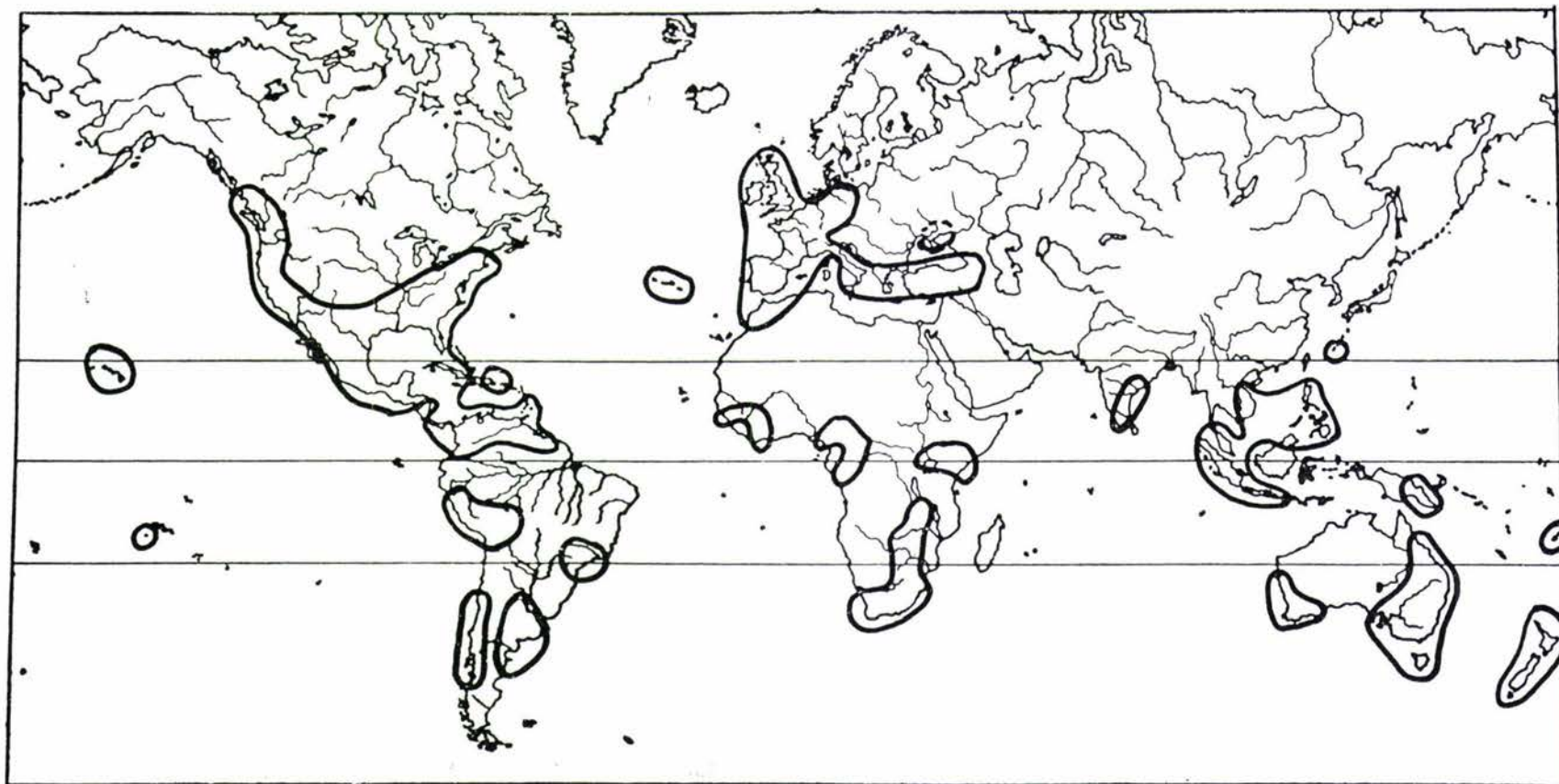
Plate 2: A 1-year old *Leucadendron* 'Wilson's Wonder' plant which is showing symptoms of 'patch death', ie *P. cinnamomi* infection. Note the collapse of the terminals and stem blackening at the base. Incipient chlorosis is also present although not so obvious from the photo. This specimen from the Lower Kaimai property of Brian and Lynette Hooper, was located in an area where the soil often remains saturated after prolonged rain.

## 2.4 HISTORY AND ORIGIN OF *Phytophthora cinnamomi*

### 2.4.1 Centre of Origin

*Phytophthora cinnamomi* was first described by Rands in 1922 after discovering a stripe canker disease extending up to 10 metres from ground level in cinnamon trees (*Cinnamomum burmannii* Blume) in Sumatra. He noted that 'the causal fungus is believed to have come into the plantings along with its natural host which is indigenous to the region'.

Considerable controversy surrounds the geographic origin of *P. cinnamomi*. Zentmeyer (1980) suggests that the majority of the available evidence leads to a region extending from Papua New Guinea through to Malaysia, down into North-East Australia. There is also a possibility of a further source in Eastern Asia. All other areas of the world (see Map 4) are considered to have had the disease introduced. This has mainly been as a result of the movement of soil attached to plant material transported by human activity, particularly in the last two centuries. As a consequence this fungus is now widely distributed throughout the world.



Map 1: Commonwealth Mycological Institute Map 302 (revised 1978) showing  
centre of origin and distribution of *P. cinnamomi* throughout the world.

= Proposed centre of origin  
*cinnamomi*

= Areas of distribution of *P.*

#### 2.4.2 Current Distribution of *Phytophthora cinnamomi*

All the major cut flower growing areas of the world now have soils which are heavily infected with *P. cinnamomi* (see Map 3). However, there is still to be some doubt as to whether the disease is indigenous in these areas or not.

The Hawaiian Islands may well have received the pathogen from Pacific Islanders transporting taro, breadfruit and other crops from the Southern Pacific islands of Fiji and Samoa centuries ago (Zentmyer, 1980). The disease could well have been spread to other regions as trade developed.

Von Broembsen and Kruger (1985) surveyed proteaceous plants in the south-western Cape Province of South Africa. Genera dying from *P. cinnamomi* included *Aulax*, *Leucadendron*, *Mimetes*, *Orothamnus*, *Protea* and *Serruria*. The dying plants were found in the mesic and wet mountain areas which receive an annual rainfall of 600-3000 mm. Given the isolated nature of these areas with access by walking track only, they concluded that *P. cinnamomi* was indigenous to the region.

Extremes in susceptibility or resistance of indigenous vegetation to *P. cinnamomi* is a criteria used world-wide to determine the origin of the pathogen. Knox *et al* (1987) suggest that the local vegetation has had little contact with the organism during its evolution. However, they do concede that its introduction to the area may well have been in prehistoric times. Crandall and Gravatt (1967) suggest that it may have been more recently in the 15th or 16th centuries when global discovery and trade was beginning to gather momentum.

Pratt and Heather (1973) have recorded a number of proteaceous species in north-eastern Australia as resistant to *P.cinnamomi*. They also noted a greater field resistance in *Eucalyptus* species in north-eastern Australia than in southern Australia. Local forests showing little evidence of the symptoms thereof in spite of widespread existence in the soil. They surveyed a vast area of the east coast recovering the organism from both infected and healthy native plants in many remote areas. They hypothesised that the region is part of the centre of origin for the fungus, as a result.

*Phytophthora cinnamomi* is widely distributed on the commercially important coastal and near coastal areas of eastern Australia where a range of cut flowers are cultivated. Eighty percent of plants of a *Leucospermum* hybrid were destroyed by the disease on a commercial property in Queensland in 1989 (Turnbull, 1992).

In contrast, there are a large number of susceptible hosts in Western Australia (Grose, 1986). He suggests that the disease was recently introduced into that area (within the last 100 years). In the infected jarrah forest areas of western Australia, 20% of cultivated *Banksia* species have succumbed to the disease. Newhook and Podger (1972) concluded that the fungus must have either evolved in Australia in the last 5,000-10,000 years or been introduced by humans since that period.

Dingley (1969) records *P. cinnamomi* as having been discovered in New Zealand on a number of species of Proteaceous genera. These include *Banksia*, *Dryandra*, *Hakea*, *Isopogon*, *Leucadendron*, *Leucospermum* and *Protea*. *Boronia megastigma* is the only recorded Rutaceous species recorded. However, he does record other *Phytophthora* species on other members of the Rutaceae eg *Phytophthora citricola* Saw., *Phytophthora*

*citrophthora* Leonian and *Phytophthora hibernalis* Carne on *Citrus* species and *Phytophthora cactorum* Lebert and Cohn on *Phebalium squameum* and *Phebalium nudum*.

#### **2.4.3 Dissemination of *Phytophthora cinnamomi***

An important characteristic of any fungal distribution is the ability of the organism to disseminate by as many means as possible. The global distribution of *P. cinnamomi* can be attributed to several varied mechanisms of dispersal to which it has adapted.

*Phytophthora cinnamomi* is a soil fungus with biflagellate zoospores, which allow ease of movement in an aqueous environment. Thus it can readily move to new areas (Newhook and Podger, 1972; Pratt and Heather, 1973; Zentmeyer, 1980) via a number of methods so long as a film of water is present.

Infected, moist soil carrying the fungus can adhere to the roots of nursery stock and on shoes, boots or animal hooves, especially where soils are heavy. Cultivation equipment, vehicle tyres, bulldozer and other heavy machinery tracks may transport the soil. The delivery of home garden soil, roadmaking soil or gravel can also harbour the pathogen.

The redistribution of water containing the zoospores and/or soil particles can occur through surface runoff. Rivers, streams and irrigation systems can rapidly distribute the pathogen over a wide area. Rainsplash from infected soils onto leaves can occur both in nurseries, field crops and wilderness areas.

A susceptible root system may allow the fungus to move both uphill and during dry periods

The pathogen may be distributed on plant material during the dispersal of infected nursery plants, the selection of infected vegetative propagating material or the use of contaminated seed.

*Phytophthora cinnamomi* is wide spread throughout the soils of both indigenous and exotic forests in New Zealand (Dingley, 1969). The water mobility of its zoospores suggest that nurseries in New Zealand are also susceptible to infection through several methods (Baker, 1957).

Distribution may occur by the importation of contaminated container media and the use of contaminated water supplies, particularly from open water sources. Rainwater splashing off contaminated soils/benches onto plant containers placed upon them can transfer the pathogen. Infected propagation material, particularly that which is low down on the mother plant may have been exposed to rain-splashed soil.

Plants for growing on and resale should be checked before purchasing from nurseries which may carry infected stock.



### 2.5.1 The Order: Peronosporales

The Order, Peronosporales, consists, in the main, of terrestrial rather than aquatic genera, the exception being the genus, *Pythium*, which contains a number of water-motile species. The Peronosporales produce only reniform zoospores and is distinguished from other related orders by its terrestrial species producing sporangia on specialised, differentiated hyphae (sporangiophores), .

### 2.5.2 The Family: Pythiaceae

The two genera, *Pythium* and *Phytophthora*, which comprise the Family, Peronosporaceae, both contribute immensely to its economic importance, causing widespread destruction in a range of important food and material crops world-wide.

### 2.5.3 The Genus: *Phytophthora*

The genus *Phytophthora* derives its name from the Greek words *phyton* = plant and *phthora* = destruction and is literally known as the plant destroyer or plant killer. The most infamous species, *P. infestans* (Mont.) de Bary, an aerial species, was responsible for the great potato famine in Ireland in 1845-6 This fungal blight caused the almost complete failure of the potato crop, on which the Irish population depended for food and caused the starvation of more than a million people.

#### 2.5.4 Life Cycle of *Phytophthora cinnamomi*

As is common to most species of this genus, *P. cinnamomi* produces four distinct spore stages, ie sporangia, zoospores, chlamydospores and oospores, each with a different form and function.

The sporangia were described by Stamps *et al* (1990) as being non-papillate, ellipsoid to ovoid and ranging from 45 to 75 µm in length. They are able to produce large numbers of zoospores rapidly thereby increasing the inoculum potential in a short period of time.

The kidney-shape zoospores are produced by the sporangia at a rate of 10 to 30 per hour per sporangium when the temperature of the surrounding liquid medium is lowered by several degrees. They become motile by the use of two flagella, the whiplash and the tinsel flagellum. Their ability to form non-motile cysts allows them to be transported passively in moving soil water. They can move from one plant to another, into irrigation ponds or streams where they re-germinate directly by forming a germ tube or indirectly by producing a secondary zoospore.

The chlamydospores do not follow the normal definition of 'thick-walled, asexual spore'. Instead, they are described by Zentmyer (1980) as being globose, terminal, thin-walled spores often found in clusters with their origins in the spherical hyphal swellings. They are formed in both the infected bark tissue and diseased roots and released into the surrounding soil as the diseased tissue decays. There they can remain in dead roots and soil for very long periods of time without encountering a living host, thus ensuring the long term survival of the fungus.

The oospores are produced as a result of the sexual reproduction of the *P. cinnamomi*. These can be initiated by both interspecific and intraspecific crosses and can be stimulated by extracts from the roots of host plants (Zentmeyer, 1980). This suggests that such plants may well use specific chemicals as a form of defence mechanism to slow the activity of the pathogen. The more rapidly disease spreading zoospores are reduced by limiting sporangia production in favour of increasing the production of dormant oospores.

## 2.6 RESISTANCE AND SUSCEPTIBILITY

Resistance is potentially one of the most effective methods of control of soil-borne pathogens (Zentmeyer, 1980). It has become a major area of investigation for the control of *P. cinnamomi* (Zentmeyer, 1980; McCredie et al, 1985; Turnbull, 1991).

The successful use of *Phytophthora*-resistant rootstocks with susceptible species grafted on top has been achieved with *Banksia* (McCredie et al, 1985). Research has been undertaken to achieve a similar result with commercial species of *Protea*, *Leucadendron* and *Leucospermum* (Von Broembsen and Brits, 1985; Turnbull, 1991). However, results have not been very conclusive.

The susceptibility of cut flower plants to *P. cinnamomi* ranges from very susceptible in species such as *Leucadendron* 'Sylvan Red' through to the very tolerant *Protea cynaroides* L. (Turnbull, 1991).

Analysis of the root bark of *Protea cynaroides* showed that it contained an antifungal compound, p-hydroxybenzoic acid. This prevented the growth of

*P. cinnamomi* at a concentration of 10% (m/v in potato dextrose agar). The root bark of a very susceptible species, *Leucadendron argenteum* R. Br. did not inhibit mycelial growth even with 15% bark in the medium (Van Wyk, 1973).

While no specific research has been undertaken for *Boronia*, *Phytophthora* has, to a large extent, been controlled in another member of the Rutaceae, *Citrus* spp, by the use of resistant rootstocks budded with improved cultivars (Stovold, source unknown).

## **2.7 ENVIRONMENTAL EFFECTS ON *Phytophthora cinnamomi***

Environmental factors, particularly soil temperature and moisture content, have a significant influence on the growth and development of *P. cinnamomi*.

### **2.7.1 Soil Temperature**

The influence of soil temperature on the incidence of *P. cinnamomi* has been well documented (Zentmeyer, 1980, Grant and Byrt, 1984, Turnbull, 1992).

The relationship between temperature, growth of the fungus and sporangia production have been closely correlated. Higher soil temperatures increase the activity of *P. cinnamomi*, thereby resulting in higher levels of root infection (Turnbull 1992).

Van Wyk (1973) reported that the maximum disease pressure occurred on *Leucadendron argenteum* R. Br. at temperatures ranging from 24 to 31°C with low disease incidence at 13 to 16°C.

Turnbull (1992) showed a relationship between numbers of field-grown *Leucospermum* 'Firewheel' and *Leucadendron* 'Sylvan Red' plants dying and soil temperatures rising from the infection-limiting 13°C to the infection-enhancing 20°C or higher.

Zentmyer (1980) examining the growth rate of 187 isolates of *P. cinnamomi*, reported little or no growth below 9 to 10°C. The optima was from 21 to 30°C, with most cultures growing best between 24 and 27°C. The maximum temperatures for growth occurring between 30 and 36°C.

However, hyphal extension within the root is also temperature dependent. The rate of extension in *Eucalyptus marginata* J. Donn ex Sm. is contained at soil temperatures of 14°C but unrestricted at 28°C (Grant and Byrt, 1984).

Turnbull (1992) suggests that the death of *Protea* species infected with *P. cinnamomi* in the field is due to stem girdling, even in plants with apparently healthy roots. The major effect of soil temperature is to change the rate of progression of the pathogen within the roots of already infected plants. She suggests that control of the disease is limited once infection has occurred and disease pressure is high. This is because the chemicals currently available, ie metalaxyl, furalaxyl and phosphonate, for *Phytophthora* control have limited effect once infection has taken place (Marks and Smith, 1990, Turnbull, 1992).

### **2.7.2 Soil Moisture**

Soil moisture also plays an important role in the distribution, infection process, growth and sporulation of *P. cinnamomi*. Ideal environmental conditions for the infection and death of susceptible plant species during

periods of medium to high rainfall and high temperatures. Low soil moisture tension combined with optimum soil temperatures produce high infection rates and rapid plant death (Turnbull, 1992).

# **Chapter 3**

SUDDEN COLLAPSE SYNDROME OF  
ORNAMENTAL PLANTS FOR  
CUT FLOWERS

### 3.0 INTRODUCTION

For a number of years local cut flower growers in the Bay of Plenty have been observed plants showing sudden wilting of the terminal shoots, discoloration of the leaves (usually either yellowing or reddening depending on the species), and root and crown rot followed closely by the death of part or whole of the plant. These symptoms bear a marked similarity to those described for *P. cinnamomi* on pineapple, *Ananas comosus* L. (Zentmyer, 1980), *Eucalyptus marginata* J. Donn ex Sm. (Podger, 1972), *Banksia* spp (Dixon et al, 1984), *Leucadendron*, *Leucospermum*, and *Telopea* spp (Forsberg, 1988). Local growers have long suspected this pathogen is the causal agent (B Hooper pers comm), but it has seldom been confirmed by a laboratory diagnosis.

These symptoms usually appear in local plants during periods of warm soil temperatures and high rainfall which is consistent with those conditions favoured by *P. cinnamomi* (Zentmyer, 1980; Turnbull, 1995).

This experiment was designed to detect the presence of *Phytophthora* in a mixed soil and root sample attained from dying plants which have displayed symptoms similar to those recorded for the disease. The lupin baiting technique used is based on that described by Forsberg (1993).

3.1 METHODS AND MATERIALS

3.1.1 Source of potentially infected soil/root samples

A sample of soil containing a portion of the root system was removed from within the drip line of the plants at a depth of 15 cm. Soil/root mixed samples were obtained from three sites (see Table 1) for the purpose of this study. The *Weinmannia racemosa* 'Kiwi Red' was added to this trial to assess whether it should be included in the study.

PROPERTY	LOCATION (see Map 5)	SPECIES
Bay of Plenty Polytechnic gardens	Windermere Drive, Tauranga	<i>Protea cynaroides</i> (1 plant)
C&M Bowyer's 1.6 ha cut flower property	12 Neewood Road, Tauranga	<i>Boronia heterophylla</i> (1 plant) <i>Weinmannia racemosa</i> 'Kiwi Red' (1 plant)
B&L Hooper's 6.4 ha cut flower property	358 McLaren Falls Road, Tauranga	<i>Leucadendron</i> 'Wilson's Wonder' (3 plants)

Table 1: The location and number of each species used in the trial.

3.1.2 Lupin Baiting Technique

A bioassay technique was used to detect infection by *Phytophthora* species. Blue lupin, *Lupinus angustifolius* L. (hereafter known as *L. angustifolius*), seeds were surface sterilised in 50% ethanol for 1 minute, rinsed twice in

distilled water, then soaked for 12 hours in distilled water. On removal, they were planted in pre-moistened vermiculite in pots which had been sterilised in 50% ethanol for 2 minutes. The pots were sealed in a plastic bag and placed in a growth chamber in a constant temperature of 25°C for 60 hours. This allowed the radicles to extend to between 1.5 and 2 cm long, at which stage they are considered to be highly susceptible to *Phytophthora* (Forsberg, 1993)

A mixture of 150 ml of soil and roots was prepared from the root zone of the trial plants (see Table 1) , ensuring that a small quantity of the plant's roots were included with each sample. A control medium was prepared using 150 ml Dalton's standard potting mix . All the samples were sterilised for 12 h at 100°C and each sample was divided into 5 x 30ml portions. Each portion was then placed in a 200ml glass jar and 120ml distilled water was added. This produced the recommended 1:5 dilution rate for optimum zoospore production (Forsberg, 1993).

Plastic lids for each jar were prepared. A heated wire was used to drill a series of 3 mm holes in a circle through each lid with one hole in the centre. The holes allowed a number of *L. angustifolius* seedling radicles to be supported with the tips in the liquid medium in the jar.

All jars and lids were sterilised using 50% ethanol for two minutes and then rinsed twice in distilled water.

For the *Leucadendron* 'Wilson's Wonder' samples , 5 *L. angustifolius* radicles were inserted through each lid. However, for the *Protea cynaroides* and *B. heterophylla* 7 radicles per lid were used due to a greater number of

seedlings with the correct radicle length being available. There were two replicates of 5 jars tested for each of the soil samples.

### 3.1.3 Assessment criteria

*Lupinus angustifolius* seedlings were observed for symptoms of *Phytophthora* infection on day 4 and day 7. Visual symptoms included stunting or absence of extension of shoots, reduced root extension and brown lesions on the roots, as noted by Forsberg (1993). The presence or absence of these symptoms was recorded.

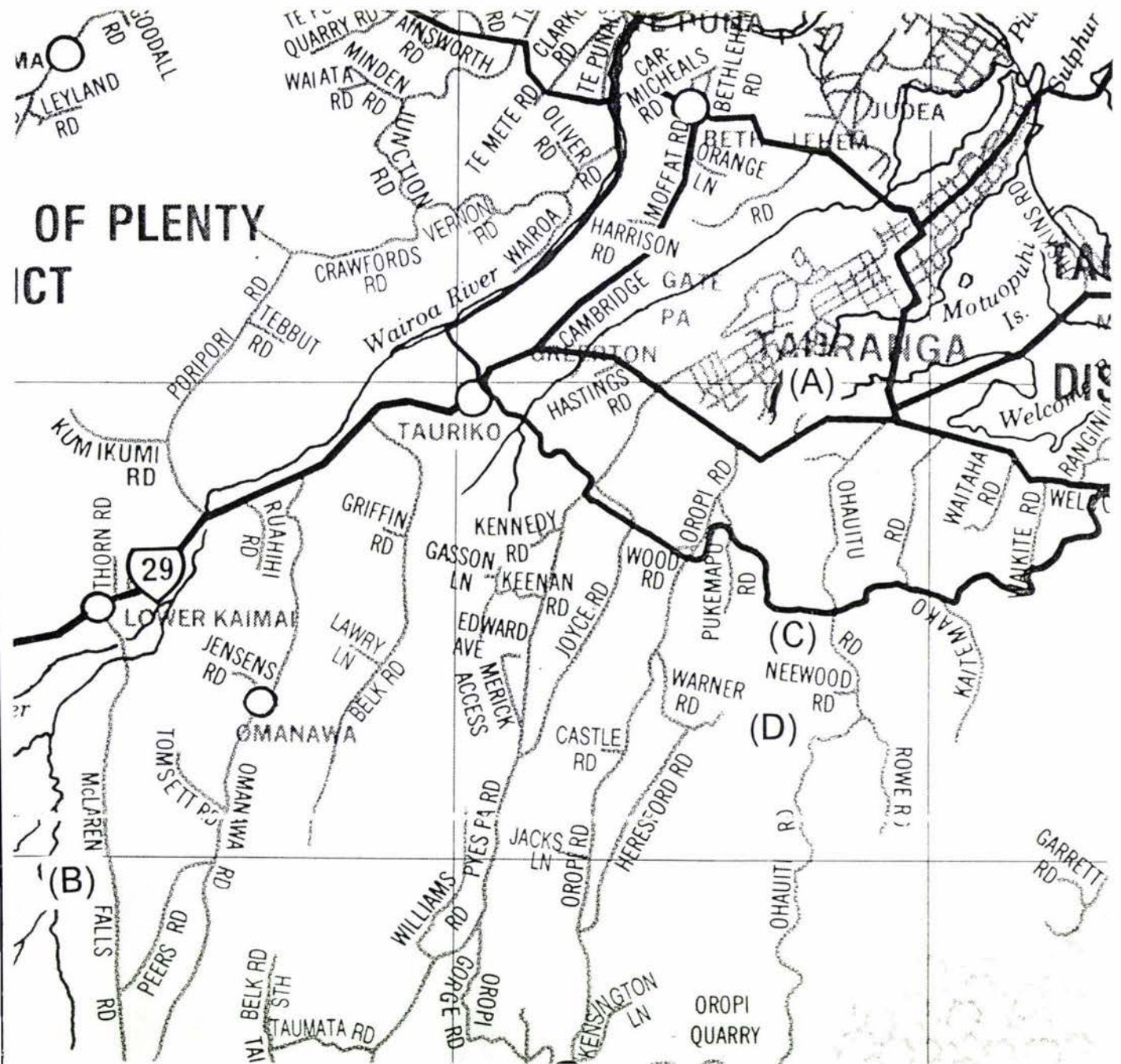
After 7 days, segments of the rotting *L. angustifolius* radicles were removed. These were surface sterilised in 50% ethanol for one minute and plated onto CMA in Petri dishes. The dishes were placed in 25°C and observed for the presence of *Phytophthora*. Root segments of the plants used in this study were also surface sterilised and plated onto CMA to allow a comparison of organisms.

## 3.2 RESULTS

The results of the lupin bioassay are presented in Table 2.

Symptoms of *Phytophthora* infection were not observed in the control by day 7 (see Plate 3).

Lupin seedlings growing in soil/root mixes from *Protea cynaroides* and *Weinmannia racemosa* 'Kiwi Red' showed no symptoms of *Phytophthora* infection by day 4 (see Plate 4) and had vigorous shoot and root growth by day 7.



Map 5: Location of the four properties from which plant material and soil/root mix samples were collected; (A) = the Bay of Plenty Polytechnic gardens and orchard, (B) = B and L Hooper's property, (C) = C and M Bowyer's property, (D) = G and G Nee's, farm.



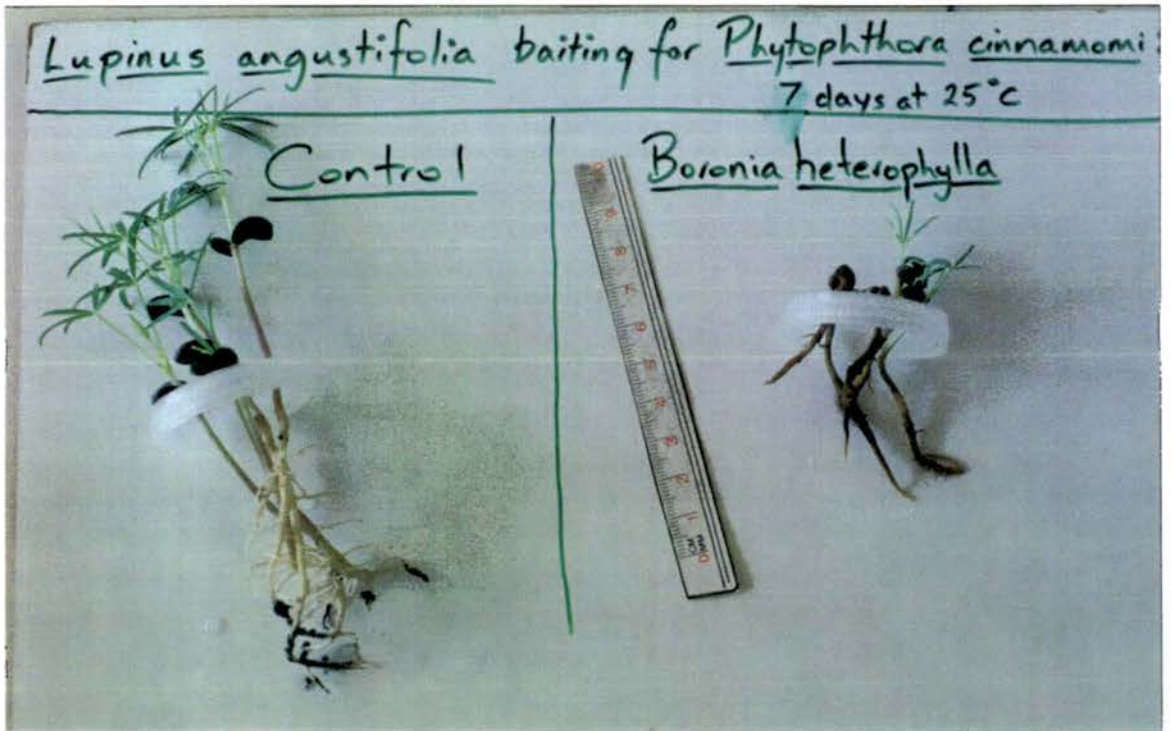


Plate 3: Blue lupin, *L. angustifolius*, baiting for *Phytophthora* species: Control and *Boronia heterophylla* plants after 7 days. *B. heterophylla* plants show severe *Phytophthora* symptoms.

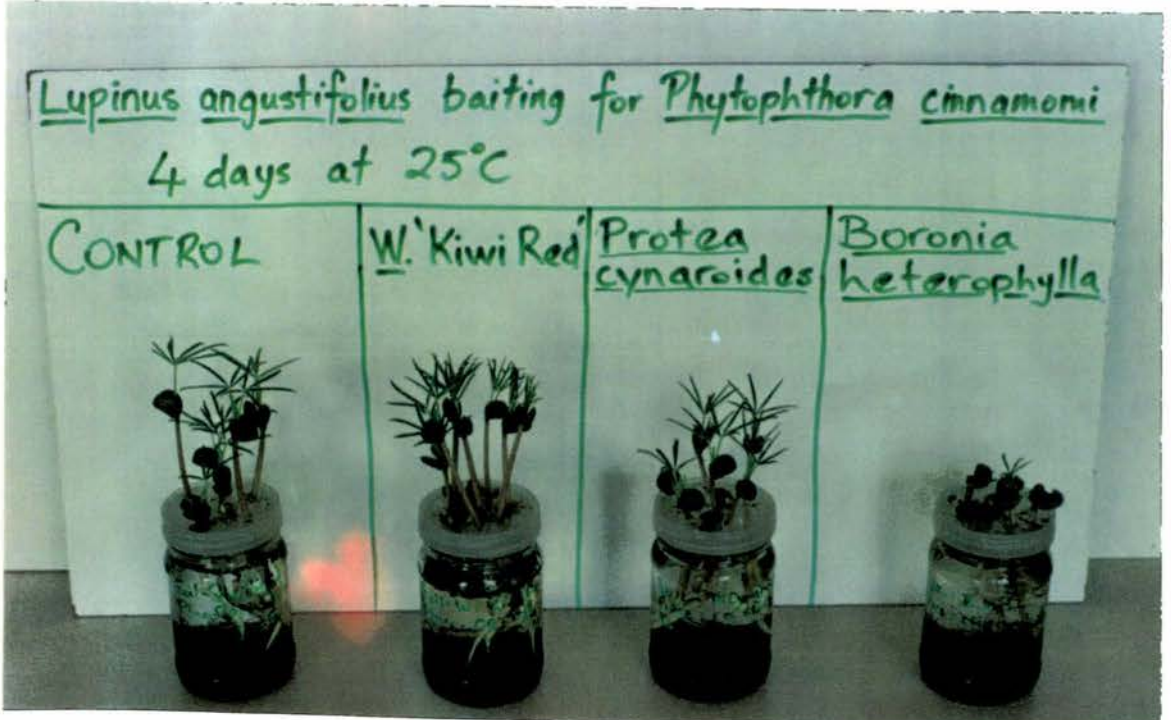


Plate 4: Blue lupin, *L. angustifolius*, baiting for *Phytophthora* species: Control, *Weinmannia racemosa* 'Kiwi Red', *Protea cynaroides* and *Boronia heterophylla* plants after 4 days. Only the *B. heterophylla* plants show symptoms of *Phytophthora*.



Plate 5: Blue lupin, *L. angustifolius*, baiting for *Phytophthora* species: Control and three different *Leucadendron* 'Wilsons Wonder' plants. All three *L.* 'Wilsons Wonder' samples showed severe symptoms of *Phytophthora* after 7 days



Plate 6: Blue lupin, *L. angustifolius*, baiting for *Phytophthora* species: Control and three different *Leucadendron* 'Wilsons Wonder' plants' root systems. All three *L.* 'Wilsons Wonder' root systems showed severe rotting after 7 days and few lateral roots formed.

### 3.3 DISCUSSION

The results show that the percentage infection in all susceptible species examined increased between days 4 and 7 when exposed on a continuous basis to the infected medium. The rate of lupin infection was probably related to the inoculum level present in the infected soil/plant extracts. More lupins were infected in the *L. 'Wilsons Wonder'* isolate extracts by day 4. This would suggest that there was more of the fungus present or that it was more virulent in these isolates.

Van Wyk (1973), demonstrated that a fungistatic chemical exists within the root bark of *Protea cynaroides* that inhibits the growth of *P. cinnamomi*. If this substance is also released into the soil in the immediate vicinity of the root system it could explain why there was a lack of *Phytophthora* in the soil/root mix of the *Protea cynaroides*.

Note that at the time of the initial experiments in this study it was presumed that *P. cinnamomi* was the cause of the sudden collapse of the cut flower plants, although further research in this study suggested that other *Phytophthora* species may also be involved. All the photographs taken were labelled with *Phytophthora cinnamomi* as it was only later discovered that one of the species isolated during the study was unnamed. Forsberg (1993), reports that this lupin baiting technique has been successful in recovering at least twelve different species. This raises the possibility that one or more different species of *Phytophthora* may have been responsible for the symptoms present on the *L. angustifolius*.

*Weinmannia racemosa* 'Kiwi Red' was included in this trial to evaluate its usefulness in other aspects of this study. Its use was not continued as it was

difficult to source adequate material and it did not differ greatly from the other plants selected for this study.

# Chapter 4

ASSESSMENT OF CHEMICAL CONTROL

OF *Phytophthora cinnamomi* ON

*Boronia heterophylla*, *B. megastigma* AND

*Leucadendron* 'Wilson's Wonder'.

#### 4.0 INTRODUCTION

The control of the root rot disease causing fungus, *Phytophthora cinnamomi*, by the use of fungicides has only been partially successful. No chemical has been found to be 100% effective against the disease in all plant species under field conditions inducing high disease pressure.

Various hypotheses have been advanced to explain the different degrees of disease expression. It has been suggested that environmental conditions contribute significantly to the disease cycle, in particular high soil moisture in warm periods and the presence or absence of water stress after periods of root death (Newhook, 1978).

The earliest efforts to combat the disease on grapes with copper gave limited protection to the areas where it came into direct contact with the fungus. The more recently developed systemic chemicals like ethazole (Terrazole®) produced variable results on avocados (Zentmeyer, 1980). No single chemical has attained a consistent response under all conditions in the field. The development in the late nineteen seventies of two new organic chemical groups, acylalanines and organic phosphonites provided a range of products which offered greater protection against *P. cinnamomi* than had been previously available.

The former group includes metalaxyl [methyl-DL-N-(2,6-dimethylphenyl)-N-(2'-methoxyacetyl)-alaninate] an important component of Ridomil® and furalaxyl [methyl-N-(2,6-dimethylphenyl)-N-furoyl-(2)-alaninate], the major component of Fongarid®. Both of these products are used as soil and growing media drenches against *Phytophthora* spp but are most effective when applied prior to infection (Marks and Smith, 1992).

The latter chemical group contains fosetyl-Al (aluminium tris-O-ethyl phosphonate) which is known as Aliette®. It is a mono-ethyl phosphonate which has exceptional phloem mobility (Ouimette and Coffey, 1989) allowing it to be applied either as a soil drench, a foliar spray or a trunk injection for effective control of *Phytophthora* species. Marks and Smith (1992) controlled *P. cinnamomi* stem infection in *Leucadendron laeololum* x *L. salignum* hybrid when fosetyl-Al was applied to runoff 10 days before infection.

Phosphonates are relatively stable within the plant and has direct antifungal activity. However, there is conjecture that modification of the phosphonate molecule may occur within the plant, possibly by altering the number and length of the alkyl side chains (Ouimette and Coffey, 1989), thereby enhancing the toxicity towards the pathogen. However, Pegg and de Boer, 1990 note that plants which have been treated with phosphonate produce phytoalexins more rapidly after challenge by a potential pathogen than untreated plants. They suggest that the response by phosphonate-treated plants to phosphonate-sensitive pathogens resembles that of a resistant cultivar.

The purpose of this trial was to investigate the activity of three chemical products which may prevent the infection and death of three plant species, *Boronia heterophylla*, *B. megastigma* and *Leucadendron* 'Wilson's Wonder' when inoculated with *P. cinnamomi*. The chemical products included Aliette® 80 SP, Foschek® 500 and Foschek® / C-408.

4.1 METHODS AND MATERIALS

4.1.1 Source of *Phytophthora cinnamomi*

Two isolates of *P. cinnamomi* were obtained from the Crown Research Institute, Landcare Research, Mt Albert, Auckland (see Table 3)

<i>Phytophthora cinnamomi</i> Isolate	Plant Source	Location of Source
Strain 7300	root of kiwifruit, <i>Actinidia deliciosa</i> (Chev.) Liang and Ferguson	Mount Albert., Auckland, New Zealand.
Strain 7749	root rot of chestnut, <i>Castanea species</i>	Kumeu, Auckland, New Zealand.

Table 3: The location and plant source of the two isolates of *Phytophthora cinnamomi* initially trialled for the experiments.

Initial testing on a number of *B. heterophylla* and *L.* 'Wilsons Wonder' cuttings, using the split wheat technique described below, indicated that strain 7749 was the more active of the 2 strains. This strain invaded the whole plant and caused death far more rapidly than strain 7300. It was also recovered more easily when infected plant material was plated out on to corn meal agar.

#### 4.1.2 Corn meal agar (CMA) preparation

Dehydrated Bacto Corn Meal Agar (ingredients per litre being 50 g of infusion from Corn Meal and 15 g Bacto Agar) from Difco Laboratories, Detroit, Michigan, USA, was rehydrated using the following method. Five hundred ml of deionised, distilled water was poured into a volumetric flask and a magnetic 'bean' added for the purpose of mixing the preparation. Dehydrated CMA was added at a rate of 17 g/l and placed on a Janke and Kunkel, IKAMAG®RH mixer for 10 minutes. The temperature setting was 5 and the motor setting 3.5. The mixture was poured into a SHOTT bottle and the pH adjusted to 4.5 with hydrochloric acid using an Solstal EPM 950 pH meter. The medium was then autoclaved for 15 minutes at 121°C.

#### 4.1.3 Chemical trial layout and treatments

Screening trials were undertaken to test the effectiveness of 3 chemical compounds at 2 different rates for their ability to improve plants' resistance to *P. cinnamomi*. The 3 plant species used were *Boronia heterophylla* and *B. megastigma*, and *L. 'Wilsons Wonder'*.

This trial consisted of 7 x 10 plants ( 70 plants) for *L. 'Wilsons Wonder'* and *B. heterophylla* and 7 x 5 (35) plants for *B. megastigma* in each replicate. Each plant was a rooted cutting freshly removed from the rooting medium when the trial was prepared. The trial was replicated twice (350 plants in total), the replicate trials were conducted concurrently.

The 3 chemicals investigated were Aliette® 80 SP (800 g/kg fosetyl-aluminium), Foschek ® 500 (500 g/l phosphorous acid as a  $K_2+/KH_2$  salt) and Foschek ® / C-408 (a combination of  $H_3PO_3$  and an additional product made

available for trial by Taranaki Nuchem Limited. This formulation was evaluated to test if it had superior performance to Foschek ® 500) The rates used in the trial were based on suggestions from the supplier.

The formulations used and the rates thereof are in Table 3

Chemical	Formulation	Rate Applied
Control (No chemical)		
Aliette® 80 SP	wettable powder	1000 ppm fosetyl-aluminium
Aliette® 80 SP	wettable powder	2000 ppm fosetyl-aluminium
Foschek ® 500	emulsifiable concentrate	1000 ppm phosphorous acid (H <sub>3</sub> PO <sub>3</sub> )
Foschek ® 500	emulsifiable concentrate	2000 ppm phosphorous acid (H <sub>3</sub> PO <sub>3</sub> )
Foschek ® / C-408	emulsifiable concentrate	1000 / 200 ppm
Foschek ® / C-408	emulsifiable concentrate	2000 / 400 ppm

Table 4: The formulations and rates of the chemicals used in the trial.

The control plants were watered with deionised, distilled water when the experiment was set up and the other plants in the trial were drenched with the chemical under test.

After infection with *P. cinnamomi*, the plants were kept in a growth chamber used for *in vitro* plant culture. The plants were grouped in blocks but the replicates were separated from each other.

The layout of the replicates is shown in Table 5.

<b>TREATMENT</b>	<b><i>L.</i>'Wilsons Wonder'</b>	<b><i>B. heterophylla</i></b>	<b><i>B. megastigma</i></b>
<b>Control (distilled H<sup>2</sup>O)</b>	10 plants	10 plants	5 plants
<b>Aliette® 1000 ppm</b>	10 plants	10 plants	5 plants
<b>Aliette® 2000 ppm</b>	10 plants	10 plants	5 plants
<b>Foschek® 1000 ppm</b>	10 plants	10 plants	5 plants
<b>Foschek® 2000 ppm</b>	10 plants	10 plants	5 plants
<b>Foschek® /C408 1000/200 ppm</b>	10 plants	10 plants	5 plants
<b>Foschek® /C408 2000/400 ppm</b>	10 plants	10 plants	5 plants

Table 5: The plants used in the trial and the rates of chemical applied to them. This layout was replicated twice.

800 ml vermiculite was placed in each of 42 x 1000 ml beakers. These were separated into 2 replicates of 7 treatments. Each replicate consisted of 3 beakers. The 3 beakers in each replicate were flooded with 1 of the 6 chemical treatments in Table 5 or with distilled water as the control and allowed to drain back to field capacity. Plants from each species were root drenched in the appropriate beaker for 7 days, with the regular addition of chemical treatment or distilled water to ensure that the samples remained constantly moist.

#### **4.1.4 Source of inoculum**

A source of the fungal inoculum was prepared using wheat seed as the substrate, which was sterilised in an autoclave at 121°C for 15 minutes before placing on an actively growing, 3-day-old, culture of *P. cinnamomi* strain 7749 growing on Corn Meal Agar (CMA) in a Petri dish. The sealed dishes were incubated at 25°C for a further 3 days, thus allowing the mycelium to penetrate the seed testa and starch within.

#### **4.1.5 Root Inoculation Technique**

A wound inoculation procedure reported by Dixon *et al* (1984) was modified to infect the root systems of susceptible rooted cuttings upon their removal from the 7-day pre-treatment described in 4.1.3.

The root bark was removed from a 3-5mm length on all the primary roots of each plant. A split, infected wheat grain was placed in contact with the wounded area approximately 5 mm from the stem or at an easily accessible site free of major lateral connections and secured with a strip of Gladwrap®

plastic cling film (see Plate 7). The control consisted of similar but uninfected, autoclaved wheat grains.

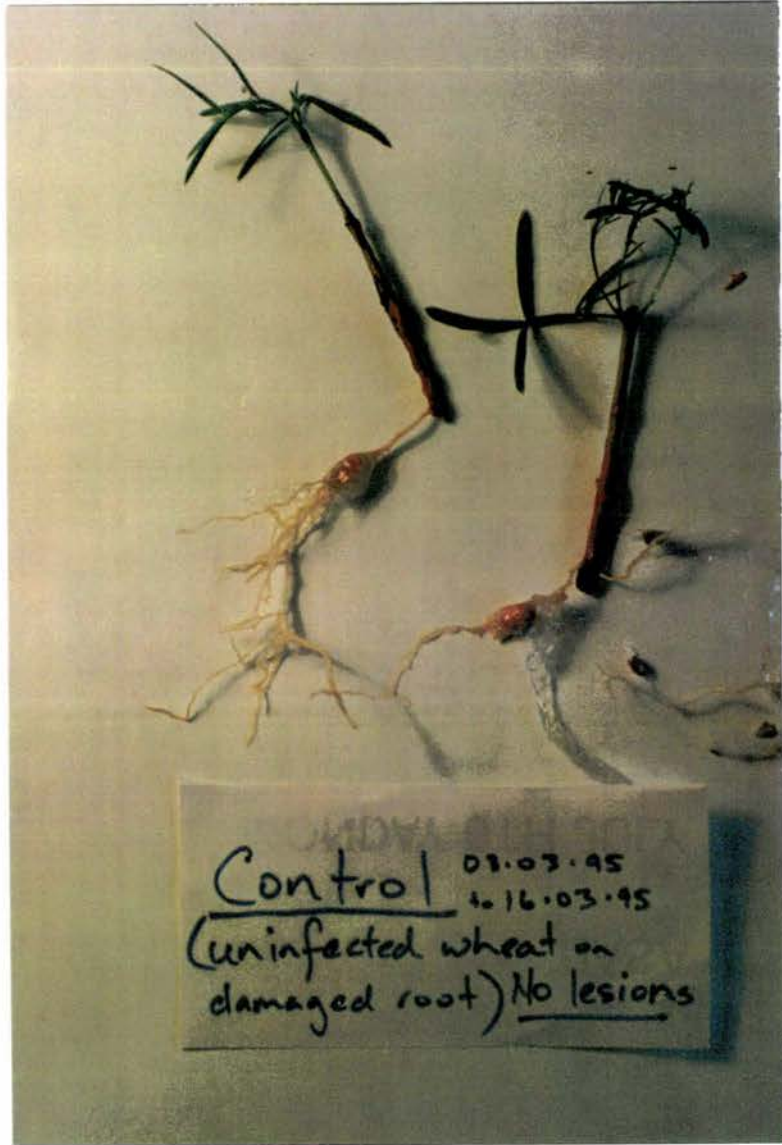


Plate 7: The wound inoculation technique using a split, wheat grain placed in contact with a wounded root of *Boronia heterophylla* and sealed with Gladwrap® plastic cling film.

This method allowed the mycelium ease of penetration into the root system. Desiccation of the wound was prevented by enclosing the wheat grain and the roots in the immediate proximity in a sealed layer of Gladwrap® plastic cling film. The whole plant was then inserted in a 200 ml glass jar, with its roots in 80 ml of the treated vermiculite. A plastic screw top lid containing a 5 mm diameter aperture into which cotton wool had been forced was used to cap the jar. The cotton wool allowed some air transfer thereby reducing excessive condensation within the jar.

The jars were then transferred to the growth chamber used for *in vitro* plant culture and subjected to high disease pressure of 25°C, with 16h light/ 8h dark periods and low substrate moisture tension. Regular watering with the appropriate treatment maintained a constantly moist root-holding medium.

The presence of *P. cinnamomi* was verified by plating out randomly selected 5 mm sections of inoculated root and stem tissue onto CMA.

#### **4.1.6 Assessment criteria**

Plants were examined for visual signs of *P. cinnamomi*, particularly in the above ground portions. The number of days to the death of the plants were also recorded. Symptoms of dying plants varied between species so criteria for the critical point at which death was inevitable were established for each and recorded as follows:

The *L.* 'Wilsons Wonder' plants exhibited severe lesioning over the length of the stem. All leaves displayed some yellowing, wilting or browning extending from the petiole.

The *B. megastigma* leaves showed leaf yellowing or sudden drop. Stem lesioning was not always easy to detect on these plants.

The *B. heterophylla* exhibited some lesioning on the stem. All the leaves displayed reddening, wilting or sudden drop.

Plants were assessed over a 5 week period for visual symptoms of infection and the rate of death was measured daily. Infection was considered at a maximum when the leaves became totally brown and began to fall. Root samples from dead plants were plated onto CMA to confirm the presence of *P. cinnamomi* in the isolates.

#### **4.1.7 The effect of Aliette® 80SP, Foschek® 500 and Foschek®/C 408 on *Phytophthora cinnamomi* growth *in vitro*.**

The following method was used to determine whether the three chemical compounds possessed fungistatic properties at the rates being trialled in the root inoculation experiment.

CMA was autoclaved at 121°C for 15 minutes and cooled slightly. Aqueous preparations of Aliette® 80 SP, Foschek® 500 and Foschek®/C 408 were mixed with the liquid CMA to bring it to the concentrations stated in 4.1.3. (When the Aliette®, Foschek® and Foschek®/C408 were added prior to autoclaving the CMA/Aliette® and CMA/Foschek®/C408 mixtures failed to solidify). While still liquid, 25 ml of the mixture was poured into each of 5 petri dishes, then allowed to cool and solidify. For each treatment 2 replicates were prepared. Each dish was inoculated in the centre with a wheat grain infected with *P. cinnamomi*. The inoculated dishes were

incubated at 25°C. The rate of growth of the fungus observed and recorded on days 2, 3, 4, 5, 6 and 7.

The control plates contained CMA without any added fungicidal compound and the trial was replicated twice.

All recorded data was analysed using the GLM model in SAS (SAS Institute)

## 4.2 RESULTS

The controls for all three species experienced a rapid rate of mortality, once symptoms became apparent and the first plant had died.

Initial deaths of *L. 'Wilson's Wonder'* were recorded 6 days after inoculation of the roots with *P. cinnamomi*. 50% of the plants were dead by day 9 (see Table 6). All the control plants were dead by day 24.

*B. heterophylla* control plant deaths occurred from day 7 (see Table 7) with 50% of the plants dead by day 15 and all of the plants dead by day 31.

The control plants of *B. megastigma* remained alive until day 20 (see Table 8) when 50% of the plants died. All of the control plants had died by day 31.

By day 20, Aliette® treated plants of *B. megastigma* began to die but, compared to the control, Aliette® at 1000 ppm and 2000 ppm reduced the number of deaths of *B. megastigma* with only 40% of plants dying by day 31.

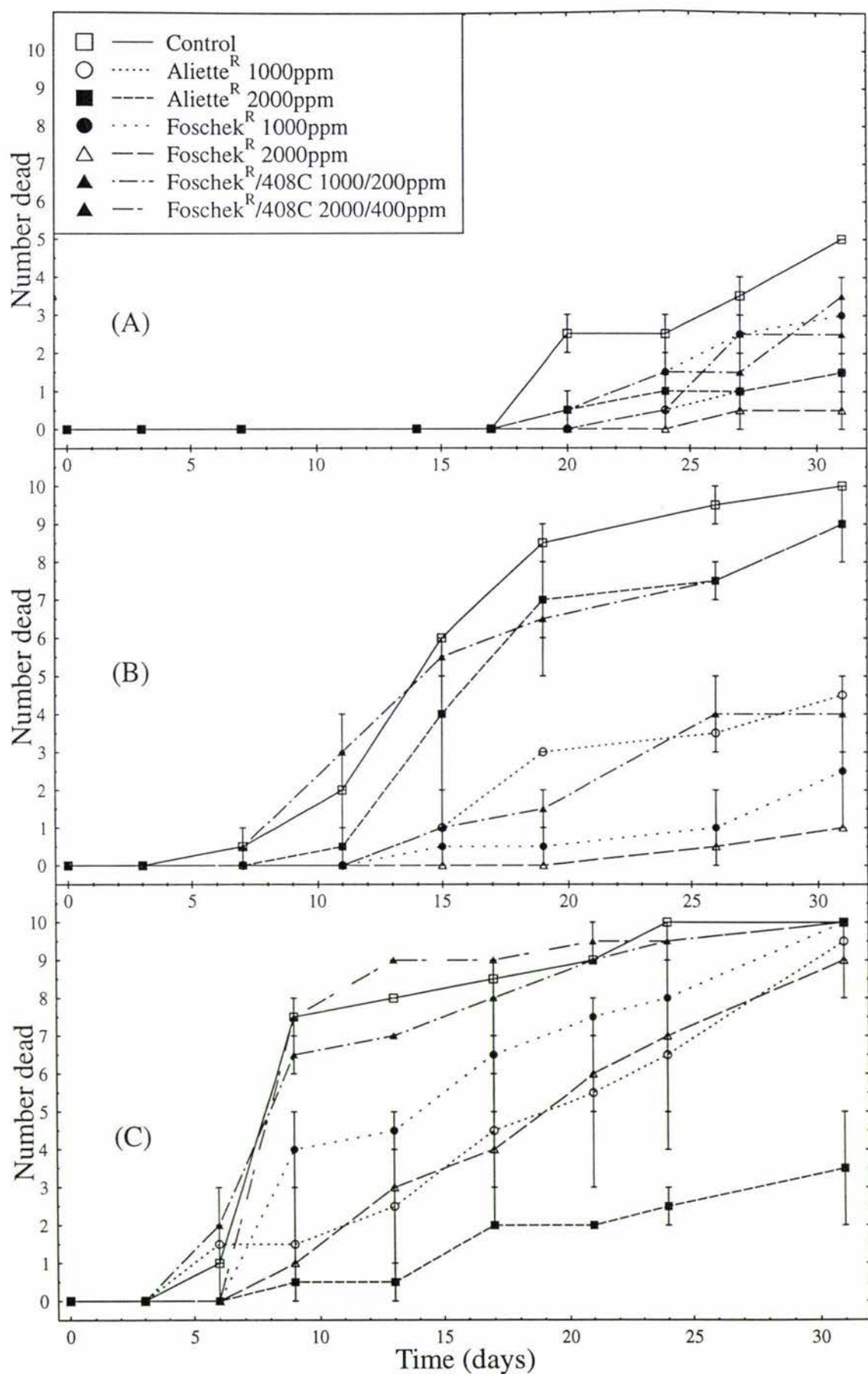


Figure 1. Effect of chemical treatments on death of (A) *Boronia megastigma*, (B) *Boronia heterophylla*, (C) *Leucadendron* 'Wilson's Wonder' plants inoculated with *Phytophthora cinnanomi*. Data were collected for one month after applying treatments. Vertical bars represent the standard error of the mean for each treatment.

Plant deaths were first recorded on day 6 on *L. 'Wilsons Wonder'* treated with the combination of compounds, Foschek®/C 408, at 1000 ppm. By day 9, 50% of the *L. 'Wilsons Wonder'* (see Table 6) plants were dead and all plants had died by day 31.

In the 2000 ppm treatment deaths were first recorded on day 9 (see Table 6) when more than 50% died and all plants had died by day 31.

The first plant deaths on the control and the 1000 ppm Foschek®/C 408 treated *B. heterophylla* plants were recorded after day 7. By day 15, 50% were dead. All of the plants in replicate 1, and 8 of the 10 plants in replicate 2 had died by day 31.

In the 2000 ppm, the Foschek®/C 408 treatment plants were protected for at least another 8 days beyond those in the control with the two deaths being recorded on day 15. On day 26, death was recorded in 50% of the plants in replicate 1 although only 30% of replicate 2. No further deaths were observed by day 31.

On *B. megastigma* plants the first deaths in the 1000 ppm and 2000 ppm Foschek®/C 408 treatments were recorded on days 24 and 20 respectively. However, 50% of the 1000 ppm treated plants were dead on day 27 but with the 2000 ppm treatment only 1 plant had died by day 31.

In Figure 1 all the main effects of day, species and chemical treatments were highly significant ( $P < 0.0001$ ). Generally the attrition rate imposed by the inoculation with *Phytophthora* was delayed by chemical treatment and generally expressed as an approximately sigmoidal response.

When the rate of plant deaths is assessed by the number dead at a particular time the most tolerant species was *Boronia megastigma* followed by *B. heterophylla* with *L. 'Wilsons Wonder'* the most susceptible to *Phytophthora* infection.

In the trial described in 4.1.7 *P. cinnamomi* grew unhindered on the two control replicates. Hyphal growth reached an average distance from the source of 35 and 36 mm respectively after 7 days. However, no hyphal growth of *P. cinnamomi* was observed on any of the plates treated with the two concentrations of Aliette® 80 SP, Foschek® 500 and Foschek®/C 408

#### 4.3 DISCUSSION

No single response function can be found to describe the rate of death of plants of *L. 'Wilsons Wonder'*, *B. heterophylla* or *B. megastigma* in response to treatment with the compounds Aliette®, Foschek® or Foschek®/C 408 (see Figure 1).

The untreated control treatments were generally inferior to any of the chemical treatments, therefore plants died most rapidly in these treatments compared with the chemical treatments. The best treatment seems to vary with species. The individual treatments can be compared by checking if there is an overlap of the standard error bars.

Aliette® is reported to have excellent systemic activity against a number of *Phytophthora* species on a range of plant species (Coffey and Joseph, 1985). However, Aliette®, at 1000 ppm, did not significantly delay plant mortality on either *B. heterophylla* or *L. 'Wilsons Wonder'*. On *B. megastigma*, at 2000 ppm (which is close to the manufacturer's recommendation of 2500 ppm for

field use) results were similar to 1000 ppm with only 30% dying after 31 days in both treatments.

TREATMENT	TIME TO DEATH (days) OF L. 'Wilsons Wonder'	
	50% DEAD	100% DEAD
Control	9	24
Aliette® -1000 ppm	21	> 31
Aliette® -2000 ppm	> 31	> 31
Foschek® -1000 ppm	17	31
Foschek® -2000 ppm	21	> 31
Foschek®/C408 -1000/200 ppm	9	31
Foschek®/C408 -2000/400 ppm 2	9	31

Table 6: Average number of days required for 50% and 100% of treated *Leucadendron* 'Wilsons Wonder' plants to die as a result of inoculation with *Phytophthora cinnamomi*.

Foschek®, at 2000 ppm, exhibited an excellent ability to reduce plant mortality in both of the *Boronia* species with only one plant death recorded in *B. heterophylla* and *B. megastima* at day 31 (see Figure1). However, on *L. 'Wilsons Wonder'*, 50% of the plants were dead on day 21 compared to control plants at day 9 (see Table 6). This suggests that the *P. cinnamomi* may be more difficult to control in *Leucadendron* species with Foschek® which is in agreement with Turnbull (1995). She noted that phosphorous acid

limited rather than prevented the development of the disease on *Leucadendron* 'Sylvan Red' under conditions of high disease pressure.

TREATMENT	TIME TO DEATH (days) OF <i>Boronia heterophylla</i>	
	50% DEAD	100% DEAD
Control	15	31
Aliette® -1000 ppm	> 31	> 31
Aliette® -2000 ppm	19	> 31
Foschek® -1000 ppm	> 31	> 31
Foschek® -2000 ppm	> 31	> 31
Foschek®/C408 -1000/200 ppm	15	> 31
Foschek®/C408 -2000/400 ppm	> 31	> 31

Table 7: Average number of days required for 50% and 100% of treated *Boronia heterophylla* plants to die as a result of inoculation with *Phytophthora cinnamomi*.

TREATMENT	TIME TO DEATH (days) OF <i>Boronia megastigma</i>	
	50% DEAD	100% DEAD
Control	20	31
Aliette® -1000 ppm	> 31	> 31
Aliette® -2000 ppm	> 31	> 31
Fosc hek® -1000 ppm	27	> 31
Fosc hek® -2000 ppm	> 31	> 31
Fosc hek®/C408 -1000/200 ppm	31	> 31
Fosc hek®/C408 -2000/400 ppm 1	31	> 31
Fosc hek®/C408 -2000/400 ppm 2	31	> 31

Table 8: Average number of days required for 50% and 100% of treated *Boronia megastigma* plants to die as a result of inoculation with *Phytophthora cinnamomi*.

The mode of action of phosphorous acid is not well understood, particularly in the plants under study. Guest and Bompeix (1990) suggest the chemical may act by stimulating the defence mechanisms of the host plant. This could explain the variation in response between the two *Boronia* species and *L. 'Wilsons Wonder'* whereby the natural defence mechanisms operating in *Boronia* may differ from those of *Leucadendron*. However, it is not known what critical levels of the compound are required in plant tissue to maintain effective disease control. Fosc hek® may, therefore, be more effective if

applied at a higher rate to the *Leucadendron*. This should be investigated as a natural extension to this work.

Foschek® was more effective at 2000 ppm than at 1000 ppm on all three plant species trialled. Foschek®/C 408 does not confer the same level of protection that was observed with Foschek® at 2000 ppm.

Foschek®/C 408 is a mixture of Foschek® and another chemical and the supplier was hoping for evidence of a synergistic effect. However, the results of this *in vitro* experiment do not provide any evidence of synergism between each component and nor appear to offer any increased protection to *L.* 'Wilsons Wonder', *B. heterophylla* or *B. megastigma* over Foschek®. It is possible, however, that the experiment was not sensitive enough to detect a difference between the two chemicals

These results are in direct contrast to field trials that suggest there is a substantial improvement in protection of *L.* 'Wilsons Wonder' when the Foschek®/C 408 mixture is used. (Mason, 1996 pers comm).

High disease pressure occurs as a result of a combination of optimum soil temperatures and low soil moisture tension (Zentmyer, 1980) and such conditions are often experienced on the cut flower properties in the Bay of Plenty in autumn and spring. Chemical control of *P. cinnamomi* on cut flower crops has been reported as inadequate under conditions conducive to high disease pressure (Marks and Smith, 1988, Turnbull, 1992).

These results confirm that some degree of delay of the symptoms of the disease can occur under these *in vitro* conditions, using Aliette® at 2000 ppm on *L.* 'Wilsons Wonder'.

Aliette® at 1000 ppm, Foschek® at 1000 and 2000 ppm, and Foschek®/C 408 at 2000 ppm all delayed the disease on *B. heterophylla* with Foschek® at 2000 ppm providing the best protection.

Aliette® at 1000 ppm and 2000 ppm, and Foschek® at 2000 ppm offered the best protection against *P. cinnamomi* on *B. megastigma*.

However, disease pressure is not always high and the growers often need control during periods of medium disease pressure. Other trial plants have shown better results with the chemicals, Foschek® and Foschek®/C 408 (Christie, 1995 pers comm). These chemicals may be re-trialled under conditions of medium disease pressure to gain information on their protective ability on a range of plant species under such conditions.

The experiment did not allow the opportunity to re-apply the chemicals during the trial. This could have been assessed by monitoring the level of active chemicals or their derivatives in the plant over time. While this would have been useful information for growers it was beyond the scope of this study.

Results of the trial described in 4.1.7 indicate that all three of the compounds, Aliette® 80 SP, Foschek® 500 and Foschek®/C 408, have the ability to check, and totally inhibit the growth of the fungus when they come into direct contact with it.

When these compounds are applied in the field at these concentrations they would be further diluted before entering the plants. This may well account for the reduction in fungicidal/fungistatic activity of the compounds. The lack of inhibition within the plants in this study suggests either phytoalexin production is not stimulated by any of these chemicals or the threshold of this

response was not reached. Applying an increased concentration of the compounds may raise the internal concentrations to those required to increase the fungistatic protection against the pathogen. However, drenching the roots of *L. 'Wilson's Wonder'* plants in 5,000 and 10,000 ppm of Foschek®/C 408 resulted in phytotoxicity and plant death (see Plates 8 and 9).



Plate 8: Toxicity symptoms on the leaves of *L. 'Wilson's Wonder'* plants after the roots were drenched in 5,000/1000 ppm of Foschek®/C 408. Note the browning of the leaf edges.



Plate 9: Toxicity symptoms on the leaves of *L. 'Wilson's Wonder'* plants after the roots were drenched in 10,000/2000 ppm of Foschek®/C 408. Note the severe leaf browning.

# **Chapter 5**

THE INFLUENCE OF  
PROTEACEOUS ROOT BARK ON  
THE GROWTH OF *P. cinnamomi*.

## 5.0 INTRODUCTION

The fungal pathogen, *Phytophthora cinnamomi*, is notorious for the wide range of species that it attacks, within these species there is a range of susceptibility and resistance to the disease. The tolerance of the disease within a single genus can be rated from resistant through to highly susceptible as recorded for *Banksia* (Dixon et al, 1984), *Eucalyptus* (Pratt and Heather, 1973) and *Protea* (Turnbull, 1991). *Protea cynaroides* is considered tolerant of *P. cinnamomi* (Turnbull, 1991) and will grow in heavy soils where other species would fail through attack by *P. cinnamomi*.

*Leucadendron* 'Wilson's Wonder' is considered to be one of the more susceptible cultivars grown in New Zealand (Hooper, pers comm). Zentmeyer (1980) reports up to 10% of *Macadamia* plantings in Queensland, Australia, are affected by the disease. Podger and Newhook (1971) noted the presence of *P. cinnamomi* in *Knightia excelsa* R. Br. Van Wyk (1974) noted the presence of an antifungal chemical compound, *p*-hydroxy-benzoylcalleryanin, in the root bark of *Protea cynaroides* and postulated that it may confer some protection to this plant.

In 1995, a number of specimens of *Protea cynaroides* on the Ohauiti property of C and M Bowyer suffered an outbreak of a disease (see Photos 9, 10 and 11) with symptoms similar to those of *P. cinnamomi*. Approximately 10% of infected plants died from the infection.

This experiment investigates the biological activity of the root bark from a range of proteaceous species to modify the growth of *P. cinnamomi*.

In this study the term root bark is as that described by Van Wyk (1974). The region from the surface of the root down to the stele was removed for use in these experiments and is hereafter referred to as root bark.



Plate 10: Symptoms of *Phytophthora* infection in field grown, 3-year-old *Protea cynaroides*. Chlorosis of the leaves on some stems is characteristic of an advanced stage of infection.



Plate 11: Advanced symptoms of *Phytophthora* infection in field grown, 3-year-old *Protea cynaroides*. Terminal shoots wilt, leaves die and the aerial portion of the plant will usually collapse. If the roots are not badly infected new shoots may grow from the lignotuber at ground level.



Plate 12: Advanced symptoms of *Phytophthora* infection in field grown, 3-year-old *Protea cynaroides*. Stem lesioning occurs and rapidly girdles the stem bark.

Samples of the diseased plant material plated on to CMA indicated the presence of a fungus which was identified as a *Phytophthora* species but did not resemble *P. cinnamomi*. Dance, 1995, (pers comm) confirmed that the pathogen was an indigenous but unnamed *Phytophthora* species found in New Zealand forests. Unlike *P.cinnamomi*, this species appears to peak in activity in a relatively narrow range of temperatures, 12-16°C, dying out at about 22-24°C and is similar to *P. cactorum* and *P. hibernalis* (Dance, 1995, pers comm). Initial infection from this species of *Phytophthora* is more likely to occur in the winter than the summer. However, the symptoms of the disease may only become visually apparent when plants are stressed over summer.

## 5.1 METHODS AND MATERIALS

### 5.1.1 Source of Root Bark

Root bark samples were collected from a range of Proteaceous species from four sites (see Table 9) for the purpose of this study. For each plant sampled roots were exposed for a distance up to 1.5 metres from the stem. The bark was removed in single sections and transferred into labelled, plastic bags.

The four *Protea cynaroides* isolates were obtained from one healthy plant (isolate i) and three plants infected with the undescribed, indigenous *Phytophthora* species (isolates ii, iii and iv).

The three *Knightia excelsa* isolates were obtained from two trees. Isolate (i) was the root bark from the first tree sampled at a distance of 1m from the trunk. Isolates (ii) and (iii) were root bark samples from the second tree and taken at 1 and 1.5m respectively from the trunk.

The bark was dried for 12 h at 100°C and ground to a powder in an Emmco Kwikmix blender as described by Van Wyk (1974).

**5.1.2 Bark powder/CMA preparation and inoculation**

Bark powder from each sample was mixed at different concentrations (5 or 10%) with the rehydrated CMA and autoclaved at 121°C for 15 minutes. Aliquots of 25 ml were poured into each of 5 petri dishes, allowed to cool, then inoculated with a *P. cinnamomi* (strain 7749) infected wheat grain. Each bark sample and concentration was replicated twice. The inoculated dishes were incubated at 25°C.

PROPERTY	LOCATION (see Map 5)	SPECIES (1 plant unless otherwise stated)
G&G Nee's farm (on a stream bank)	588 Ohauti Road, Tauranga	<i>Knightia excelsa</i> (2 trees)
C&M Bowyer's 1.6 ha cut flower property	12 Neewood Road, Tauranga	<i>Protea cynaroides</i> (3 dying plants)
Bay of Plenty Polytechnic orchard	Windermere Drive, Tauranga	<i>Macadamia</i> 'Beaumont'
Bay of Plenty Polytechnic gardens	Windermere Drive, Tauranga	<i>Protea cynaroides</i> ,

Table 9: The location and number of each species used to source the Proteaceous root bark.

### 5.1.3 Assessment criteria

All plates were examined for the presence of *P. cinnamomi*. The distance grown by the fungus from the source of inoculation on the CMA was measured and recorded on days 2, 3, 4, 5, 6 and 7 (or until the maximum possible distance was reached, given the constraint of the maximum plate size (40 mm radius).

### 5.1.4 Determination of 'zone of inhibition'

The *Protea cynaroides* plants, that died from infection by an unnamed, indigenous *Phytophthora* species, may have lacked or contained a lower concentration of the antifungal chemical, *p*-hydroxy-benzoylcallerynin than is present in a plant which is not infected by *Phytophthora*.

The following experiments were undertaken to compare the root bark of the dying *Protea cynaroides* with that of the uninfected plant.

The first part of the experiment was to determine whether the root bark of the dying *Protea cynaroides* possessed the ability to inhibit the growth of *P. cinnamomi* at a similar extent to that of an uninfected plant. The fatal infection may have occurred as a result of a reduction or absence of chemical resistance.

The second part of the experiment was to determine whether the root bark of *Protea cynaroides* possessed the ability to create a zone of inhibition or protection against the *P. cinnamomi* and was the size of the zone of inhibition related to the quantity of root bark.

#### **5.1.4.1 *Protea cynaroides* root bark powder influence on growth rate of *Phytophthora cinnamomi*: (I)**

Alignots of 25 ml of CMA were poured into Petri dishes and allowed to cool. Dried root bark powder was prepared as described in 5.1.2. from the disease-free *Protea cynaroides* in the Bay of Plenty Polytechnic gardens and an infected, dying *Protea cynaroides* from C and M Bowyer's property. Small quantities (0.05 or 0.5 g) of the dried root bark powder was evenly distributed over an area of 1000 mm<sup>2</sup> (20 x 50 mm) on the CMA (see Table 7). A wheat grain infected with *P. cinnamomi* was placed on the surface of the CMA 13-14 mm from the edge of the root bark powder. The lid was placed on each dish and the rim of the lids sealed with Gladwrap® cling foil. All the dishes were incubated at 25°C and the growth of the *P. cinnamomi* towards the root bark powder recorded daily from day 2.

The control dishes contained only CMA and were infected in the same manner as the other treatments.

#### **5.1.4.2. Influence of *Protea cynaroides* root bark powder influence on growth rate of *Phytophthora cinnamomi* (II)**

Alignots of 25 ml of CMA were poured into Petri dishes and allowed to cool. Dried root bark powder samples were prepared as described in 5.1.2. from the uninfected *Protea cynaroides* in the Bay of Plenty Polytechnic gardens and an infected, dying *Protea cynaroides* from C and M Bowyer's property. For each of the plants, three samples of dried, root bark powder (0.01, 0.05 and 0.1 g) were placed on a CMA dish 10 mm from the rim and 15 mm apart.

TREATMENT	0.05g/ 1000 mm <sup>2</sup>	0.5g/ 1000 mm <sup>2</sup>
Control - CMA only	4 Petri dishes	4 Petri dishes
Root bark powder of dying, <i>Phytophthora</i> infected <i>Protea cynaroides</i>	4 Petri dishes	4 Petri dishes
Root bark powder of uninfected <i>Protea cynaroides</i>	4 Petri dishes	4 Petri dishes

Table 10: Layout of the 'zone of inhibition' trial using two measured amounts of dried, root bark powder of two *Protea cynaroides* plants, one fatally infected with an undescribed, indigenous *Phytophthora* species, the other plant was not infected with this pathogen.

The control dishes contained CMA without any root bark powder. Each trial was replicated twice.

A *P. cinnamomi* infected wheat grain was placed on the surface of the CMA equi-distant from the edge of each sample of root bark powder. The lid was placed on each dish and the rim of the lids sealed with Gladwrap® cling foil. All the dishes were incubated at 25°C. The distance grown by the *P. cinnamomi* towards the three samples was recorded daily from day 2.

The recorded data was analysed using the GLM model in SAS (SAS Institute)

## 5.2 RESULTS

### 5.2.1 The effect of bark extracts on the time course of *P. cinnamomi* hyphal growth on CMA agar.

The results of the experiment described in 5.1.2 are shown in Table 11.

In the control the growth of *P. cinnamomi* followed a linear pattern from day 2 to day 6 after which the maximum possible growth was achieved (see Figure 2). Progress ranged from 5 to 8 mm per day

No significant different difference was noted between *K. excelsa* at 5 or 10% concentrations. Only the 5% treatments of *K. excelsa* have been shown in Figure 2 while only one of the *Protea cynaroides* treatments is shown as they all prevented the growth of *P. cinnamomi*.

In Figure 2 there were highly significant differences ( $P < 0.0001$ ) between hyphal growth at each day and between species. The interaction between species and time was also significant ( $P < 0.0001$ ) as can be seen in Figure 2 by the wide divergence in the lines for two sets of treatments. The data collected in 5.1.2 and means thereof (presented in Figure 2) were analysed by treating the means as linear functions and comparing the slopes and intercepts for data recorded from day 2. It became clear that there were no significant differences in the value of the intercept for all treatments except where *Protea cynaroides* and *Macadamia* 'Beaumont' were used. The individual treatments can be compared by checking if there is an overlap of the standard error bars.

Species and Concentration Root Bark Powder Used		Distance Grown (mm)	
		Day 4	Day 6
<b>Control</b>		17.4	29.8
<b><i>K. excelsa</i></b>			
isolate i	5%	22.5	39.6
isolate ii	5%	22.6	40.0
	10%	20.8	40.0
isolate iii	5%	22.0	39.8
	10%	20.9	37.3
<b><i>M. 'Beaumont'</i></b>		13.4	26.4
<b><i>P. cynaroides</i></b>			
isolate i	5%	0	0
	10%	0	0
isolate ii	5%	0	0
	10%	0	0
isolate iii	5%	0	0
	10%	0	0
isolate ii	5%	0	0
	10%	0	0

Table 11: Vegetative growth of *Phytophthora cinnamomi* on CMA enriched with different concentrations of root bark extracts of *Knightia excelsa*, *Macadamia' Beaumont'*, and *Protea cynaroides*.

The slopes of the *Knightia* treatments (see Figure 2) were not significantly different from one another but were significantly different ( $P < 0.05$ ) from the control treatment. The average distance grown by the *P. cinnamomi* on *K. excelsa* was 129% of that of the control on day 4.

With *Protea cynaroides* the difference in the intercept at day 2 (see Figure 2) was significant ( $P < 0.001$ ). Similarly the slope of the *Protea cynaroides* treatments was significantly different ( $P < 0.001$ ) from the the other treatments including the control.

The slope of the 5% *Macadamia* 'Beaumont' treatment (see Figure 2) was also significantly different ( $P < 0.05$ ) from the the other treatments and the control. The average distance of extension growth of the hyphae on day 4 was 77% lower than that of the control.

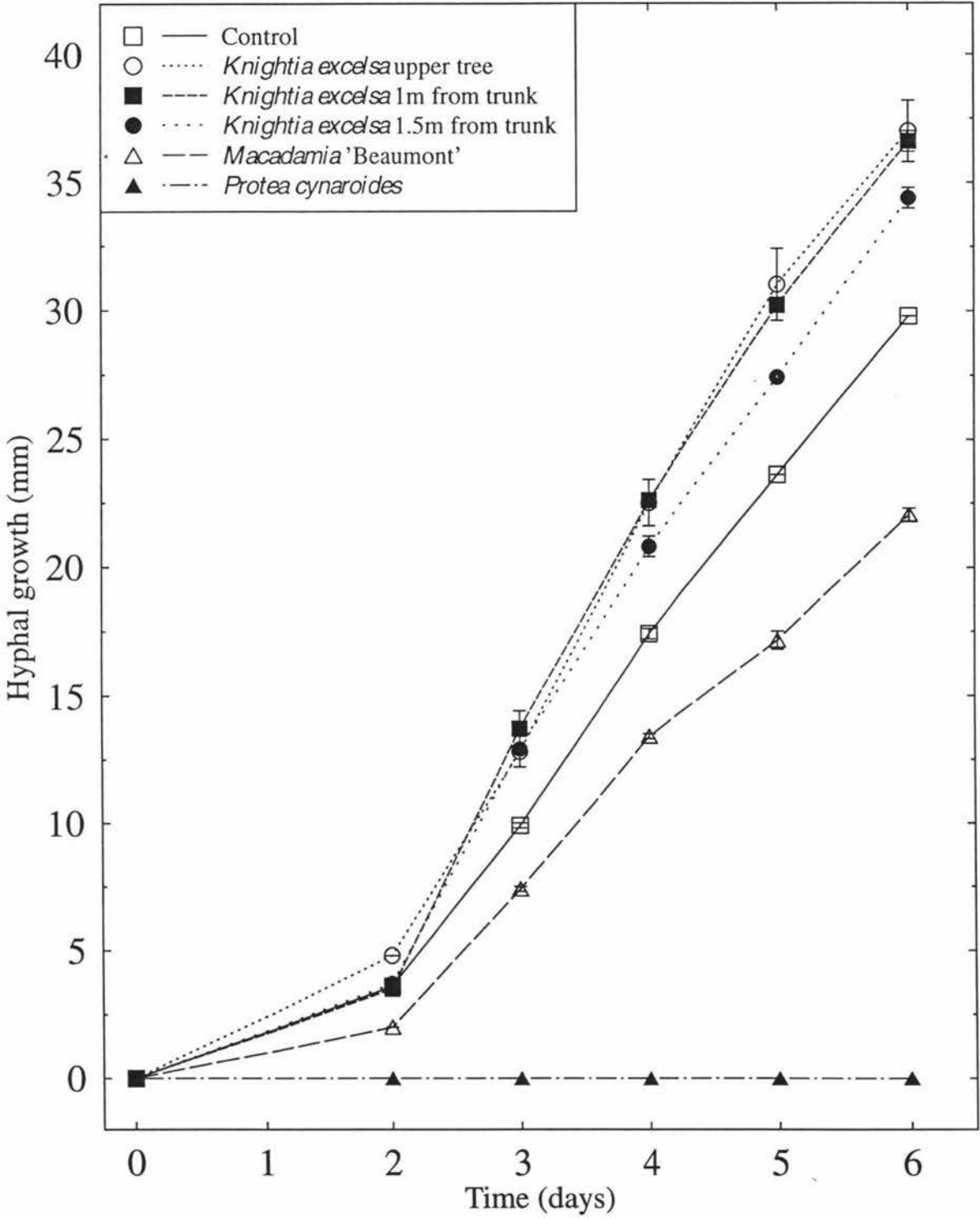


Figure 2. Effect of bark extracts on time course of *Phytophthora cinnanomi* hyphal growth on CMA agar. Data for *Protea cynaroides* bark extracts from healthy and infected plants is represented by a single line. Vertical bars represent the standard error of the mean for each treatment.

### 5.2.2 The effect of *Protea cynaroides* root bark infected with an undescribed *Phytophthora* species on the growth of *Phytophthora cinnamomi* on CMA.

The results of the experiment described in 5.1.4.1 (effect of small quantities [0.05 or 0.5 g distributed over an area of 1000 mm<sup>2</sup>] of the dried *Protea cynaroides* root bark powder from healthy and infected plants on the time course of hyphal growth from an undescribed *Phytophthora* species on CMA agar) are shown in Figure 3.

The growth rate of the *Phytophthora. cinnamomi* on the 0.05 g/1000 mm<sup>2</sup> root bark powder samples of both the *Phytophthora*-infected and the uninfected plants was similar to that of the controls. This concentration of root bark powder did not produce a markedly visible zone of leachate (orange-brown discoloration of the CMA) beyond the the 1000 mm<sup>2</sup> area. The *P. cinnamomi* grew through the area of root bark powder and beyond unhindered (see Plate 13).

Figure 3 shows that there were highly significant differences ( $P < 0.0001$ ) between hyphal growth at each day and between the root bark treatments. The 0.5 g root bark powder sample from the uninfected *Protea cynaroides* reduced the rate of growth of *P. cinnamomi* compared to the controls and the 0.05 g sample results. *Phytophthora. cinnamomi* on the 0.05 g samples reached the edge of the 1000 mm<sup>2</sup> area on day 5 (see Figure 3). However it required 8 days on the 0.5 g samples to reach the edge.

The interaction between time and treatment effects was also significant ( $P < 0.0001$ ) as can be seen by the wide divergence in the lines for two sets of treatments.

When the data was collected in 5.1.4.1 and the means were presented in Figure 3 the influence of high root bark application rate was evident by the markedly different response curves relative to the other treatments including the control. The infected root bark produced the highest level of influence on the growth of *Phytophthora* hyphae.

The 0.5 g root bark powder sample from the *Phytophthora*-infected, dying *Protea cynaroides* plant reduced the growth of *P. cinnamomi* markedly. By day 4 the distance measured was only 2 mm whereafter no further growth was recorded. This cessation of growth coincided with the fungus reaching a dark orange-brown zone of leachate (see Photo 13) which is termed the 'zone of inhibition' in this study.

### **5.2.3 The effect of different rates of *Protea cynaroides* root bark infected with an undescribed *Phytophthora* species on the growth of *Phytophthora cinnamomi* on CMA.**

On the CMA dishes containing the 0.01, 0.05 and 0.1 g samples of dried root bark powder from the uninfected *Protea cynaroides* the growth rate of *P. cinnamomi* was similar to that observed on the controls (see Figure 4). There were, however, highly significant differences ( $P < 0.0001$ ) between hyphal growth at each day and between type of bark treatment.

The rate of growth of the *P. cinnamomi* towards the undescribed, indigenous *Phytophthora*-infected root bark was reduced by all three samples (see Plate 15). A slower rate was recorded near 0.05 and 0.1 g than near 0.01 g. By day 3 the growth rate was reduced when compared to the control and this interaction between time and treatment was also significant ( $P < 0.0001$ ) as can

be seen in Figure 4 by the wide divergence in the lines for two sets of treatments.

The data collected in 5.1.4.2 and the means presented in Figure 4 were analysed by treating the means as linear functions and comparing the slopes and intercepts for data recorded from day 0. It was clear that there were no significant differences in the value of the intercept for all treatments.



Plate 13 *Phytophthora cinnamomi* growing towards the root bark powder of a *Protea cynaroides* dying from an indigenous *Phytophthora* species. On agar < 0.05g/1000 mm<sup>2</sup> root bark powder did not reduce the growth of *Phytophthora. cinnamomi*.

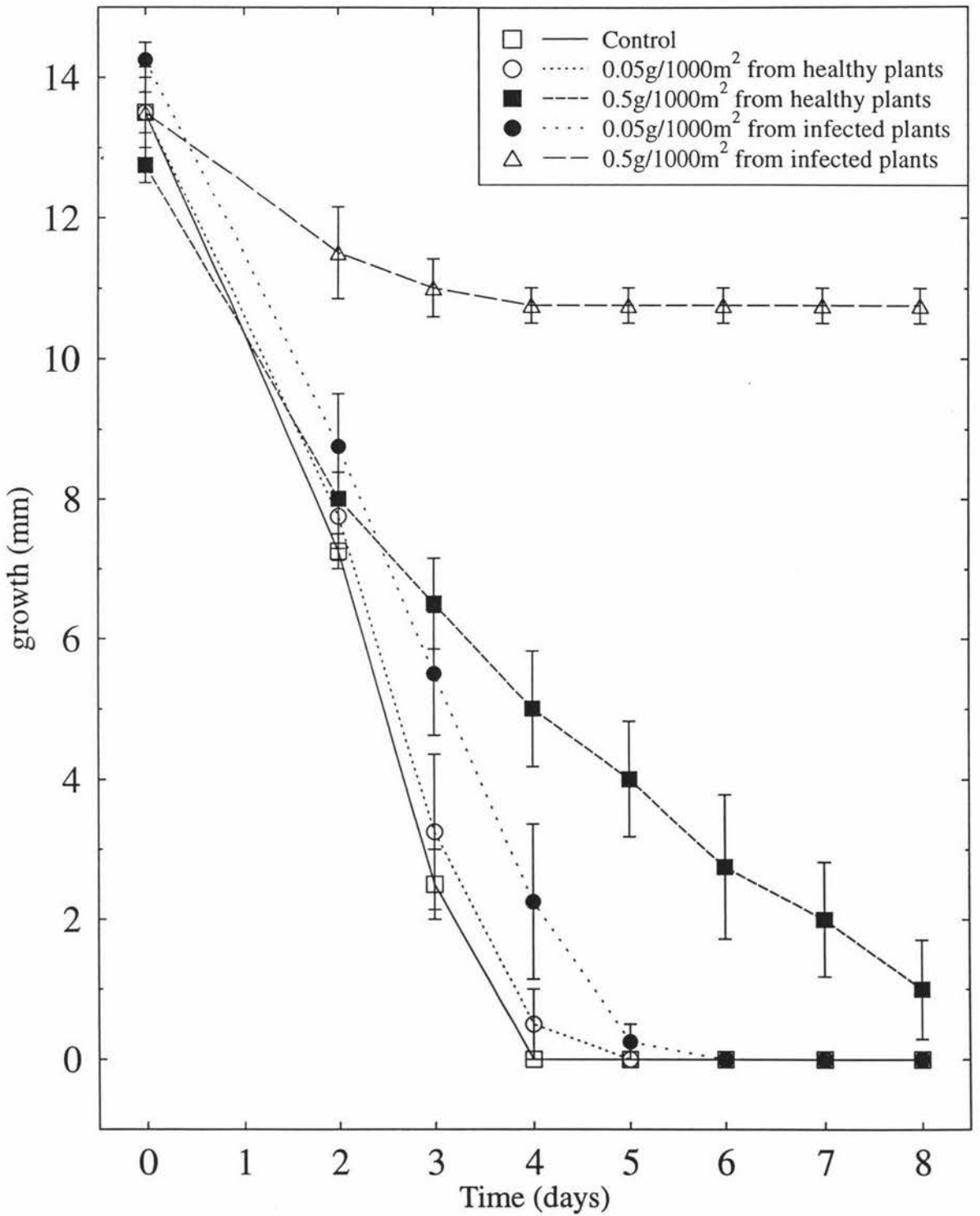


Figure 3. Effect of *Protea cynaroides* root bark from healthy and infected plants on time course of an undescribed *Phytophthora* species hyphal growth (mm) on CMA medium. Vertical bars represent the standard error of the mean for each treatment.

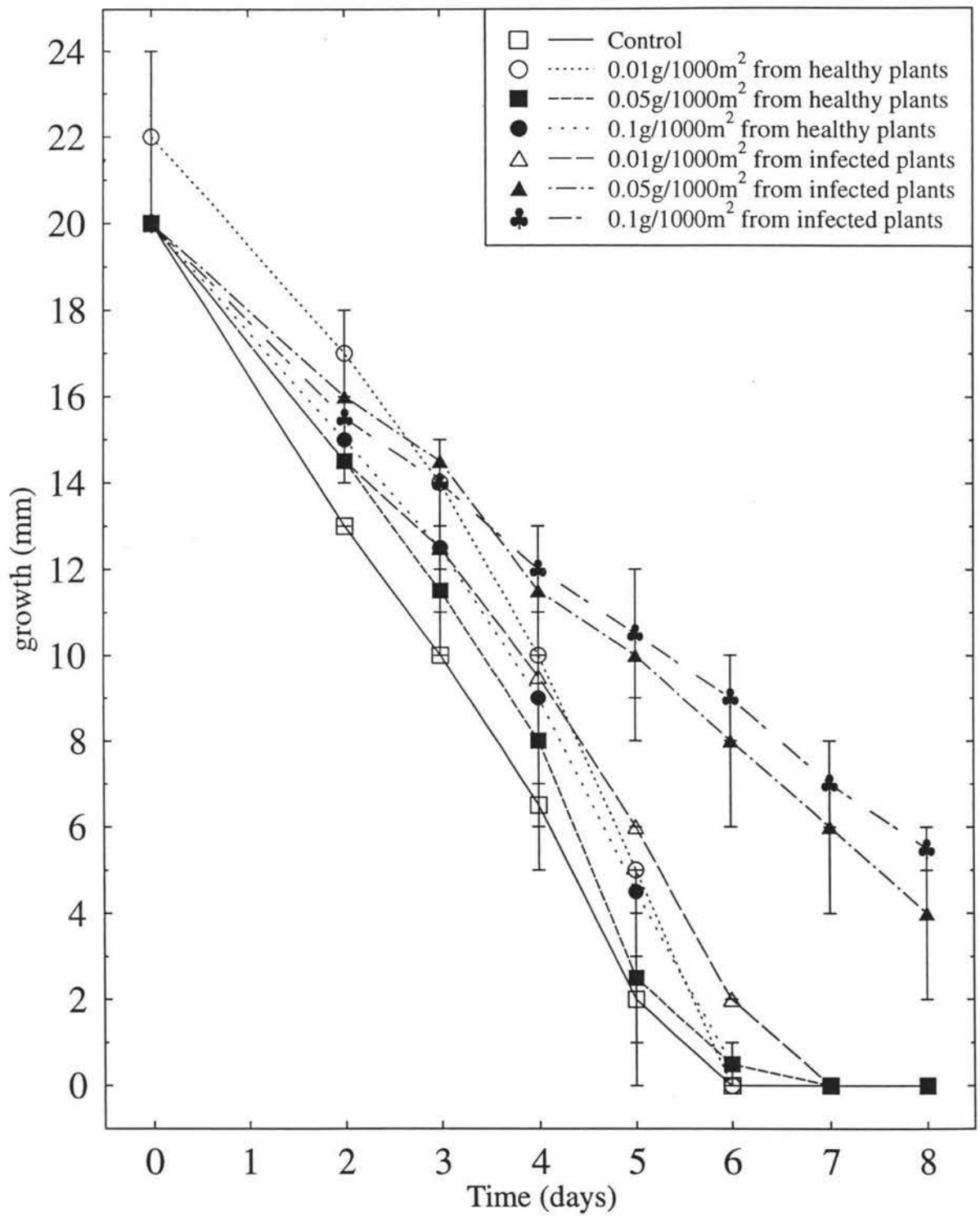


Figure 4. Effect of *Protea cynaroides* root bark from healthy and infected plants on time course of an undescribed *Phytophthora* species hyphal growth (mm) on CMA medium. Vertical bars represent the standard error of the mean for each treatment.

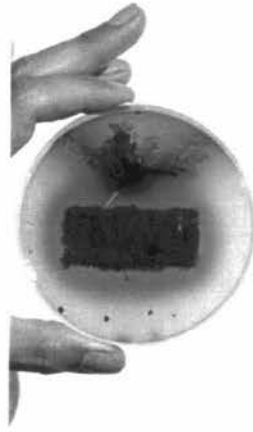


Plate 14: *Phytophthora cinnamomi* growing towards the root bark powder of a *Protea cynaroides* dying from an indigenous *Phytophthora* species. At 0.5g/1000 mm<sup>2</sup> the growth of *P. cinnamomi* was inhibited by the orange-brown leachate from the dried root bark powder. This leachate produced a 'zone of inhibition' which prevents the growth of *P. cinnamomi*.

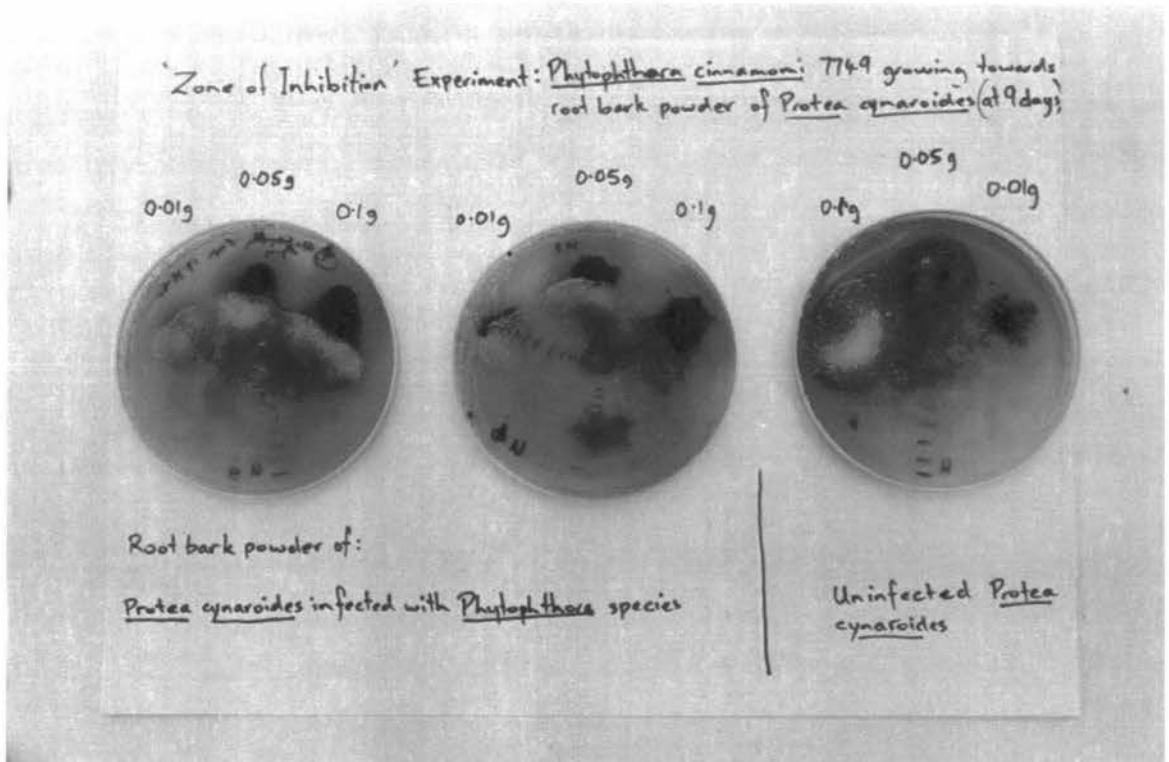


Plate 15: The effect on the growth of *Phytophthora cinnamomi* of the different concentrations (0.01, 0.05 and 0.1g) of root bark extract from a *Protea cynaroides* infected with an undescribed, indigenous *Phytophthora* species and an uninfected *Protea cynaroides*.

### 5.3 DISCUSSION

The slopes of the lines in Figure 2 indicate a similar set of factors may have been operating in *Knightia. excelsa* but a rather different response was elicited by the *Protea cynaroides* and *Macadamia* 'Beaumont'.

The inhibition of the vegetative growth of *P. cinnamomi* by the 5% dried, powdered bark of the uninfected *Protea cynaroides* root agrees with the results of Van Wyk (1974). However, a factor was present in the root bark of the dying plants which continued to inhibit the growth of *P. cinnamomi* in spite of those plants having suffered a lethal infection of the pathogen. This suggests that the factor, which is assumed to be *p*-hydroxyl-benzoylcalleryanin as reported by Van Wyk (1974), is a relatively stable compound which remains unaltered by the interaction with the invading fungus.

However, the compound is unable to prevent the development of the pathogen under conditions of extreme and sustained disease pressure. This may be due to a dilution factor when the root system is constantly immersed in water during periods of high, sustained rainfall. This may result in reduced root and microbial exudates from the rhizosphere. These may regulate the presence of *P. cinnamomi* to tolerably low levels which the plant's defence mechanisms can counter.

Under periods of high disease pressure, the root system may not accumulate a adequate chemical quickly enough to inhibit the rapidly accelerated mycelial growth. These results suggest that *p*-hydroxyl-benzoylcalleryanin or a related phytoalexin-eliciting chemical is already present in the root bark of an unstressed *Protea cynaroides* before infection occurs. *Phytophthora*

*cinnamomi* is a relatively recent arrival in South Africa (Crandall and Gravatt, 1967; Knox *et al.*, 1985) and there has scarcely been enough time for the plant to develop a substance to specifically resist this pathogen. Therefore, it can be assumed that this is only a secondary role of this fungistatic compound or that it is broad spectrum in its action

The compound, *p*-hydroxyl-benzoylcalleryanin or related fungistatic substance, may not be produced in cells which are directly adjacent to the area of invasion. It may, for example, be produced in the leaves and translocated to the roots. This could be investigated using reciprocal grafts of *Protea cynaroides* and other *Protea* species which do not produce this compound to determine where the production occurs and if it is influenced by the ordering of the graft components.

The pathogen may enter the plant through an area of damaged root bark directly into the stele thereby circumventing any contact with the inhibitory substance. This could explain why plants which sustain broken or damaged roots from what growers know as 'windrock'. During periods of high or gusty winds plants often observed to suffer the sudden collapse and death associated with *P. cinnamomi* infection. Similar root damage can occur for a number of reasons including surface cultivation, rabbits, and mechanical weeding, all of which may lead to the exposure of tissue below the bark.

The pathogen may also enter the plant through the stem or leaves rather than the root system. Resistance in *Protea cynaroides* may be conferred by the chemicals like *p*-hydroxyl-benzoylcalleryanin restricting or preventing entry of *P. cinnamomi* to the root system. However, to date, there is no evidence of chemical resistance in the stems or leaves. This could be investigated in future studies in this area.

The rapid rate of growth of *P. cinnamomi* on the *Knightia* enriched CMA contrasts with the reported tolerance of *Knightia excelsa* to the disease (Christie, 1995 pers comm). The observed, rapid growth rate suggests that the pathogen, should it be present in the surrounding soils, would rapidly invade and distribute itself throughout these forest trees. The rate of growth of *P. cinnamomi* on CMA enriched with powdered *Knightia* root bark suggests that *K. excelsa* provides an excellent medium for the growth of the fungus. However, the wide distribution of the pathogen throughout New Zealand forest soils (Newhook, 1968) including *K. excelsa* forests (Podger and Newhook, 1971) indicates that the tree has an alternative, non-chemical resistance mechanism against the fungus. Podger and Newhook (1971) also suggest that the effect of the fungus may be limited in some indigenous communities where soil temperature and moisture content are considered favourable for the pathogen. This may be due to three factors.

Firstly, that the indigenous hosts may have the ability to regenerate roots at a rate at least equal to that of destruction by the *P. cinnamomi*.

Secondly, that the damaged hosts are not exposed to any environmental stress.

Thirdly, that there exists within the soil a state of equilibrium which keeps the levels of *P. cinnamomi* low.

The soil samples from the root regions of the 2 trees sampled failed to respond to the lupin baiting technique. *Phytophthora cinnamomi* may be present in only low levels, being kept in check perhaps, as Newhook and Podger (1972) suggest, by in the rhizosphere (Newhook, 1978).

In the forest area where the *Knightia* root samples were sourced, all the mature tree specimens were located on ridges and steep slopes away from the valley floors and creeks. This allows the roots to be surrounded by well-drained soils although the leaf litter below the trees was usually quite moist as is favoured by *P. cinnamomi*. Therefore the soil beneath these trees is likely to contain mycorrhiza or other soil factors which are regulating the presence of *P. cinnamomi* to tolerably low levels.

*Macadamia* 'Beaumont' is a hybrid resulting from an Australian cross between *M. integrifolia* Maiden and Betche and *M. tetraphylla* L.A.S. Johnson (Menninger, 1977). Zentmeyer (1980) reported that inoculation tests demonstrated *M. tetraphylla* to be far more resistant to *P. cinnamomi* than *M. integrifolia*. This degree of resistance may have been transferred to *M. 'Beaumont'* from its resistant parent. If this resistance is conferred by a compound in the bark it could explain the reduced rate of *P. cinnamomi* growth on the CMA containing 5% *M. 'Beaumont'* bark powder.

The root bark from the *Protea cynaroides* plants which were infected with the undescribed, indigenous *Phytophthora* strongly influenced the growth of the *P. cinnamomi* hyphae. Van Wyk (1974) recorded a reduction in the growth rate of *P. cinnamomi* at low concentrations of *p*-hydroxi-benzoylcalleryanin (0.25% mixed with potato dextrose agar). This suggests that the leachate in the 0.05g/1000 mm<sup>2</sup> samples from the infected plants, which did not prevent or reduce the growth of *P. cinnamomi*, contained concentrations even lower than 0.25%.

The leachate from the 0.5 g root bark powder sample from the *Protea cynaroides* plant infected with the undescribed, indigenous *Phytophthora*

prevented the growth of *P. cinnamomi*. This cessation of growth coincided with the fungus reaching the dark orange-brown zone of leachate (see Plate 13) which is termed the 'zone of inhibition' in this study. In this zone the concentration of anti-fungal compound must be higher than that surrounding the 0.5 g root bark powder of the uninfected *Protea cynaroides* plant. This suggests that the roots of a *Protea cynaroides* plant invaded by a *Phytophthora* species have the potential to produce greater concentrations of the anti-fungal compound as a result of that invasion.

This is a similar response to that of a phosphonate treated plant which, when challenged with a phosphonate sensitive pathogen resembles a resistant cultivar (Guest and Bompeix, 1990). Phosphonate-treated plants produce phytoalexins more rapidly after challenge with a potential pathogen than those not so treated.

*Protea cynaroides* does not contain naturally high concentrations of the compound *p*-hydroxyl-benzoylcalleryanin (Van Wyk, 1974) Neither does it grow in an area where *P. cinnamomi* is indigenous so the degree of resistance the compound confers on the species may well be coincidental. It is known that many plant species have resistance to parasites to which they have never been exposed (Baker and Cook, 1974). However, the occurrence of the compound may well result from the *Protea cynaroides* contact with another indigenous *Phytophthora* species which does fatally infect the plant.

The increased concentration of the *Phytophthora*-inhibiting compound in *Protea cynaroides*, which have become infected by a *Phytophthora* species, could be further investigated. This could be achieved by determining whether a harmless *Phytophthora* species can be introduced into the root system of *Protea cynaroides* to increase the resistance of the plant to *P. cinnamomi*.

Alternatively the root bark powder could be applied to potting media to determine whether it offers protection to plants in the containers against *P. cinnamomi*.

The *P. cinnamomi* resisting compound could be isolated from the root bark of *Protea cynaroides* in sufficient quantity to allow it to be applied to other plant species. This would offer the opportunity to observe whether it confers resistance to *P. cinnamomi* in other treated plants.

There is the opportunity to synthetically reproduce the *P. cinnamomi*-inhibiting compound and apply it to a range of plant species to observe whether it confers resistance to *P. cinnamomi* to them.

New gene transfer technology could possibly be used to transfer the gene responsible for the production of this resistance-conferring compound to other species more susceptible to *P. cinnamomi*. At this stage the value of the crop does not justify this approach.

The effect of the different rates (0.01, 0.05 and 0.1 g) of bark powder of the *Protea cynaroides* infected with the undescribed, indigenous *Phytophthora* was to reduce the growth of *P. cinnamomi* significantly ( $P > 0.0001$ ).

The slopes of the lines in Figure 4, if treated as linear regressions up to day 6, were generally not significantly different except where  $>0.01\text{g}/1000\text{ mm}^2$  of infected *Protea cynaroides* was used. This would suggest infected plant material was showing a higher tolerance to *P. cinnamomi* infection.

The reduced growth of *P. cinnamomi* by day 3 indicates that the inhibiting chemical diffused out from the root bark powder very rapidly. Strongly coloured leachate was clearly visible surrounding each of the root bark powder samples (see Plate 15)

In future experimentation the agar containing the leachate could be analysed to determine the presence and concentration of a *P. cinnamomi* inhibiting compound. In the previous experiment (5.1.4.1) on the dish containing the 0.5 g root bark powder *P. cinnamomi* grew further from the wheat grain behind a crack in the agar (see Plate13). This opened up when the wheat grain was placed in the agar. It prevented the leachate from reaching the agar on the far side from the root bark powder further reinforcing the proposal that the leachate contains a *P. cinnamomi* inhibiting compound.

The ability of the leachate to diffuse out from the *Protea cynaroides* root bark suggests that resistance could be occurring at the surface of the root when initial contact is established with the *Phytophthora*. However, the compound may diffuse beyond the root to produce a zone of inhibition around the root. This could be the basis of future investigation into the resistance mechanism of *Protea cynaroides*.

# **Chapter 6**

CONCLUSION

## 6.1 THE EFFECT OF THE INTRODUCTION OF *Phytophthora cinnamomi* TO CUT FLOWER SPECIES HABITATS.

The cut flower crops grown commercially in New Zealand often originate from areas of South Africa and Australia have harsh, hot and arid climates and ancient weathered soils of low nutrient and moisture content. The colonisation of these marginal regions for plant growth has occurred as a result of the development of specialised adaptations by the Proteaceous and Rutaceous species involved.

An important consideration for a commercial grower, when deciding which species to establish on his/her property is the micro-climate of the habitat where a species naturally grows. A comparison between the micro-climate of the grower's site and the natural requirements of the species may help the grower decide whether modifications to the site are needed or whether alternatives should be considered. Some Proteaceous and Rutaceous species may grow well in conditions quite different to those found in the wild. However, such comparison may be critical when considering the range of diseases which can be encountered as the microclimate varies from that of the natural habitat of the species.

The natural habitats of many cut flower plants have often been free of the root rot diseases caused by *Phytophthora* species, in particular *Phytophthora cinnamomi*. However, through human activity in these regions, *P. cinnamomi*, has become relatively widespread and the many Proteaceous plants have exhibited tolerance levels which vary from highly susceptible (3 of 5 plants dead in 90 days) to tolerant (3 of 5 plants alive after 365 days) (Turnbull, 1991).

## 6.2 THE EFFECT OF *Phytophthora* ON CUT FLOWERS IN THE BAY OF PLENTY

On the commercial properties of the Bay of Plenty cut flower crops experience conditions which, during some periods of the year, are extremely conducive to fungal diseases. Root rot diseases of the type associated with the presence of *Phytophthora* species have often been reported by local growers in the region. These cases have usually been attributed to the pathogen *P. cinnamomi*. This is probably because the affected plants develop symptoms closely resembling those of *P. cinnamomi*. These symptoms have been featured and acknowledged in grower literature in Australia and South Africa as the major pathogen responsible for many plant deaths. Lupin baiting of the roots and the surrounding soil of plants exhibiting these symptoms often provides a positive recovery of a pathogenic organism.. However, at least twelve different common species of *Phytophthora* have been recovered using this method (Forsberg, 1993).

In New Zealand, a number of, undescribed, indigenous *Phytophthora* species exist. At least one of them is responsible for plant deaths in *Protea cynaroides* and no trials have been undertaken to determine the recovery rate of indigenous New Zealand species using the lupin baiting technique. Further investigation into the identification, taxonomy and pathological effects of the indigenous species could provide valuable information to aid 'protea' growers.

## 6.3 CHEMICAL CONTROL OF *Phytophthora cinnamomi*

The development of foliage sprays and soil drenches are considered essential for the control of *P. cinnamomi* where the high disease pressure

often experienced in autumn and spring following a combination of optimum soil temperatures and low soil moisture tension.

Under such conditions favouring the pathogen, chemical control has always been difficult to achieve (Marks and Smith, 1988, Turnbull, 1992). These results are in agreement with this study although some degree of delay of the symptoms of the disease occurred.

When Aliette® was applied to the three species trialled the optimum rate varied from 2000 ppm on *L. 'Wilson's Wonder* and 1000 ppm on *Boronia heterophylla* and *B. megastigma* providing the best protection. The variation in optimum rate may be a reflection of species ability to absorb, translocate and assimilate the chemical.

Foschek® at 2000 ppm provided useful protection to both species of *Boronia* but not to the *Leucadendron*. The results with Foschek® at 2000 ppm on *B. heterophylla*, with only one plant death after 31 days of high disease pressure, warrant further investigation into the use of this chemical by field trials on this important economic cut flower crop.

The combination product, Foschek®/C 408, did not provide additional protection to the three species trialled under conditions of high disease pressure.

All three chemicals were applied as a soil drench and when mixed with CMA were shown to have fungicidal properties by preventing the growth of *Phytophthora cinnamomi*. As Aliette® is normally recommended and applied as a foliage spray commercially, the products Foschek® and Foschek®/C 408 may also show improved performance when applied as a foliage spray.

#### 6.4 *Phytophthora cinnamomi*, A RECENT PATHOGEN OF THE PROTEACEAE

*Phytophthora cinnamomi* grows at different rates on the dried root bark powder of a number of Proteaceous species ranging from enhanced growth on the root bark of *Knightia excelsa* to totally inhibited growth on the root bark of *Protea cynaroides* suggesting that few, if any of the Proteaceae originated in habitats where *P. cinnamomi* was indigenous. Only *Protea cynaroides* exhibited strong tolerance of *P. cinnamomi* which agrees with Turnbull (1991) and suggests that the inhibition of the growth of *P. cinnamomi* may be a secondary and coincidental function of the compound responsible.

#### 6.5 FUTURE DIRECTIONS FOR *Phytophthora cinnamomi* RESEARCH

The compound from the root bark of *Protea cynaroides* which inhibits the growth of *P. cinnamomi* is produced at higher concentrations by plants which are infected with other, less lethal species of *Phytophthora*. This offers the opportunity for further research into the mechanism of enhanced production of the compound and whether increased tolerance of plants can be stimulated by infection with 'mild' strains of *Phytophthora*.

*Phytophthora cinnamomi* remains a major root rot disease in plants throughout the world but this study has provided further information about the disease, potential chemicals for its control and how it affects some of the plants it is known to attack. Further possibilities for research into the disease have been suggested and will, hopefully, be able to be examined in the future.

## ABBREVIATIONS

cm	centimetre(s)
°C	degrees Celsius
g	gramme(s)
h	hour(s)
kg	kilogramme(s)
l	litre(s)
m	metre(s)
ml	millilitre(s)
mm	millimetre(s)
pers comm	personal communication
ppm	parts per million

## PLANTS USED IN THIS STUDY

*Boronia heterophylla*

*Boronia megastigma*

*Knightia excelsa*

*Leucadendron 'Sylvan Red'*

*Leucadendron 'Wilson's Wonder'*

*Macadamia 'Beaumont'*

*Protea cynaroides*

*Weinmannia racemosa 'Kiwi Red'*

**All the data from the experiments on these plants is  
available on disk from the author.**

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