PHYTOPHTHORA CROWN ROT OF ALMOND AND CHERRY TREES: PATHOGENS, ROOTSTOCK AND SCION SUSCEPTIBLITY AND CONTROL

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SUMMARY

Soils from naturally infested almond and cherry orchards were sampled over two years to determine the seasonal variation in the activity of *Phytophthora*. Root and soil samples were also collected from around declining almond and cherry trees to determine the incidence of *Phytophthora* species in South Australian orchards.

P. cambivora, *P. cryptogea*, *P. megasperma* and *P. syringae* were the main species isolated from the regularly sampled orchards, whereas *P. cactorum*, *P. citricola*, *P. citrophthora* and *P. nicotianae* var *parasitica* were isolated from other orchards. *P. citricola* and *P. syringae* were isolated from cherry and *P. nicotianae* var *parasitica* and *P. syringae* from almonds for the first time in South Australia.

Phytophthora were not detected in new almond plantings in the Riverland of South Australia and the likely reasons for this are discussed.

P. cambivora was isolated from almond orchards in all seasons but showed peak activity in spring - early summer and low activity at other times. The incidence of *P. cambivora* was low in the cherry orchard under study, but showed activity in late summer/early autumn. Both the A^1 and A^2 mating types of *P. cambivora* were isolated from almond and cherry orchards, but most of the isolates were of the A^1 type.

Phytophthora was difficult to isolate from infected tissue and although the reasons for this remain unclear, the fungus was readily recovered from the margin of expanding cankers and there was no seasonal effect on the recovery.

The pathogenicity of the main *Phytophthora* species and of different isolates of some of these species was tested on almond and cherry using excised twigs, stem and shoot inoculations as well as root inoculations. These studies confirmed *P. cambivora* and *P. syringae* as virulent pathogens of almond and cherry. The A^1 isolate of *P. cambivora* was most virulent to almonds whereas both mating types were pathogenic to cherry, although the A^2 isolate was more virulent than the A^1 isolate on Mazzard but not on Mahaleb root stocks. *P. megasperma* although isolated frequently from orchards, was not a serious pathogen of almonds or cherries. Of the other species tested, some isolates of *P. citricola*, *P. citrophthora*, *P. cryptogea* and *P. nicotianae* var *parasitica* caused cankers on inoculated almond stems. Although there was a trend for isolates of *P. cambivora* to be most pathogenic to the original host, the virulence of apple, cherry and pear isolates to almond as well as almond and cherry isolates to cherry shows that *P. cambivora* has a wide host range and that isolates are not host specific.

Root inoculation studies showed that almond roots were attacked by several species of *Phytophthora*; mainly *P. cambivora* and to a lesser extent *P. cryptogea* caused crown cankers.

Since the use of resistant rootstocks is the preferred means of controlling *Phytophthora* - related soil - borne diseases, a number of experiments were conducted to evaluate the susceptibility of almond and cherry rootstocks to *P. cambivora*. Almond seedlings of 'Mission' and 'Chellaston' which are commonly used as almond rootstocks, were susceptible to *P. cambivora* whereas 'Nemaguard' rootstocks were resistant. Cherry rootstocks were not resistant to *P. cambivora* but Mahaleb rootstocks were more susceptible than were Mazzard.

Inoculating grafted rootstocks above and below the scion showed that the inherent susceptibility of either almond or cherry rootstocks did not change after they had been grafted to a more susceptible cultivar.

Other factors that could have affected the susceptibility of the host to *Phytophthora* were examined. These included seasonal variation, type of host tissue, waterlogging, growth hormones (gibberellins) and soil amendments such as lime and urea. Of these only the stage of plant growth had a major effect on the development of disease. For example in almonds, *P. cambivora* colonised twigs most rapidly in spring whereas in cherries this occurred in late summer. On the other hand, *P. syringae* colonised almonds in autumn and winter but not in spring. Although flooding severely inhibited plant growth in almond and cherry seedlings, flooding did not increase their susceptibility to attack by *P. cambivora*.

Fungicides, particularly metalaxyl and phosphorous acid, were evaluated in vitro, in potted plants and in field experiments for the control of *P. cambivora* in almond and cherry. Both fungicides inhibited mycelial growth in vitro, but the production of sporangia was most sensitive to metalaxyl with inhibition almost complete at 1 mg a.i./l.

Metalaxyl and phosphorous acid drenches inhibited the development of cankers on inoculated stems, but phosphorous acid was phytotoxic to almonds causing marginal leaf necrosis and elongated brown necrotic lesions on young shoots.

Dipping severely infected almond seedlings in either metalaxyl, fosetyl-Al or phosphorous acid before planting or applying the fungicides after planting did not prevent plant deaths or improve plant growth compared with uninfected plants.

In naturally infested orchards metalaxyl broadcast once on to the soil surface immediately after planting did not prevent infection of *P. cambivora* in almond seedlings. On the other hand, regular broadcast applications of metalaxyl controlled *P. cambivora* in cherry seedlings.

In mature almond orchards, metalaxyl applied in a shallow trench around the trunk protected trees from natural infection but many trees declining at the time of treatment were not rehabilitated or saved from further decline. The inhibition of cankers on shoots inoculated with *P. cambivora* showed that metalaxyl or a breakdown product was taken up and translocated for at least 96 days after applying metalaxyl to soil around almond trees.

In artificially inoculated mature almond and cherry trees, established infections were not controlled with soil applications of metalaxyl which could have been because of lack of uptake of metalaxyl. Levels of metalaxyl in treated soils were shown by chemical analysis and bioassays to be concentrated in the 0 to 5 cm soil depth and to persist at high levels in some soils for up to 34 to 50 weeks after applications.

Solarization, the technique of heating soil by covering the soil with transparent plastic sheeting, was evaluated as a means of controlling *P. cambivora*. Laboratory experiments showed that brief exposure to 45°C was lethal to mycelial growth and inhibitory to the production of sporangia of *P. cambivora* whereas several periods of exposure at 35 and 40°C were necessary to inhibit the fungus to the same degree. In field experiments soil temperatures greater than 40°C were rarely recorded at a depth of 15 cm in solarised soil, yet the treatment controlled *P. cambivora* in cherries for more than 12 months. Solarization was not effective in almond orchards and the possible reasons for this are discussed.

Solarization had no effect on the development of disease in plants infected at the time of treatment but growth of plants in solarized areas was significantly greater than that of plants in untreated or metalaxyl treated areas.

Overall, these studies have shown that *P. cambivora* is a serious pathogen of almond and cherry in South Australia. In almonds the disease can be controlled by using 'Nemaguard' rootstocks, and in cherries the problem decreased by using Mazzard rootstocks. In infested orchards the spread of the disease can be controlled with metalaxyl applied in a shallow trench around the tree trunk and in replant situations solarization may be useful in establishing young trees.

STATEMENT

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

I give consent to this copy of my thesis, when deposited in the university library, being available for loan and photocopying.

T.J. Wicks

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CHAPTER 1

GENERAL INTRODUCTION

(a) The South Australian Almond and Cherry Industries.

In South Australia the almond and cherry industries are small but valuable horticultural industries. Over 80% of the Australian almond crop is produced in South Australia and was valued in 1985/86 at $5.9 million (Australian Bureau of Statistics). Almonds are grown mainly on the Adelaide plains within 60 km north and south of Adelaide with some young plantings less than 10 years old near the River Murray 30 km east of Renmark.

The cherry industry valued at $1.7 million in 1985/86 (Australian Bureau of Statistics), supplies local and interstate markets and in recent years has expanded into the South East Asian market. Cherries are grown predominantly in the Adelaide Hills 30 km east of Adelaide.

Tree deaths have contributed to the decline of plantings in the main almond and cherry growing areas of South Australia. In the Willunga area, 60 km south of Adelaide dead and declining almond trees are common and the cause has been attributed to increased susceptibility to *Pseudomonas syringae* Van Hall caused by waterlogging (Baker and Gathercole 1977). While the role of *P. syringae* in death of almond trees has not been investigated, recent studies have shown that *Phytophthora* spp. are widespread in the area and are associated with dead and declining trees (Wicks and Lee 1986). In severely affected almond orchards up to 17% of mature trees were either dead, or in a serious state of decline.

In cherry orchards tree losses are common and on many orchards young trees fail to establish on replant sites. While diseases such as those caused by Prunus Nerotic Yellows virus, *Pseudomonas syringae* and *Armillaria luteobubalina* are present in some orchards and contribute to tree decline, recent surveys (Bumbieris et al, 1982) have indicated that *Phytophthora* spp. are also involved. Bumbieris *et al.*, (1982) reported *Phytophthora* spp. associated with dead and declining cherry trees in 21% of the 87 orchards sampled. The extent of tree losses in cherry orchards has not been accurately determined, but limited surveys in eight orchards showed that losses of between 1 to 5% were common (T. Wicks, unpublished).

In both almond and cherry orchards even a low incidence of dead trees results in an economic loss to growers. These include losses attributed to the establishment cost and the trees' annual production as well as losses due to chemicals, fertilisers and irrigation water which are wasted when they are applied to dead trees or vacant sites. Further losses occur when replanting is attempted and trees fail to establish.

(b) The Disease and Pathogens.

The symptoms developed by almond (Wicks and Lee 1986) and cherry (Mircetich and Matheron 1976) trees infected with *Phytophthora* spp have been described and are similar for both crops. Infected trees either fail to grow out in spring or they produce small chlorotic leaves in spring and weak growth late in the season. Some trees suddenly collapse and die during the first period of hot weather in late spring or early summer Plate 1 (a) and (c). Trunk cankers that originate just below ground level are found on infected trees Plate 1 (b) and (d). These

Plate 1 Symptoms of *Phytophthora* crown rot on naturally (a to d) and artifically (e and f) infected almond and cherry trees.

(a) Almond trees collapsed in mid summer.

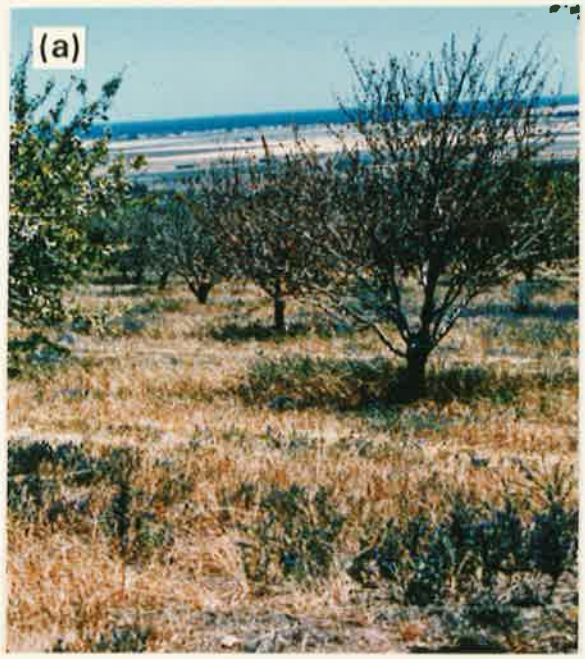
(b) Crown canker on a three year old almond tree.

(c) Mature cherry tree collapsed early summer.

(d) Crown canker extending up the trunk of a declining cherry tree.

(e) Crown canker on Mazzard cherry inoculated with *P. cambivora*.

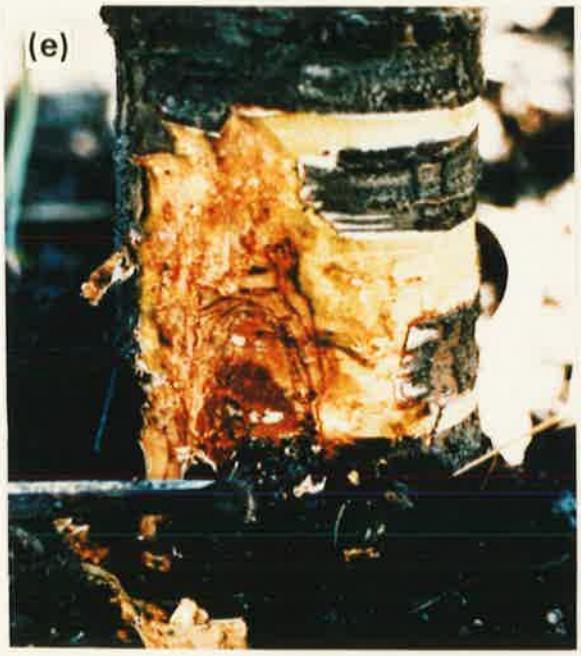
(f) Gum exudation from an almond branch inoculated with *P. cambivora*.


(a)


(b)


(c)


(d)


(e)


(f)

cankers frequently extend into the main scaffold branches and in both almond and cherry are associated with gum exudations Plate 1 (f). In the absence of gum exudate, almond trunk cankers are difficult to detect unless the bark is removed. On cherries, the extent of trunk cankers is defined by the area of sunken dead and discoloured bark. In rapidly expanding cankers a distinct margin between healthy and infected tissue usually appears in both almonds and cherry as banded stripes beneath the bark (Plate 1 (e).

Several *Phytophthora* species have been reported from almond and cherry growing areas throughout the world. In California several species of *Phytophthora* have been implicated in root and crown rots of almond and so far *P. cambivora* (Petri) Buisman *P. drechsleri* Tucker var drechsleri, *P. megasperma* Drechsler and *P. syringae* (Klebahn) Klebahn have been identified (Miretich et al 1974, Moller and Teviotdale 1978). In addition to these species Kouyes and Chitzanidis (1968) isolated *P. cactorum* (Lebert and Cohn) Schroeter, *P. citricola* Sawada and *P. citrophthora* (Smith and Smith) Leonian from almond in Greece. Fatemi (1980) has also reported that *P. cryptogea* Pethybridge and Laffery and an unidentified species of *Phytophthora* are associated with declining almond trees in Iran.

In Australia *P. cambivora*, *P. citrophthora*, *P. cryptogea* and *P. megasperma* have also been associated with almond trees affected with crown rot (Wicks and Lee 1986).

Although a number of *Phytophthora* species have been associated with declining almond trees few of these have been identified as pathogens of almonds. *P. cambivora* was the most pathogenic of the

Phytophthora species isolated from South Australian orchards. Almond seedlings grown in soil artificially inoculated with *P. cambivora* produced crown cankers similar to those in naturally infected trees (Wicks and Lee 1986) whereas no crown rot developed in plants grown in soil inoculated with either *P. citrophthora*, *P. cryptogea* or *P. megasperma*. Pathogenicity tests conducted by Fatemi (1980) with *P. cryptogea* and an unidentified species of *Phytophthora* showed that both species caused root rotting and decline in young almond trees but the development of crown cankers was not reported. Inoculation studies by Bostock and Doster (1985), and Mircetich *et al.*, (1974) showed *Phytophthora syringae* to be pathogenic to almonds. In the studies of Bostock and Doster (1985), branch segments inoculated with *P. cactorum* and *P. citricola* produced rapidly expanding cankers at 12°C to 20°C but not at 2°C where *Phytophthora syringae* caused disease.

The *Phytophthora* species recovered from Californian cherry trees have included *P. cambivora*, *P. megasperma* and *P. drechsleri* (Mircetich and Matheron 1976) and an unidentified *Phytophthora* sp. (Wilcox and Mircetich 1985a). *P. megasperma* and an unidentified *Phytophthora* sp. have been isolated from New York State (Hayes and Aldwinkle 1983) as well as *P. cactorum*, *P. cambivora* and *P. cryptogea* (Wilcox et al., 1985).

In South Australia *P. cambivora* was isolated frequently from declining cherry trees whereas *P. cryptogea*, *P. megasperma* and an unidentified *Phytophthora* sp. (Bumbieris et al., 1982), were isolated infrequently.

The pathogenicity of *Phytophthora* spp. to cherry trees was first reported in detail by Mircetich and Matheron (1976) who showed *P. cambivora* and *P. megasperma* induced severe root and crown rot on cherry whereas *P. cryptogea* caused only feeder root necrosis.

These workers also showed that a synergistic interaction such as a more rapid rate of disease development, higher incidence of trunk canker and greater mortality of Mahaleb rootstocks occurred in soil infested with both *P. cambivora* and *P. drechsleri* than in soil infested with either *P. cambivora* or *P. drechsleri* alone.

Studies by Wilcox and Mircetich (1985a) also showed *P. cambivora*, *P. cryptogea* and *P. megasperma* were serious pathogens of cherry as well as walnut isolates of *P. cinnamomi* Rands and *P. citricola* and isolates of *P. megasperma* from unrelated hosts (Wilcox and Mircetich, 1982). Although few details are given, McIntosh (1964) reported *P. cactorum*, *P. cambivora* and *P. cryptogea* pathogenic to cherry seedlings, and *P. drechsleri* and *P. megasperma* var *sojae* Hildebrand less virulent.

(c) Survival and spread of the pathogens.

The *Phytophthora* species attacking almond and cherry are soil borne hence such long term survival of these fungi is likely to be as resistant spores free in the soil or in colonised root or trunk tissue (Weste 1983). Mycelium, sporangia and zoospores of most *Phytophthora* are relatively short lived in soil as they are lysed by bacteria and are sensitive to low soil moisture. Thick - walled

chlamydpores and oospores are the propagules most likley to survive in soil although with many *Phytophthora* species causing disease of fruit trees these propagules have not been observed in soil or host roots or if they have their role in the development of disease is uncertain.

The survival of *Phytophthora* in decaying host roots such as demonstrated with *P. cinnamomi* (Old et al., 1984, MacKay et al., 1985, Shea *et al.*, 1980) may be important in the survival of propagules throughout the summer in Australian orchards.

Another means of survival may be alternative hosts such as weeds that are common in most orchards. While there is little information on this aspect, the work of Cother (1975) showed that *P. drechsleri* infected and formed chlamydospores in a number of weed species and Weste (1975) showed that *P. cinnamomi* infected wheat seedlings. Thus both studies indicate that alternative hosts may be important in the survival of *Phytophthora* in orchards.

Apart from introducing *Phytophthora* on infested nursery stock (Julius *et al.*, 1978 and Jeffers 1985) soil borne progagules of *Phytophthora* can be spread from infested areas by soil adhering to agricultural equipment, shoes, tyres or animals (Kliejunas and Ko 1976, Weste 1983). Surface-run off water carrying infested root pieces or soil particles or, more probably, zoospores, disseminate *Phytophthora* down slopes or in drainage channels and is a major factor in the spread of *Phytophthora* induced diseases in orchards (Zentmyer 1980, Wicks and Lee 1986). Furthermore, water used for irrigating orchards from dams constructed to store run off water may

also serve as an important source of inoculum. McIntosh (1966) detected the fruit tree pathogens *P. cactorum*, *P. cambivora* and *P. citricola* in water from dams and ponds used to irrigate orchards in British Columbia and suggested that repeated irrigations with the contaminated water could result in the rootstocks becoming infected. Further evidence for the irrigation water to be the original source of inoculum in British Columbia was the widespread occurrence of *P. cactorum* in only the irrigated orchards of the region. A similar range of *Phytophthora* species was detected in surface waters in orchard districts of Idaho (Helton *et al.*, 1984) and from irrigation canals in Victoria (Taylor 1977) all of which indicate that contaminated water may be an underrated source of inoculum.

(d) Predisposing factors.

The development of *Phytophthora* induced root and crown rots of tree crops is frequently associated with excess soil moisture usually in poorly drained soils (Mircetich and Matheron 1976, Zentmyer 1980). Most of the data on a number of *Phytophthora* species show that flooding and associated cyclic changes in soil moisture are conducive to the formation of sporangia, zoospore release, transmission and germination although there is a complex interaction between these aspects and temperature, soil aeration and *Phytophthora* species (Duniway 1983).

Such relationships are illustrated by the work of Wilcox and Mircetich (1985b) who showed that *P. cambivora* formed few or no sporangia on colonised cherry leaf disks after 3 days at a soil water matric potential of -25mb whereas they formed within 30-60 minutes of flooding and released zoospores 3 to 6 hours later. In addition *P. cambivora*

sporangia that were produced in flooded soils continued to develop in the soil that was drained and too dry for them to be initiated. By way of contrast *P. cryptogea* formed numerous sporangia within 3 days at a soil water matric potential of -25mb but released zoospores only after flooding.

Flooding, in addition to promoting the discharge and dispersal of zoospores, also depletes oxygen levels in soil resulting in changes to the host physiology (Rowe and Beardsell 1973). In some cases flooding increases the susceptibility to infection as shown in alfalfa (Kuan and Erwin 1980) and rhododendron (Blaker and MacDonald 1981).

This was not the case with *Phytophthora* root rot of Fraser fir seedlings where flooding increased mortality by promoting the production and dispersal of inoculum rather than increasing the susceptibility of the host.

Other factors such as salinity stress also predisposed plants to infection as shown with Chrysanthemum root rot caused by *P. cryptogea* (MacDonald 1982). The extent of the colonisation of host tissue by *Phytophthora* is also influenced by many factors some of which are related to the stage of plant growth (Sewell and Wilson 1973a, Jeffers and Alwinkle 1986) while others are associated with moisture content of the host (Tippett and Hill 1983).

The objects of the studies reported in this thesis were to investigate practical methods to control *Phytophthora* crown rot of almond and cherry. The first aspect studied was to determine the incidence of various *Phytophthora* species in South Australian orchards and also

to determine if these fungi exhibited any marked seasonal variation in soil activity. The latter being important in relation to the timing of fungicide applications.

Once species were identified, the pathogenicity of various isolates was tested so the most pathogenic isolates could be used to evaluate the susceptibility of various almond and cherry rootstocks to *Phytophthora*. The effect of factors such as seasonal variation in susceptibility, water logging, growth hormones and soil amendments such as lime and urea were also investigated since they could have important implications in the management of disease.

A major part of the investigations involved the evaluation of fungicides as these were likely to be the most convenient and economic means of controlling the disease in established orchards. These studies were concerned mainly with metalaxyl and phosphorous acid.

Solarization was also evaluated as a means of controlling crown rot as the technique of heating soil seemed suited to the control of *Phytophthora* induced diseases.

CHAPTER 2.

GENERAL EXPERIMENTATION AND RESULTS

This section describes preliminary experiments conducted to evaluate techniques as well as materials and methods that were frequently used throughout these studies.

(a) Sensitivity of Pear Baiting

A pear baiting technique (McIntosh, 1964) utilising ripening c v. Packham pears was used to recover species of *Phytophthora* from soil. Although other baiting techniques using juvenile apple seedlings or apple cotyledons are reported more sensitive than fruit in recovering *Phytophthora*, (Harris and Bielenin, 1986; Jeffers and Aldwinkle 1983), pears were used in these studies as they were convenient to use considering the large numbers of soil samples that were tested. In addition a cheap and reliable supply of pears was available throughout the investigation.

To check the sensitivity of the pear baiting technique, soil and roots from artificially inoculated almond seedlings as well as soil inoculated with 1g vermiculite infested with *Phytophthora* and naturally infested cherry orchard soil were mixed separately with various dilutions of pasteurised soil. Dilutions of up to 1/64 (Tsao, 1960) were prepared for each of the three main samples. Three sub samples each of 200 ml were taken from each soil dilution, placed in plastic containers, baited with pears and flooded with tap water. Three samples of pasteurised soil were also baited with pears. All samples were incubated on a laboratory bench for two weeks where temperatures ranged from 10° to 27°C. Pears that developed lesions were swabbed with alcohol and flamed. Pieces approximately 2 to 3 mm^2 were excised from the margin of the lesion and placed on corn meal agar (CMA).

Plates were incubated in the dark at 22°C for at least two weeks during which they were examined microscopically for the presence of *Phytophthora*.

Pear baiting was an effective technique as it recovered *Phytophthora* from all dilutions of both samples of artificially inoculated soil and from undiluted, 1/2 and 1/4 dilutions of naturally infected soil (Table 2.1). No *Phytophthora* was recovered from the peat/sand mixture used to dilute the infected soil.

(b) Soil Sampling

To determine the most appropriate depth to sample, soil was collected from depths of 0 to 5 cm, 10 to 15 cm and 20 to 25 cm at different sites from three almond and one cherry orchard previously shown to be naturally infested with *Phytophthora*. This was achieved by digging a 40 cm wide trench within 30 cm of the base of infected trees and collecting soil from the side of the trench closest to the tree. Care was taken to prevent soil from the upper levels contaminating lower levels. Soil from different depths and sites was collected in plastic bags, returned to the laboratory and baited with pears.

Phytophthora was recovered from most sites in the three almond orchards sampled and three out of four sites of the cherry orchard (Table 2.2). In both the almond and cherry orchards *Phytophthora* was recovered most frequently from 10 to 15 and 20 to 25 cm depths. As a result of these studies most soil samples were collected from depths of at least 10 cm.

(c) Identification

Phytophthora species isolated from soil and cankers were transferred to CMA, V8 agar and lima bean agar (LBA) and incubated at 22°C unless otherwise

Table 2.1.

Sensitivity of pear baiting for recovering *Phytophthora* from soil.

Origin of *Phytophthora* inoculum	Recovery of *Phytophthora**						
	Soil dilution**						
	0	1/2	1/4	1/8	1/16	1/32	1/64
Soil and roots from	+	+	+	+	+	+	+
artificially inoculated	+	+	+	+	+	-	+
almond seedlings	+	+	+	+	+	+	+
Soil inoculated with 1%	+	+	+	+	+	+	+
vermiculite infested	+	+	+	-	+	+	-
with *Phytophthora*	+	+	+	-	-	+	-
Naturally infested	+	+	+	-	-	-	-
cherry soil	+	+	+	-	-	-	-
	-	-	-	-	-	-	-

*Three replicates per sample.

*Diluent consisted of a steam pasteurised mixture of 75% peat and 25% sand.

+*Phytophthora* recovered

-*Phytophthora* not recovered.

Table 2.2

Recovery of *Phytophthora* from three depths of soil in naturally infested orchards.

Depth (cm)	Almond orchards									
	A		B		C					
	Sites									
	a	b	a	b	a	b	c	d		
0 to 5	+	-	-	+	-	-	-	-		
10 to 15	-	+	-	+	+	+	+	-		
20 to 25	-	+	-	-	+	+	+	+		

Depth (cm)	Cherry orchard			
	Site			
	a	b	c	d
0 to 5	-	-	-	-
10 to 15	+	+	+	-
20 to 25	+	-	+	-

A,B,C, = different orchards.
a,b,c,d = different sites within an orchard.

+*Phytophthora* recovered

- No *Phytophthora* recovered.

stated. Sporangia were produced on agar plugs removed from the margins of five to seven day old colonies grown on LBA. The plugs were placed in plastic petri dishes and flooded with either distilled water or a soil extract prepared from almond or cherry soil. The flooded mycelial plugs were incubated on the laboratory bench in diffuse daylight for two to three days before they were examined for the production of sporangia.

Cardinal temperatures for mycelial growth were determined on CMA using four replicate plates for each treatment. Mating types and the morphology of sexual reproduction structures were studied on cleared V8 media supplemented with β-sitosterol (Mircetich and Matheron 1976).

The mating types of unknown isolates was determined by pairing it with known mating types. This was done by placing a 4 mm diameter disk of the unknown isolate in the centre of a plate and placing equidistant from this, three disks of either known A^1 or A^2 cultures. Each combination of mating types was repeated once. All plates were sealed with parafilm, wrapped in aluminium foil to exclude light and incubated for two weeks at 22°C. After incubation the plates were inverted and examined microscopically at the approximate area of junction of the mating types.

Where sexual structures were obvious small pieces of agar were removed and mounted in lactophenol cotton blue before the dimensions on at least 50 randomly chosen sexual structures were measured.

The isolates of *Phytophthora* were identified from the descriptions of Waterhouse (1970) and Newhook *et al* (1978), and Ribeiro (1978).

Table 2.3: Mating patterns of some *Phytophthora* isolates recovered from almond and cherry orchards

Unknown Species		Known species																	
		P. cambivora						*P. cinnamani*			*P. cryptogea*					*P. dreschleri*			
Isolate no.	Associated host	A1			A2			A1	A2		A1			A2			A1	A2	
		[5	79	80]	[6	9	130]	[96]	[136	157]	[137	140	142]	[4	8]		[144]	[143]	
87	Apple	-	-	-	>*	>*	>*	-	.	.	-	-	-	*	*		_	*	
101	Cherry	-	-	-	>*	>*	>*	-	-	<*	-	-	-	-	<.		-	*	
108	Almond	-	-	-	>*	>*	>*	-	<*	<*	-	-	-	.	.		-	.	
190	Almond	-	-	-	>*	>*	>*	-	-	.	-	-	-	-	.		-	.	
192	Almond	-	-	-	>*	>*	>*	-	-	.	-	-	-	.	-		-	<.	
195	Almond	-	-	-	>*	>*	>*	-	.	.	-	-	-	<.	-		-	-	
204	Almond	-	-	-	>*	>*	>*	-	-	.	-	-	-	-	<.		-	<.	

>* = abundant bullate oogonia

<* = few bullate oogonia

* = sparse bullate oogonia

. = non bullate oogonia

<. = sparse non bullate oogonia

- = no oogonia detected

Isolates recovered from almond trees and soil were *P. cambivora* (Petri) Buisman, *P. citrophthora* (R.E. Smith and E.H. Smith) Leonian, *P. cryptogea* Pethybridge and Lafferty., *P. megasperma* Drechsler var. *megasperma*, *P. nicotianae* (Breda de Haan) var. *parasitica* (Dastur) Waterhouse and *P. syringae* (Klebahn) Klebahn. Those from cherry were identified as *P. cactorum* (Lebert and Cohn) Schroeter, *P. cambivora*, *P. citricola* Sawada, *P. cryptogea*, *P. megasperma* var. *megasperma* and *P. syringae*.

Of the 60 *P. cambivora* isolates checked for mating type, all were of the A^1 type since these formed numerous large bullate two celled amphigynous oogonia only when crossed with known A^2 mating types of the same species. Typical mating patterns of some of these isolates are shown in Table 2.3. All seven isolates of *P. cryptogea* were of the A^2 mating type as oogonia developed when these were mated with known A^1 isolates of *P. cambivora* *P. cryptogea* or *P. dreschleri*.

(d) *Phytophthora* isolates

The *Phytophthora* isolates used in these studies are shown in Table 2.4 and include local isolates recovered from soil and naturally infected plants as well as isolates received from colleagues interstate and overseas.

(e) Soil medium

Plants were grown in 10 cm diameter, x 20 cm long, black plastic bags containing a mixture of 75% peat and 25% sand that had previously been treated for 35 minutes with a mixture of steam and air. (Baker 1972).

Table 2.4

List of *Phytophthora* isolates

Species	Mating Type	Culture* No.	Area of origin	Host association
P. cactorum		P58	Lenswood, South Australia (S.A.)	Apple
P. cambivora	A_1	P5	Willunga, S.A.	Almond
		P59	Perth, W.A.	Apple
		P67	Willunga, S.A.	Almond
		P79	Lenswood, S.A.	Apple
		P80	Montacute, S.A.	Cherry
		P87	Lenswood, S.A.	Apple
		P89	Perth, W.A.	Pear
		P99	Basket Range, S.A.	Cherry
		P100	Willunga, S.A.	Almond
		P101	Lenswood, S.A.	Cherry
		P104	Willunga, S.A.	Almond
		P108	Willunga, S.A.	Almond
		P116	Lenswood, S.A.	Cherry
		P117	Lenswood, S.A.	Apple
		P131	New York, U.S.A.	Cherry
		P171	Willunga, S.A.	Almond
		P188	Willunga, S.A.	Almond
		P190	Willunga, S.A.	Almond
		P192	Willunga, S.A.	Almond
		P195	Willunga, S.A.	Almond
		P204	Willunga, S.A.	Almond
	A_2	P6	Willunga, S.A.	Almond
		P9	Norton Summit, S.A.	Cherry
		P130	New York, U.S.A.	Cherry
P. cinnamomi	A_1	P96	unknown, S.A.	unknown
	A_2	P136	unknown, S.A.	unknown
		P157	unknown, S.A.	unknown
P. citricola		P70	Angle Vale, S.A.	Almond
		P158	Waikerie, S.A.	Citrus
		P163	Blackwood, S.A..	Erica
		P176	Adelaide, S.A.	Citrus
P. citrophthora		P3	Willunga, S.A.	Almond
		P164	Loxton, S.A.	Citrus
P. cryptogea	A_1	P137	New York, U.S.A.	Apple
		P138	New York, U.S.A.	Apple
		P140	New York, U.S.A.	Cherry
		P142	Ireland	Tomato
	A_2	P4	Willunga, S.A.	Almond
		P8	Houghton, S.A.	Apple
		P68	Willunga, S.A.	Almond
		P69	Willunga, S.A.	Almond
		P169	Boundary Bend, Vic.	Almond

Species	Mating Type	Culture No.	Area of origin	Host association
P. cryptogea		P188	Willunga, S.A.	Almond
		P189	Lenswood, S.A.	Apple
		P199	Willunga, S.A.	Almond
		P205	Angle Vale, S.A.	Almond
P. dreschleri	A_1	P144	California, U.S.A.	Cherry
	A_2	P143	California, U.S.A.	unknown
P. megasperma		P7	Willunga, S.A.	Almond
		P10	Paracombe, S.A.	Pear
		P109	McLaren Flat, S.A.	Cherry
		P185	Angle Vale, S.A.	Almond
		P186	Angle Vale, S.A.	Almond
		P196	Angle Vale, S.A.	Almond
		P197	Willunga, S.A.	Almond
P. nicotianae var. *parasitica*		P73	Angle Vale, S.A.	Almond
		P200	Angle Vale, S.A.	Almond
P. syringae		P61	Willunga, S.A.	Almond
		P81	Angle Vale, S.A.	Almond
		P161	Basket Range, S.A.	Cherry

* South Australian Department of Agriculture.

(f) Soil inoculum and flooding

In preliminary experiments with almond seedlings, three types of inoculum were evaluated: (1) macerated mycelia of *Phytophthora* previously grown in a mixture of 200 ml of V8 juice and 800 ml of sterile distilled water; (2) autoclaved fodder beet seed (*Beta vulgaris* L. ssp. *Vulgaris*) inoculated with a *Phytophthora* isolate and incubated for 4 weeks at 22°C; and (3) autoclaved vermiculite, moistened with V8 juice, inoculated with a *Phytophthora* isolate and incubated for 4 weeks at 22°C. With each type of inoculum 50 ml was poured into 2.5 cm deep holes made in the soil within 2 cm either side of the stem.

Most inoculated plants were killed and there appeared to be no difference between the types of inoculum. Vermiculite inoculum was therefore used as the standard technique since it was the most convenient to prepare and handle.

In some experiments, instead of pouring the inoculum into holes, 100 to 150 ml of soil was scraped away from the surface of a pot and the inoculum poured into the area near the stem and the soil was replaced. Before each batch of inoculum was used at least 25 ml was plated on to CMA to check viability of the *Phytophthora* and for the presence of contaminants. Contaminated inoculum was discarded.

Between 7 to 14 days after inoculation, plants were flooded for 48 hours by enclosing the potted plants in a larger plastic bag (2 per bag) which was filled with water until 2 cm of water covered the soil surface. Bags were stapled or tied together to prevent water from escaping or the bag from falling over. After flooding the plants were removed from the large bags and allowed to drain. Flooding was repeated once a week for at least 3 weeks and

to avoid possible cross-contamination new large bags were used for each flooding period.

(g) Growing conditions

Plants were grown in either a shade house where temperatures ranged from a minimum of 2°C in the winter to a maximum of 27°C in the summer or in a growth cabinet where temperatures were maintained at 25°C with 12 hours of light and at 20°C for the 12 hours of darkness. In the shade house, plants were watered individually with an automatic dripper system that applied water for 3 minutes twice a day. This period of watering usually resulted in water draining from the bottom of each bag. The plants were placed on galvanised iron mesh benches 10 cm above a sloping cement floor. Contamination by inoculum splashing between plants was considered unlikely and therefore plants of various treatments were randomised along the benches.

Plants in the growth cabinet were placed in 15 cm deep plastic trays containing randomised groups of either inoculated or non inoculated plants. These were watered as required by flooding the trays with tap water until the bottom 2 cm of each bag was covered.

(h) Description of main field sites.

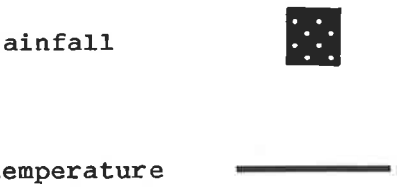
Soil sampling and field experiments were conducted in almond orchards in the Willunga area which is situated approximately 60 km south of Adelaide. Soils in this area are predominantly loamy sands which in many orchards overlies impermeable clay. Soil samples were also taken from almond orchards in the Angle Vale area 40 km North of Adelaide where soils are described as clay loams.

Some almond experiments were conducted in an abandoned almond orchard at Pooraka, 20 km north of Adelaide where the soils are loamy sands.

For cherries, intensive soil sampling as well as field experiments were conducted in cherry orchards at the Lenswood Research Centre which is in the main cherry growing area of the state 30 km east of Adelaide. Soils at this site are predominantly sandy loam. The physical properties of the top 10 cm of soils used in these studies are shown in table 2.5.

The climate in all these areas is mediterranean, as shown by the monthly average rainfall and the average daily mean temperature for each month recorded at Lenswood, Pooraka and Willunga (Figure 2.1).

Figure 2.1 The monthly average rainfall and average daily mean temperature recorded at Lenswood, Pooraka and Willunga from September 1983 to April 1986.



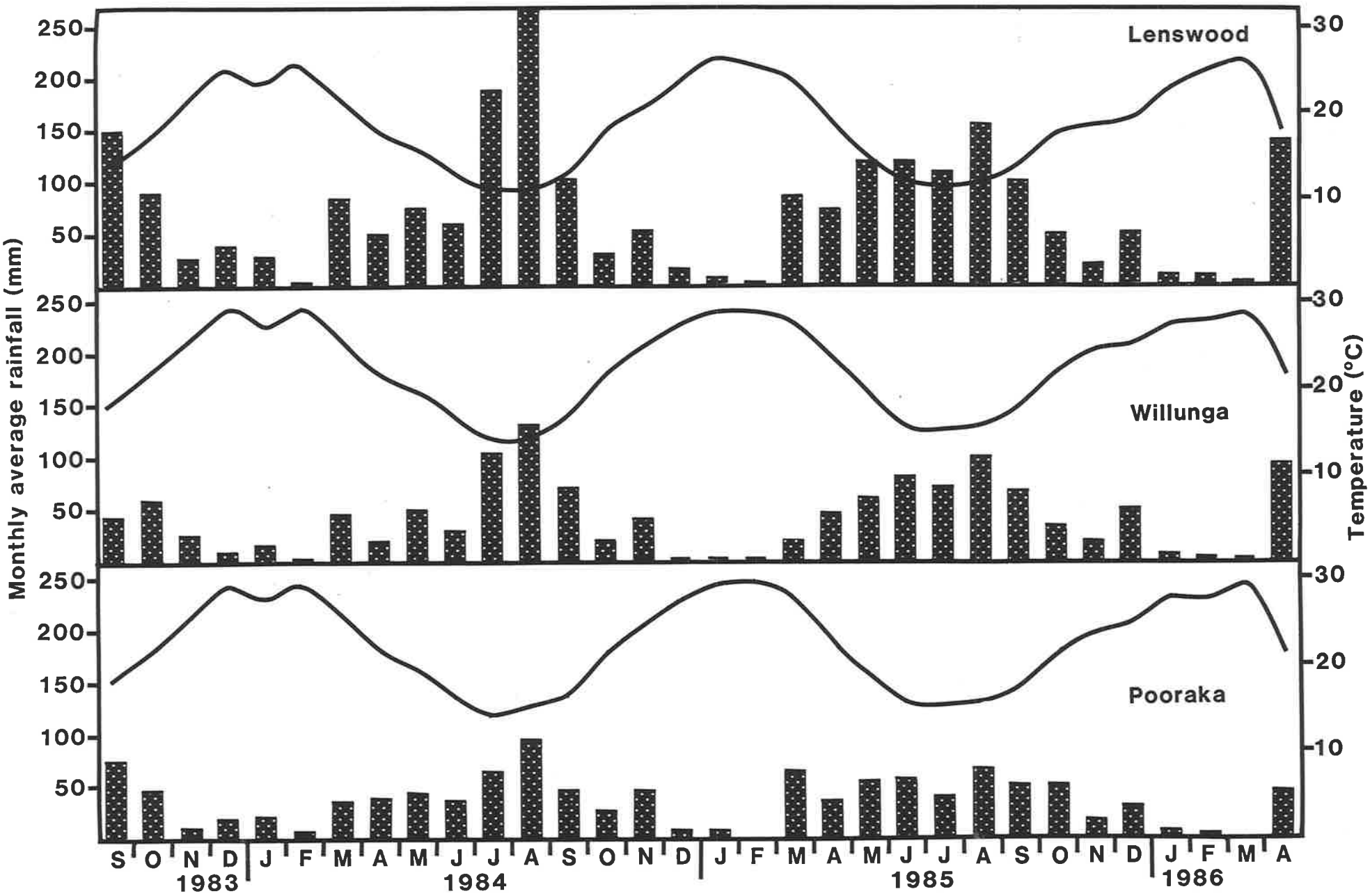

Lenswood
Willunga
Pooraka
Monthly average rainfall (mm)
Temperature (°C)
250
200
150
100
50
30
20
10
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A
1983
1984
1985
1986

Table 2.5

Physical properties of soils used in almond and cherry experiments

	Composition (%)			Soil pH	Total soluble salts
Locality	Sand	Silt	Clay		
Angle Vale	58	20	22	8.1	0.051
Willunga	57	28	15	6.7	0.112
Lenswood	85	8	7	4.7	0.105
Pooraka	91	3	6	6.6	0.023

CHAPTER 3.

RECOVERY AND SEASONAL VARIATION OF PHYTOPHTHORA SPECIES IN SOUTH AUSTRALIAN ALMOND AND CHERRY ORCHARDS

INTRODUCTION

Previous studies (Bumbieris *et al.*, 1982, Wicks and Lee, 1986) showed that *Phytophthora* species were widespread in South Australian almond and cherry orchards. The objective of those studies was to determine the incidence and distribution of *Phytophthora* species within orchard soils. They were not concerned with seasonal effects on the fungi although samples were collected mainly during spring and summer when conditions were considered most favourable for fungal growth and reproduction.

Intensive sampling of selected sites in naturally infected orchards was therefore undertaken over a two year period to determine any seasonal variation in the recovery of *Phytophthora*. The objective was to determine the most appropriate time to apply fungicides to the soil.

In addition, soil samples were collected from a number of almond and cherry orchards and nurseries not included in the surveys to determine if *Phytophthora* species other than those already recorded were present in South Australian orchards

Another aspect studied, was to determine whether there was a seasonal affect on the recovery of *Phytophthora* from infected tissue. Previous experience (Wicks and Lee 1986) had frequently shown that *Phytophthora* were difficult to recover from naturally and artificially inoculated plants.

Investigations were therefore carried out to determine whether *Phytophthora* were more readily recovered from infected tissue in one season rather than another.

MATERIALS AND METHODS

(a) Seasonal Activity - Soil

Naturally infested orchards were sampled to determine seasonal variation in recovery of *Phytophthora* spp. Soil samples were collected from five almond orchards in the Willunga area and one at Angle Vale as well as the cherry orchard at Lenswood.

At each site approximately 500 ml of soil from a depth of 10 to 15 cm was collected from within 30 cm from the base of a dead or declining tree or trees with obvious trunk cankers. At the Lenswood site all trees in the sampling area appeared healthy at the early sampling times and soils were collected at random within the tree rows. At all sites soils from individual trees were labelled and baited separately. This enabled trees to be identified and re-sampled if *Phytophthora* was recovered. Soils from each site were thoroughly mixed in a sealed plastic bag and within 48 hours of collection two sub samples each of approximately 200 ml were baited.

During the course of these studies many declining and dead almond and cherry trees from orchards that had not been sampled previously were bought to my attention by orchardists and farm advisers. Soil samples collected from the bases of these trees as well as soil samples from nurseries were also baited with pears. In some orchards, trees were found with cankers on the main trunk near soil level. Small bark and wood pieces, less than 0.5 cm diameter, were removed from the canker margin and immediately plated onto

$P_{10}VP^{+}$ agar. This selective medium consisted of pimaricin 10ppm, vancomycin 200 ppm pentachloronitro benzene 100 ppm and hymexazol 50 ppm incorporated into CMA (Tsao and Guy 1977).

(b) Seasonal activity - Plants

Previous experience (Wicks and Lee 1986) had frequently shown that *Phytophthora* species were difficult to recover from cankers on both naturally and artificially inoculated plants. Investigations were therefore carried out to determine if there was a seasonal influence on the recovery of *Phytophthora* from infected tissue.

In the first experiment twenty, two year old shoots of an unknown almond cultivar were wound inoculated in May (leaf fall) with an almond isolate of *P. cambivora* (P5). The rate of canker expansion was measured at approximately 14 day intervals for at least 60 days. At each time of assessment small wood chips of approximately 4 to 9 mm^2 were removed from the advancing margins of each canker and planted into $P_{10}VP^{+}$ selective media. After at least 7 days incubation at 25°C plates were examined microscopically to determine the proportion of chips yielding *Phytophthora*.

Two months later the experiment was repeated using shoots of the same cultivar and of similar age as those used in the May experiment. In some samples, wood chips were either washed in running tap water for 24 hours or ground with water in a mortar and pestle for 5 minutes before they were plated out. Similar experiments were conducted on mature Mazzard cherry trees at Lenswood where 3 to 5 cm diameter stems were inoculated with *P. cambivora* (P9) in August (winter) and October (spring).

RESULTS

(a) Seasonal activity

Over the two year period of sampling *Phytophthora* were isolated from 16 per cent of the 1 400 soil samples tested. The fluctuations in the frequency of recovery for the main species show that at some sampling times *Phytophthora* were recovered from more than 40 per cent of the soil samples (Fig 3.1). In the Angle Vale orchard, *P. megasperma* was recovered most frequently and was also detected at all times of sampling including those collected in the summer. *P. cambivora*, *P. cryptogea*, *P. syringae* and an unidentified *Phytophthora* sp. were also recovered from this orchard.

In the Willunga orchards *P. cambivora* was detected in all seasons but was isolated with the greatest frequency in spring. *P. megasperma* was also frequently isolated from these orchards but with the highest frequency in the winter period.

The range of *Phytophthora* species recovered from the cherry orchard was similar to those recovered from the Willunga almond orchards although the frequency of recovery was lower at Lenswood. *P. cambivora* was recovered from this orchard in all seasons and showed a trend of increasing frequency of recovery with time that is probably associated with the increase of crown rot in the cherries during this period (see Chapter 6).

Phytophthora species recovered from soil samples collected from other almond and cherry orchards at various times during this study are shown in Table 3.1. All sites yielded *Phytophthora* although most sites contained the fungus in only one sample.

Figure 3.1 Isolation of *Phytophthora* species from soil collected over various seasons in naturally infested almond and cherry orchards.

P. cambivora 

P. cryptogea 

P. megasperma 

P. syringae 

P. sp. 

Sampled but no *Phytophthora* isolated 

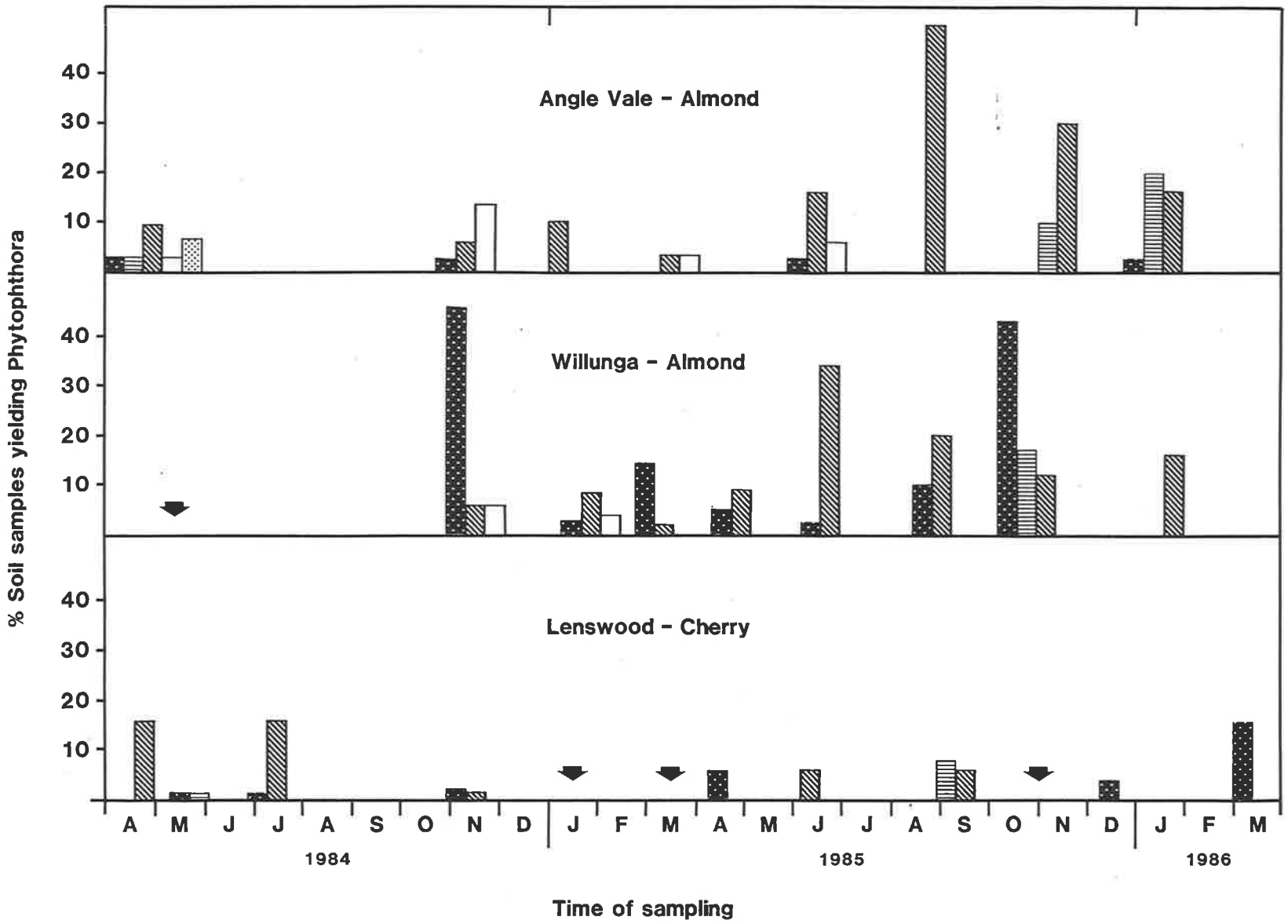

Angle Vale - Almond
Willunga - Almond
Lenswood - Cherry
% Soil samples yielding Phytophthora
40
30
20
10
A M J J A S O N D J F M A M J J A S O N D J F M
1984
1985
1986
Time of sampling

Table 3.1

Recovery of *Phytophthora* species from South Australian almond and cherry orchards.

Date	Locality	Associated host	No. samples	Species isolated	Number and (%) yielding Phytophthora	
June 1984	Adelaide Hills	Cherry	12	*P. cactorum*	1	(8)
				P. citricola	1	(8)
				P. megasperma	1	(8)
June 1984	Blewett Springs	Cherry	20	*P. megasperma*	1	(5)
Nov. 1984	Adelaide Hills	Cherry	60	*P. cambivora*	2	(3)
				P. megasperma	1	(2)
Nov. 1984	Angle Vale	Almond	20	*P. citrophthora*	1	(5)
Nov. 1984	Willunga	Almond	15	*P. syringae*	1	(6)
Jan. 1985	Adelaide Hills	Cherry	10	*P. megasperma*	1	(10)
June 1985	Adelaide Hills	Cherry	13	*P. cambivora*	1	(8)
				P. megasperma	1	(8)
Oct. 1985	Adelaide Hills	Cherry	10	*P. cambivora*	1	(10)
Oct. 1985	Willunga	Almond	50	*P. cryptogea*	1	(2)
				P. megasperma	1	(2)
Oct. 1985	Angle Vale	Almond	25	*P. megasperma*	1	(4)
				P. parasitica	1	(4)
Dec. 1985	Angle Vale	Almond	20	*P. megasperma*	4	(20)

(b) Seasonal activity - Plants

P. cambivora rapidly colonised almond shoots producing cankers up to 60 and 70 mm long, 18 days after inoculation in May and July respectively (Figure 3.2). Around 40 days after inoculation cankers ceased expanding rapidly and by 60 days the cankers had extended on average 70 mm in shoots inoculated in May and over 100 mm in shoots inoculated in July. At both times of inoculation *P. cambivora* was recovered most frequently during the early and most rapid stages of colonization. As the rate of canker expansion declined *P. cambivora* was recovered less frequently. Over all *P. cambivora* was recovered more readily from shoots inoculated in July rather than May.

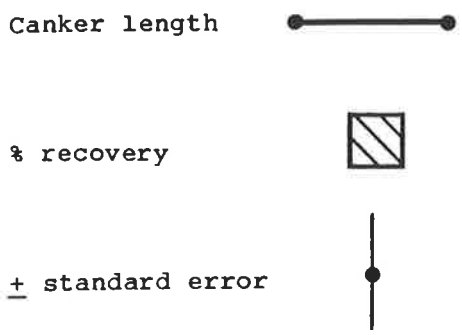
Similar trends in canker expansion occurred in Mazzard cherry stems as in almonds. In cherry however *P. cambivora* was rarely recovered from canker margins even during the period of rapid expansion. For example in stems inoculated in August, *P. cambivora* was recovered three weeks after inoculation and in less than 10% of the wood chips removed from the margins of the cankers.

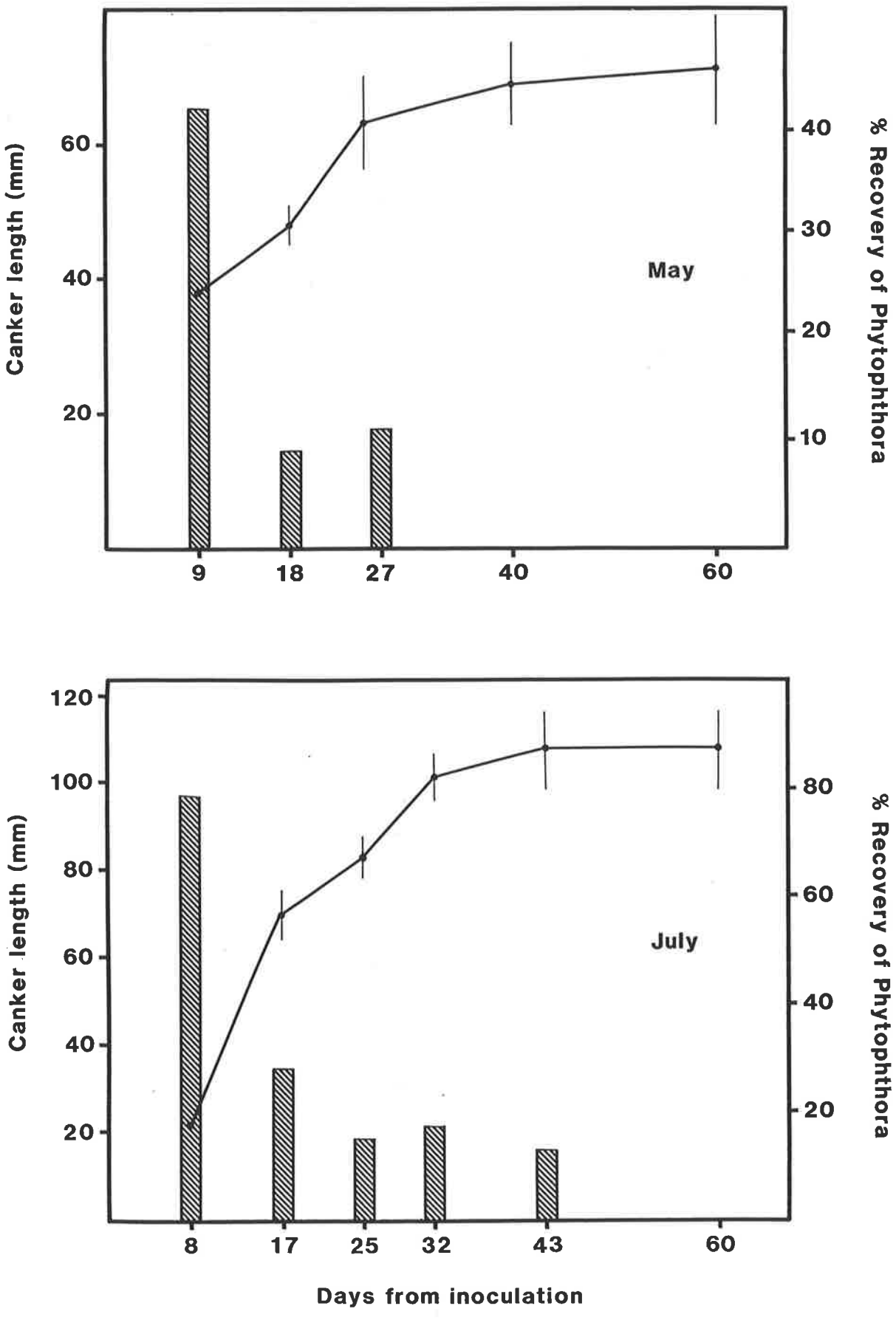
DISCUSSION

These studies confirm earlier work (Bumbieris *et al.*, 1982, Wicks and Lee 1986) showing that *Phytophthora* species are widespread in almond and cherry orchards of South Australia. In addition to those species already recovered, these studies detected *P. parasitica* from an almond orchard, *P. syringae* from both almond and cherry orchards and *P. citricola* from a cherry orchard.

The recovery of *P. syringae* from an almond orchard in November was unexpected as it is most active only during the cooler months (Bostock and

Figure 3.2 Recovery of *Phytophthora cambivora* from the margins of cankers on almond shoots inoculated in either May or July.




Canker length (mm)
% Recovery of Phytophthora
May
July
9
18
27
40
60
8
17
25
32
43
60
Days from inoculation

Doster 1985) and in the United Kingdom it was only isolated at temperatures below 16°C (Sewell *et al.*, 1974). *P. syringae* has been isolated from almond in California (Bostock and Doster 1985,) and Greece (Kouyeas and Chitzanidis 1968) but this is the first report of the pathogen from almond and cherry in South Australia.

Of the *Phytophthora* spp. isolated from almond orchards in South Australia *P. cambivora* is the most serious pathogen (Wicks and Lee 1986). It was frequently recovered with *P. megasperma* on most occasions the soils were collected from the Willunga orchards. The frequent isolation of *P. megasperma* from the almond orchard at Angle Vale and a number of Willunga orchards suggested that further pathogenicity testing of this species was warranted even though previous studies with one isolate showed it to be the least virulent of *Phytophthora* spp, recovered from almonds (Wicks and Lee 1986).

When determining the seasonal variation in the activity of *Phytophthora* spp in soil from apple orchards, Sewell et al., (1974) showed that the isolations of *Phytophthora* differed markedly between those incubated at 20°C and those incubated at variable seasonal temperature conditions. Their work showed the importance of minimizing as far as possible the artificial conditions that may distort the results when isolating for soil borne organisms.

In the South Australian studies, large numbers of soil samples were collected from seven orchards and it was not possible to incubate baited soils at temperatures similar to those occurring in the field. However, all soils were incubated on shelves in an open shed where temperatures were no more than 5°C higher in the winter and 5°C to 8°C less than ambient temperatures

in the summer. In these conditions *P. cambivora* in the Willunga orchard soils showed activity in all seasons but with marked peaks of activity in spring/early summer and low activity at other times. Similar seasonal variations were detected in the level of *P. cinnamomi* populations in soils from Victoria (Weste and Ruppin 1977) and Western Australia (Shea *et al.*, 1980). In these situations the increase in population levels in the field was often associated with high soil temperatures and soil moisture levels.

In the present studies the soils were sufficiently wet for the production of sporangia at both spring sampling times although the temperature was not suitable for their optimum production. Apart from adequate soil moisture conditions other reasons for these peaks are unclear. It could be that large numbers of susceptible young feeder roots present at that time of the year stimulated resting propaqules to grow. This in turn would have resulted in wide spread root infection and subsequent sporulation from these roots. A more likely explanation is that the frequent occurrence of winter rain produced surface runoff in the Willunga orchards that may have spread infective propagules downhill from initial infected sites. These would have been detected frequently in soil samples collected in spring but less frequently later when soils dried out and the soil moisture and temperature were unsuitable for their survival.

In the Angle Vale and Lenswood orchards changes in seasonal variation of *P. cambivora* could not be detected because of the low level of recovery. In the Angle Vale orchard the level of recovery was low at all times of sampling and may have been due to low levels of propagules in the soil. In this sampling area, dead almond trees had been removed before sampling began and the area replanted with Nemaguard rootstocks at the start of sampling.

Reproduction and survival of *P. cambivora* may have been inhibited on the roots of the Nemaguard rootstocks which appear resistant to this pathogen (Wicks and Lee, 1985, 1986).

In contrast, the recovery of *P. cambivora* from the cherry orchard was initially low with the rate of recovery gradually increasing during the sampling period. This is most likely associated with the infection, reproduction and survival of *P. cambivora* on the roots and crowns of Mahaleb rootstocks that were planted in the area at the start of sampling.

P. cambivora was not detected in almond orchards in the Riverland area of South Australia and North Western Victoria although dead and declining trees were occasionally observed in some orchards. Soils in these orchards are sandy and generally free draining and are therefore not conducive to the spread of *Phytophthora*. Since *P. cinnamomi*, *P. citrophthora* and *P. parasitica* are known to occur in the area, *P. cambivora* could also be expected to survive in these soils if it was introduced into the area.

That *P. cambivora* was not detected could be attributed either to the low incidence of the pathogen that prevented its detection in our samples, or that the pear baiting technique was not sensitive enough to detect low levels in the soil samples. The more likely explanation is that the pathogen had not been introduced into the area. Most almond plantings in the Riverland area are less than fifteen years old and rootstocks of these trees originated from local nurseries or from nurseries in the Angle Vale area which had been fumigated before planting. The likelihood of *P. cambivora* being introduced on infected rootstock is therefore remote. In any case had this occurred the free draining soils and the extensive use of herbicides rather

than cultivation for weed control would not be conducive to the spread of the disease in the Riverland orchards.

The isolation of *P. cambivora* from some soils in summer months was not unexpected as many of the sampled orchards were irrigated for 12 hours every fourth day during this period which would have provided soil moisture conditions favourable for growth and possibly reproduction.

However *P. cambivora* was also isolated from a non irrigated orchard during summer when the soil was dry and the moisture content unfavourable for growth and the formation of sporangia.

The propagules capable of surviving these conditions were not found although chlamydospores and oospores are the propagules most likely to tolerate the dry soils. Further studies are required to determine first if chlamydospores or oospores or some other survival structures are produced in the soil or within the host tissue and second the survival of these structures in soils in various seasons.

Although none was observed in any cultures of the present studies, Haasis *et al.*, (1966) reported *P. cambivora* formed chlamydospores in culture which were similar to those produced by *P. cinnamomi*.

It may be that *P. cambivora* behaves similarly to *P. cinnamomi* in forming chlamydospores in small decaying roots (Weste and Vithanage 1978) in response to dry soil conditions. The persistence of *P. cambivora* in dry soils could also be due to thick walled oospores that are considered likely to survive low soil water potentials. The importance of these structures in the survival of *P. cambivora* is unknown. While both the A^1 and A^2 mating

types of *P. cambivora* have been isolated from soil, more than 98% of the isolates have been of the A^1 type. This is similar to the results of Suzui and Hoshino (1979) who found isolates of *P. cambivora* from apple orchards to be predominately the A^1 mating type. However it may be that the sexual stage is not important in the field. Old *et al.*, (1984) showed that there was little evidence for recombination between A^1 and A^2 mating types of *P. cinnamomi* and suggested that two populations of the fungus may co-exist independantly.

Also Weste and Vithanage (1978) reported that oospores of *P. cinnamomi* were never found in soil or roots despite an extensive search.

Whether species of *Phytophthora* were introduced into the orchards of these studies or whether they occur naturally in the soils could not be determined from these investigations. *Phytophthora* were not detected in soils from three almond orchard nurseries surveyed in these studies, but this was probably due to the soil fumigants that had been applied to the nursery sites in recent years. Contaminated nursery stock either as infected nursery plants or infested soil adhering to plants are the most likely potential sources of primary inoculum in orchards and has been demonstrated as such with *P. cactorum* and *P. cambivora* on apple rootstocks (Julius *et al.*, 1979, Jeffers 1985). Infected nursery stock is likely to be the means of introducing the pathogens into cherry orchards as evident with the isolation of *P. cambivora* from roots and crown cankers of cherry rootstocks (Bumbieris *et al.*, 1982). This is also likely to be the case with almonds although the recovery of *Phytophthora* from almond root stocks obtained from nurseries has not been reported.

It is unclear whether *P. cambivora* occurs in non agricultural soils in South Australia. Although only orchard soils were sampled in this present study, extensive sampling of South Australian natural forest soils (Lee and Wicks 1977) and other soils (Davidson and Bumbieris 1973) has failed to detect *P. cambivora*. On the other hand Gerrettson - Cornell (1978) isolated *P. cambivora* from a native *Eucalyptus* forest in New South Wales.

In the recent experiments there was no obvious seasonal effect on the recovery of *P. cambivora* from infected almond and cherry tissue. After all times of inoculation, *P. cambivora* was difficult to recover from lesions except when isolations were made within a few weeks from inoculation, the time when cankers were expanding rapidly.

These results partially explain why there was no consistent pattern in the recovery of *P. cambivora* from naturally infected trees. In these situations it is unlikely that trees in an orchard or district would be infected at the same time and variations of weeks or months in the time of infection could occur between infected trees. Thus *P. cambivora* would have only been recovered from trees where the isolations were made within a few weeks from infection.

The reasons for the low level of recovery from cherry tissue is difficult to explain. Grinding and leaching infected tissue to release any water soluble materials that may have been inhibitory to *P. cambivora* did not increase the rate of recovery from tissue taken from the margin of expanding cankers. Mircetich and Matheron (1976) have also reported the difficulty of recovering *Phytophthora* from cherry trunk cankers above ground level although the

pathogens were more readily recovered from the same tree from the margin of cankers below ground level.

Histological studies similar to those described by Tippet *et al.*, (1983) with *P. cinnamomi* lesions in *Eucalyptus marginata* need to be conducted in almond and cherries to determine the mechanism(s) inhibiting the spread of *P. cambivora* in host tissue.

Although many *Phytophthora* species were recovered from orchards in these studies their significance as pathogens of almond and cherries was in most cases uncertain.

A number of studies described in Chapter 4 were undertaken to determine which of these species were likely to be serious pathogens in almond and cherry orchards.

CHAPTER 4.

PATHOGENICITY AND RELATIVE VIRULENCE TO ALMOND AND CHERRY OF PHYTOPHTHORA SPECIES ISOLATED FROM ORCHARDS

INTRODUCTION

Studies in the previous chapter showed that a number of *Phytophthora* species occur in soils of almond and cherry orchards in South Australia. As a result the number of reported *Phytophthora* species associated with almonds (Wicks and Lee 1986) and cherries (Bumbieris *et al.*, 1982) was increased as well as the number of isolates of species already identified. Overseas, several *Phytophthora* species are reported as pathogens of almonds (Bostock and Doster 1985, Fatemi 1980, Kouyeas 1971) and cherries (Mircetich and Matheron 1976, Wilcox and Mircetich 1985a) but little work has been done to test the pathogenicity of Australian isolates.

Wicks and Lee (1986) tested the pathogenicity of *P. cambivora*, *P. citrophthora*, *P. cryptogea* and *P. megasperma* on almonds, but none of the Australian cherry isolates has been tested against cherry trees.

Studies were therefore undertaken to test the pathogenicity on almond and cherry of the isolates recovered from or associated with these crops. Also a number of *Phytophthora* isolates associated with fruit trees that were obtained from colleagues both interstate and overseas, were included for comparison.

The objective of these studies was to compare the virulence (relative capacity to cause disease) of different *Phytophthora* species on almond and cherry and to determine if virulence varied significantly between

isolates of a species; the aim being to select the most virulent isolates for use in future resistance screening programs and related work.

MATERIALS AND METHODS

Several different methods were used to test the pathogenicity of the *Phytophthora* species isolated from soil and infected trees. The first utilised the excised twig method of Jeffers *et. al.*, (1981), the second involved inoculating the stems of potted seedlings or 1 or 2-year old shoots of mature trees and a third method, growing almond or cherry seedlings in artificially infested soil.

(a) Excised twigs

Shoots between 5 to 8 mm diameter from the previous seasons' growth were removed from 'Nemaguard' peach seedlings grown in a shade house and from mature almond trees (*Prunus dulcis* (Miller) D.A. Webb c v. 'Jordan') and another of unknown variety. The shoots were soaked in a 1% sodium hypochloride solution for 2 minutes and after 2 rinses in tap water were dried and cut into sections approximately 7 cm long. The base of each section was cut at an angle to facilitate placing it upright in a plastic tub containing 30 ml of $P_{10}VP$ agar inoculated 14 days previously with an isolate of *Phytophthora*. Eight twigs were placed around the margin of the culture in each tub. Replicates of 3 tubs were used for each treatment. After 7 days' incubation at 24°C the length of the stem necrosis was measured on each twig. The same technique was used with shoots from mature Mazzard (*Prunus avium* L) and Mahaleb (*Prunus mahaleb* L) cherry trees collected after leaf fall in experiment one and from summer growth in experiment two.

(b) Stem or shoot inoculation

The stems of four to 18 months old potted seedlings or similarly aged shoots of mature almond cv. 'Mission', 'Chellaston' and an unknown cv. as well as Mahaleb and Mazzard cherry shoots were inoculated by using a hollow punch to remove a 5 mm diameter disk of bark to expose the cambium. A mycelial plug taken from the margin of an expanding colony growing on CMA or V8 agar was inserted into the wound which was then sealed with parafilm®. After a specified incubation period the bark was removed with a scalpel to expose the zonation which was used to define the extreme margins of the canker. The extent of canker expansion was measured on at least 10 stems per treatment.

(c) Effect of temperature on growth rate in culture

An experiment was carried out to measure the growth rates of four of *P. cambivora* isolates (P9,P79,P80,P87) over temperatures ranging from 5°C to 35°C to determine if lower growth rates were associated with low virulence. For each isolate 5 mm diameter disks were taken from the margin of an actively growing colony and placed in the centre of a 9 cm diameter Petri dish of C.M.A. Replicates of four plates were used for each treatment. To prevent the plates drying out, all plates were sealed with parafilm. Over a period of seven to twelve days colony diameters were measured twice at right angles for each plate. Although they were not used in inoculated shoot and soil infestation studies because of quarantine restrictions, the American A^1 (P 131) and A^2 (P130) mating types of the cherry isolate of *P. cambivora* were included for comparison.

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(d) Root inoculation

Several root inoculation experiments were conducted using 'Chellaston' and 'Mission' almond seedlings and Mahaleb and Mazzard cherry seedlings between three to six months old. At least five, but mostly 10 plants were used for each treatment.

Plants were inoculated by adding infested vermiculite to the soil and flooding the soil as described in the General Experimention Section.

At harvest, soil was washed from the roots and the degree of root rotting, fresh root weight and the presence or absence of crown cankers assessed. A number of rotted root pieces were removed at random from at least half the plants in each treatment. The roots were washed several times in tap water blotted dry and then placed into P_{10} VP^{+} agar. After 7 to 10 days incubation at 22°C, plates were examined microscopically for the presence of *Phytophthora*.

In some treatments soil samples were collected at harvest and baited with pears to determine the presence of viable propagules.

RESULTS

(a) Excised twigs

(i) Almond

This experiment was conducted in collaboration with Mr. T.C. Lee of the Adelaide Botanic Garden and the results have been published elsewhere (Wicks and Lee 1986). Briefly all isolates caused necrosis on excised twigs of almond and 'Nemaguard' peach (Table 4.1), but with most isolates the length of necrosis was greater in almond than 'Nemaguard'. Both A_1 (P 5) and A_2 (P

Table 4.1

Virulence of *Phytophthora* spp. to excised almond and 'Nemaguard' peach twigs

Phytophthora species	Origin	Necrosis length[A] (mm)		
		Almond		Nemaguard peach
		Cultivar Jordan	Cultivar unknown	
P. cambivora (A_1)	soil	30.9	21.0	18.5
P. cambivora (A_2)	trunk canker	33.2	20.1	23.1
Phytophthora sp.	trunk canker	33.5	17.0	16.7
P. citrophthora	soil	33.5	26.2	19.1
P. cryptogea	trunk canker	25.3	18.7	12.3
P. megasperma	trunk canker	17.9	12.0	11.9
Nil		0	0	0
l.s.d. (0.05 level)		6.1	4.8	4.5

[A] Mean necrosis length measured after 7 days' incubation at 24°C on 24 Jordan twigs, 16 twigs of the unknown cultivar and 45 twigs of Nemaguard peach. All twigs collected in late summer.

6) mating types of *P. cambivora* caused lesions of similar length on almond and 'Nemaguard' twigs. *P. cryptogea* (P 4) and *P. megasperma* (P 7) were least virulent on all twigs and the length of necrosis caused by these fungi was significantly less than that caused by most of the other isolates of *Phytophthora*.

(ii) Cherry

The first in vitro screening experiment utilising excised twigs compared the virulence of A^1 (P 80) and A^2 (P 9) mating types of *P. cambivora* from cherries with an A^1 (P 79) isolate of the same species from apple and an isolate of *P. syringae* (P 81) from almond. All *Phytophthora* isolates colonised Mahaleb and Mazzard twigs but there were significant differences in the extent of colonisation between isolates and between cherry cultivars inoculated with the same isolate (Table 4.2 Experiment 1). For example the A^2 mating type of *P. cambivora* was the most virulent of the cherry isolates and colonised significantly more Mahaleb than Mazzard tissue. The virulence of *P. syringae* was similar in both cherry cultivars.

In the second experiment the virulence of local A^1 and A^2 cherry isolates of *P. cambivora* was compared with similar mating types (P 130 and P 131) of the same species obtained from Dr. S. Jeffers, University of Wisconsin, USA.

Canker lengths were longer in Mazzard than in Mahaleb when inoculated with the local A^2 isolate whereas they were longer in Mahaleb rather than Mazzard when inoculated with the local A^1 isolate (Table 4.2 Experiment 2). Similar differences between mating types were not apparent with the American isolates.

Table 4.2

Relative virulence of *Phytophthora cambivora* isolates and *P. syringae* to excised Mahaleb and Mazzard cherry twigs.

Isolate and associated host	Mating type and origin	Canker length (mm) Mahaleb	Mazzard	
EXPERIMENT 1				
P. cambivora				
P9 - cherry	A^2 - SA	15.6	42.8	
P79 - apple	A^1 - SA	7.9	2.6	
P80 - cherry	A^1 - SA	12.6	2.7	
P. syringae				
P81 - almond		9.0	8.1	n.s.
Control		0	0	
l.s.d. (0.05 level)		3.0	3.1	
EXPERIMENT 2				
P. cambivora				
P9 - cherry	A^2 - SA	34.8	43.9	
P80 - cherry	A^1 - SA	49.9	37.1	
P130 - cherry	A^2 - USA	43.6	44.8	n.s.
P131 - cherry	A^1 - USA	44.1	43.6	n.s.
Control		0	0	
l.s.d. (0.05 level)		6.9	6.1	

(b) Stem and shoot inoculations

(i) Almond

Almond isolates of *P. cambivora*

The pathogenicity and relative virulence of various *P. cambivora* isolates recovered from different almond orchards and sites within orchards was compared by inoculating either shoots of mature trees or stems of seedlings in two separate experiments. In the first experiment one-year old shoots of mature 'Chellaston' and 'Mission' trees were inoculated in the winter with isolates recovered from five different orchards. Assessments 12 weeks after inoculation showed that all isolates except one produced cankers of similar length on both 'Chellaston' and 'Mission' almond shoots (Table 4.3 Experiment a). In comparing an apple and a cherry isolate both produced similar canker lengths to that of the almond isolates. Isolates recovered from different orchards and from various sites within an orchard were evaluated by inoculating the stems of one year old 'Mission' seedlings growing in a shade house. Plants were inoculated in early summer and assessed four weeks later. All isolates rapidly colonised almond some producing extensive cankers (Table 4.3 Experiment b). In both experiments significant differences in canker length occurred between some isolates; most isolates produced cankers larger than 60 mm. On 'Chellaston', all except one isolate girdled and killed more than 60% of the inoculated shoots.

Table 4.3

Virulence on almond tree shoots and seedling stems of *Phytophthora cambivora* isolates recovered from soils of different almond orchards and from soil collected from various sites within an orchard.

Orchard* origin and site	Canker length (mm) 'Chellaston'	'Mission'
Experiment (a)	Tree shoots	
almond - Willunga - A	107	87
almond - Willunga - B	114	105
almond - Willunga - C	91	93
almond - Willunga - D	40	39.7
almond - Willunga - E	134	107.7
apple	93	77
cherry	115	99
l.s.d. (0.05 level)	33.6	22.8
Experiment (b)		Seedling stems
almond - Willunga B_1		124
almond - Willunga B_2		98
almond - Willunga B_3		78
almond - Willunga C_1	not tested	77
almond - Willunga C_2		62
almond - Willunga D_1		94
almond - Willunga D_2		94
almond - Willunga D_3		87
l.s.d. (0.05 level)		31

* *P. cambivora* recovered from:

A to E = different orchards

B_1 to B_3 etc = different sites of the same orchard

Almond, apple and cherry isolates of *P. cambivora* and other isolates of *Phytophthora*

Almond and other fruit tree isolates of *P. cambivora* as well as other species of *Phytophthora* were compared by inoculating shoots of an unknown mature almond cultivar in two separate experiments. In the first experiment all isolates produced cankers but those of *P. cactorum*, (P 58) *P. cryptogea* (P 4) and *P. megasperma* (P 7) produced cankers less than 25 mm long (Fig. 4.1).

Except for the A^2 (P 6) almond isolate, most cankers on *P. cambivora* - inoculated shoots were longer than 75 mm. The apple (P 79) and cherry (P 80) isolates produced the largest cankers with some extending more than 140 mm within eight weeks from inoculation. Most of the shoots inoculated with either of these isolates were girdled and killed.

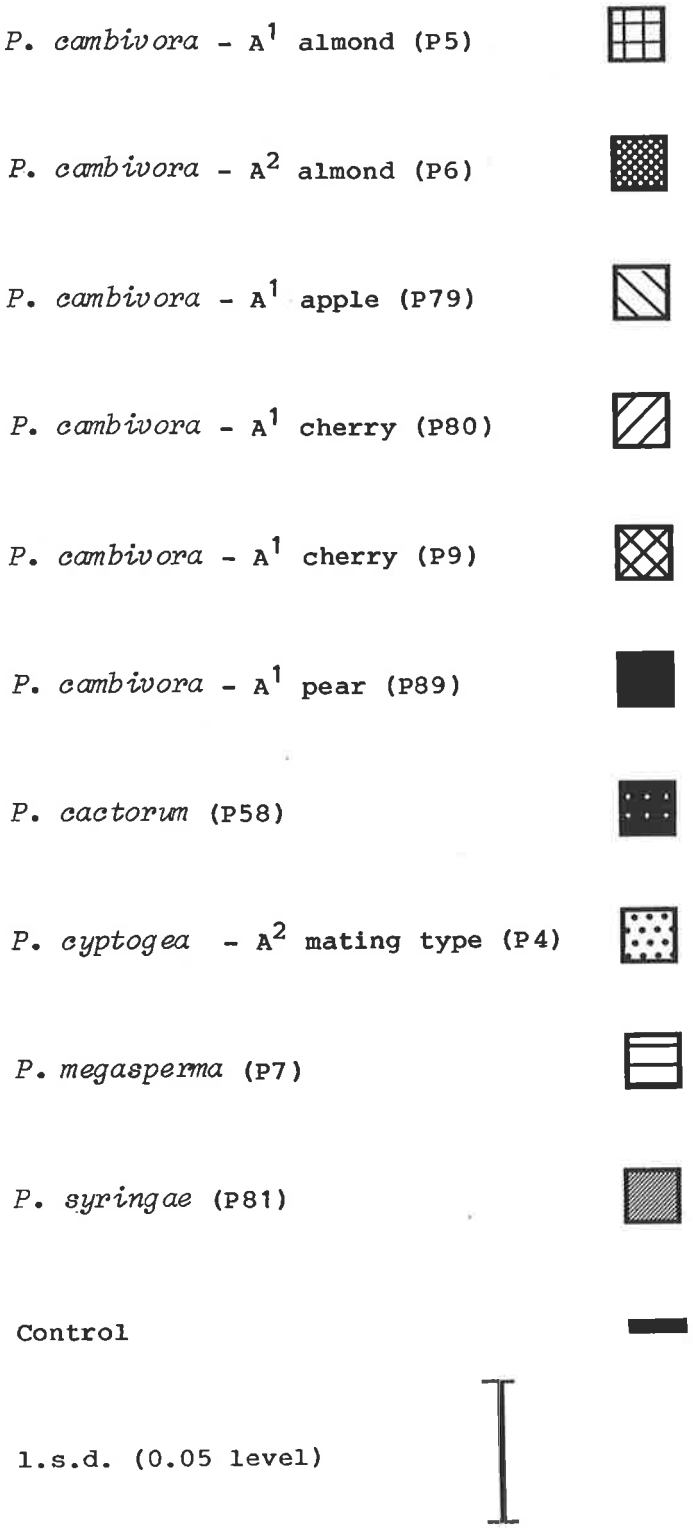
The almond isolate of *P. syringae* (P 81) also produced extensive cankers and killed most of the inoculated shoots.

In the second experiment the rate of canker extension was measured on shoots inoculated with various *P. cambivora* isolates and *P. syringae*. Except for the A^2 almond (P 6) and the apple isolate of *P. cambivora* (P 79) all isolates produced large cankers, some more extensive than others (Fig 4.2). *P. syringae* cankers extended most rapidly and extensively resulting in the death of all inoculated shoots.

Almond isolates of *P. megasperma*

Although cankers did not develop on almond shoots inoculated with *P. megasperma* in previous studies (Wicks and Lee 1986) the frequent isolation of this species from almond orchards (Fig. 1 Chapter 3) and its recovery from

Figure 4.1 The relative virulence of six *Phytophthora cambivora* isolates from different hosts and four other *Phytophthora* spp.to almond shoots.



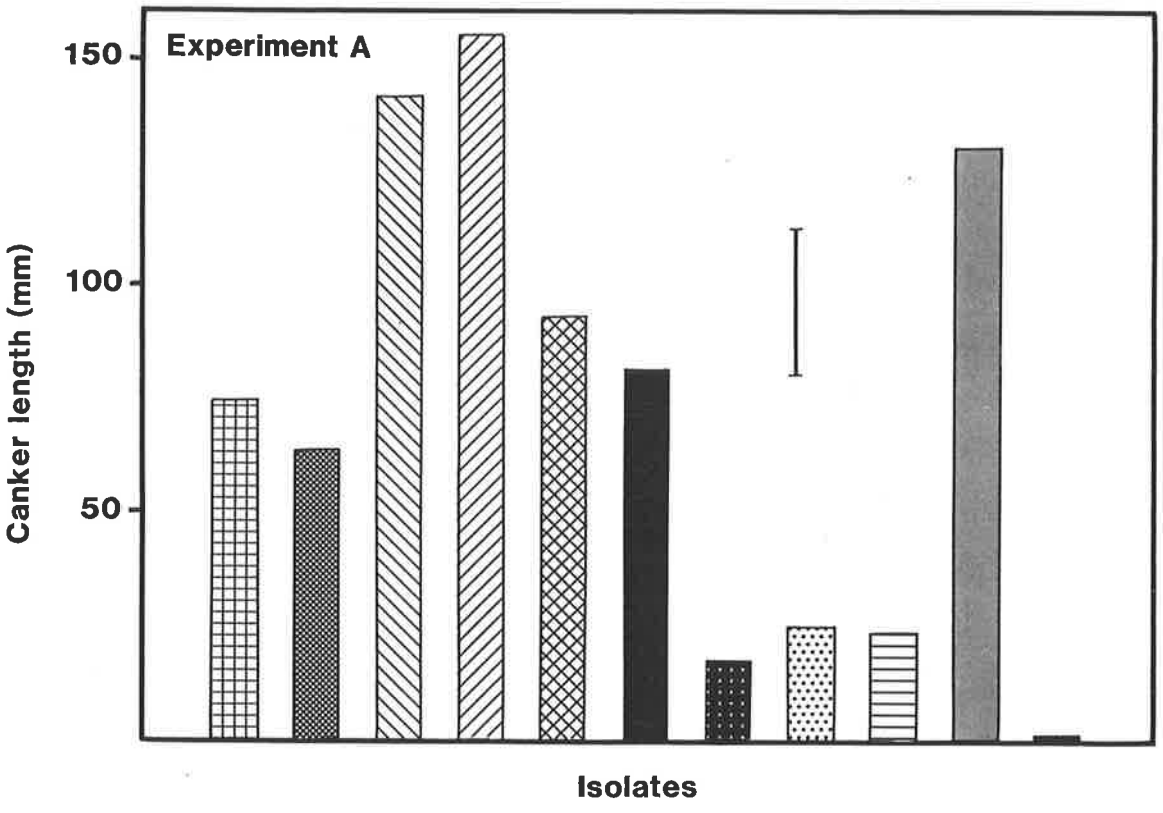
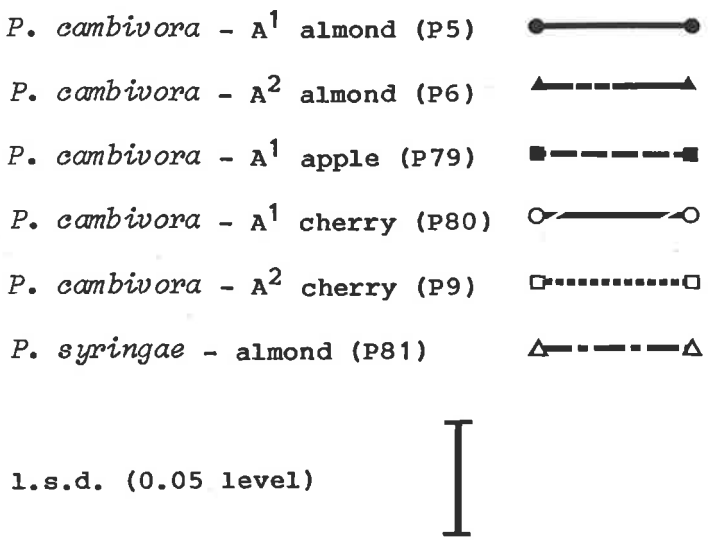

Experiment A
150
100
50
Canker length (mm)
Isolates

Figure 4.2 The rate of canker extension on almond shoots inoculated with *Phytophthora cambivora* isolates from different hosts and *P.syringae*.



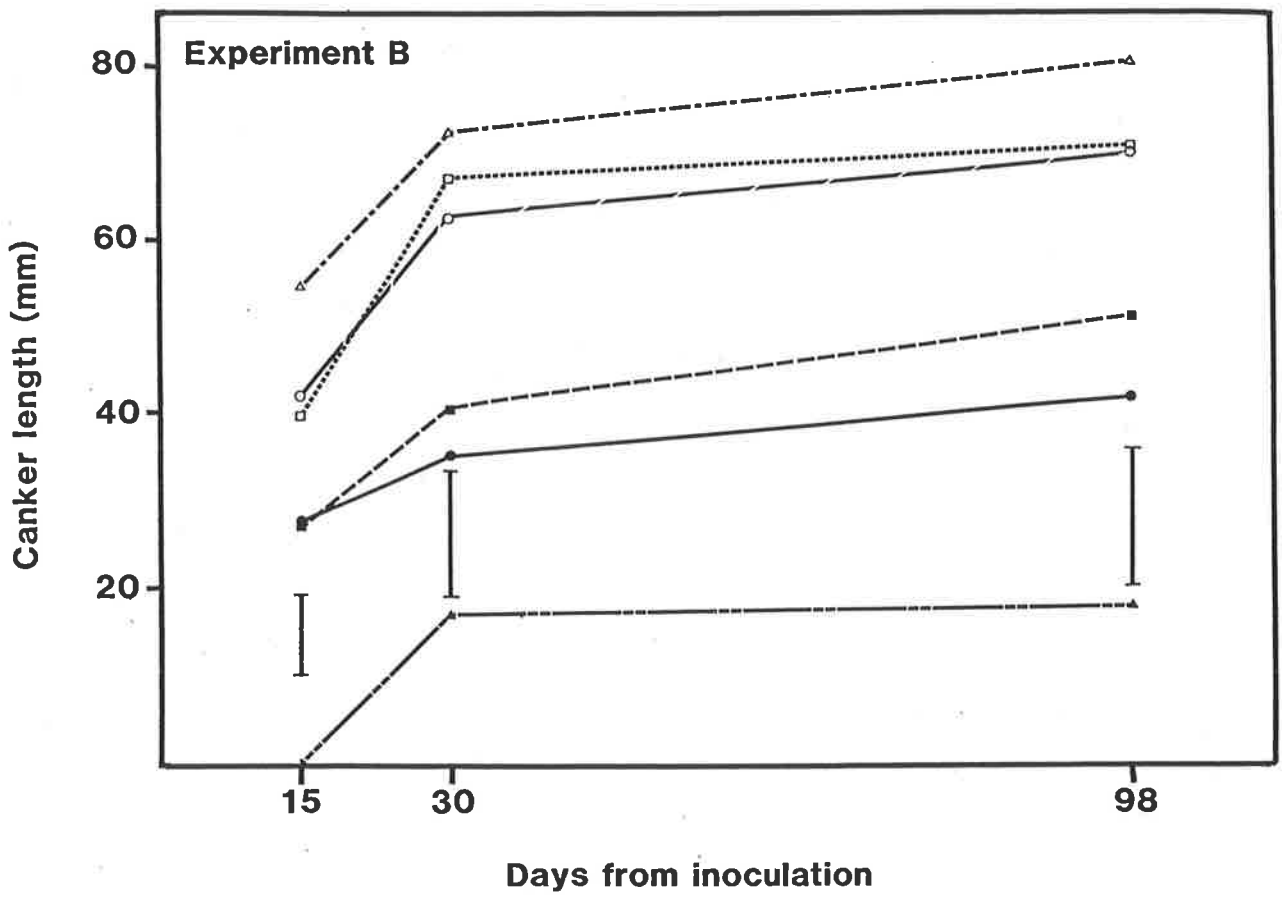
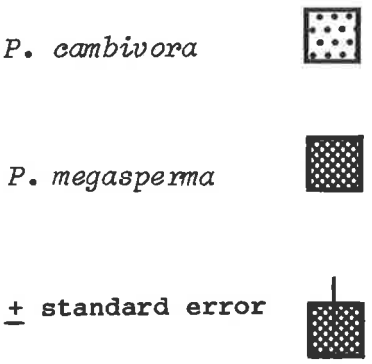
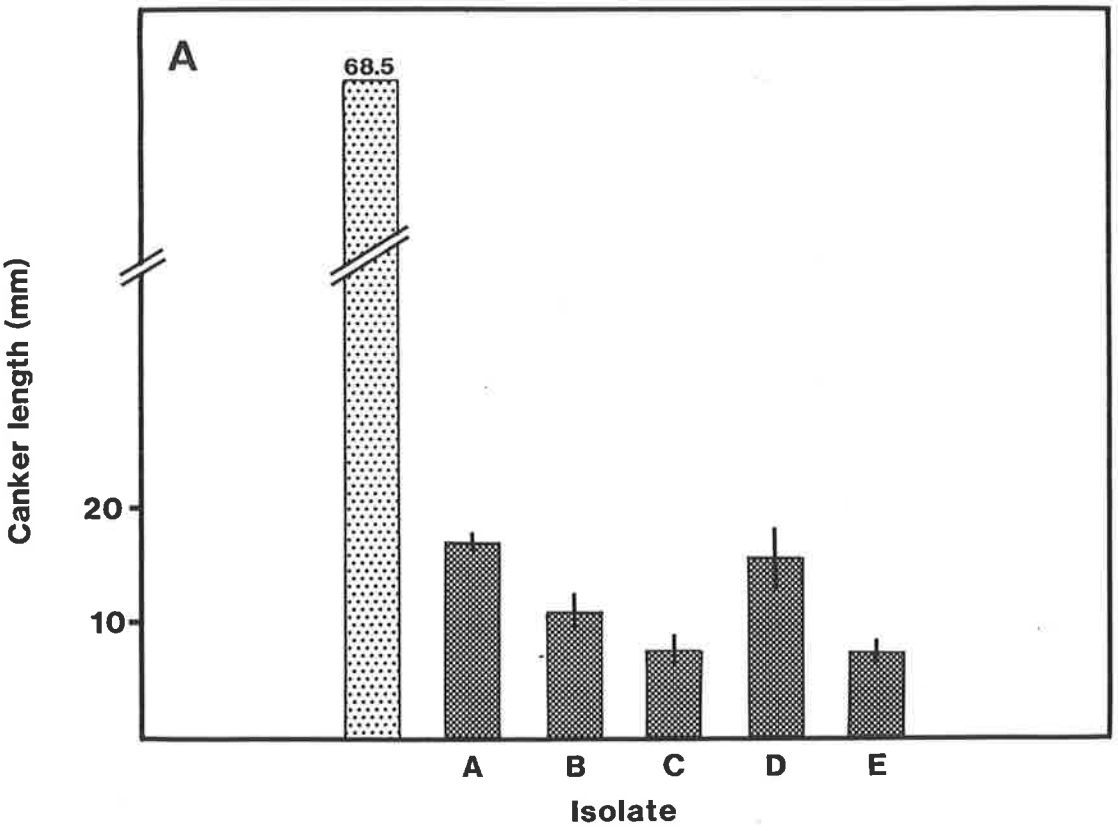
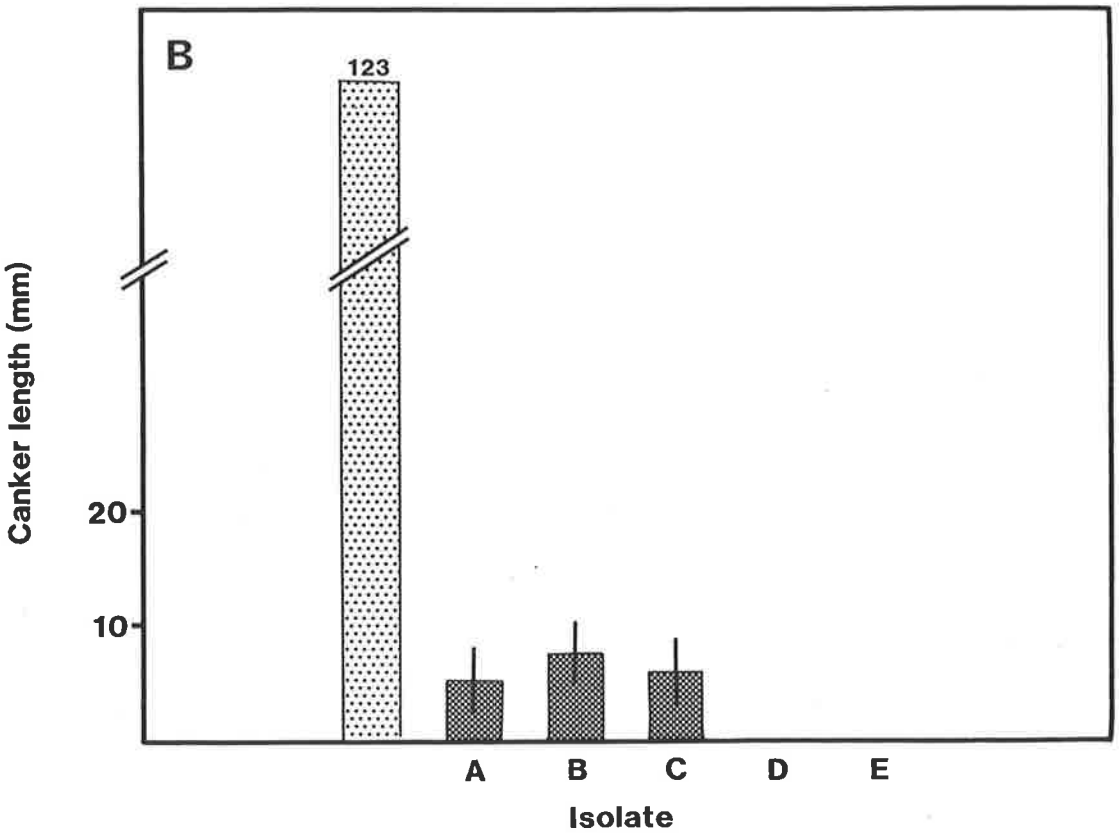

Experiment B
80
60
40
20
Canker length (mm)
15
30
98
Days from inoculation

Figure 4.3 Relative virulence on almond shoots of *Phytophthora cambivora* and *P. megasperma* isolates from various almond soils.




A
68.5
Canker length (mm)
20
10
A
B
C
D
E
Isolate


B
123
Canker length (mm)
20
10
A
B
C
D
E
Isolate

naturally infected almond roots suggested that further pathogenicity tests were warranted.

In these studies, three experiments were conducted to compare the pathogenicity and virulence of *P. megasperma* isolates recovered from different almond orchards with that of an almond isolates of *P. cambivora* (P 5) and *P. cryptogea* (P 4).

In the first experiment, one year old shoots of 'Mission' almond trees were inoculated with *P. megasperma*. Cankers developed on inoculated shoots and the lengths were significantly less than those caused by *P. cambivora* (Fig 4.3a). Similarly in the second experiment where four month old Chellaston seedlings were inoculated, cankers less than 10 mm long developed on the stems inoculated with *P. megasperma* whereas cankers measuring 123 mm developed on stems inoculated with *P. cambivora* (Fig 4.3b).

In the third experiment of this series, isolates of *P. megasperma* recovered from the Angle Vale orchard were inoculated onto the stems of twelve month old 'Chellaston' seedlings and compared with two isolates of *P. cryptogea* (P 4 and P 199) recovered from almond. Cankers did not develop on shoots inoculated with *P. megasperma* whereas nine weeks after inoculation, cankers ~~of~~ with a mean length of 20 mm developed on stems inoculated with either isolate of *P. cryptogea*.

Almond and other isolates of *P. citricola*, *P. citrophthora* and *P. nicotianae* var parasitica

Isolates of *P. citricola* (P 70, P 163 and P 176), *P. citrophthora* (P 3 and P 164) and *P. nicotianae* var *parasitica* (P 73 and P 200) previously recovered from almond soil and isolates recovered from citrus and other soils were inoculated on to the stems of one year old 'Chellaston' seedlings. Ten weeks after inoculation significant differences in canker lengths were obvious between isolates of the same *Phytophthora* species and between some isolates of different species (Table 4.4).

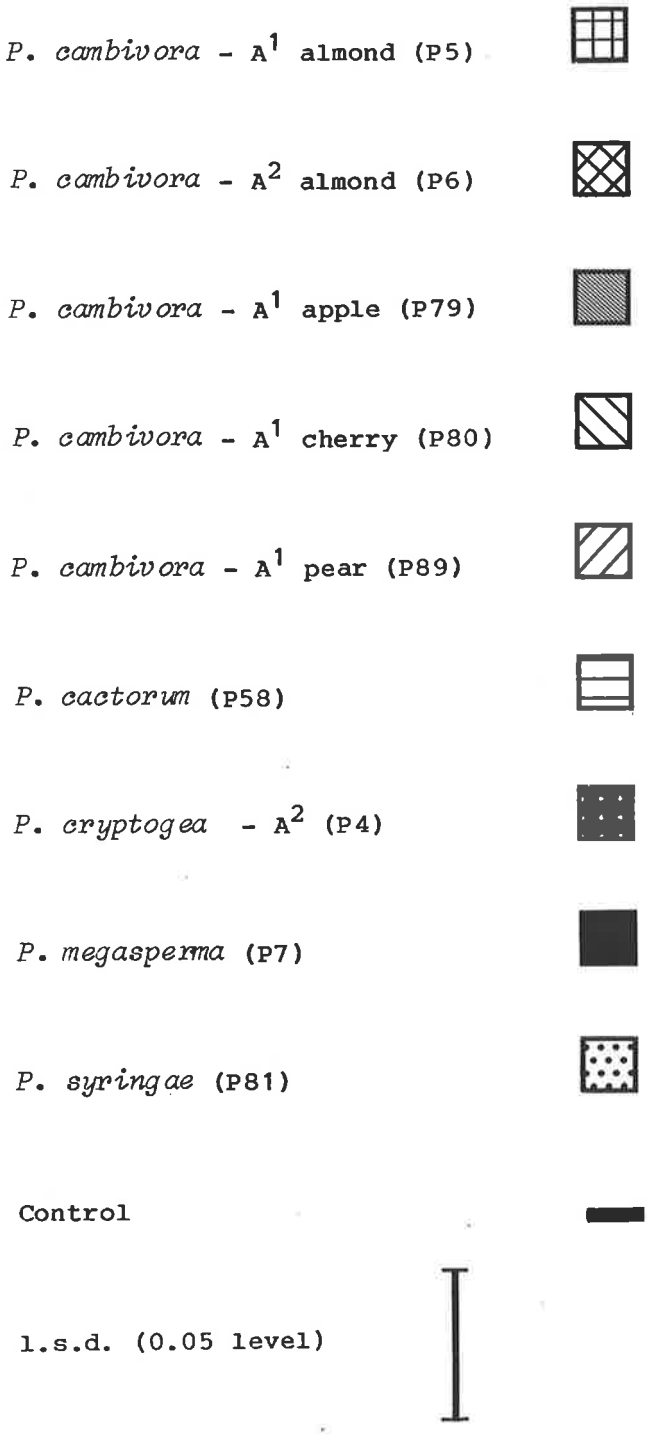
(ii) Cherry

Almond, apple and cherry isolates of *P. cambivora*

The relative virulence of a number of *P. cambivora* isolates associated with various fruit crops was evaluated in three experiments by inoculating one year old shoots of mature cherry trees growing at the Lenswood Research Centre. In the first experiment, Mahaleb shoots were inoculated in spring and assessed six weeks later. Of the isolates tested, *P. syringae* (P 81) and the cherry isolate of *P. cambivora* (P 80) were the most virulent (Fig. 4.4). Both the A^1 (P 5) and A^2 (P 6) mating types of the almond isolate of *P. cambivora* and the apple (P 79) and pear (P 89) isolates were weakly pathogenic in this experiment. *P. cryptogea* (P 4) induced cankers on all inoculated shoots, but these were significantly shorter than those on shoots inoculated with either the cherry isolates of *P. cambivora* or *P. syringae*.

The experiment was repeated with a similar range of *Phytophthora* species as above, but inoculated on to shoots of both Mahaleb and Mazzard with the rate of canker development measured up to 12 weeks after inoculation.

Figure 4.4 The relative virulence of five *Phytophthora cambivora* isolates from different hosts and four other *Phytophthora* spp. to Mahaleb cherry shoots.



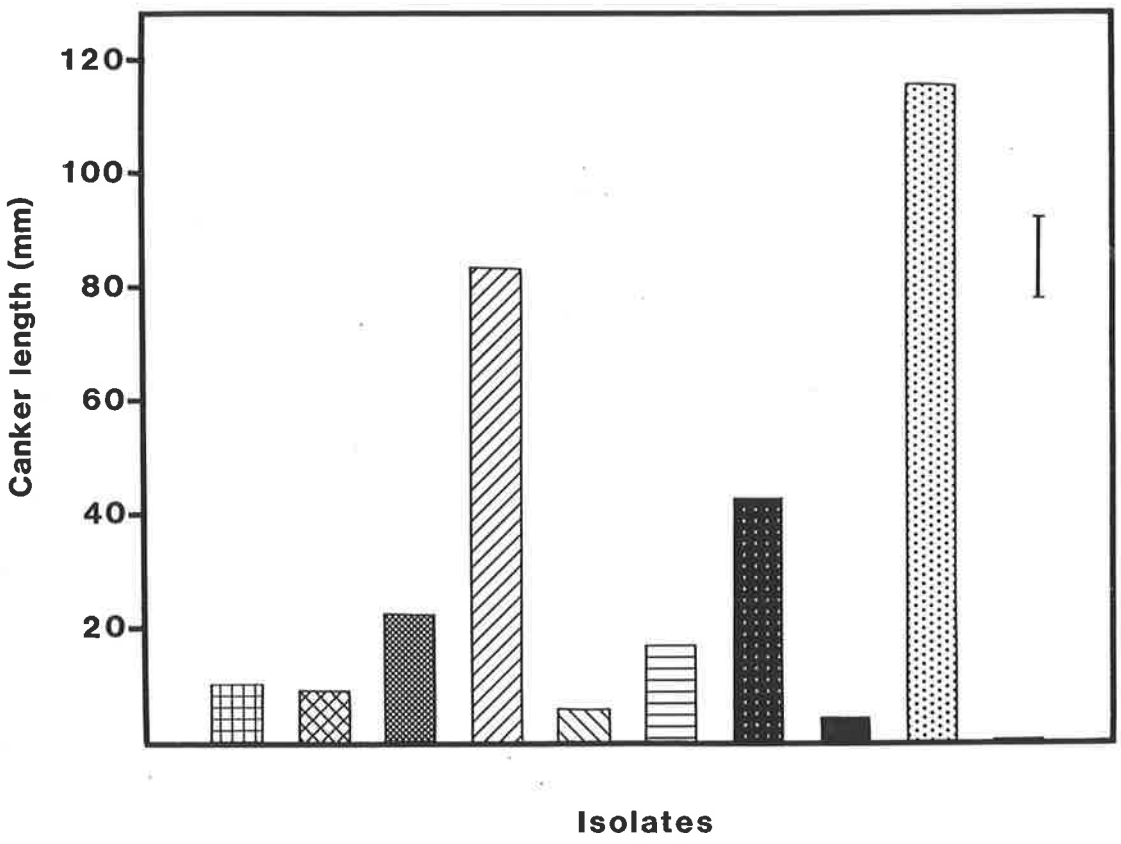

120
100
80
60
40
20
Canker length (mm)
Isolates

Table 4.4

Relative virulence of *P. citricola*, *P. citrophthora* and *P. nicotianae* var *parasitica* isolates to Chellaston almond stems.

Species	Associated host	Almond Canker length (mm)
P. citricola (P 176)	citrus	92
P. citricola (P 70)	almond	21
P. citricola (P 163)	soil	90.2
P. citrophthora (P 164)	citrus	65.6
P. citrophthora (P 3)	almond	7.6
P. nicotianae (P 73) *var parasitica*	almond	100.4
P. nicotianae (P 200) *var parasitica*	almond	48.3
l.s.d. (0.05 level)		31.0

With some isolates, canker development was greater on Mahaleb than Mazzard (Fig. 4.5). However with the A^2 (P 9) mating type of the cherry isolate of *P. cambivora* and *P. syringae*, cankers were longest on Mazzard. Almond and ~~pear~~ apple isolates of *P. cambivora* were also weakly pathogenic in this experiment compared to other isolates of this species.

During the course of these studies, more isolates of *P. cambivora* were recovered from orchards and in order to compare the virulence of these a number were inoculated on to both Mahaleb and Mazzard shoots. Plants were inoculated in winter and the rate of canker development measured for 13 weeks. Cankers developed on all inoculated shoots and except for the A^2 mating type, they were longest on Mahaleb (Fig. 4.6). In this experiment there was significant variation in virulence of isolates. On Mahaleb, an almond isolate was more virulent than cherry isolates.

(c) Growth rate in culture

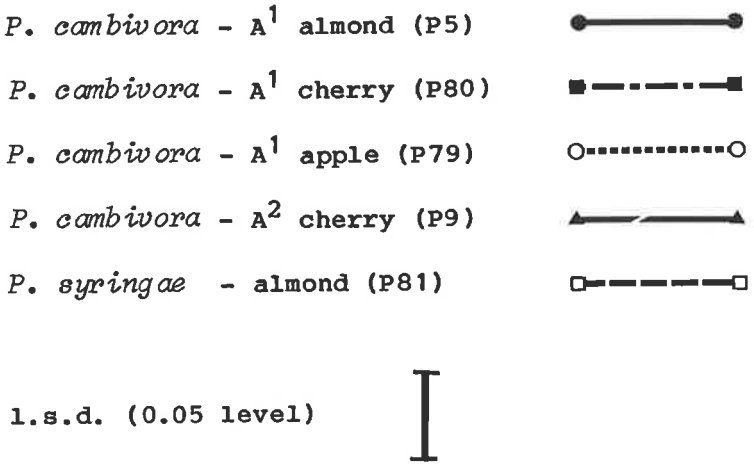
The growth studies showed that the optimum growth rate for most isolates was near 25°C but the isolates could be separated into two distinct groups; one with maximum growth rates near 3 mm per day and the other with maximum growth rates near 9 mm per day (Fig 4.7). These differences were not related to different mating types as both A^1 and A^2 mating types occurred in each group. Isolates in the lower growth rate group were no less virulent than isolates in the higher growth rate group in some experiments.

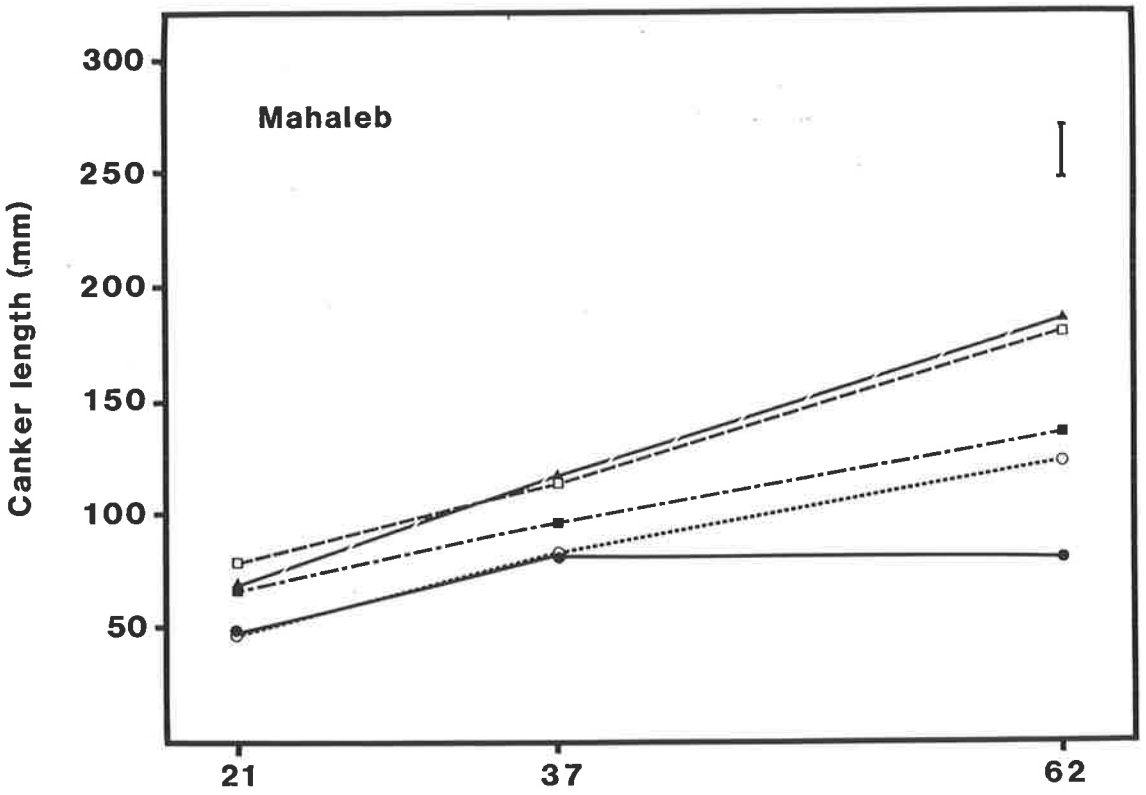
(d) Root inoculation

(i) Almond

Two experiments were conducted to compare the virulence of *P. cambivora* isolates with that of other *Phytophthora* species.

Figure 4.5 The rate of canker extension on Mahaleb and Mazzard cherry shoots inoculated with *Phytophthora cambivora* isolates from different hosts and *P. syringae*.




Mahaleb
Canker length (mm)
300
250
200
150
100
50
21
37
62

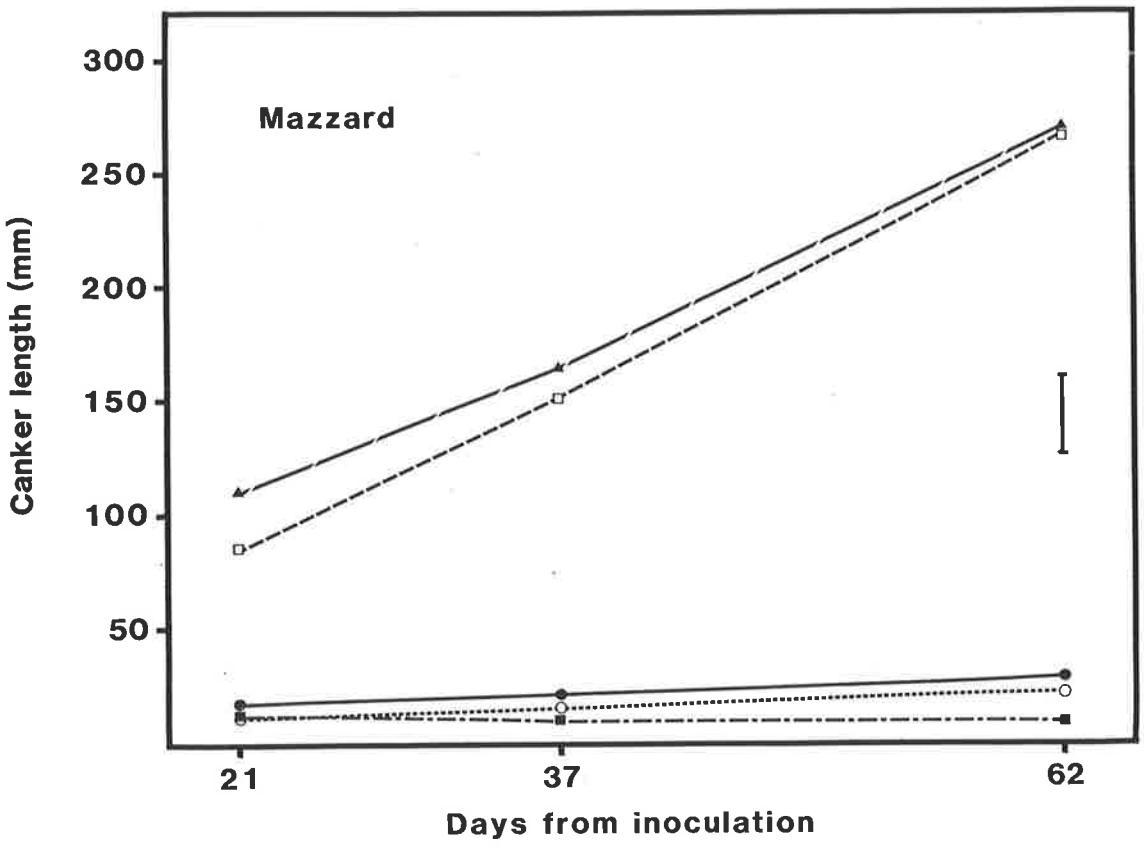
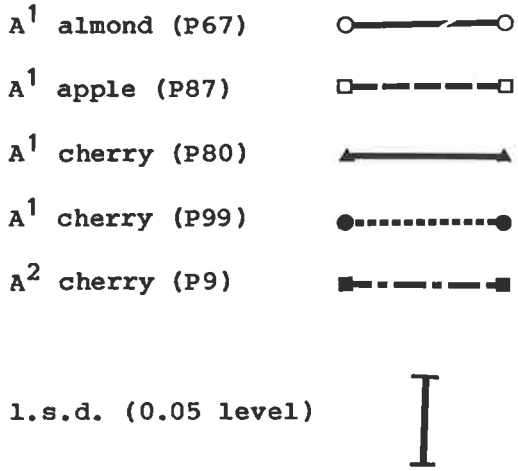
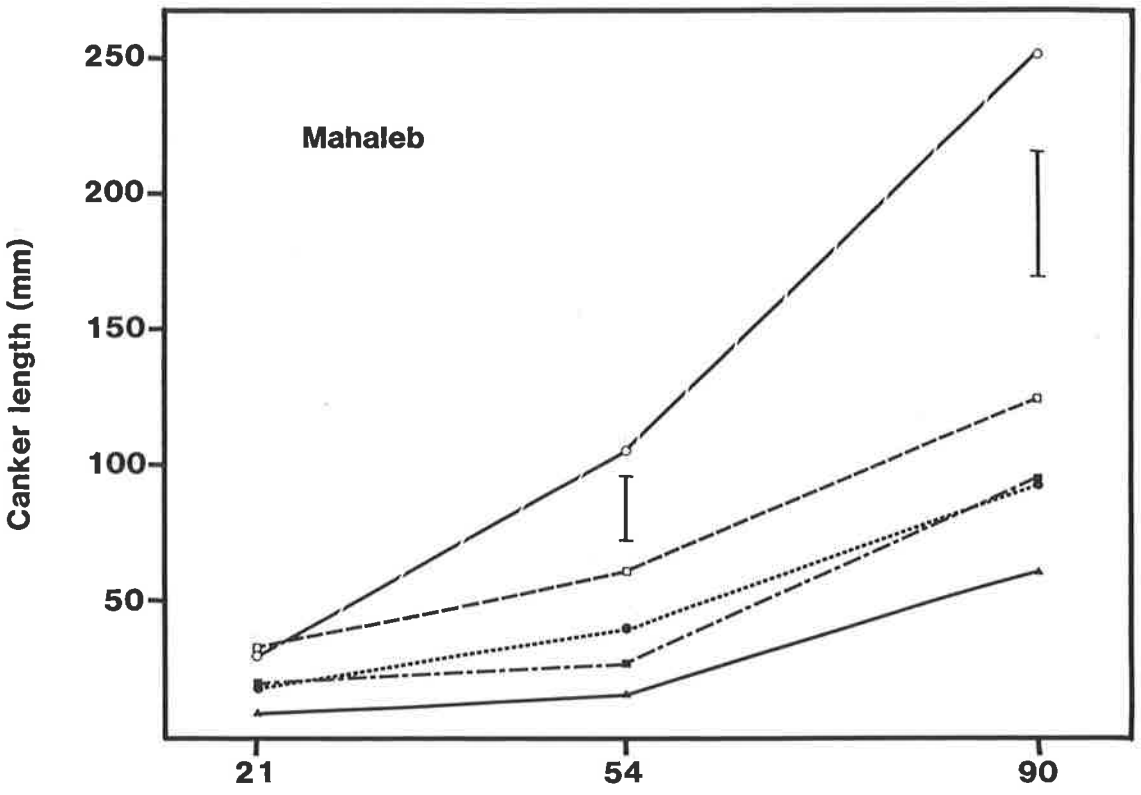

Mazzard
Canker length (mm)
300
250
200
150
100
50
21
37
62
Days from inoculation

Figure 4.6 Relative virulence of almond and cherry isolates of *Phytophthora cambivora* to shoots of Mahaleb and Mazzard cherry rootstocks.




Mahaleb
Canker length (mm)
250
200
150
100
50
21
54
90

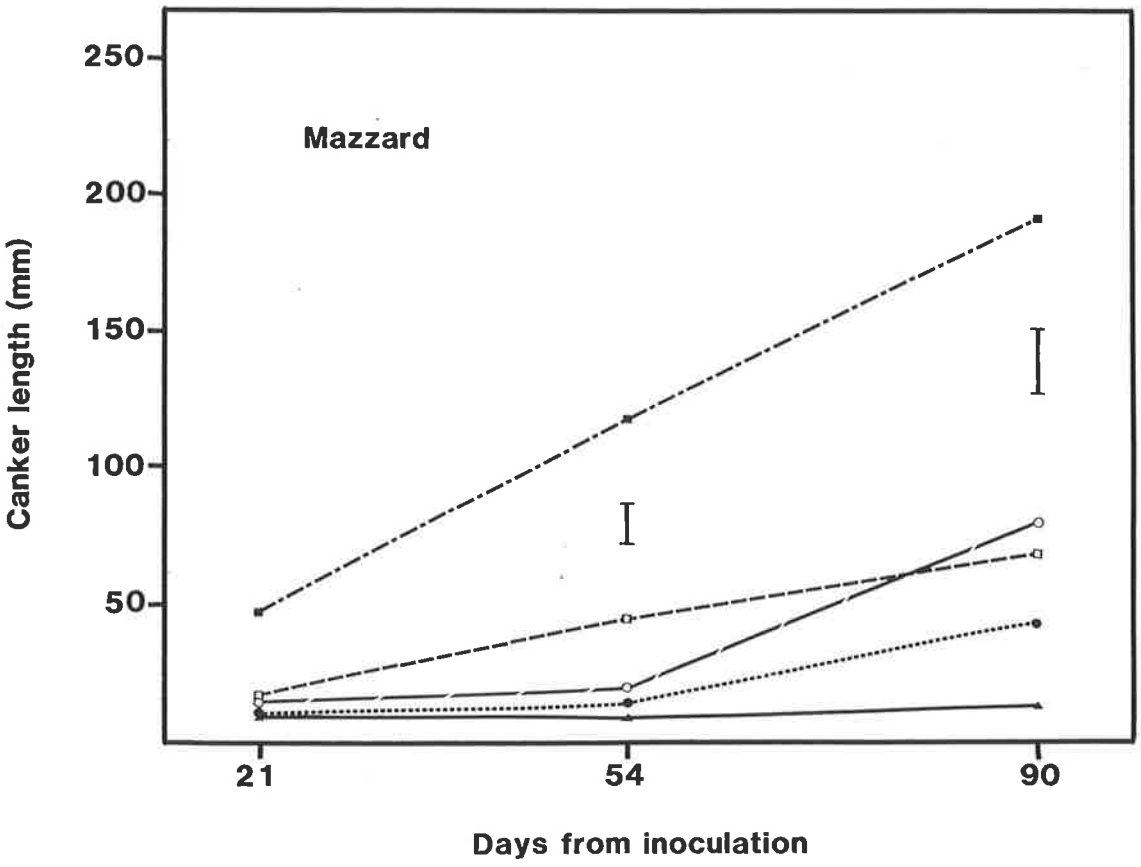
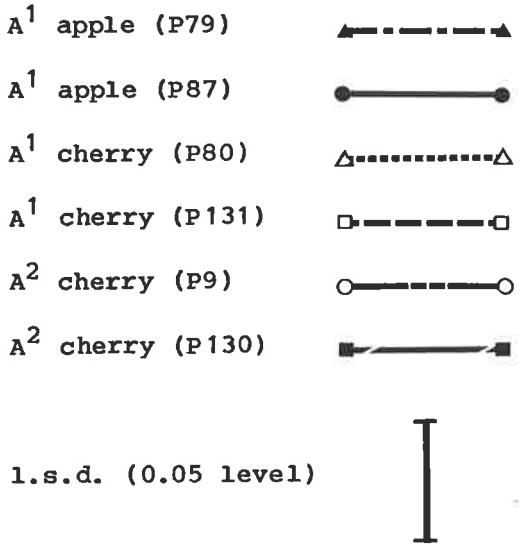
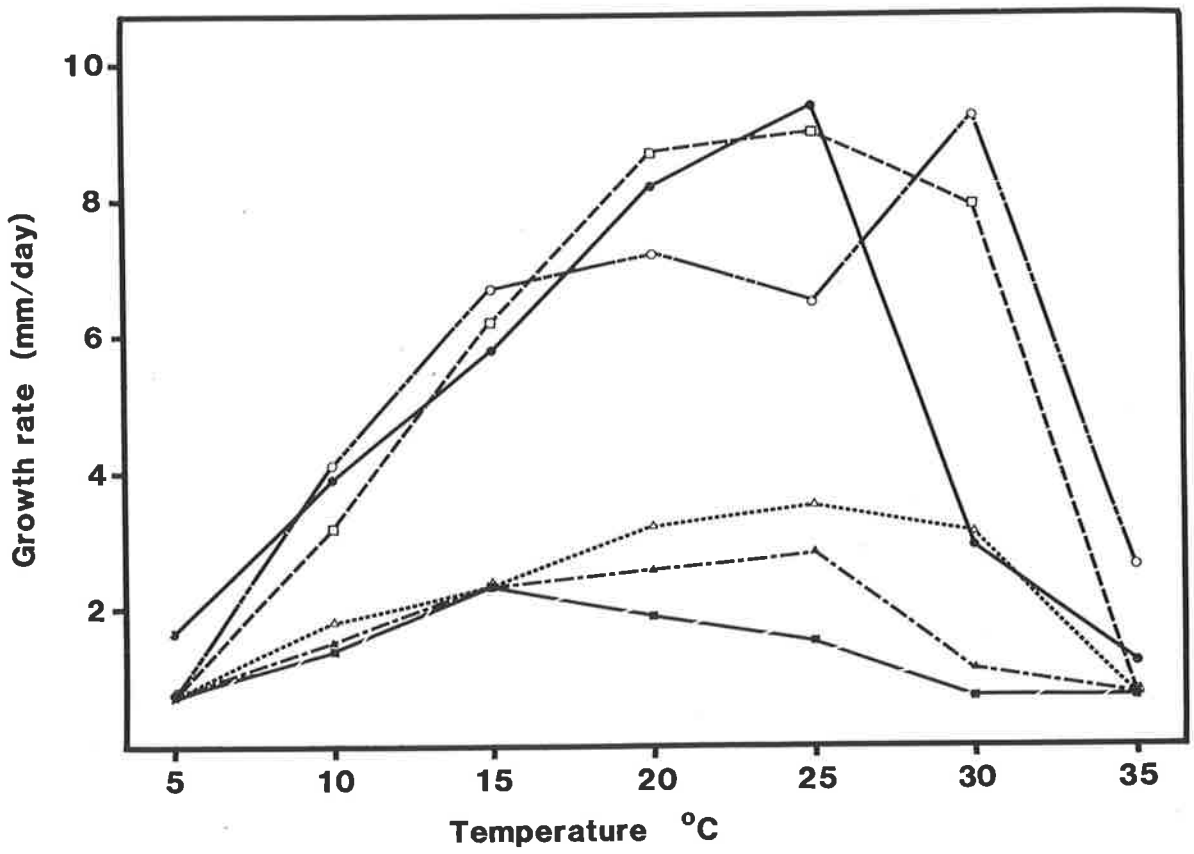

Mazzard
Canker length (mm)
250
200
150
100
50
21
54
90
Days from inoculation

Figure 4.7 The effect of temperature on the growth rate of six isolates of *Phytophthora cambivora* on agar cultures.




Growth rate (mm/day)
10
8
6
4
2
5
10
15
20
25
30
35
Temperature °C

In the first experiment, almond (P 5) and apple (P 79) isolates of *P. cambivora*, were compared with almond (P 81) and cherry (P 161) isolates of *P. syringae* by growing six months old 'Chellaston' seedlings in artificially infested soil for 12 weeks. These plants were grown in a growth cabinet.

Crown cankers and extensive root rotting developed in all plants grown in soil infested with the almond isolate of *P. cambivora* (Table 4.5).

All isolates severely rotted roots compared to the control but only plants inoculated with the almond isolate of *P. cambivora* developed crown cankers on all plants. *Phytophthora* was recovered from roots and stem cankers of plants inoculated with both isolates of *P. cambivora*, but not from plants inoculated with *P. syringae*.

In the second root inoculation experiment the pathogenicity of a number of isolates of *P. cambivora*, *P. cryptogea* and *P. megasperma* from almond orchards was compared. In this experiment six months old 'Mission' almond seedlings were grown in inoculated soil, which was flooded one day every week for the first 12 weeks after inoculation. The plants which were grown in a shade house were watered as required and harvested 12 months after inoculation. Eight plants were assessed for each treatment.

Almond isolates of *P. cambivora* rotted the most roots and induced crown cankers on more plants than other isolates (Table 4.6). An apple and one cherry isolate of *P. cambivora* induced crown canker, rotted roots and caused a significant reduction in root weight compared to the uninoculated treatment. *P. cryptogea* and *P. megasperma* isolates were less

Table 4.5

Virulence of *Phytophthora cambivora* and *P. syringae* isolates to 'Chellaston' almond seedlings grown in artificially infested soil.

Treatment and original host association	Plants with crown canker %	Roots rotted %	Fresh root weight (g)	Recovery Cankers	Recovery Roots
Control	0	20	80.6	-	-
P. cambivora (almond - P5)	100	100	36.1	+	+
P. cambivora (apple - P79)	20	88	43.2	+	+
P. syringae (almond - P81)	0	95	30.9	-	-
P. syringae) (cherry - P161)	20	85	37.6	-	-
l.s.d. (0.05 level)			20.2		

+ = *Phytophthora* recovered.

- = *Phytophthora* not recovered.

Table 4.6

Virulence of *Phytophthora cambivora*, *P. cryptogea* and *P. megasperma* isolates to 'Mission' almond seedlings grown in artificially infested soil.

Inoculum	Plants with crown canker %	Roots rotted %	Fresh root weight (g)	Recovery from roots
Control	0	46	74.0	-
P. cambivora (P 5) almond	37	75	15.8	+
P. cambivora (P 9) cherry	0	55	62	+
P. cambivora (P 80) cherry	37	74	28.5	-
P. cambivora (P 87) apple	37	63	34.4	-
P. cambivora (P 188) almond	62	76	27.8	+
P. cryptogea (P 188) almond	0	30	55.4	-
P. cryptogea (P 199) almond	12	61	43.9	-
P. megasperma (P 185) almond	0	46	55	+
P. megasperma (P 196) almond	0	31	53	+
P. megasperma (P 197) almond	0	43	42.5	+
l.s.d. (P 0.05 level)			19.7	

\+ *Phytophthora* recovered
\- No *Phytophthora* recovered

virulent although some isolates rotted roots significantly and one isolate of *P. cryptogea* caused crown cankers on some plants.

(ii) Cherry

Two experiments were conducted to compare the virulence of *P. cambivora* isolates to cherry rootstocks when grown in infested soil. In the first experiment Mazzard rootstocks were used and these were grown in soil infested with either the A^1 (P 80) or A^2 (P 9) mating types of *P. cambivora* added to soil either singly or combined. Also included for comparsion was a cherry isolate of *P. syringae* (P 161).

Three weeks after inoculation plants appeared to wilt and 7 weeks later 50% of the plants in soil infested with the A^2 mating type were dead (Table 4.7 Experiment a). One plant of each of the other inoculated soils had also died at this stage and none had died in the flooded uninoculated soils. Root rot ratings showed the highest incidence of root rot in the A^2 infested soil with root rot in most other treatments being no greater than that in the control plants. *Phythophthora* was readily recovered from the roots of all plants grown in infested soil, but was not recovered from uninoculated plants.

In the second experiment, the virulence of A^1 (P 87 and P 80) apple and cherry and A^2 (P 9) cherry isolates of *P. cambivora* was compared on Mazzard and Mahaleb rootstocks. Sixteen weeks after inoculation, dead plants with stem cankers were found in each of the inoculated Mahaleb treatments and in the Mazzard treatment inoculated with the A^2 mating type (Table 4.7 Experiment b). In Mahaleb plants, cankers varied in length from 28 mm ($\pm$15.9) to 86 mm ($\pm$13.9) for each of the P80 and P87 (A^1) and to 80 mm ($\pm$ 11.3) for the P9 (A^2) isolates respectively. On Mazzard plants, cankers extended 115 mm

Table 4.7

The virulence of *Phytophthora cambivora* isolates to Mazzard and Mahaleb cherry rootstocks grown in artificially infested soil.

Inoculum and mating type	Fraction of plants dead	Fraction of plants with cankers	Root rot* % (exp. a) or root weight (g) (exp. b)	Recovery from roots %
Experiment (a)		Mazzard		
P. cambivora A^1 (P80)	1/8	1/8	30	15
P. cambivora A^2 (P9)	4/8	7/8	70	48
P. cambivora $A^1 + A^2$ (P80 + P9)	1/8	1/8	18	55
P. syringae (P161)	1/8	5/8	46	60
Control flooded	0/8	0/8	30	0
Experiment (b)		Mahaleb		
P. cambivora A^1 (P80)	3/10	6/10	35	4
P. cambivora A^1 (P87)	2/10	5/10	42.4	40
P. cambivora A^2 (P9)	1/10	6/10	35.5	32
Control Flooded	2/10	0/10	83	0
l.s.d. (0.05 level)			17.3	
		Mazzard		
P. cambivora A^1 (P80)	0/10	0/10	124.3	4
P. cambivora A^1 (P87)	0/10	0/10	149.1	6
P. cambivora A^2 (P9)	3/10	3/10	90.4	4
Control Flooded	2/10	0/10	99.1	0
l.s.d. (0.05 level)			43.7	

(±10.4) along the stems. With all isolates root rotting was more severe in Mahaleb compared to Mazzard.

DISCUSSION

The virulence of *P. cambivora* on almond and cherry was demonstrated with excised stem, stem inoculation and soil inoculation studies which confirms other reports showing *P. cambivora* to be a serious pathogen of almond (Wicks and Lee 1986) and cherry (Mircetich and Matheron 1976). Of the other *Phytophthora* species tested only *P. syringae* extensively colonised almond and cherry shoots more than *P. cambivora*. The development of severe root rotting and crown cankers in almonds and cherry seedlings grown in soil infested with *P. syringae* indicates that this fungus may also be an important pathogen of almond and cherry in South Australia.

P. cryptogea has also been reported as a pathogen of almond (Fatemi 1980), but in the recent studies and those reported previously (Wicks and Lee 1986) few of the isolates were virulent pathogens of almond.

P. megasperma which was isolated repeatedly from various almond orchards did not form extensive cankers on inoculated almond shoots nor did it rot almond roots extensively or form crown cankers on almond seedlings grown in infested soil. Although *P. megasperma* was recovered from roots of naturally infected plants (see chapter 5) and those grown in artificially inoculated soil, the results here and those reported elsewhere (Wicks and Lee 1986) show that *P. megasperma* is unlikely to cause serious problems in almonds.

Some isolates of *P. citricola*, *P. citrophthora* and *P. nicotianae* var *parasitica* caused extensive cankers on inoculated almond shoots

suggesting that further root inoculation studies should be made to determine if these isolates also cause severe root rot and crown cankers. An isolate of *P. citrophthora* recovered from almond soil was shown previously to rot roots, but it did not form crown cankers on plants grown in infested soil (Wicks and Lee 1986). Overall these results show that many species of *Phytophthora* are capable of severely rotting the feeder roots of almond, but it is only *P. cambivora* and occasionally *P. cryptogea* that attack the crown resulting in tree deaths when the infections girdle the trunk.

Although *P. cryptogea* and *P. megasperma* were isolated from South Australian cherry orchards and have been reported as pathogens of cherry (Miretich and Matheron 1976, Wilcox and Mircetich 1985a) none of the South Australian isolates of these species colonised shoots of cherry rootstocks (see also Chapter 3) as extensively as either *P. cambivora* or *P. syringae*. Because of this the main emphasis of cherry root inoculation studies were with isolates of *P. cambivora*. None of the local isolates of *P. cryptogea* or *P. megasperma* appeared as pathogenic to cherries as those reported from California (Mircetich and Matheron 1976, Wilcox and Mircetich 1985a).

The use of excised twigs and stem inoculations to compare relative virulence is unrealistic as the physiology of excised twigs or wounded stems may differ from that of naturally infected crown tissue. This is evident from the work of Matheron and Mircetich (1985) who have shown with walnut rootstocks that disease was greatest with excised stems than with stem inoculations and that the canker lengths were higher with these than with soil infestation. Similar results were obtained with almond where colonisation of excised twigs showed that A^1 and A^2 mating types of almond isolates of *P. cambivora* were equally virulent to almond whereas soil infestation studies with the

same isolates showed the A^1 mating type to be the most pathogenic isolate (Wicks and Lee 1986). Similarly excised 'Nemaguard' peach stems were readily colonised by *P. cambivora*, but root inoculation studies showed 'Nemaguard' to be resistant (Wicks and Lee 1985, 1986).

Nevertheless the use of excised twigs and inoculated stems were shown in the recent experiments to be useful techniques to select the most virulent isolates for further pathogenicity testing. For example most isolates that extensively colonised shoots also rotted roots and induced crown cankers whereas those *Phythphthora* species that did not rapidly colonise shoots rotted fewer roots and rarely induced crown canker. The virulence of *P. cambivora* isolates to different cherry rootstocks was similar with either excised twigs, inoculated shoots or soil infestation. The cherry studies showed that there was marked interaction between the mating type of the local cherry isolates of *P. cambivora* and the rootstock type. Thus the A^1 isolate was the most virulent when tested on Mahaleb rootstocks whereas the A^2 isolate was most virulent on Mazzard rootstocks. The reasons for this interaction cannot be explained as similar interactions were not obvious with A^1 and A^2 mating types of cherry isolates of *P. cambivora* obtained from the U.S.A. It is unlikely that this interaction has any practical significance however as most isolates of *P. cambivora* recovered from cherry orchards both here and elsewhere are of the A^1 mating type (see Chapter 3 and Miretich and Matheron 1976) and Mahaleb appears the most susceptible rootstocks in commercial cherry orchards.

These results show that before rootstocks are evaluated for resistance to *Phytophthora* the incidence of mating types of heterothallic species should be determined as well as the virulence of the predominant mating type occurring in affected orchards.

These results have also indicated that isolates of *P. cambivora* vary markedly in their degree of virulence and consequently any system to screen rootstocks should use a wide range of isolates or else selected isolates which are the most virulent. Variations in the virulence of other *Phytophthora* species have been reported, such as *P. cactorum* to apple seedlings (Aldwinkle *et al.*, 1975, Harris and Tobutt 1986 and Sewell and Wilson 1959), *P. cactorum* and *P. drechsleri* to walnut rootstocks (Matheron and Mircetich 1985b), isolates of *P. cinnamami* (Zentmyer 1980) and in the current studies isolates of *P. cambivora*, *P. citricola* and *P. citrophthora* showed large variations in their virulence to almonds.

In some cases variations in virulence of isolates may have been due to loss of virulence in culture as has been reported with isolates of *P. cactorum* (Harris and Tobutt 1986). In the present studies loss of virulence is considered unlikely as except for isolates obtained from interstate, the pathogenicity of most isolates was determined within six months from the recovery from soil or host tissue. In addition isolates such as P5, P9 and P80 that were used throughout these studies were regularly recovered from inoculated host tissue rather than sub-cultured from the original type cultures.

Although there was a trend for isolates of *P. cambivora* to be most pathogenic to the original host, the virulence of apple, cherry and pear isolates to almond as well as almond and apple isolates to cherry shows that *P. cambivora* has a wide host range and that isolates are not host specific. Since *P. cambivora* was a serious pathogen of almonds (Wicks and Lee 1960) and common in South Australian cherry orchards (Bumbieris *et al.*, 1982) studies to test the susceptibility of almond and cherry

rootstocks were undertaken mainly with this species and are described in Chapter 5.

CHAPTER 5.

SUSCEPTIBILITY OF ALMOND AND CHERRY ROOTSTOCKS AND SCIONS TO PHYTOPHTHORA

INTRODUCTION

The relative susceptibility of different rootstocks and scions to *Phytophthora* is of considerable importance to orchardists particularly where these fungi have killed trees and the sites require replanting.

The major studies evaluating rootstocks for resistance to *Phytophthora* has been confined to apples and *P. cactorum* and there are few critical studies on the resistance of stonefruit rootstocks to diseases caused by *Phytophthora*. In cherries, Mircetich and Matheron (1976) showed that Mahaleb rootstocks were more susceptible than Mazzard to *P. cambivora* and *P. megasperma* and in almonds, Wicks and Lee (1986) showed almond seedling rootstocks to be highly suceptible to *P. cambivora* whereas the Nemaguard peach rootstock was resistant.

One of the reasons why extensive screening programmes have not been conducted with *Phytophthora* in deciduous fruit and nut trees may be that these fungi have only relatively recently been recognised as causing significant problems in these crops.

Other possible reasons are the complex nature of the problem. For example, rarely are single species of *Phytophthora* recovered from dead or declining trees. Therefore in screening programmes, ideally a range of species and combination of species needs to be tested against each rootstock. Further complicating factors are the need to evaluate several isolates of

each species as there is often wide variations in the virulence of the same species, and interactions between the scion and stock may alter the susceptibility of the rootstock. Environmental factors may also affect rootstock susceptibility, and some of these will be considered in chapter 6. In this chapter studies on the susceptiblity of almond and cherry rootstocks and scions to a range of *Phytophthora* species are reported.

MATERIALS AND METHODS

(a) Almond rootstocks

Several experiments were conducted to compare the relative susceptibilities of almond, 'Nemaguard' peach and 'Nemaguard' peach x almond hybrid rootstocks to *P. cambivora*.

The first experiment compared 'Titan', a late flowering selection of the almond cultivar 'Nonpareil' (Jones, 1972) with the almond cultivars, 'Chellaston' and Mission. Ten, four-month old seedlings of each cultivar were inoculated by adding *P. cambivora* (P5) infested vermiculite to the soil in which each plant was growing. All plants were flooded for 24h once a week for six weeks. After a further four weeks growth in a shadehouse, the experiment was terminated and soil washed from the roots of all plants to expose any stem cankers. Where cankers were obvious the extent of colonisation was measured and small pieces of tissue were taken from the margins of the cankers and placed on to selective $P_{10}VP^{+}$ agar.

Further experiments compared the susceptibility of 'Mission' almond with 'Nemaguard' peach and selections of a 'Nemaguard' peach x 'Nonpareil' almond hybrid rootstock. Numbered selections (72, 120 and 123) of the hybrid material that originated from source trees at the Loxton Research Centre were propagated using tissue culture techniques. All plants were approximately

30 cm high when they were inoculated with *P. cambivora* (P5) and flooded as described previously. For each cultivar, a minimum of six plants was used. A similar number of uninoculated plants was also flooded for the same period. Two weeks after the final flooding, soil was washed from the roots of the inoculated plants and two of the uninoculated plants of each cultivar. Fresh root weight and canker lengths were measured and rotted root pieces placed on to selective $P_{10}VP^{+}$ agar. The experiment was repeated when further selections of hybrids became available from tissue cultured plants. In this experiment a minimum of nine plants were used for each treatment.

Field evaluation of 'Chellaston' almond, 'Nemaguard' and hybrid rootstocks was also carried out in an orchard naturally infested with *P. cambivora* in the Willunga district. Since this experiment also incorporated fungicides and solarisation treatments, further details and results are given in Chapter 7.

The susceptibility of plums to *Phytophthora* was also investigated since plum rootstocks have been recommended for almonds particularly in heavy soils (Baker and Gathercole 1977, Hartman and Kester 1983). Plum rootstocks were evaluated first by inoculating two year old shoots of mature trees. Ten shoots on each of the cultivars 'Brompton', 'Marianna' and 'Myrobalan' were inoculated in autumn and again in spring with either an A^1 mating type of an almond isolate (P5) or an A_2 mating type of cherry isolate (P9) of *P. cambivora*. The extent of colonisation was examined several times up to 40 weeks after inoculation.

In the second plum experiment, six months old rooted 'Marianna 2624' cuttings grown in peat/sand were inoculated with the almond isolate of *P. cambivora* (P5). Two days after inoculation the soil was flooded for 2 days

and then drained. Further flooding periods of one day every 14 days were imposed for another two months. Other treatments included for comparison, were unflooded and not inoculated (control), flooded not inoculated, flooded and inoculated, and inoculated not flooded. Two weeks after the final flooding, the fresh root and shoot weights were measured on 12 plants per treatment.

(b)Cherry rootstocks

Shoots of mature cherry rootstock trees and vegetatively propagated rootstocks were used to evaluate the susceptibility of various rootstocks to *Phytophthora*. In the first experiment one-year-old shoots on mature trees of either Mahaleb, Mazzard, or 'Stockton Morello' cultivars growing at Lenswood were inoculated by wounding the stem and placing a mycelial plug of either *P. cambivora* (P5) or (P9), *P. cryptogea* (P8) or *P. megasperma* (P10) into the wound. In these experiments the inoculated sites were sealed with coloured ribbon used to identify the different treatments. Ten shoots per treatment were inoculated in autumn and assessed 14 weeks later by measuring of the extent the canker development.

In a second series of experiments, eight to 10-month-old 'Colt', Mahaleb, Mazzard, and 'Stockton Morello' plants produced by tissue culture were grown in a peat/sand mixture inoculated in spring with vermiculite infected with either an A^1(P80) or A^2 (P9) mating type of *P. cambivora* recovered from cherry and an A^1 (P5) mating type of the same species recovered from almond.

All inoculated plants and a series of uninoculated controls were flooded for 48 hours every week for 10 weeks. For each cultivar, a series of uninoculated and non-flooded plants were included for comparison. Ten plants were used for each treatment. Six weeks before the completion of the

experiment approximately 50 ml of soil was removed from five plants chosen at random from each treatment. The soil was bulked and mixed for each treatment and then separated into two subsamples which were baited with ripening Packham pears. The experiment was terminated after the plants had grown for 22 weeks in a shadehouse where temperatures did not exceed 27°C. Shoot growth was measured on each plant and after soil was washed from the roots they were blotted dry with paper towels and weighed.

For each treatment 60 rotted root pieces were selected at random from at least five plants, cut into sections of 2.5 to 5 mm long and placed on $P_{10}VP^{+}$ selective medium. Plates were incubated for two weeks at 25°C and during this time fungal growth originating from the roots was examined microscopically for branched hyphae with swellings typical of *P. cambivora*.

Another experiment was conducted with tissue cultured plants of the same cultivars used above, but plants between 5 to 10 cm high were transplanted into a peat/sand mixture into which was added 20 ml of vermiculite inoculum per litre of soil mix. The vermiculite had previously been inoculated with both A^1 (P80) and A^2 (P9) isolates of *P. cambivora* recovered from cherry. The potted plants were grown in a shadehouse for two weeks to allow them to establish. After this period the plants were flooded for 24 hours once a week for eight weeks. A minimum of six plants were used for each treatment. The same number of similarly sized plants of each cultivar was potted into a peat/sand mix containing vermiculite but no *Phytophthora* inoculum. These plants were also flooded for the same periods as the inoculated plants. Plants were grown in a shade house and plant height measured at five, 10 and 15 weeks after transplanting. Soil was washed from the roots of plants to expose rotted roots and crown cankers. Small pieces of rotted roots and

pieces of tissue excised from the margins of cankers were plated on to $P_{10}VP^+$ selective medium. Once *Phytophthora* colonies were identified, the mating type of selected isolates from a number of roots and cankers was determined by crossing them with other *P. cambivora* isolates of known mating type. After 4 weeks incubation at 25°C in the dark the plates were examined for the presence of bullate oogonia.

(c)Stock-scion interaction

(i) Almond

To determine whether the scion cultivar influenced the susceptiblity of the stock and vice versa, an experiment was conducted using two year old almonds cultivar 'Nonpareil' grafted on to 'Nemaguard' rootstocks. The graft union was between 20 to 25 cm above the crown area of each rootstock. Plants were wound inoculated as previously described with a A^1 mating type of an almond isolate of *P. cambivora* (P5). The areas inoculated were either the scion stem approximately 2 cm above the graft union, the stem of the stock 2 cm below the union or at least 3 of the main roots of the rootstock. Control plants were wounded and inoculated with a plug of CMA on the stem of the scion and stock as well as the roots of each plant. Fifteen plants for each treatment were inoculated immediately before planting in the field at Lenswood in winter. A randomised block design of five rows was used with plants 1 m apart within the row and 1.5 m apart between the rows.

Fifteen weeks after planting the plants were dug up, and the roots washed with running tapwater before the extent of colonisation and shoot growth were assessed. Tissue from the margins of colonised scion or rootstock stems was placed on to $P_{10}VP^+$ selective medium.

(ii) Cherry

An experiment similar to that described for almond was conducted on cherry shoots to determine if the scion cultivar affected the rootstock susceptibility.

In this experiment 1-year-old shoots of Mazzard trees growing at Lenswood were grafted with the scion cultivars 'Stella', 'Napoleon' and 'Montmorency'. Successful grafts were only achieved with 'Stella' and experiments were initially confined to that cultivar. One year after grafting, shoots were inoculated with an A^2 mating type of a cherry isolate of *P. cambivora* (P9). This was done by removing a disk of bark and placing a mycelial plug into the wound. Ten shoots per treatment were inoculated within 10 to 15 cm either above or below the graft union.

A similar number of ungrafted rootstock shoots were also inoculated at the same time. Six weeks after inoculation the extent of colonisation was measured after the bark was removed with a scalpel to expose the typical zonate pattern of *Phytophthora* infection.

The experiment was repeated the following year using Mazzard rootstock shoots grafted with 'Merton Bigarreau'. Both stock and scion-wood were inoculated with the same isolate of *P. cambivora* used the previous season.

RESULTS

(a) Almond rootstocks

Experiment 1

Stem cankers originating in the crown area developed extensively in 'Chellaston' and 'Mission' almond seedlings. Most plants were killed when the cankers girdled the stem (Table 5.1 Experiment 1). Fewer cankers formed on

'Titan' plants and these were significantly smaller (34 mm long x 1 to 5 mm wide) than those on other almond plants.

Phytophthora was not recovered from canker margins on 'Mission' or 'Titan' plants, but was recovered from 40% of the 'Chellaston' cankers.

Experiment 2

All plants of the hybrid peach x almond selections, numbers 72, 120 and 123 developed extensive stem cankers (Table 5.1 Experiment 2). Crown cankers developed on inoculated Mission but not inoculated 'Nemaguard' plants. *Phytophthora* was readily recovered from cankers and rotted roots of Mission but was isolated less frequently from 'Nemaguard' roots and not from peach x almond hybrid roots. When the experiment was repeated with selections 64, 116 and 123, crown cankers developed on less than half of the plants of each selection. Most of these plants were killed when the cankers girdled the stem.

P. cambivora caused severe root rotting in these hybrids as the mean root weight of flooded, uninoculated plants was 31.4 g (± 8.5) compared with that of 14.7 g (± 5.8) for flooded inoculated plants. This was also reflected by the 20%, 27% and 45% recovery of *Phytophthora* from rotted roots selected at random from the selections 64, 116 and 123 respectively.

In the plum experiments cankers did not develop on any of the plum shoots inoculated in either autumn or spring.

In the root inoculation experiment regular periods of flooding either killed 'Marianna' rootstocks or severly inhibited growth (Table 5.2). Most plants

Table 5.1

The relative susceptibility of 'Chellaston', 'Mission' and 'Titan' almond, Nemaguard peach and hybrid almond ('Nemaguard' x 'Nonpareil') seedlings to *Phytophthora cambivora*.

Host	Fraction* of plants dead	Fraction* of plants with crown cankers	Canker length (mm) (± S.E.)
Experiment 1			
'Chellaston'	7/10	9/10	95 (± 16.8)
'Mission'	8/10	10/10	147 (± 7.7)
'Titan'	0/10	4/10	34.1 (±3.6)
Experiment 2			
'Mission'	0/10	5/10	30.8 (± 3.2)
'Nemaguard'	0/10	0/10	0
'Hybrids' (72, 120 & 123)	0/17	17/17	112 (± 9.9)

* Number of plants dead or with crown cankers per number of plants in the treatment.

Table 5.2: Growth of 'Marianna' plum rootstocks in soil inoculated with *Phytophthora cambivora*.

Treatment	Dead Plants %	Fresh weight (g) Root	Shoot
Not flooded or inoculated	0	38.9	55.6
Flooded not inoculated	25	9.2	15.6
Inoculated not flooded	0	29.0	34
Inoculated and flooded	75	3.3	7.2
l.s.d. (0.05 level)		10.6	8.9

were killed when grown in flooded infested soil whereas no plants died in infested soil that was not flooded.

(b) Cherry rootstocks

(i) Shoot inoculation

The cherry isolate of *P. cambivora* was the most virulent of the isolates tested on all rootstocks (Fig. 5.1). However, the extent of colonisation was more variable on Mahaleb than other rootstocks resulting in significant differences only between the cherry isolate of *P. cambivora* and *P. megasperma*. Colonisation by the cherry isolate of *P. cambivora* was at lest five times greater than that of *P. cryptogea* and *P. megasperma* on Mazzard and nearly three times greater than other isolates on 'Stockton Morello'.

Comparing the relative susceptibility of each rootstock against each isolate, there was no significant difference (P = .05) between rootstocks inoculated with either *P. cryptogea* and *P. megasperma*. However the cherry isolate of *P. cambivora* colonised Mahaleb significantly less (P = .05) than either Mazzard or 'Stockton Morello'. On the other hand the almond isolate of *P. cambivora* colonised significantly less Mazzard tissue than that of either Mahaleb or 'Stockton Morello'.

(ii) Root inoculation

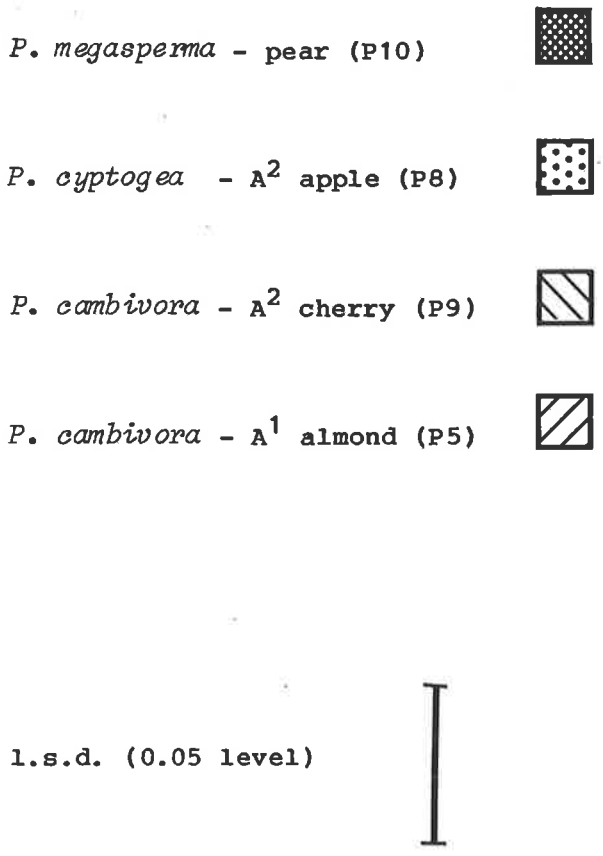
In this experiment all Mahaleb rootstocks died within 9 weeks of inoculating with cherry isolates of either the A^1 or A^2 mating types of *P. cambivora*. Similarly 40% of Mahaleb inoculated with the almond isolate of *P. cambivora* were killed within the same period. None of these isolates killed other rootstocks tested. Measurements of shoot length and root weight showed that most rootstocks were susceptible to both the A^1 and A^2 cherry isolates of *P. cambivora* (Table 5.3). The roots of Mahaleb rootstocks

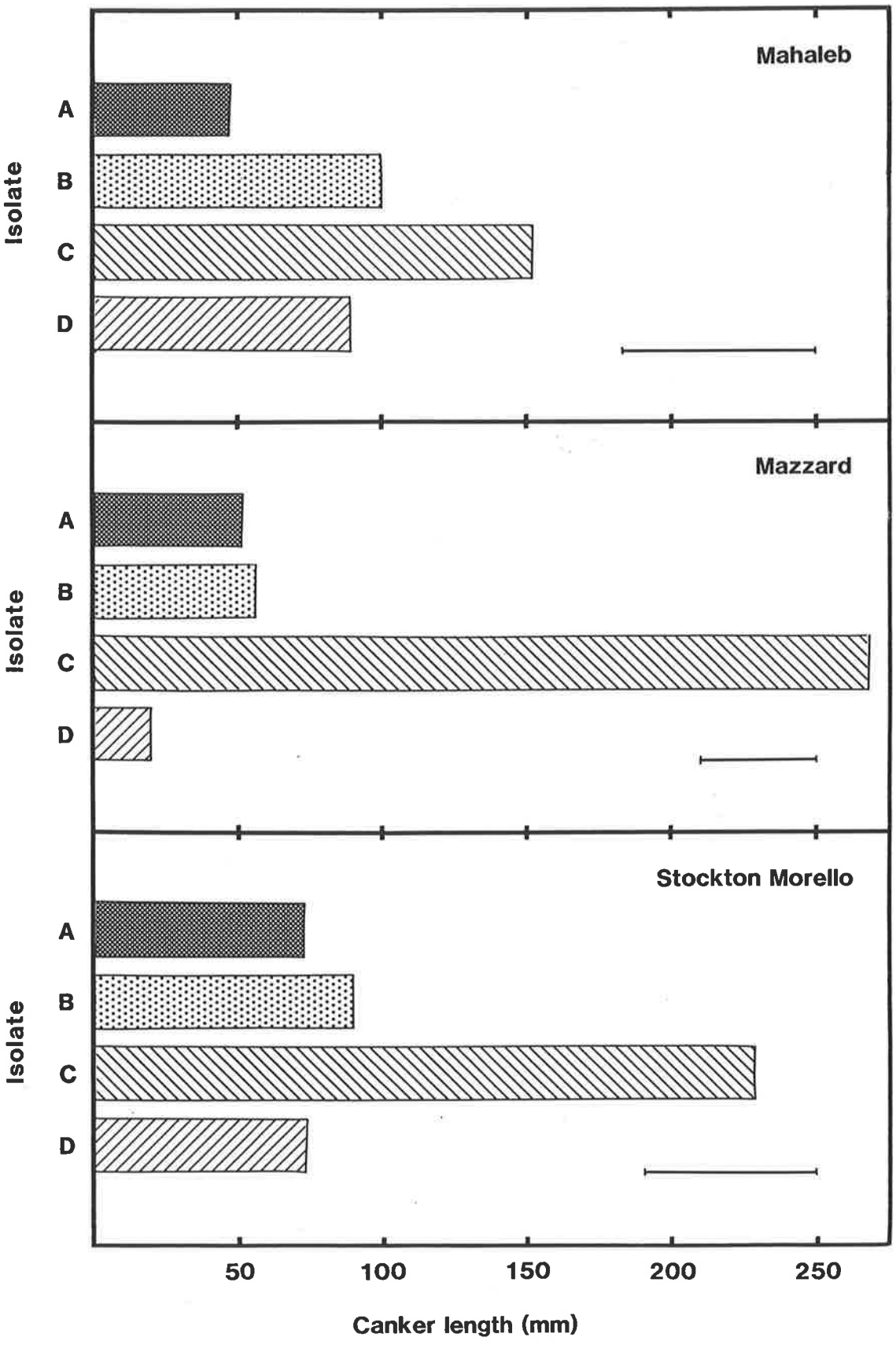
Table 5.3: Susceptibility of cherry rootstocks to isolates of *Phytophthora cambivora*

	Shoot length (cm)				Root weight (g)				*Phytophthora* recovered from roots			
	C	Mh	Mz	S	C	Mh	Mz	S	C	Mh	MZ	S
Control - not flooded or inoculated	119	117	100	105	215	220	180	135	0	0	0	0
Flooded - not inoculated	109	92	93	104	86	115	122	63	0	0	0	0
flooded and inoculated with												
P. cambivora - A[1] (almond, isolate P5)	80	71	81	107	51	32	97	88	40	8	3	5
P. cambivora - A[1] (cherry isolate P80)	92	60	70	114	91	27	85	99	38	28	3	5
P. cambivora (A[2]) (cherry isolate P9)	91	40	65	99	63	10	104	127	1	10	0	13
l.s.d. (0.5 level)	8	19	15	17	25	34	39	30				

C = 'Colt' Mh = Mahaleb Mz = Mazzard S = 'Stockton Morello'

Figure 5.1 The relative colonisation of shoots of three cherry rootstocks inoculated with either almond or cherry isolates of *Phytophthora cambivora*, *P. cryptogea* or *P. megasperma*.




Mahaleb
Mazzard
Stockton Morello
Isolate
A
B
C
D
50
100
150
200
250
Canker length (mm)

were extensively rotted by all isolates, more so than any other rootstock. *Phytophthora* was recovered from the roots of all inoculated plants, except those of Mazzard inoculated with P9. With other plants the recovery was less from Mazzard and Stockton Morello rootstocks.

In the second root inoculation experiment the growth of all cherry rootstocks was severely retarded when grown in infested soil (Fig. 5.2). Although no rootstocks were killed, crown cankers developed on at least 40% of all the rootstock cultivars. Most of the fine feeder roots of the rootstocks were severely rotted.

Both the A^1 and A^2 mating types were recovered from the soil, roots and cankers of infected plants. However the A^1 mating type was frequently recovered alone from plant tissue or soil samples whereas the A^2 mating type was only recovered in combination with the A^1 mating type from those sources.

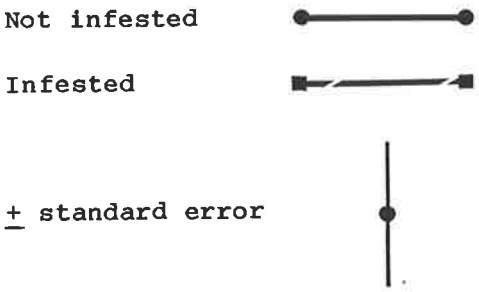
(c) Stock-scion interaction

(i) Almond

Twelve weeks after inoculation, *P. cambivora* had extensively colonised 'Nonpariel' stems producing cankers more than 11 cm long in most plants. Although the cankers girdled the stems and killed 93% of the scion shoot growth (Table 5.4), most of these plants produced new shoots from the rootstock. Cankers less than 4 cm long and 0.5 cm wide developed on smiliar plants inoculated on the Nemaguard rootstock stem. Six per cent of these plants died, but none produced new shoots from the rootstock.

Root lesions did not develop on inoculated 'Nemaguard' roots and no plants of this treatment or those of the uninoculated control plants died. Inoculating

Figure 5.2 Growth of four cherry rootstocks in soil artificially infested with *Phytophthora cambivora*.



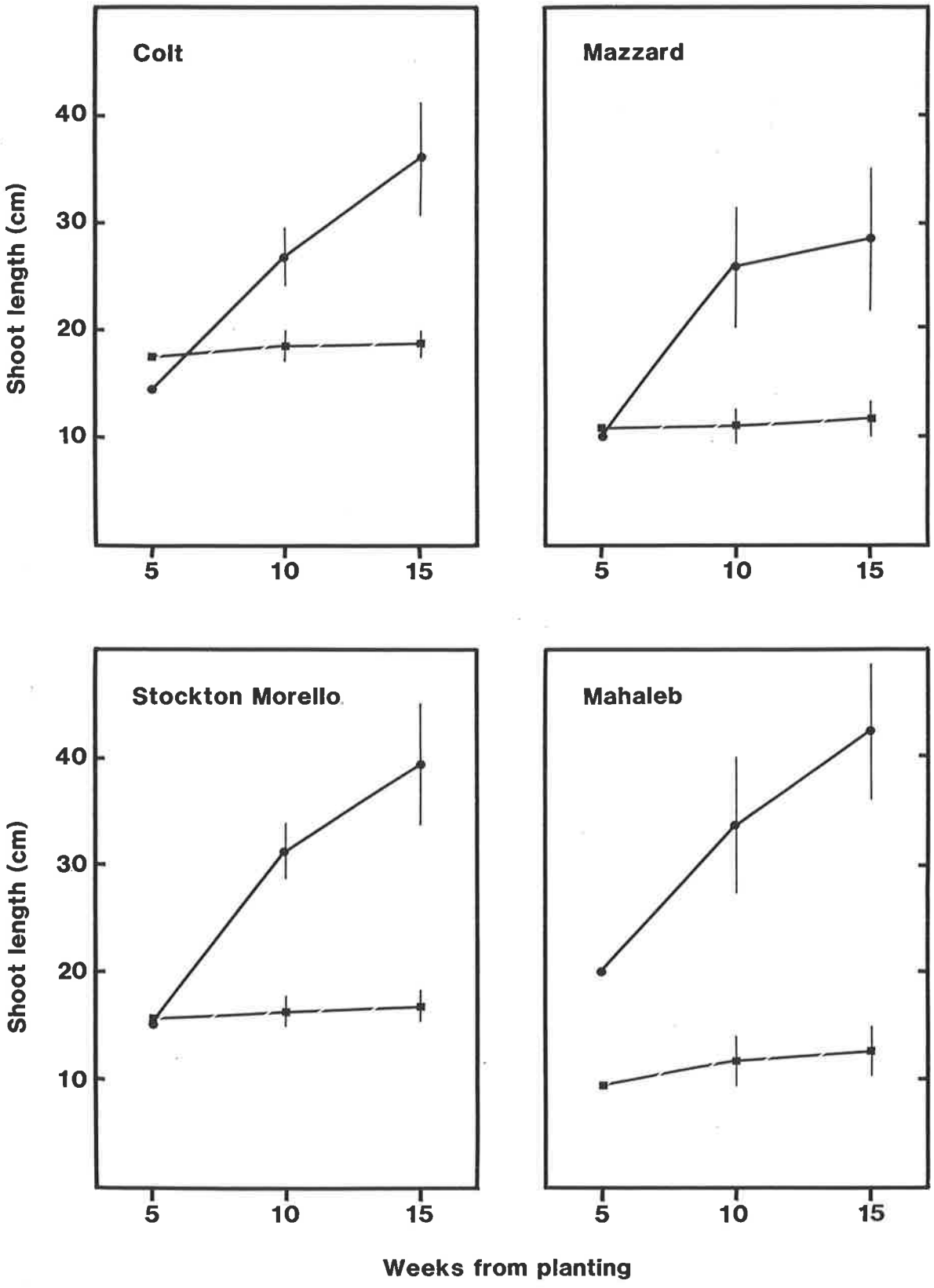

Colt
Mazzard
Stockton Morello
Mahaleb
Shoot length (cm)
10
20
30
40
5
10
15
Weeks from planting

Table 5.4

Colonisation of 'Nonpariel' scions and Nemaguard rootstocks inoculated with *Phytophthora cambivora*.

Tissue inoculated	% plants with dead scion	% plants with dead stock	Shoot growth (cm) (± SE)	Canker length (cm) (± SE)
Control (wounded not inoculated)	0	0	9.4 (±1.4)	0
Scion	93	14	4.7 (±0.4)	11.3 (±0.8)
Rootstock				
- stem	6	6	11.0 (±1.8)	3.7 (±0.5)
- roots	0	0	16.3 (±0.9)	n.a.

n.a. = not assessed.

Table 5.5

Effect of scion cultivar on the susceptibility of Mazzard cherry rootstock to *Phytophthora cambivora*.

Tissue inoculated	Canker length (mm) (± SE)	
	Experiment A 'Stella' scion	Experiment B 'Merton Bigarreau' scion
Stock - ungrafted	52.2 (±9.6)	84 (±17.6)
Stock - grafted	71.1 (±10.9)	97.2 (±9.1)
Scion - grafted	102 (±3.4)	157 (±7.2)

the rootstock stem did not reduce shoot growth when compared to growth of the control plants. On the other hand shoot growth was greatest in plants where the roots had been inoculated (Table 5.4). *P. cambivora* was not recovered from the margins of stem cankers of either stock or scion tissue nor was it recovered from inoculated roots.

(ii) Cherry

Canker lengths of both 'Stella' and 'Merton Bigarreau' grafted scions were nearly double that on ungrafted Mazzard stock. Table 5.5). However the extent of colonisation of Mazzard stock was not increased markedly by grafting the stock with a more susceptible scion cultivar.

DISCUSSION

These results confirm previous studies (Wicks and Lee 1986) showing almond seedlings to be most susceptible and 'Nemaguard' peach resistant to *P. cambivora*. Of the almond rootstocks tested, 'Titan', a late flowering mutant of Non pareil almond appeared most resistant to *P. cambivora* as no plants were killed, and fewer and smaller crown cankers developed compared to the other almond cultivars.

In these tests the plants were exposed to high levels of inoculum and conditions favourable for sporulation and presumably infection. Thus plants such as 'Titan' that showed low levels of infection, warrant further testing as these may exhibit "field resistance" that may be of benefit to growers.

Although 'Nemaguard' rootstocks are resistant to *Phytophthora*, other alternative almond rootstocks need to be evaluated as trees on 'Nemaguard' rootstocks are susceptible to lime induced chlorosis (F. Gatherole - personal communication), are generally shorter lived than those on almond stocks

(Hartman and Kester 1983) and are sensitive to waterlogging (Wicks and Lee 1985). None of the Nemaguard x almond hybrid selections showed resistance to *P. cambivora* in these tests as the fungus caused severe root rotting and extensive crown cankers on plants of all selections.

The clonal plum rootstock 'Marianna 2624' is also recommended as a rootstock suitable for almonds in "heavy wet soils" (Hartman and Kester 1983) but in the present experiments regular periods of flooding with or without *P. cambivora* killed plants and severly inhibited growth of the surviving plants. Despite this further testing of plum rootstocks in naturally infested orchards needs to be conducted as the flooding imposed in the experiment may have been unrealistically too frequent and not related to that occurring in the field.

Recently released rootstocks 'Hansen 2168' and 'Hansen 536' suitable for almonds need to be evaluated for tolerance to *Phytophthora* since "Hansen 536" is reported to be the most tolerant of this group of hybrid clones to *P. syringae* (Kester and Asay 1986).

The susceptibility of various cherry rootstocks was first tested by inoculating shoots and using an unknown mating type of *P. cambivora* as inoculum. The results obtained showed Mazzard were more susceptible than Mahaleb which differed from the Californian work of Mircetich and Matheron (1976) and Wilcox and Mircetich (1985a) who showed Mazzard to be significantly more resistant than Mahaleb to root and crown rot caused by *P. cambivora*.

These differences could not be explained until the inoculum was identified as an A^2 mating type and which was subsequently shown to differ markedly in virulence to the A^1 isolate on some hosts (see Chapter 4).

In the root inoculation experiments only Mahaleb rootstocks were killed by both mating types of *P. cambivora* which supports the Californian work showing Mahaleb to be more susceptible than Mazzard. Only *P. cambivora* isolates were used in the cherry root inoculation studies as this species was the most pathogenic of the *Phytophthora* species tested in stem inoculation studies (Chapter 3) and was the most common *Phytophthora* species found in local cherry orchards (Bumbieris et al. 1982). Other species such as *P. cryptogea* were not included although Wilcox and Mircetich (1985a) have recently shown this species to be a serious pathogen of both Mahaleb and Mazzard.

Apart from Mahaleb, 'Colt' also appeared susceptible to *P. cambivora* as this rootstock showed a significant reduction in root weight when grown in flooded and infested soil. This was also reflected by the high rate of recovery of *P. cambivora* from the roots of 'Colt' rootstocks.

In other studies Mircetich and Matheron (1981), reported 'Colt' as susceptible as Mazzard.

In the root inoculation experiments, the failure of the A2 (P9) isolate of *P. cambivora* to rot Mazzard roots severely was unexpected since the same isolate was highly pathogenic to Mazzard in other soil infestation experiments (see table 2.6 - Chapter 2) and also to inoculated stems. The lack of rotted roots and dead Mazzard plants as well as the inability to recover *Phytophthora* from the roots of inoculated plants is difficult to

explain especially ~~when~~ since the fungus was recovered from the soil of the same plants.

In the present studies root weight of all the cherry rootstocks was significantly reduced by periodic flooding in the absence of *Phytophthora*. The same flooding treatment did not inhibit shoot growth to the same degree as root growth. However in the experiment where rootstocks were planted in infested soil, the flooding treatment severely inhibited shoot growth when compared to growth in flooded not inoculated soils.

In this experiment the growth of all cherry rootstocks was inhibited by *P. cambivora*. Mahaleb was the rootstock most severly inhibited with the effect appearing five weeks after planting.

Overall these results show that none of the cherry rootstocks tested are resistant to *P. cambivora* and that severe growth reductions could occur when these rootstocks are used in soils subject to waterlogging.

These studies have also shown that root and crown rotting is more likely to occur in Mahaleb rather than on other cherry rootstocks when used in soils where *P. cambivora* is the main pathogen.

The rootstocks used in these studies are the most common stocks used in cherries (Hartmann and Kester 1983) which shows there is a challenge to develop horticulturally acceptable cherry rootstocks that are also resistant to *Phytophthora*.

Although the rootstocks of most tree crops are grafted or budded with a scion there are few published reports (Sewell and Wilson 1973b and Dakwa and Sewell 1981) on the interaction of stock and scion on trunk and crown rots caused by *Phytophthora*. Both these studies involved inoculating the scion and determining the effect of the rootstock on canker development. These workers showed that in apples there was an effect of rootstock on the scion susceptibility but this was related to its effect on tree vigour and not to the inherent rootstock resistance. This was also demonstrated in the present study with almond, where the resistant rootstock, 'Nemaguard' did not impart resistance to the almond scion. In this case most of the almond plants on Nemaguard stock died when the scion was inoculated and the resulting cankers girdled the stem. This has important practical implications in that the graft union on 'Nemaguard' rootstocks should be high to reduce the possibility of burying the susceptible scion at or after planting which would increase the risk of infection.

In the cherry experiments' the development of similar sized cankers on either grafted or ungrafted stock shoots indicated that the presence of the scion did not influence the susceptibility of the stock.

These results suggest that the susceptibility of rootstocks to *Phytophthora* is unlikely to change after they are grafted to a scion cultivar. Therefore in programmes to screen rootstocks for resistance to *Phytophthora*, the extra expense of using budded or grafted rootstocks is not justified. If the scion material is infected with virus however this may alter the rootstock susceptibility as has been shown with MM104 apple rootstocks and *P. cactorum* (Campbell 1969).

Other environmental factors may also affect host susceptibility to diseases caused by *Phytophthora* and some of these are discussed in Chapter 6.

CHAPTER 6

FACTORS AFFECTING THE SUSCEPTIBILITY OF ALMOND AND CHERRY TO PHYTOPHTHORA

INTRODUCTION

Numerous environmental factors affect the susceptibility of the host to diseases caused by *Phytophthora* (Shea and Broadbent 1983). For example in some plants, factors such as waterlogging (Kuan and Erwin 1980), water stress (Blacker and MacDonald 1981) and salinity stress (MacDonald 1982 and 1984) and heat stress (MacDonald and Shapiro 1985) increase root rot caused by *Phytophthora* pathogens. In other plants the susceptibility of the host varies with the season or growth stage. Apples for example are most susceptible to infection by *P. cactorum* in late spring early summer (Sewell and Wilson 1974, Jeffers and Aldwinkle 1986). The nutrition of the host also influences disease development as shown with nitrogenous fertilizers that significantly increased infection by *Phytophthora* (Utkhede 1984a). Other fertilizers such as those containing calcium are reported to suppress disease (Boughton *et al.*, 1978, Shea and Broadbent 1983).

Investigations were therefore undertaken on many of these factors since they could have an important bearing in the management of disease particularly in relation to time of planting, fungicide application, frequency of irrigation and fertiliser use.

MATERIALS AND METHODS

Several experiments were conducted to investigate the effect of factors such as seasonal variation, host tissue type, water logging, plant hormones

and plant nutrition on the susceptibility of almond and cherry to *Phytophthora*.

(a) Seasonal variation

To determine if the susceptibility of almond and cherry exhibited any seasonal variation, shoots were inoculated at various stages of plant growth and the extent of colonisation measured at different times after inoculation.

In the first experiment one year old shoots of an unknown almond cultivar were inoculated with an A^1 mating type of an almond isolate of *P. cambivora* (P^5) at either dormancy (June 14), early flowering (July 13), petal fall or four weeks after petal fall (August 4). The extent of colonisation was measured from three to 11 weeks after inoculation.

The experiment was repeated the following season using the same isolate of *P. cambivora* as well as an almond isolate of *P. syringae* (P81) and inoculating the same cultivar at similar growth stages as used previously. In the latter experiment the rate of colonisation for each stage of inoculation was measured for at least 60 days after inoculation.

Similar experiments were carried out on shoots of Mahaleb and Mazzard inoculated in autumn and spring with the A^1 (P80) and A^2 (P9) mating types of cherry isolates of *P. cambivora*. Shoots were also inoculated with *P. syringae* for comparison.

(b) Susceptibility of host tissue

Several experiments were set up to determine the relative susceptibility of root, crown and trunk tissue to *P. cambivora*.

In the first series of experiments, dormant one year old 'Mission' almond seedlings were knocked from their containers to expose the roots and crown areas. At least three roots approximately 0.4 mm diameter were inoculated per plant and the inoculated area of roots sealed with 'parafilm' before the plants were repotted. Another group of almond seedlings was inoculated on the stem in the area which was buried at least 5 cm deep. A final group of almond seedlings was inoculated on the stem approximately 10 cm above soil level. Ten plants were inoculated in each group using an almond A^1 isolate of *P. cambivora* (P5). Canker extension was measured approximately six months after inoculation.

The experiment was repeated with one year old Mahaleb and Mazzard cherry root stocks inoculated in the same areas as the almonds but using the cherry A^2 isolate of *P. cambivora* (P9).

In another similar experiment the roots of three year old dormant 'Chellaston' almond seedlings growing in 20L containers were exposed by gently removing the top 10 cm of soil. Roots 0.5 cm and larger were inoculated in areas that were at least 10 cm away from the trunk. On the same seedlings the crown area was inoculated on opposite sides of the trunk. Both roots and crowns were inoculated with the almond isolate of *P. cambivora* (P5) and the wounded areas sealed with 'parafilm' before the soil was replaced. The development of cankers was measured on five plants, six months after inoculation.

The roots of mature almond trees were also inoculated to determine if they responded similarly to roots of juvenile plants. In this experiment ten roots of mature almond trees of an unknown cultivar growing at Pooraka were

exposed by carefully removing soil from around the base of several trees. Roots between 4 to 14 mm diameter were inoculated in spring with an almond isolate of *P. cambivora* (P5). The inoculated areas were sealed with 'parafilm', and the roots covered with the original soil. Seven weeks after inoculation the roots were exposed and the length of cankers measured. For comparison 6 to 10 cm diameter scaffold limbs of these trees were also inoculated with the same isolate and the canker development measured.

In both almond and cherry experiments the rate of colonisation was measured on 10 shoots per treatment.

(c) Waterlogging

A number of experiments were conducted to determine if periods of waterlogging altered the susceptibility of almond and cherry plants to attack by *Phytophthora*. In these experiments one year old 'Mission' almond seedlings were inoculated in spring with an A^1 mating type of an almond isolate of *P. cambivora* (P5) three weeks before the flooding regimes were imposed. The plants were wound inoculated as previously described on the main stem two to five cm above soil level. The potted plants were flooded by placing them in plastic bins or buckets filled with tap water so that the soil surface was covered with at least 2 cm of water.

Plants were watered normally and allowed to drain or either flooded one or three days every one or two weeks. Immediately after flooding, the plants were drained. The flooding periods were maintained for four weeks after which the extent of colonisation and shoot length was measured on 10 plants per treatment.

The experiment was repeated in summer using similar treatments and replicates as above but extending the time of assessment until eight weeks after inoculation.

In both experiments wood chips were taken from the margins of cankers and plated onto P_{10} VP selective medium and soil samples from each treatment were baited with pears.

Similar flooding experiments were conducted with cherry rootstocks. The main stems of one year old Mahaleb rootstocks were inoculated in late summer with an A^2 mating type of a cherry isolate of *P. cambivora* (P9). One week later, plants were flooded 3 days per week for six weeks. Uninoculated flooded plants and plants inoculated but not flooded were not included for comparison. Canker length and shoot growth were measured on each of 10 plants per treatment after the final flooding period.

The experiment was repeated with Mahaleb seedlings of similar age inoculated in autumn and flooded over a period of four weeks. Shoot growth was not measured in the later experiment since the plants were dormant.

(d) Growth hormones

Cytolin, a commercial formulation of gibberellins GA_4 and GA_7 and benzyladenine is applied to cherries to promote vegetative growth from axillary buds which increase the fruiting capacity per tree.

To determine whether the use of this material alters the susceptibility of cherry wood to *Phytophthora* three experiments were conducted. In the

first experiment conducted in late spring, cytolin was mixed with equal volumes of Agseal® a commercial pruning wound sealant consisting of 1%, 9-hydroxy-quinoline sulphate and 1%, captafol in a polyvinyl acetate (PVA) based paint. The resultant mixture was applied with a brush to wounds made at the base of the stem of one year old Mahaleb seedlings by removing a section of bark 2 to 3 cm long and 0.5 cm wide. Another treatment included spraying leaves of Mahaleb seedlings to "run off" with a mixture of 4 ml of cytolin per litre of water which is three times the recommended rate. After the sprayed leaves and treated stems had dried, each plant was inoculated with the A^2 mating type of the cherry isolate of *P. cambivora* (P9). Uninoculated control plants were sprayed with water and the stems wounded.

The experiment was repeated in late summer on another batch of Mahaleb seedlings of similar age but in this case cytolin was mixed with an equal volume of tap water and brushed onto unwounded stems. The inoculum and other treatments were similar to the previous experiment. In both experiments ten plants were used for each treatment.

The extent of colonisation was measured eight weeks after inoculation in the first experiment and four weeks later in the second experiment.

Treatments similar to above were applied to mature trees of c v. 'Stella' grafted onto Mazzard rootstocks to determine if the response to cytolin differed between mature trees and seedlings. In this experiment cytolin was mixed with an equal volume of PVA based paint and applied by brush to all buds on the shoot up to 30 cm below the point of inoculation. On other shoots cytolin was also applied to buds and an area of shoot 2 to

3 cm long by 0.5 cm wide where the bark had been removed. Other treatments included painting buds and wounded areas with paint not containing cytolin.

All treatments including untreated shoots were inoculated with an A^2 mating type of a cherry isolate of *P. cambivora* (P9) immediately after the cytolin-paint mixture had dried.

Each treatment was applied to 10, two year old shoots randomised amongst four trees. The extent of colonisation was measured at various times up to 48 days from inoculation.

(e) Soil amendments

An experiment was set up to determine whether soil amendments of either urea or lime altered the susceptibility of cherry rootstocks to *Phytophthora*. In this experiment one year old Mazzard rootstocks previously grown in a pasteurised peat/sand mixture were planted in the field at Lenswood in spring. The rootstocks were planted in eight rows 2.5 m apart with treatments randomised and replicated once in each row. Replicates consisted of two plants 0.5 m apart and separated 1 m in the row from other treatments. The soil amendments were (a) none, (b) 10 g urea/m^2 (100 kg/ha), (c) 20 g urea/m^2, (d) 750 g hydrated lime/m^2 (7.5 ton/ha), (e) 1500 g hydrated lime/m^2 and (f) 20 g urea plus 1500 g hydrated lime/m^2 applied at planting, 4 weeks and 12 weeks later.

In autumn the main stem of each plant was inoculated approximately 30 cm above soil level with an A^2 mating type of cherry isolate of *P. cambivora* (P9) and the extent of colonisation measured eight weeks later.

The treatments were also applied in the next growing season when all plants were reinoculated in spring and assessed 8 weeks later.

RESULTS

(a) Seasonal variation

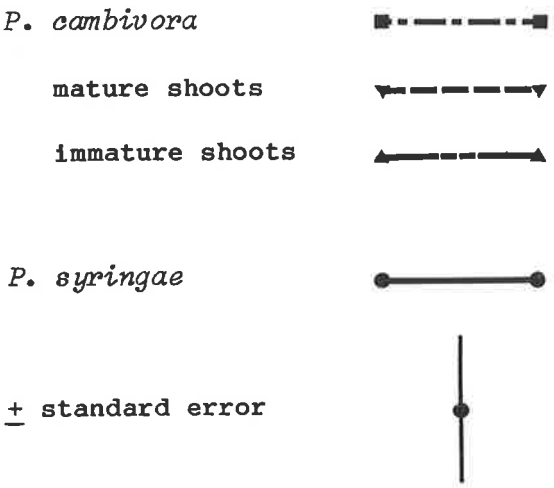
In the first almond experiment, cankers developed in all inoculated shoots but they were significantly shorter in shoots inoculated during dormancy when compared to those inoculated after flowering (Table 6.1). Cankers developed most rapidly on shoots inoculated during shoot extension. This was also reflected in the numbers of dead shoots; 20% were killed following dormant inoculations whereas up to 90% were killed when shoots were inoculated after flowering.

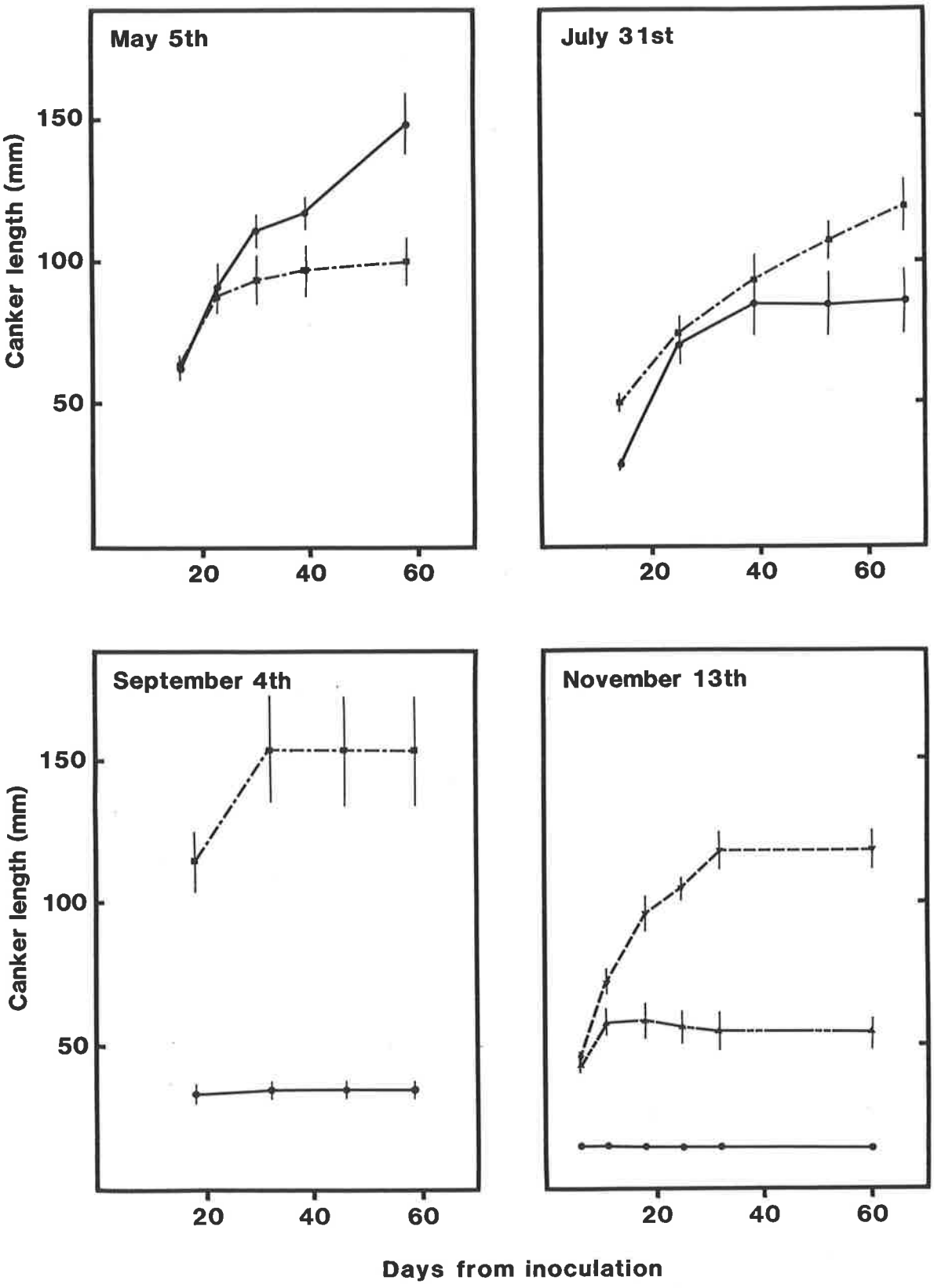
In the second almond experiment cankers induced by *P. cambivora* were generally longer than those produced the previous season. However a similar pattern emerged in that cankers developed most rapidly in spring (Fig. 6.1). With the November treatment both one year old and new developing 'green shoots' on the same tree were inoculated with *P. cambivora* for comparison. Canker lengths were significantly smaller and the cessation of canker expansion much earlier on the immature shoots compared to the older shoots.

Table 6.1 Colonisation of almond shoots infected with *Phytophthora cambivora* at various stages of growth.

Date of inoculation and growth stage	Canker length (mm) (±SE)	Time of assessment (weeks from inoculation)	% of shoots killed
June 14 - dormant	31.4 (±6.9)	11	20
July 13 - early flowering	80.4 (±8.1)	7	70
August 4 - petal fall	76.6 (±5.5)	4	70
October 5 - shoot extension	102.1 (±5.4)	3	90

Figure 6.1 Seasonal variation in colonisation of almond shoots inoculated with either *Phytophthora cambivora* or *P. syringae*.




May 5th
July 31st
September 4th
November 13th
Canker length (mm)
150
100
50
20
40
60
Days from inoculation

Extensive cankers formed rapidly on shoots inoculated with *P. syringae* in winter and before flowering, but they did not extend following the spring inoculations.

On Mazzard cherry, extensive cankers formed in both autumn and spring when shoots were inoculated with the A^2 mating type of *P. cambivora* or *P. syringae* (Fig. 6.2). Cankers rarely developed on Mazzard shoots inoculated with the A^1 mating type of *P. cambivora*. Cankers were less developed on Mahaleb but formed on all shoots inoculated with either isolate of *P. cambivora* or *P. syringae*. In both Mahaleb and Mazzard the development of cankers was greatest in shoots inoculated in May rather than September.

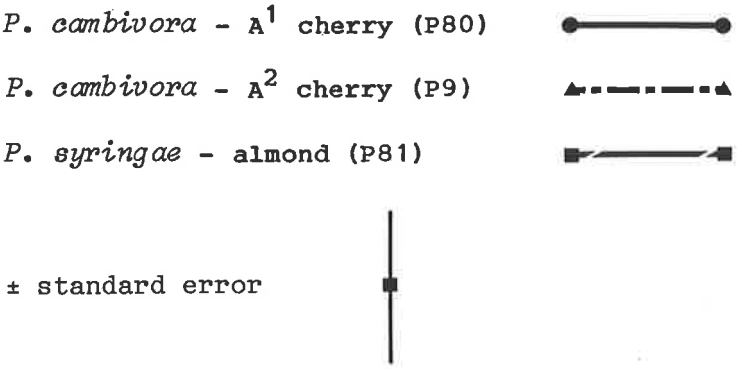
(b) Susceptibility of host tissue

In almond and cherry seedlings, cankers rapidly established in stem tissue and in most cases developed to a similar extent whether inoculated in the crown area below soil level or above soil level (Table 6.2). Cankers developed on inoculated almond roots but were not extensive and were approximately one fifth of the length of stem cankers. No cankers formed on inoculated cherry roots.

On three year old 'Chellaston' seedlings no cankers formed on inoculated roots whereas cankers up to 12 cm long formed from inoculated crowns and in most cases these girdled at least half of the trunk diameter.

On mature trees the development of root cankers was variable as they ranged from 8 mm long on the smaller roots to 170 mm on the largest roots.

Figure 6.2 Seasonal variation in colonisation of cherry rootstocks inoculated with either *Phytophthora cambivora* or *P. syringae*.



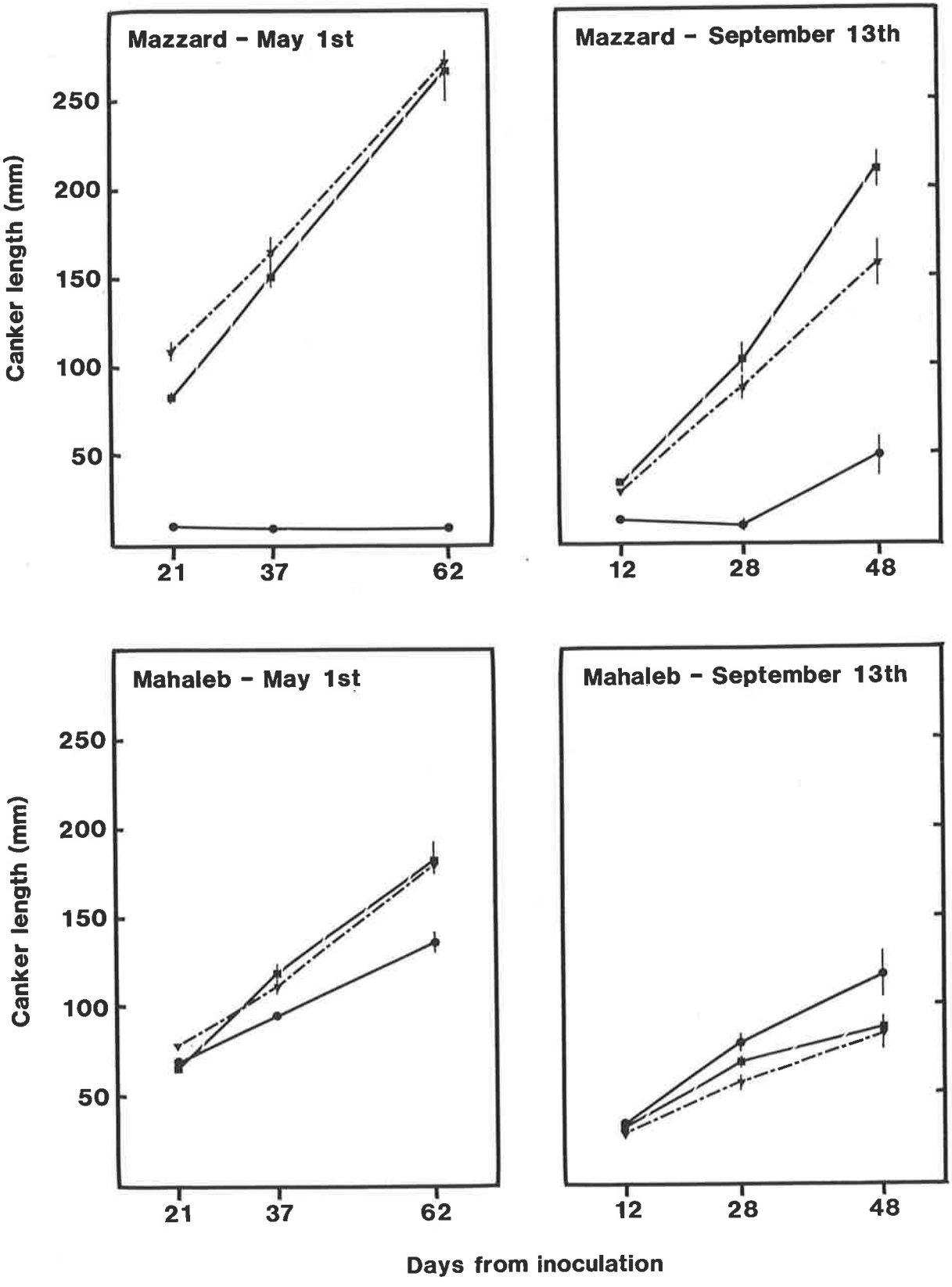

Mazzard - May 1st
Mazzard - September 13th
Mahaleb - May 1st
Mahaleb - September 13th
Canker length (mm)
250
200
150
100
50
21
37
62
12
28
48
Days from inoculation

Table 6.2 Relative susceptibility of almond and cherry stem, crown and roots to *Phytophthora cambivora*

Area Inoculated	Mean canker length (mm) (± SE)		
	Almond	Cherry	
		Mahaleb	Mazzard
Stem	67.5 (±15.8)	48.5 (±2.2)	61.9 (±4.8)
Crown	75 (±15)	32.0 (±7.6)	61.7 (±5.8)
Root	12.1 (±1.5)	0	0

Eight weeks after inoculation, cankers averaging 37 cm (±2.3) developed on inoculated limbs of the same root inoculated trees.

(c) Waterlogging

In experiment A, cankers developed in all inoculated almond seedlings and although the cankers in most flood treatments were larger than those of the un-flooded treatments these differences were not significant (Table 6.3). Cankers developed more rapidly in plants inoculated in summer (experiment B), but most cankers in the flooded treatments were not significantly longer than those in the un-flooded plants. In experiment A, flooding for three days and in experiment B, flooding for 1 day ~~per~~ every 2 weeks inhibited the development of cankers.

In both experiments flooding retarded shoot growth but the effect was more inhibiting in summer than in spring. For example three days flooding per week in summer reduced shoot growth to nearly half of that in the unflooded plants whereas the same flooding period in spring did not have a similar effect. *P. cambivora* was recovered from cankers in all treatments of both experiments except for plants flooded in summer for 3 days every 14 days. Pear baiting detected *P. cambivora* in each of 2 flooding treatments in experiments A and B as well as the unflooded treatment in experiment B.

In the cherry experiments canker lengths were similar in both flooded and control treatments where plants were inoculated in either January or April (Table 6.4). Shoot growth however was significantly reduced by the flooding periods.

Table 6.3 Effect of waterlogging on colonisation and shoot growth of 'Mission' almond seedlings inoculated with *Phytophthora cambivora*

Days flooded every 2 weeks	Canker length (mm)	Shoot length (mm)	Fresh shoot weight (g)
Experiment A*			
0	14.2	1224	14.5
1	21.0	1076	17.5
2 (1 day per week)	21.8	1151	19.7
3	9.7	613	10.4
6 (3 days per week)	24.2	967	16.0
l.s.d. (0.05 level	10.6	396	6.4
Experiment B**			
0	91.6	1005	14.3
1	60.0	788	8.9
2 (1 day per week)	106.0	554	5.8
3	87.0	651	6.5
6 (3 days per week)	93.7	581	6.1
l.s.d. (0.05 level)	21.1	358	4.2

* Inoculated September

** Inoculated January

Table 6.4 Effect of waterlogging on the colonisation of cherry seedlings infected with *Phytophthora cambivora*

Treatment	Canker length (mm) (±SE) Mahaleb*		Shoot growth (cm (±SE)	
	a.	b.	a.	b.
Control - normal watering	84.1 (±6.6)	76.1 (±11.3)	77.6 (±6.5)	n.m.
Flooded 3 days in 7	89.8 (±6.6)	75.3 (±6.6)	5.6 (±1.8)	n.m.

* Mahaleb inoculated, (a) January and (b) April

n.m. not measured

(d) Growth hormones

Neither painting the stems nor spraying the leaves of Mahaleb seedlings with cytolin significantly increased the extent of colonisation of plants inoculated in either November or March (Table 6.5). In the November experiment, cankers were significantly smaller on plants that had been sprayed with cytolin.

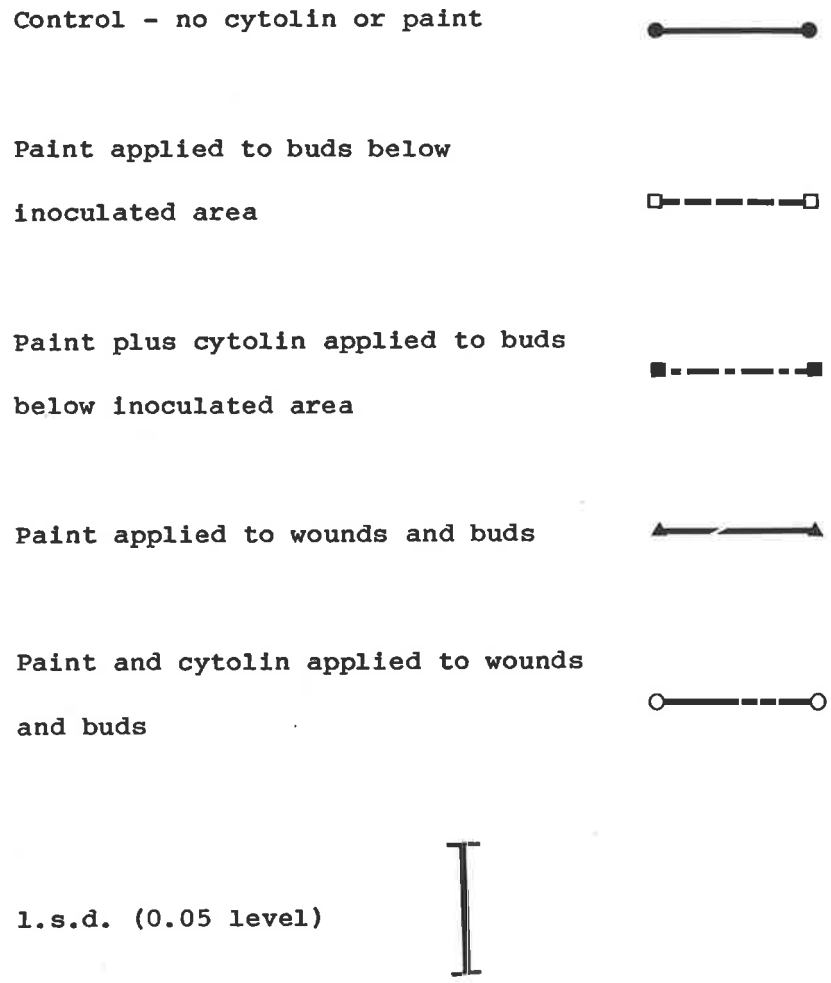
On mature trees, cankers were largest on shoots that had been wounded and painted with cytolin (Fig. 6.3) but these were not significantly larger than those treated with paint alone. The application of cytolin to buds of unwounded shoots did not increase canker lengths as they were not significantly larger than those on the shoots treated with paint or the untreated shoots.

(e) Soil amendments

Cankers developed on plants in all treatments and although there was a trend for cankers to be larger (29.2 mm) in plants receiving the highest rate of urea, the mean length was not significantly greater than that of the other treatments including that of the controls. When the treatments were repeated another ~~season~~ year and the plants inoculated in spring, cankers on plants treated with urea were not significantly longer from those of other treatments.

Measurements of calcium and nitrogen levels in cherry stem tissue were not made in this experiment as marked differences in colonisation were not obvious. However plants treated with urea were more vigorous and produced shoots with leaves greener than those of other treatments.

Figure 6.3 The effect of cytolin on the rate of canker expansion on 'Stella' cherry shoots inoculated with *Phytophthora cambivora*.



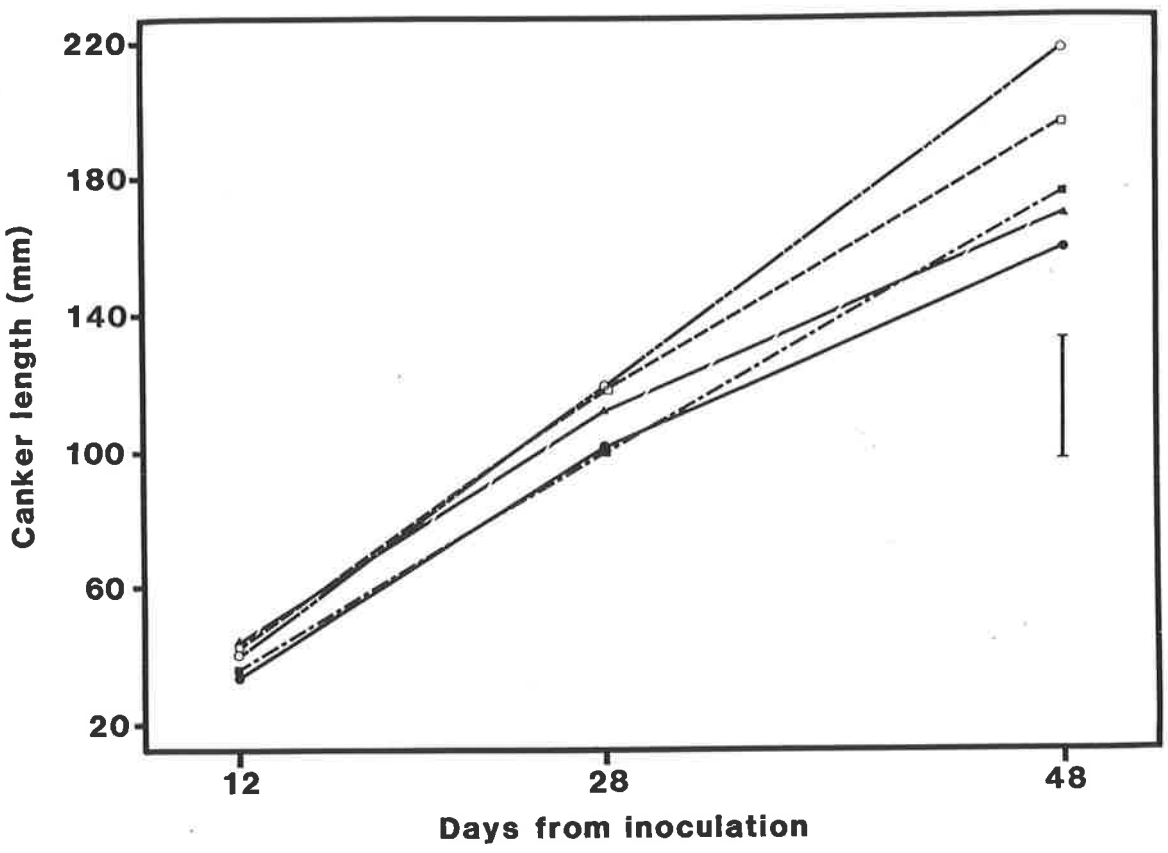

220
180
140
100
60
20
Canker length (mm)
12
28
48
Days from inoculation

Table 6.5 Effect of cytolin on colonisation of 1 year old Mahaleb cherry seedlings stems inoculated with *Phytophthora cambivora*

Treatment		Canker length (mm) Experiment	
Cytolin	Phytophthora	A*	B**
-	-	0	0
+ paint	-	-	-
+ paint	+	147.5	48.5
-	+	127.2	55.6
+ spray	+	83.7	51.8
+ spray	-	0	0
l.s.d. (0.05 level)		41.9	10.1

* Inoculated in November and assessed 8 weeks later.

** Inoculated in March and assessed 4 weeks later.

DISCUSSION

These experiments show that the stage of plant growth at the time of infection has a major effect on the development of canker diseases caused by *Phytophthora*. In almonds, *P. cambivora* colonised shoots most extensively during periods of rapid extension growth in spring whereas *P. syringae* failed to colonise almond at this time. Although the growth of *P. syringae* is severely inhibited at temperature above 25°C (Bostock and Doster 1985) such temperatures were rarely recorded at the times that almonds were inoculated indicating that other factors were inhibiting the fungus. Sewell and Wilson (1973a) showed similar effects in apples where *P. syringae* produced aggressive lesions only when inoculated in the dormant season. Several attempts have been made to identify the biochemical factors in host tissue that control the variation in host susceptibility. Borecki *et al.*, (1970) and Gates and Millikan (1972) isolated substances from apple bark tissue that stimulated *P. cactorum* growth in vitro, but the exact nature of these chemicals and their role in host susceptibility has not been elucidated.

In cherries, *P. cambivora* colonised both Mahaleb and Mazzard shoots most rapidly at the start of dormancy rather than spring and in contrast to the almond experiments, *P. syringae* continued to colonise cherry shoots inoculated in spring.

In both almond and cherry the times for optimum susceptibility correspond roughly to peaks in soil activity of the fungus as indicated in the soil baiting studies and with the development of field symptoms. For example

extensive colonization of inoculated almond tissue occurs in spring (September to November); the period when most *P. cambivora* isolates were baited from soil and the time when cankers on naturally infected trees are most obvious. While no pattern of soil activity was evident in the cherry studies, the highest frequency of isolation of *P. cambivora* from cherry orchards occurred in autumn which was also the period when cankers were first observed on naturally infected trees.

While further detailed studies are needed on periods of susceptibility, particularly with cherries, the present studies have shown that the susceptibility of stone fruits to *Phytophthora* differs from that of other tree fruit crops such as apples. Several workers such as Gates and Millikan (1972), Sewell and Wilson (1973a) and Jeffers and Aldwinckle (1986) inoculated excised twigs or shoots and showed apple trees to be most susceptible to *Phytophthora* in spring around the blossoming period and in most cases to be highly resistant to infection at other times. Jeffers and Aldwinckle (1986) also showed that the seasonal patterns of susceptibility varied with the rootstock type and the *Phytophthora* species, Peak periods of colonisation occurred with *P. cactorum* and *P. cambivora* in late spring and summer whereas peaks for *P. cryptogea* and *P. megasperma* occurred during summer and winter.

Artificially inoculating stems may provide unrealistic results however as this takes no account of the defence mechanisms that may be operating in the bark and other layers of tissue that are removed at the time of inoculation. Such resistance mechanisms would be operating in orchards yet the wound inoculation test only evaluates resistance after the pathogen has gained entry into the host. Matheron and Mircetich (1985a) have shown in

walnuts that stem inoculations with *P. citricola* indicated a higher disease severity than that achieved with soil infestation.

Another factor not examined in these studies was the effect of tree age on susceptibility. Most of the inoculation studies have been carried out on one or two year old wood and this tissue may react differently from that of tissue of mature trees. Sewell and Wilson (1974) report that in apples the period of susceptibility lengthens as trees grow older and this may explain the increased incidence of *P. cactorum* collar rot in older apple trees described by McIntosh and MacSwan (1966).

In the present inoculation studies, almond and cherry showed no period where they were resistant to infection by *P. cambivora*. Whether the susceptibility of almond and cherry trees shows any seasonal variation to natural infection is unknown. In the root inoculation studies with almond, (Chapters 4 and 5) almonds were planted in infected soil during March, April, October, December and January and crown cankers developed on plants at all planting times. This suggests that large variations in season susceptibility as shown with other plants is unlikely to occur in almonds. Whether this is also the case with cherries is unknown as root inoculation studies were only conducted during spring and early summer.

The root inoculation experiments comparing canker development on roots with that of stems showed that the roots of both almond and cherry are less susceptible to infection than stems. Taylor (1980) also showed that peach roots were more resistant than trunks to infection by *P. cactorum* and *P. cinnamani*.

These results have also been confirmed by field observations (Wicks unpublished) of roots and trunks on naturally infected almond trees that had been 'grubbed out' in a declining orchard at Willunga. In these trees root cankers had developed on some roots, but cankers longer than 150 mm were rare. Few cankers extended from the roots into the trunk and on declining trees, trunk cankers developed near soil level and completely or partially girdled the trunk.

Fine feeder roots of almond and cherry are extensively rotted by several *Phytophthora* species as observed in the root inoculation experiments conducted in these studies and elsewhere (Wicks and Lee 1985). The loss of fine feeder roots severely debilitated but did not kill plants which suggests that in the field, trees presumably recover from these infections when roots regenerate.

The root and stem inoculation studies have shown that plants are killed when the infections girdle the stem. These infections are likely to arise from direct penetration of the trunk at soil level or below rather than infections extending into the trunk from primary roots. Trunk infections are likely to occur following prolonged periods of soil saturation as a result of natural flooding or poor irrigation management. These conditions are suitable for zoospore release and infection as shown with *Phytophthora* crown rot of cherry (Wilcox and Mircetich 1985 b and c). Saturated soil conditions also result in a rapid decrease in soil oxygen concentration that affects respiration and may ultimately increase the plants' susceptibility to infection.

In the flooding experiments most flooding periods inhibited the growth of both almond and cherry seedlings. However none of the flooding treatments significantly altered the susceptibility of stem tissue to infection. These plants were inoculated before the flooding periods were imposed whereas in the field the flooding periods are more likely to coincide with the time of infection. Nevertheless in these experiments no significant increase in colonisation occurred following flooding. Davison and Tay (1986b) inoculated seedlings of *Eucalyptus marginata* Donn ex Sm. with *P. cinnamoni* and found that flooding did not predispose the plants to infection. They showed that the increased root infection produced by waterlogging was due to increased numbers of root lesions rather than an increase in lesion length. The present results confirm other work where almonds were flooded and inoculated with *P. cambivora* (Wicks and Lee 1985) but differs from the work of Blacker and MacDonald (1981) who showed that flooding predisposed a relatively resistant rhododendron cultivar to infection by *P. cinnamoni*. Although Davison and Tay (1986a) showed reduced growth of *P. cinnamomi* at low oxygen concentrations, it is unlikely that the flooding in the almond and cherry experiments inhibited the growth of *P. cambivora* since the inoculated stem area was not flooded.

The periods of water logging used in the almond and cherry experiments were similar to those observed in almond orchards following heavy rainfall in spring. Thus cankers developing on trees following these periods are likely to be a result of soil conditions suitable for infection resulting from the production and dispersal of inoculum rather than waterlogging increasing the susceptibility of trunk tissue to infection.

Although there are few reports of growth hormones such as gibberellins altering the host susceptibility to infection by *Phytophthora* spp, this aspect was investigated because of the increasing use of these materials in South Australian cherry orchards in recent years. In these experiments there was no clear evidence of commercial formulations of GA_4 and GA_7 significantly changing the host susceptibility even at rates which were greater than those recommended.

The effect of soil amendments such as lime on the susceptibility of host tissue was investigated as increasing the calcium level in soils has in many cases reduced the incidence of root rot caused by *P. cinnamomi* (Shea and Broadbent 1983). Furthermore such a treatment if effective would be a relatively cheap and simple procedure. Urea treatments were also investigated as nitrogenous fertilisers are frequently applied in almond and cherry orchards and such treatments have been shown to increase the percentage of apple trees affected by *P. cactorum* (Utkhede 1984a). Also it was considered that the use of nitrogenous fertilisers in the early establishment phase may result in plants with succulent bark tissue susceptible to infection by *Phytophthora*.

Lee and Zentmyer (1982) reported seedlings of *Persea indica* (L) Spreng to be more resistant to *P. cinnanomi* when grown in nutrient solutions containing high levels of calcium. They reported that roots were more resistant to zoospores and root lesions were smaller in plants grown in the high levels of calcium (160 mg/l). Bryt *et al.*, (1982) also reported that calcium cations occurred in soil at concentrations sufficient to stimulate encystment of *P. cinnamomi* zoospores and suggested that the encystment of zoospores could influence the rate of dispersion of the pathogen.

In the present studies, although calcium levels in cherry tissue were not measured, it is likely that they were high considering the frequent applications of hydrated lime. In addition depletion of calcium cations from the soil was probably negligible considering the high exchange capacity of the soil. Calcium is an important component in the structure of cell walls and increasing the concentration in the tissue of some plants has significantly increased the resistance of that tissue to fungal infection (Conway 1982, McGuire and Kelman 1984).

In the present studies however the application of high rates of lime did not reduce the susceptibility of cherry to infection by *P. cambivora*. Different effects may have resulted if the treated plants had been subjected to natural infection or artificial inoculation with zoospores. Similarly high applications of urea had no affect on the susceptibility of cherry to *P. cambivora*. This differs from the work of Utkhede (1984a) where the application of urea and other forms of nitrogenous fertilisers such as ammonium sulphate, ammonium nitrate, calcium nitrate and sewage sludge, significantly increased the percent of apple trees infected with *P. cactorum*. On the other hand Lee and Zentmeyer (1982) found plants with stem cankers caused by *P. cinnamomi* were fewer in soils treated with the highest rate (300 mg N/kg soil) of ammonium sulphate.

Although there is a complex interaction of host environment and pathogens operating in natural infections, the current series of experiments did not identify any environmental factor that predisposed almond or cherry plants to infection. However these studies, as well as field observations on the occurrence of symptoms, indicated that fungicide applications were most

effective if first applied in early spring in almonds and in early autumn in cherries. Furthermore fungicides were more effective if applied in a circular zone close to the trunk rather than widely broadcast under the tree canopy.

Fungicides applied in this manner were evaluated in both almond and cherry and the results of these experiments are described in Chapter 7.

CHAPTER 7

EFFECT OF FUNGICIDES ON THE CONTROL OF PHYTOPHTHORA CAMBIVORA

INTRODUCTION

The current studies have shown that the rootstocks of mature almond and cherry plantings in South Australia are susceptible to *P. cambivora*. The pathogen is widespread in many orchards and control measures are required to prevent further losses. Many of the plantings are in soils subject to frequent waterlogging and although improving soil drainage is likely to reduce further spread of the disease, in most established orchards this would be difficult and often costly to implement. In these cases the use of fungicides is considered the most economic and convenient means of controlling the disease.

Prior to the release of metalaxyl and fosetyl-Al in the late 1970's, few soil-applied fungicides except fenaminosolf (Dexon) were effective against root or collar rots caused by *Phytophthora* (Zentmyer 1980). Since that time however numerous reports have shown these materials effectively control *P. cactorum* in apple (Ellis *et al.*, 1982; Utkhede 1984b), *P. cinnamomi* in avocado and pineapple (Allen *et al.*, 1980; Coffey *et al.*, 1984b; Darvas *et al.*, 1984; Pegg *et al.*, 1985) and *P. parasitica* and *P. citrophthora* in citrus (Farih *et al.*, 1981). Both metalaxyl and fosetyl-Al are effective against numerous species of *Phytophthora*, on a wide range of crops, but there are few reports on the field evaluation of these materials on *P. cambivora* on stone fruits.

Preliminary studies by Wicks and Lee (1985) showed metalaxyl drenches inhibited *P. cambivora* on container grown almond seedlings. Investigations were carried out in vitro, on glasshouse grown plants and in orchards to further evaluate the efficacy of fungicides for the control of *P. cambivora* in almond and cherry trees.

MATERIALS AND METHODS

(a) In vitro studies

The initial in vitro studies were conducted with metalaxyl, but following the report of Fenn and Coffey (1984, 1985) on the inhibition of *Phytophthora* species with phosphorous acid (H_3PO_3), this material as well as fosetyl-Al was also included in some studies.

(i) Linear growth

The sensitivities of A^1 (P5 and P80) and A^2 (P6 and P9) mating types of *P. cambivora* to metalaxyl and P5 and P9 to H_3PO_3 was determined by growing the isolates on fungicide amended CMA.

Either metalaxyl formulated as Ridomil® a 25% active ingredient (a.i.) wettable powder or H_3PO_3 adjusted to pH of between 6 and 6.5 with KOH were added to the agar media just before pouring to give final concentrations ranging from 0.01 to 10 mg a.i./l metalaxyl and 2.5to 100 mg a.i./l H_3PO_3. A 4 mm diameter disk taken from the margin of an actively growing colony was placed centrally on each agar plate which was then incubated at 24°C for 7 days. Replicates of four plates were used for each treatment.

Two diameters at right angles to each other were measured for each colony. Control treatments consisted of CMA without fungicide. Growth on these plates compared to the amended agar was used to determine the degree of inhibition. Neither isolate had been exposed to metalaxyl or H_3PO_3 in the field.

(ii) Sporangium production

The effect of fungicides on the formation of sporangia was also studied with the A^1 (P5) mating type of the almond isolate of *P. cambivora*. In this experiment the fungus was grown for 3 to 5 days on LBA before 3.5 mm diameter disks were removed from the margin of several colonies. Disks with mycelia were placed in small Petri dishes and flooded with a suspension of metalaxyl, fosetyl-Al or H_3PO_3 at concentrations of 0, 1, 10, 100 and 1000 mg a.i./l.

Two H_3PO_3 treatments were used; in the first, all concentrations were adjusted to pH within the range of 6.2 to 6.5 with KOH, but with the second treatment, pH was not adjusted. This part of the experiment was conducted separately from that with the other fungicides. A soil extract was used to dilute the fungicide suspensions from the stock solutions. The extract was prepared by mixing 15 g of almond orchard soil in one litre of water, agitating this overnight and then filtering the suspension through 6 layers of paper tissue. Flooded mycelial disks were incubated for 3 days on a laboratory bench beneath a window where the temperatures ranged from 20 to 25°C.

Four disks were placed in each petri dish which was replicated three times for each metalaxyl and fosetyl-Al treatment and twice for the H_3PO_3 treatments. Mycelial disks were examined microscopically and the numbers of both mature and discharged sporangia produced around the margin of the disks were counted. The disks were left in the fungicide suspension for a further five days before the viability of each disk was tested. Disks were removed from the suspension, blotted dry on paper tissue and placed on $P_{10}VP^+$ selective medium. The presence or absence of mycelial growth from the disks was recorded after 10 days' incubation at 25°C.

(b) Glasshouse studies

(i) Almond

Previous studies by Wicks and Lee (1985) showed that metalaxyl inhibited the colonisation of inoculated almond stems when applied as a drench before inoculation. Experiments similar to these were conducted on one year old 'Chellaston' almond seedlings where drenches of either 250 mg a.i./l metalaxyl or 3200 mg a.i./l fosetyl-Al were compared with drenches of 100, 10^3 or 10^4 mg a.i./l of H_3PO_3. Drenches each of 200 ml per plant were applied to actively growing plants two days before the stem of each plant was inoculated with an A^1 (P5) type of an almond isolate of *P. cambivora*. Control plants were watered with 200 ml of tap water. Immediately after inoculation all plants were transferred to a growth cabinet maintained at 25°C.

Fourteen days after inoculation the extent of colonisation was measured on each of 5 plants per treatment and small wood chips were removed from the canker margin and plated on to $P_{10}VP$ selective medium. Another fungicide drench experiment was conducted in spring using eight to twelve months old

Mission seedlings previously shown to be infected with *P. cambivora*. These plants were obtained from inoculation experiments where *P. cambivora* was recovered from either the stem or roots of each plant. All plants were potted into soil which had been artificially infested with *P. cambivora* (P5) several months previously and from which the fungus was readily recovered just before the fungicide drenches were applied. The soil of each plant was drenched with 500 ml of either 250 mg a.i./l metalaxyl, 3200 mg a.i./l fosetyl-Al, or 10^4 mg a.i./l H_3PO_3 immediately after planting and six weeks later. Eight plants were used for each treatment. All plants were pruned to two shoots at the start of the experiment and shoot growth was measured at eight, 11 and 14 weeks from inoculation.

(ii) Cherry

A soil drenching experiment was also conducted using cherry seedlings. In this experiment 12 months old potted Mahaleb seedlings were drenched with 200 ml per plant of the same fungicides, times of application and concentrations used in the Chellaston almond experiment. All plants were inoculated with an A^2 (P9) mating type of a cherry isolate of *P. cambivora* 2 days after applying the first fungicide drench.

An additional treatment of applying 10^3 mg a.i./l H_3PO_3 as a foliar spray at 10 to 15 ml per plant was included in this experiment. Sheets of plastic were wrapped around the base of these plants to prevent run off from the foliar spray falling on to the soil. Canker lengths were measured on 8 plants per treatment 11 days after inoculation.

(c) Field experiments

Several field experiments to evaluate fungicides were conducted in naturally infested almond orchards at Willunga and in cherry plantings at the Lenswood Research Centre.

(i) Fungicide applications at planting

Experiments were set up to evaluate fungicide applications at planting. The first experiment compared the efficiency of metalaxyl, 500 mg a.i,/l; fosetyl-Al, 3200 mg a.i./l and H_3PO_3, 10^4 mg/l as a pre-planting dip, or a post-planting soil drench on infected and non-infected eight month old 'Mission' almond seedlings. The plants in this experiment had been infected six months previously by growing them in a peat/sand mixture inoculated with 1% vermiculite infected with an A^1 (P5) mating type of an almond isolate of *P. cambivora*. Healthy plants were grown in a similar mixture not inoculated with the fungus. Both healthy plants and infected plants were knocked out of the growing bags and the roots washed under running tap water. Initially the roots of bundles of healthy followed by the diseased plants were each dipped for 30 minutes in a 20 l suspension of fungicide before planting. The H_3PO_3 solution was adjusted to pH 6.5 with KOH before the roots were immersed. The other treatments in this experiment were applied immediately after planting. Fosetyl-Al and H_3PO_3, were applied at the rates used above at 2 l per plant whereas 'Ridomil' granules (5% a.i. metalaxyl) were applied at 25 g/plant. In this experiment approximately 250 ml of soil infested with *P. cambivora* was placed in the planting hole immediately before planting each infected plant.

Alternate rows of healthy and infected plants in a total of eight rows each separated 1.5 m apart were planted at the Lenswood Research Centre in winter. Treatments were randomised and replicated twice within a row with

plants 1 m apart. All plants were pruned to approximately 30 cm high and only the two youngest shoots retained for growth measurements at various intervals. To determine if other *Phytophthora* species occurred naturally in the trial area fifteen 250 ml soil samples were collected just before planting and baited with Packham pears. Samples were taken from a "W" shaped transect across the trial site. Soil samples from several dead or declining plants in the infected rows and from several plants chosen at random from the healthy rows were collected and baited with pears on several occasions throughout the growing season and at the completion of the experiment.

Metalaxyl was also applied to soil around Mahaleb cherry seedlings planted in soil naturally infested with *P. cambivora* at Lenswood. The trial designed primarily to evaluate solarization included fungicides for comparison and is described in detail in Chapter 8. The fungicide treatments comprised 50 g of 'Ridomil' granules applied in a $1m^2$ area around the trunk of each tree at planting and then in autumn and spring for the following two years with a total of five separate applications. The incidence of trunk cankers was recorded at various times up to 43 months after planting.

(ii) 'Nemaguard' rootstocks

Two separate experiments were conducted to determine whether fungicide applications were warranted when 'Nemaguard' rootstocks were planted in soils naturally infected with *P. cambivora*. The first experiment was conducted in an area of an orchard at Willunga where two rows of mature 'Chellaston' almonds had been removed from an area heavily infested with *P. cambivora*. Before replanting, the area was 'deep ripped' to a depth of approximately 60 cm to improve soil drainage. Two rows each of 40, two year old 'Nemaguard' rootstocks grafted to 'Chellaston' were planted in winter at 7 m

between rows and within the row. At the time of planting either 25 g of 'Ridomil' granules, 2 l of 3200 mg a.i./l suspension of fosety-Al, or 2 l of 50 or 100 g a.i./l of H_3PO_3 was applied to the soil surface in an area of approximately 1 m^2 around each tree. Additional treatments included urea at either 40 or 80 g per plant, hydrated lime at 250 or 500 g per plant, 40 g urea plus 250 g lime per plant and untreated control plants. Within each row, treatments were replicated four times and randomised. Butt diameter was measured, 15 to 20 cm above soil level at the time of planting and 10 months later. Soils from depths of 5 to 10 cm within 30 cm from the base of each plant were collected from untreated plots six weeks after planting and baited with pears. A second experiment was conducted on another naturally infested almond orchard at Willunga situated approximately 6 kilometres from the orchard used in the previous experiment. In this orchard, dead or severely declining mature 'Chellaston' trees were removed and two year old 'Nemaguard' seedlings replanted in the old tree sites. Within one metre of each of these plants one or more of six to 12 month old seedlings of hybrid ('Non Pariel' almond x 'Nemaguard'), 'Chellaston' and 'Nemaguard' were planted in autumn. 'Ridomil' granules, (5% a.i. metalaxyl) at 50 g/m^2 per site were applied to 12 sites and nine sites were left untreated.

Four sites that had previously been covered with plastic mulch (solarised) were also planted with similar seedlings in this orchard. Further details are given in Chapter 8.

Six months after planting all except the original control 'Nemaguard' plant of each site was dug up and returned to the laboratory where most of the soil adhering to the roots was washed away with running tap water. Plants were then examined for the presence of crown cankers, assessed for the degree of

root rotting and samples of rotted roots plated onto $P_{10}VP^{\dagger}$ selective medium. Plates were incubated at 25 C for at least 14 days during which time '*Phytophthora* like' mycelia that developed were transferred to CMA or LBA to aid identification of the species.

(iii) Mature almond trees

Extensive sampling of the almond orchard where *P. cambivora* was first discovered showed that the fungus was widespread within the orchard (Wicks and Lee 1986). 'Chellaston' trees within the most heavily infested area of this orchard were rated as either declining (one of the initial symptoms of crown rot) or vigorous and showing no decline symptoms or obvious cankers. Trees within each category received no fungicide or either 100 g or 200 g per tree of 'Ridomil' granules (5% a.i. metalaxyl), applied around the butt of each tree in a shallow trench 5 to 10 cm deep and within 10 to 15 cm from the trunk. Treatments were randomised and between 15 to 20 trees were included in each treatment. Over two years fungicides were applied on four occasions. These were in spring 1983, autumn and spring 1984 and autumn 1985. At the time of the final application, fungicide granules from the previous treatment were present on the soil surface and because of this the rate for each of the final treatments was reduced by half. Tree health was examined periodically with the final assessment made 12 months after the last fungicide treatment. Trees were rated as either dead, no further decline from the original rating, presence of gumming on the butt or scaffold branches and presence of new or old crown cankers. Attempts to recover *P. cambivora* from the margins of these cankers were not undertaken as previous studies (Chapter 3) had shown that the likelihood of recovering the fungus was low. The presence of cankers was confirmed by using an axe to remove the bark around a suspect area to expose the margin between healthy and diseased areas. Most cankers were just above ground but many were only obvious when soil to depths of 5

to 10 cm was removed from around the crown. New cankers had well defined margins between diseased and healthy tissue and usually showed a typical zonate pattern. These were readily distinguished from old cankers which had ill defined margins. Soil samples were collected occasionally from at least 5 treated and untreated trees of each category and baited with pears.

To determine if metalaxyl or a fungitoxic breakdown product was taken up and translocated within almond trees, shoots of untreated and metalaxyl treated trees were inoculated with the A^1 (P5) mating type of *P. cambivora* recovered from the orchard. For each treatment, 10 one year old shoots randomised amongst two trees were inoculated 27 days and again 96 days after the 1984 spring application of metalaxyl. The extent of colonisation on each shoot was measured after six weeks and used to determine the degree of inhibition by comparing the canker length of the untreated to that of the metalaxyl treated trees.

A similar number of shoots were inoculated the next season in May, 17 days after applying metalaxyl. Lesion lengths were measured 5 weeks after inoculation. A similar experiment was conducted in another Willunga almond orchard where *P. cambivora* had also been isolated from trunk cankers and soil. In this orchard 'Ridomil' granules (5% a.i. metalaxyl) were applied either in a furrow around the base of the trees at 200 g/tree or spread evenly on the soil surface at 100 g/m^2 in a 4 m^2 area around each tree. Treatments including an untreated control were randomised amongst two rows of healthy and declining mature 'Mission' trees with a minimum of 12 trees per treatment. Metalaxyl was applied on three occasions; the first in winter, the second eight weeks later in early spring and the third in autumn, 32 weeks after the previous application. As in the previous experiment shoots of metalaxyl treated and non treated trees were inoculated with

P. cambivora to detect the presence of metalaxyl. This was also done at 27 and 96 days after the second application of metalaxyl. Another series of shoots were inoculated 30 and 71 days after the final application of metalaxyl. AT least 10 shoots were inoculated for each treatment. On each occasion canker lengths were assessed on all shoots six weeks after inoculation.

(iv) Established infections

Experiments in almond and cherry trees were set up to determine the effect of metalaxyl soil applications on established infections. The almond experiments were situated at Pooraka 20 km north of Adelaide in an abandoned orchard of an unknown cultivar. In this orchard, trees were inoculated on opposite sides of the trunk at soil level, on scaffold stems 6 to 9 cm diameter and on one year old shoots four weeks after flowering. Trees were wound-inoculated as previously described with an A^1 (P5) mating type of an almond isolate of *P. cambivora*. At 0, 2, 4 and 6 weeks after inoculation 'Ridomil' granules (5% a.i. metalaxyl) were applied at 100 g per tree in a 5 to 10 cm deep trench 30 cm from the butt of the tree. At least 3 limbs and 5 shoots per tree were inoculated on each of the 6 tree replicates of each treatment which were randomised within uniform trees of one row. The extent of colonisation on butts, stems and shoots was measured four weeks after inoculation. Small wood chips were also taken from the margins of expanding cankers and plated on to $P_{10}VP^{\dagger}$ selective medium. To determine if there was a delay in the uptake of metalaxyl into the plants another group of almond trees in this orchard were treated at the same time as above with either 0, 25, 50 or 100 g of 'Ridomil'. Two year old shoots were inoculated with *P. cambivora* three weeks after applying the fungicide. The canker lengths were measured 14 weeks after inoculation on five shoots on each of four replicate trees per treatment which were randomised throughout the orchard.

The cherry experiments were conducted at Lenswood Research Centre where metalaxyl was applied in September to mature cherry trees that had been artificially inoculated. The section of the orchard used in this experiment consisted of the cultivars 'English Morello', 'Montmorency', 'Kansas Sweet', and 'Vega', grafted on to Mazzard rootstocks and grouped into pairs of each cultivar. In spring the rootstocks of all trees were inoculated at soil level with a A^2 (P9) mating type of a cherry isolate of *P. cambivora*. This was done by drilling a hole 1 cm deep and 0.6 cm wide into the trunk of the tree, and moistening the exposed tissue with approximately 0.5 ml of deionised water before placing two 0.5 cm diameter mycelial disks into the hole. A tightly fitting wooden dowel was hammered into the hole to prevent the inoculated area from drying out. The protruding dowel also served as a marker for the point of inoculation. Immediately after inoculation and seven months later in autumn 100 g of 'Ridomil' granules (5% a.i. metalaxyl) were spread on the soil surface in a 1 m^2 area around the trunk of one tree of each cultivar pair.

The rate of canker expansion was measured at various times up to 6 months after inoculation. Where the canker margins were not obvious, small areas of bark above the inoculated "point" were removed with a scalpel or small chisel until the typical zonate pattern of *Phytophthora* infected tissue was exposed.

One week after the second metalaxyl application, 20 shoots on each of four pairs of trees were inoculated with *P. cambivora* to determine if metalaxyl was translocated within the trees. Canker lengths on both untreated and treated trees were measured three and eight weeks after inoculation.

(d) Metalaxyl residues

Soils treated with 'Ridomil' granules were collected at various times after application and used for qualitative and quantitative measurements on the metalaxyl residues in the soil.

(i) Chemical analysis

Samples were collected from treated soils around almond trees at Pooraka and three orchards at Willunga and around cherry trees at the Lenswood Research Centre. The 'Ridomil' treatments from which the samples were collected, the sampling depth and time of sampling are shown in Table 7.8. At all the sites 250 ml of soil was collected from around the base of each of six trees in a treated area. Where granules had been placed in a furrow, samples were collected from positions beneath this area. Soil from each treatment was bulked, mixed thoroughly, air dried, sieved and ground. Subsamples each of 25 g were stored at -18°C before the metalaxyl residues were analysed using a gas chromatograph.

(ii) Soil leachates

Since the production of *P. cambivora* sporangia was severely inhibited by low concentrations of metalaxyl, soil samples treated with 'Ridomil' granules were tested for metalaxyl residues by determining the effect of soil leachates on sporangium production. Soil leachates were obtained from 'Ridomil' treated soils from the Lenswood Research Centre (Blocks E and K) and almond orchards A and C from Willunga. The soil samples used were some of those collected for metalaxyl residue analysis except that leachates were extracted before the samples were frozen. For each soil sample, four 25 g subsamples were each mixed with 100 ml of tap water and vigorously shaken for 30 minutes. The suspensions were left to settle for a further 30 minutes after which the supernatant was filtered through six sheets of paper tissue.

The filtrates from the four subsamples were mixed together and used to flood mycelial disks of *P. cambivora* taken from the margins of expanding cultures growing on L.B.A. Four disks per dish were replicated twice for each soil sample. An A^2 mating type of a cherry isolate of *P. cambivora* was used for the soils from Lenswood and an A^1 almond isolate (P5) for the Willunga soil samples. In some tests, mycelial disks were flooded in tap water only. As a check on the extraction of metalaxyl from the soil, 5 g of 'Ridomil' granules were mixed with 100 g of each soil sample before the sample was shaken with water. Flooded mycelial disks were incubated on the laboratory bench beneath a window where the temperature varied between 15 to 25°C.

After two days incubation, the numbers of sporangia produced around the margin of the disks were counted. The viability of each disk was also checked after they were incubated for a further five days in the soil leachates. After this period, each disk was removed from the leachate, blotted dry, placed on $P_{10}VP^{+}$ selective medium and incubated at 25 C for at least 10 days.

(iii) Soil bioassay

A method similar to that described by Bailey and Coffey (1984) was used to detect metalaxyl levels in the soil that were inhibitory to mycelial growth of *P. cambivora*. The soils were collected from various depths at the Pooraka site where trees had been treated five weeks previously with 25, 50 or 100 g of Ridomil granules per tree. Samples used for this test were the same as those collected for chemical analysis. For each site an air dried, ground sub sample of 10 g was shaken with 100 ml of water for an hour. The suspension was filtered through Whatman No. 1 paper and a 10 ml sample of the filtrate was added to 90 ml of CMA and autoclaved. Cornmeal agar containing

the soil extract was poured into plates which after cooling were inoculated with 5 mm diameter disks taken from the margin of actively growing colonies of an almond A_1 (P5) mating type of *P. cambivora*. Colony diameters were measured after seven days' incubation at 25°C. Soil samples from the same site that had not received 'Ridomil' granules were used as controls to determine the degree of inhibition of mycelial growth.

The bioassay procedure of Bailey and Coffey (1984) was also used to determine the levels of metalaxyl in the 'Ridomil' treated soils from Pooraka. Known quantities of metalaxyl were added to previously untreated soil, the leachate extracted and then added to CMA which was inoculated with *P. cambivora* and used to calculate a standard response curve. By referring the degree of inhibition on agar prepared from 'Ridomil' treated soil to the standard curve, the actual metalaxyl concentration in the soil was determined.

RESULTS

(a) In vitro studies

(i) Linear growth response

Linear regression of probit percent inhibition of mycelial growth and log concentration of metalaxyl showed that all isolates of *P. cambivora* were sensitive to metalaxyl (Figure 7.1a) some more so than others. The A_2 mating types were more sensitive than the A_1 mating types in both the almond and cherry isolates.

For example at 1 mg/l, metalaxyl inhibited the A^2 almond isolate by nearly 90% whereas the same concentration inhibited the A^1 isolate by 77%. Most isolates however were inhibited 70% or more by 1 mg/l of metalaxyl.

Similar regression analysis with phosphorous acid showed that both the almond A_1 and the cherry A_2 mating type of *P. cambivora* were sensitive to phosphorous acid (Figure 7.1b). The almond isolate was the most sensitive at all concentrations with nearly complete inhibition at 100 mg/l.

The ED_{50} values calculated from the regression equations were as follows:

Almond - A_1 (P5) isolate - 0.16 mg/l metalaxyl and 7.0 mg/l H_3PO_3;

A_2 (P6) isolate - 0.03 mg/l metalaxyl

Cherry - A_1 (P80) isolate - 0.11 mg/l metalaxyl

A_2 (P9) isolate - 0.006 mg/l metalaxyl and 83 mg/l H_3PO_3

(ii) Sporangium production

The production of sporangia was severely inhibited by 10 mg/l metalaxyl and at 100 mg/l or greater inhibition was complete (Table 7.1)

Sporangium production was less sensitive to fosetyl-Al and phosphorous acid as complete inhibition was reached at 1000 mg/l. Also at 1 mg/l, of either metalaxyl, fosety-Al or phosphorous acid (adjusted), sporulation was inhibited by 92%, 26% and 40% respectively. Mycelial growth was inhibited completely after the disks were incubated in either 100 or 1000 mg/l metalaxyl. Mycelia grew from 50% and 25% of disks treated with 1 and 10 mg/l metalaxyl, respectively but growth was severely inhibited being less than half the rate of that for disks incubated in untreated leachates. Mycelial disks were viable after incubation in all rates of either fosetyl-Al or phosphorous acid.

Figure 7.1(A and B) Dose response curves of *Phytophthora cambivora* on corn meal agar containing either metalaxyl or H_3PO_3.

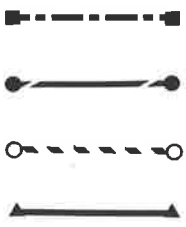
A Linear regression of probit percent inhibition of mycelial growth against log concentration of metalaxyl.

A^1 almond (P5)

A^2 almond (P6)

A^1 cherry (P80)

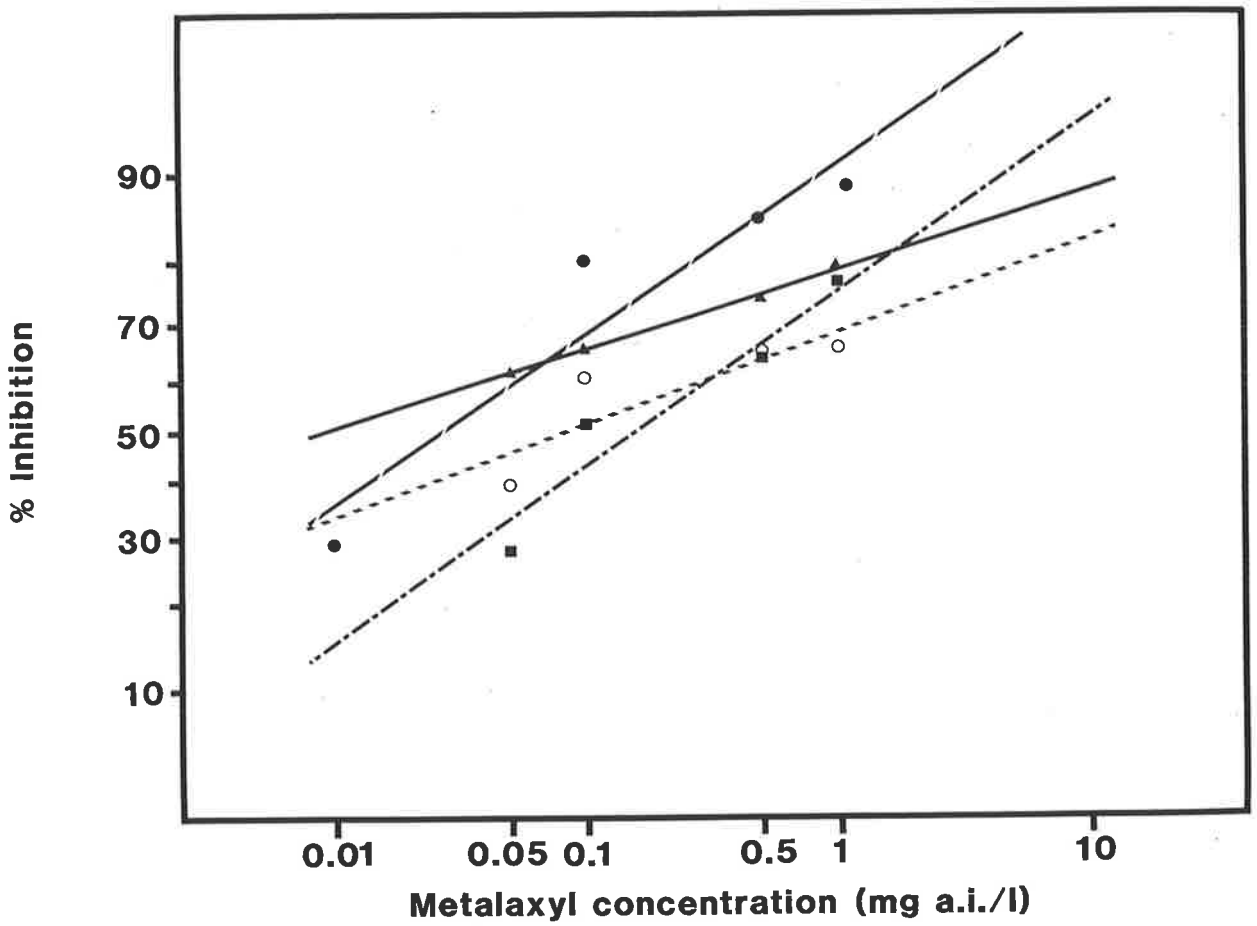
A^2 cherry (P9)



B Linear regression of probit percent inhibition of mycelial growth against log concentration of H_3PO_3.

A^1 almond (P5)

A^2 cherry (P9)


% Inhibition
90
70
50
30
10
0.01
0.05
0.1
0.5
1
10
Metalaxyl concentration (mg a.i./l)

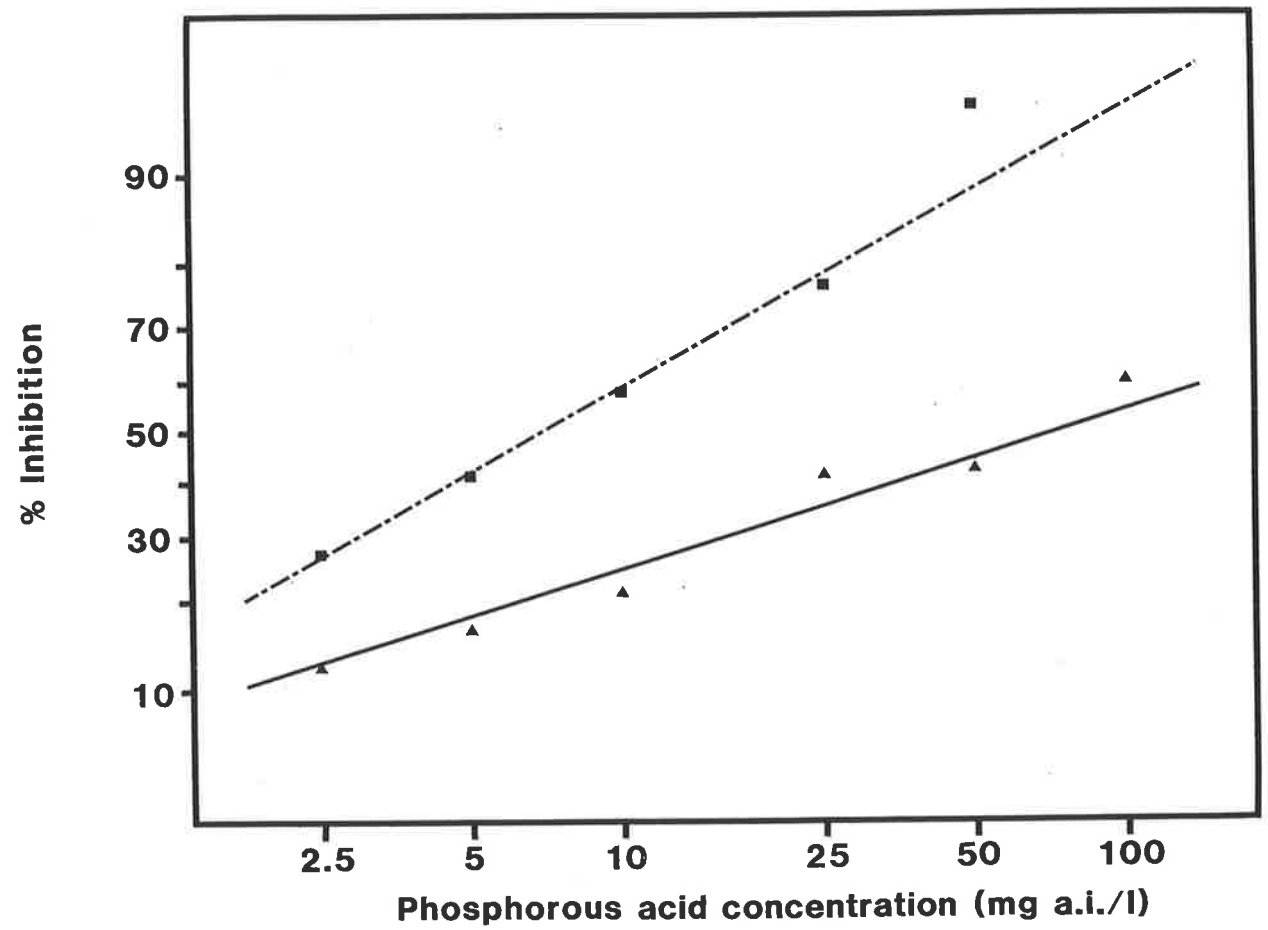

% Inhibition
90
70
50
30
10
2.5
5
10
25
50
100
Phosphorous acid concentration (mg a.i./l)

Table 7.1 The formation of *Phytophthora cambivora* sporangia on disks of lima bean agar flooded with fungicide suspensions

Concentration mg/l a.i.	Mean sporangia production per disk (±SE) Metalaxyl	Fosety-Al	Phosphorous acid pH* adjusted	Phosphorous acid pH+ not adjusted
0 (soil extract)	165 (±25.4)	165 (±25.4)	23.4 (±2)	23.4 (±2)
1	13.2 (±2.3)	121 (±9.4)	13.9 (±1.9)	21.8 (±2)
10	3.2 (.0)	95.8 (±10.1)	5.6 (±1.2)	9.5 (±1.1)
100	0	48 (±6.9)	5.1 (±1.1)	0
1000	0	0	0	0

* pH between 6.5 and 6.2

+ pH ranged from 5.8 to 2.6

(b) Glasshouse studies

(i) Almond

All fungicide drenches significantly inhibited the development of cankers although none eradicated the fungus from infected tissue (Table 7.2). Phosphorous acid at 10^4 mg/l inhibited canker development completely, but was phytotoxic causing brown necrotic lesions on young shoots and inhibiting shoot growth compared to most other treatments. Although not significantly different from most other fungicide treatments, canker lengths were smallest and the shoot growth greatest in plants treated with metalaxyl. Canker lengths in metalaxyl treatments were significantly shorter than those with the phosphorous acid treatment at 100 mg a.i./l, but not with other fungicide treatments.

Table 7.2 Effect of fungicide drenches on colonisation of stems of 'Chellaston' almond seedlings inoculated with *Phytophthora cambivora*.

Fungicide mg a.i./l	Canker length (mm)	Shoot growth (cm)	% shoots dead	% Recovery of *Phytophthora*
Nil	99.5	24.5	80	60
Metalaxyl 250	9.5	111	0	25
Fosetyl-Al 3,200	14.2	91	0	38
Phosphorous acid 100	32.8	64	30	40
Phosphorous acid 1,000	26.0	103	0	54
Phosphorous acid 10,000	0	62	0	6
l.s.d. (0.05 level)	18.8	29		

Table 7.3 Effect of fungicide drenches on the colonisation of stems of Mahaleb cherry seedlings inoculated with *Phytophthora cambivora*.

Fungicide mg a.i./l	Canker length (mm)	% Recovery of *Phytophthora*
Nil	75	8
Metalaxyl 250	0	20
Fosetyl-Al 3,200	10.7	40
Phosphorous acid 100	57.1	21
Phosphorous acid 1,000	9.3	26
Phosphorous acid 10,000	0	59
Phosphorous acid 1,000 - spray	44.5	23
l.s.d. (0.05 level)	10.8	

In the second experiment using infected plants in heavily infested soil, no substantial shoot growth occurred in any of the fungicide treated plants (Figure 7.2). Phosphorous acid was again phytotoxic and severely inhibited shoot growth.

(ii) Cherry

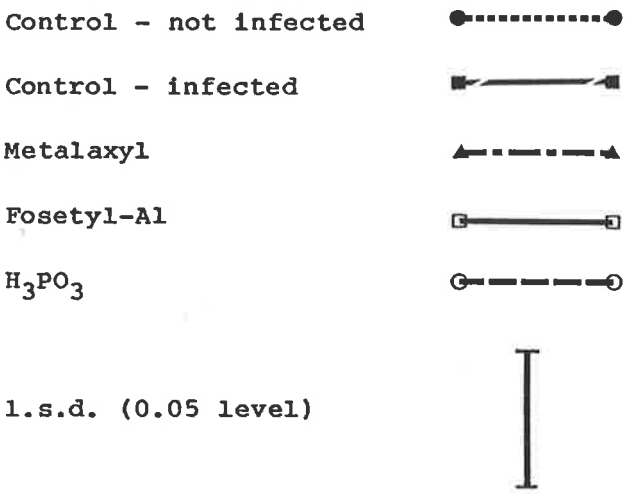
All fungicide treatments significantly inhibited canker development compared to the control, but only metalaxyl and the 10^4 mg/l rate of phosphorous acid inhibited cankers completely (Table 7.3). *P. cambivora* was recovered from all treatments including those where no cankers developed. Phosphorous acid drenches were not phytotoxic to cherries, but foliar sprays caused marginal leaf burn.

(c) Field experiments

(i) Fungicides at planting

The growth of severely infected plants was not improved by either dipping plants in fungicides before planting or applying fungicides immediately after planting (Figure 7.3). Within each batch of either infected or uninfected plants there was no significant difference between any fungicide and the untreated control plants. There were however significant differences between the growth of infected and uninfected plants. This occurred in all the fungicide treatments as well as the untreated plants. Measurements of growth rates on the untreated plants showed that the growth of infected plants was severely retarded compared to that of uninfected plants (Figure 7.4). Five weeks after planting, between one to three plants were dead in each of the treatments except those treated with either metalaxyl or phosphorous acid

Figure 7.2 Effect of fungicide drenches on the growth of 'Mission' almond seedlings in soil artificially infected with *Phytophthora cambivora*.



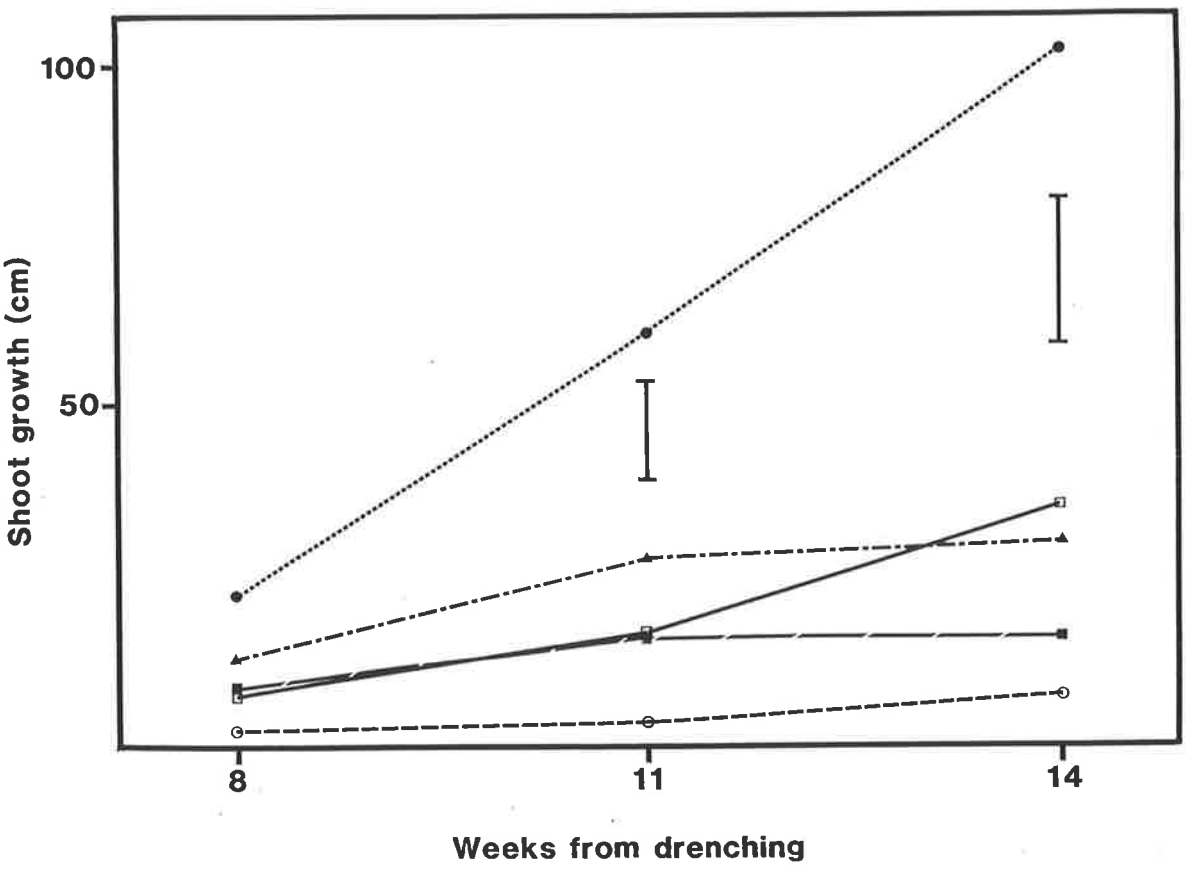

100
50
Shoot growth (cm)
8
11
14
Weeks from drenching

Figure 7.3 Effect of fungicides on the growth of 'Mission' almond seedlings with and without *Phytophthora cambivora* inoculation.

Fungicide treatments

A = Nil

B = Metalaxyl - 500 mg/l (dip)

C = Fosetyl-Al - 3200 mg/l (dip)

D = H_3PO_3 - 10^4 mg/l (dip)

E = Metalaxyl (granules) - 25 g

F = Fosetyl-Al - 3200 mg/l (drench)

G = H_3PO_3 - 10^4 mg/l (drench)

l.s.d. (0.05 level)

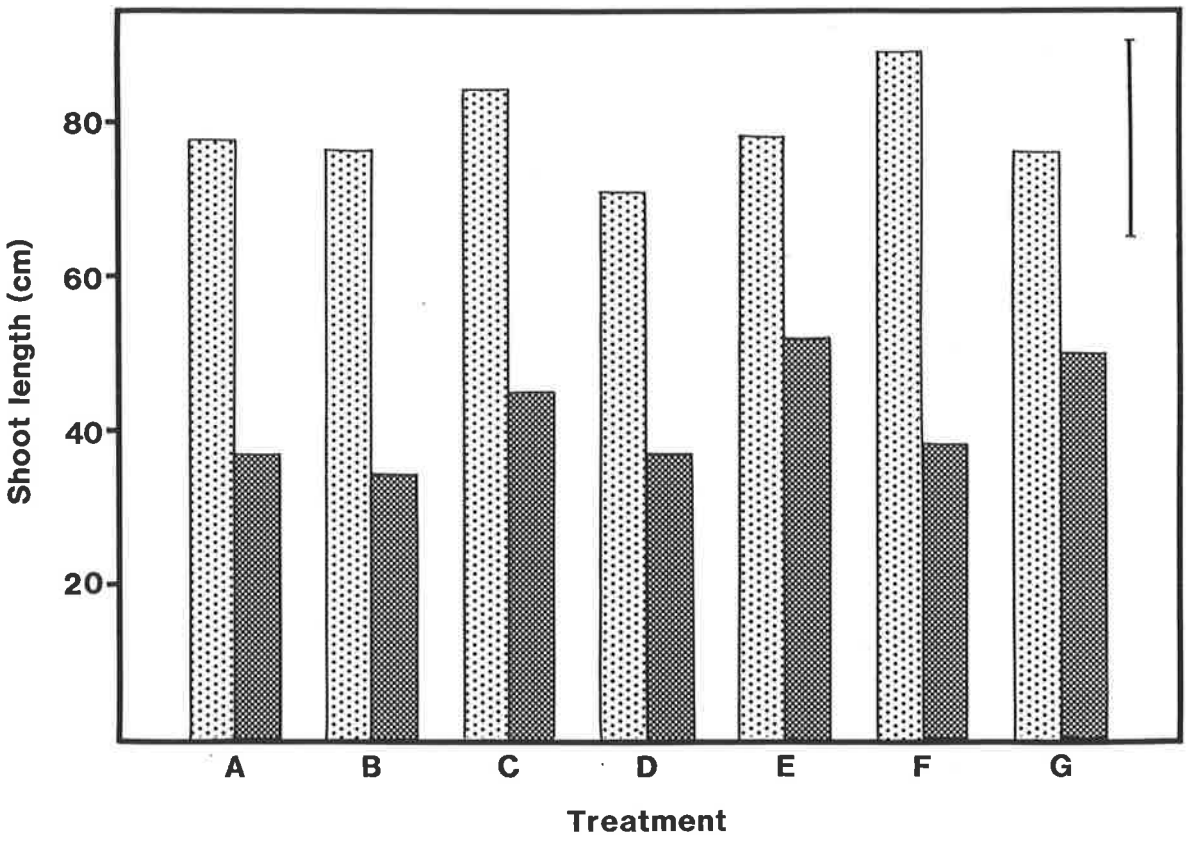
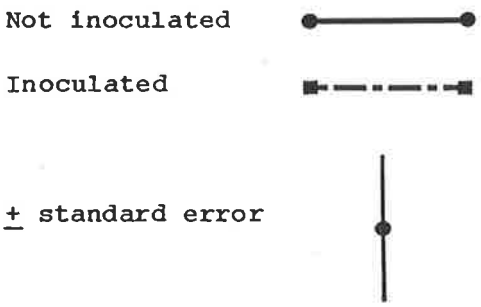
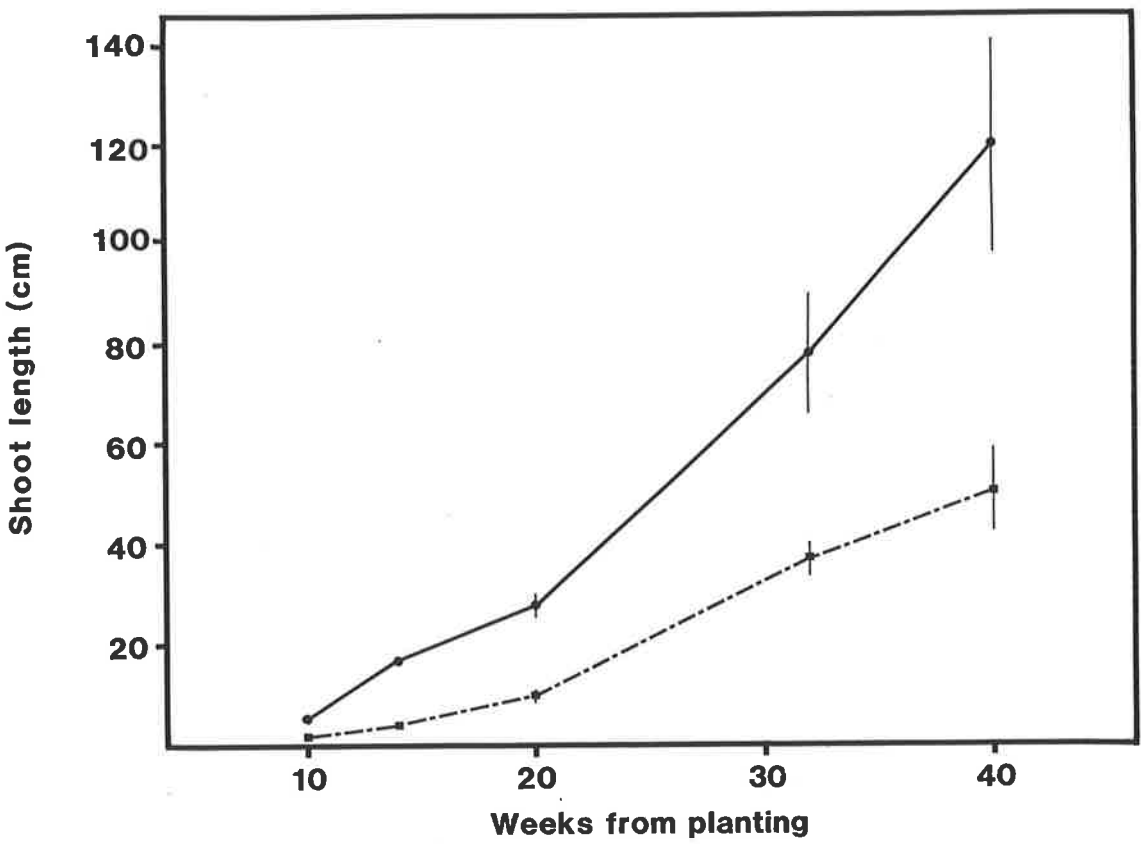

Shoot length (cm)
80
60
40
20
A
B
C
D
E
F
G
Treatment

Figure 7.4 Effect of *Phytophthora cambivora* on the growth of 'Mission' almond seedlings.




140
120
100
80
60
40
20
Shoot length (cm)
10
20
30
40
Weeks from planting

post-planting or the fosetyl-Al pre- and post-planting treatment. *P. cambivora* was recovered from most of the dead plants.

In the cherry experiment, infected trees were first detected in the untreated area seven months after planting and within a further 18 months 25% of these plants developed crown cankers. In contrast no crown cankers developed in trees grown in soil treated with metalaxyl during this time. However infected trees were found in these plots 20 months after the final application of metalaxyl (Fig 6 Chapter 8).

(ii) 'Nemaguard' rootstocks

Using butt diameter as a measure of plant growth, no fungicide treatment significantly increased the growth of 'Chellaston' almond on 'Nemaguard' rootstock in infested soil. Butt diameters were greatest in plants grown in soil treated with urea. At the 80 g/plant rate of urea, the average butt diameter of 3.94 cm was significantly greater than that in any of the fungicide treatments. Both rates of H_3PO_3 were phytotoxic producing brown necrotic lesions on the internodes of young green shoots and also marginal leaf burn and occasional tip die back.

P. megasperma was recovered from 50% of the 25 soil samples collected from the untreated plots in winter. *P. cambivora* was not recovered from the site at this stage although the fungus had been detected in soil samples taken from the site prior to planting.

In the second experiment butt diameters of 'Nemaguard' rootstocks eight months after planting were 1.1 ($\pm$.04) cm in metalaxyl treated plots and 1.1($\pm$0.06) cm in the untreated plots. Of the 'Chellaston', Hydrid and 'Nemaguard' seedlings dug up from the replant sites, dead plants and plants

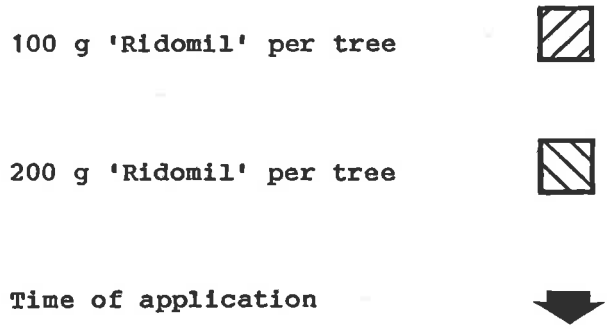
with crown cankers were found in only the 'Chellaston' seedlings. These occurred in the control and metalaxyl treated plots as well as those covered with plastic mulch (i.e. solarized). For example four out of 15 in the untreated, three out of 22 of the metalaxyl treated and seven out of 10 'Chellaston' seedlings in the solarized plots were either dead or cankered. This was also reflected in the recovery of fungi from the roots. Apart from *Pythium* species, *P. cambivora* and *P. megasperma* were the only fungi recovered and the latter were most frequent on 'Chellaston' seedlings compared to Hybrid and 'Nemaguard' (Table 7.4).

Both *P. cambivora* and *P. megasperma* were recovered from 'Chellaston', Hybrid and 'Nemaguard' plants in the metalaxyl treated soil and from 'Chellaston' and Hybrid plants in the solarised soil.

(iii) Mature almond orchards

In both 'Chellaston' and 'Mission' almond orchards, fewer dead trees and trees with cankers developed where they were treated regularly with metalaxyl (Table 7.5). Trees declining at the time of application were not protected by either high rates of metalaxyl applied in a furrow or broadcast over the soil surface. On 'Chellaston' trees new cankers were detected on both declining and vigorous trees not treated with metalaxyl. No new cankers were found on trees treated with metalaxyl. Metalaxyl or a fungitoxic breakdown product was taken up and translocated to the shoots of almond trees and severely inhibited the extension of cankers 27 days after the application of 'Ridomil' granules (Figure 7.5) in the two orchards tested. At 96 days after application colonisation of inoculated shoots was still inhibited but to a lesser degree. A further application of metalaxyl, inhibited colonization 17 days after application in orchard A with the extent of inhibition greatest

Figure 7.5 Effect of 'Ridomil' soil applications on the colonisation of almond shoots inoculated with *Phytophthora cambivora*.



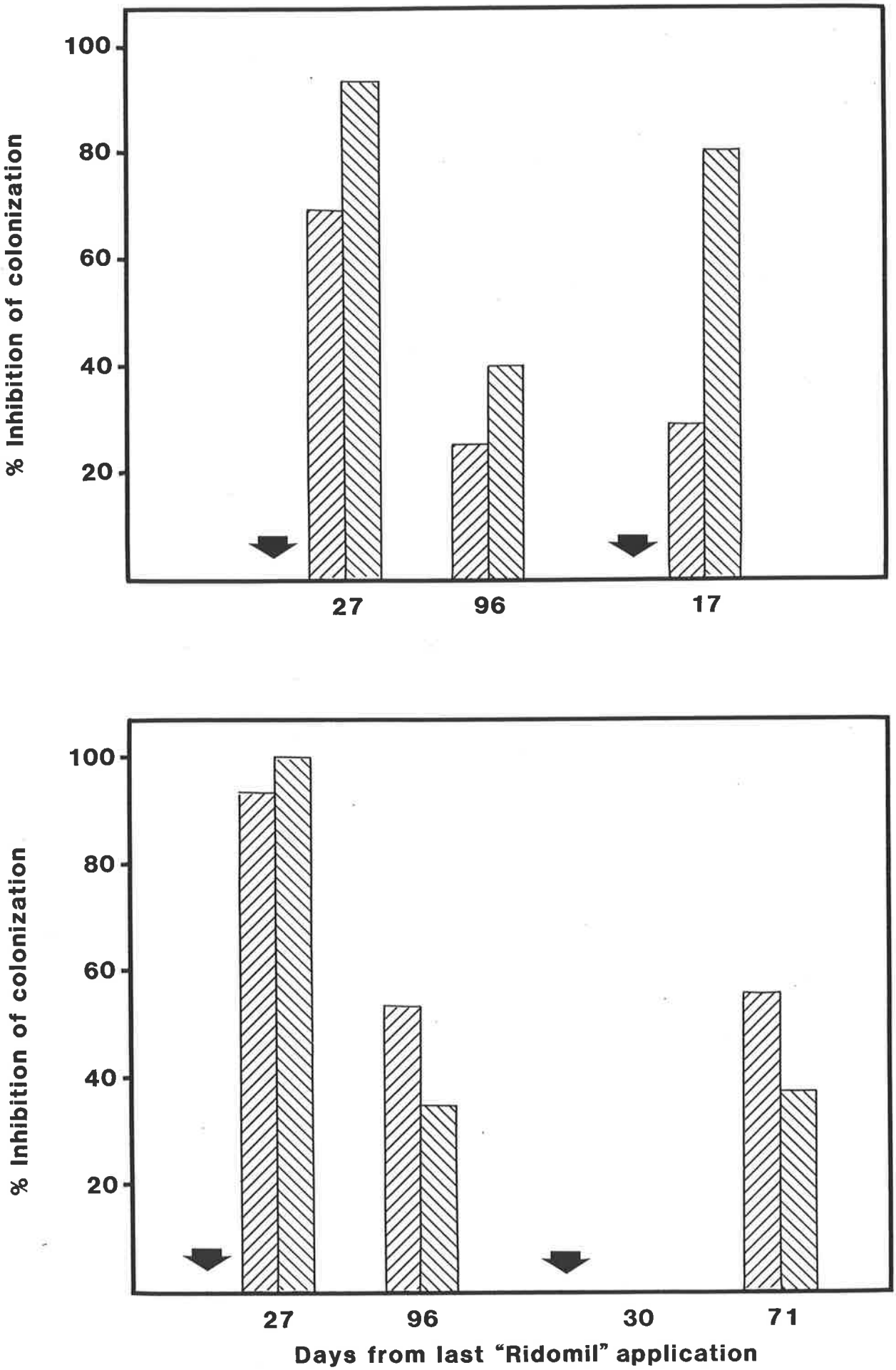

% Inhibition of colonization
100
80
60
40
20
27
96
17
% Inhibition of colonization
100
80
60
40
20
27
96
30
71
Days from last "Ridomil" application

Table 7.4 Fungi recovered from the roots of 'Chellaston', Hybrid ('Non-pareil' x 'Nemaguard') and 'Nemaguard' rootstocks grown in soil naturally infected with *P. cambivora*.

	Plants from which fungi were recovered (%)								
	Treatment								
	Control			Metalaxyl			Solarised		
Fungi recovered	H	C	N	H	C	N	H	C	N
Nil	20	25	66	53	34	62	0	12	25
Pythium spp.	50	25	16	33	26	12	25	12	50
P. cambivora	10	16	16	6	3	6	12	25	0
P. megasperma	20	25	0	26	30	6	37	25	0

H = Hybrid

C = Chellaston

N = Nemaguard

Table 7.5 Effect of 'Ridomil' soil treatments on the decline of 'Chellaston' and 'Mission' almond trees in orchards naturally infested with *Phytophthora cambivora*

Initial tree health	'Ridomil' (rate per tree)	% trees in each category			
		Gumming on trunk	Trunk Canker Old	New	Dead
Orchard A - Chellaston					
Declining	100 g	21	26	0	0
Declining	200 g	31	31	0	7
Declining	Nil	57	15	31	5
Vigorous	100 g	20	20	0	0
Vigorous	200 g	37	12	0	0
Vigorous	Nil	50	21	14	7
Orchard B - Mission					
Declining	200 g	100	*		0
Declining	400 g (broadcast)	87	*		25
Declining	Nil	83	*		33
Vigorous	200 g	0	*		0
Vigorous	400 g (broadcast)	0	*		0
Vigorous	Nil	0	*		22

* not assessed

in the 200 g treatment. In orchard B, inhibition was detected 71 days but not 30 days after the application of metalaxyl.

(iv) Established infections

Since cankers of similar lengths developed on all inoculated almond trees only those of the untreated and metalaxyl applied at the time of inoculation were measured in detail. These measurements showed that the colonisation of either butts, branches or shoots was not inhibited by metalaxyl (Table 7.6). This was also reflected by the recovery of *P. cambivora* from the margin of most cankers in both the untreated and metalaxyl treated trees.

Table 7.6 Effect of 'Ridomil' on colonisation of butts, limbs and shoots of almond trees inoculated with *Phytophthora cambivora*

	Canker length (cm)			% Recovery of *Phytophthora*		
	Trunk	Branch	Shoot	Trunk	Branch	Shoot
Untreated	27.5	15.9	10.6	33	75	80
'Ridomil' 100 g/tree	26.4	15.2	9.9	57	78	92
SE	6.3	0.98	1.08			

In the second experiment, the application of metalaxyl before inoculation did not prevent colonisation of inoculated shoots as there was no significant difference in canker lengths between any of the treatments and the control (Table 7.7).

In the cherry experiment *P. cambivora* was recovered from the trunk of most control cherry trees, 16 days after inoculation but it was not recovered at any other stage even though trunk cankers continued to extend. There was no consistent trend in the development of cankers in the various treatments and in some pairs of trees cankers were longest in those treated with metalaxyl. At the final assessment cankers in the trunks of control and metalaxyl treated trees had extended 19.1 cm (±SE 5.8) and 18.6 (±SE 5.3) respectively. The failure of metalaxyl to inhibit the development of cankers was also reflected on inoculated shoots. At both times of assessment the expansion of cankers on inoculated shoots was similar on untreated and metalaxyl treated trees. For example approximately eight weeks after the final application of metalaxyl the mean canker length on each of 80 shoots per treatment was 9.8 cm in the untreated trees and 10.2 cm on those treated with metalaxyl.

(d) Metalaxyl residues

(i) Chemical analysis

Residue analysis of several soil types showed that the highest levels of metalaxyl were confined to the top 10 cm of soil (Table 7.8).

In the Pooraka soils which were the most free draining of the treated soils, metalaxyl levels of 100 mg/kg of soil were detected at 0 to 5 cm depths 5 weeks after applying 100 g of 'Ridomil' granules. Metalaxyl residues in

Table 7.7 Effect of 'Ridomil' applied before inoculation on colonisation of almond shoots inoculated with *Phytophthora cambivora*

Rate of 'Ridomil' (g/tree)	Canker length (cm)
100 (broadcast)	13
100	16.7
50	13.7
25	15.4
0	12.9
l.s.d. (0.05 level)	4.4

Table 7.8 Concentration of metalaxyl in soils sampled up to 80 weeks after the application of 'Ridomil'* to the soil surface.

Rate of last Ridomil application per tree	Sampling depth (cm)	Weeks from last metalaxyl application	Metalaxyl concentration (mg/kg of soil)
Pooraka			
25 g	0- 5	5	16
	10-15	5	1.7
	20-25	5	0.35
	0- 5	37	0.33
	5-10	37	0.92
	0- 5	80	Not detected
50 g	0- 5	5	33
	10-15	5	1.3
	20-25	5	0.27
	0- 5	80	0.44
100 g	0- 5	5	100
	10-15	5	2.6
	20-25	5	0.76
	0- 5	37	1.4
	0- 5	80	1.2
Lenswood			
Block E			
50 g	0- 5	33	7.9
	0- 5	48	0.69
	0- 5	55	Not detected
Block K			
100 g	0- 5	12	169
	0- 5	27	84
	0- 5	34	13
Willunga			
Orchard A			
50 g	5-10	31	11.0
	5-10	46	16.0
	5-10	50	3.7
100 g	5-10	31	55
	5-10	46	20
	5-10	50	7.5
Orchard B			
100 g (furrow)	5-10	31	83
	5-10	46	22
200 g (broadcast)	5-10	31	189
Orchard C			
25 g	0- 5	21	0.42
	0- 5	37	0.27
	0- 5	41	Not detected

* (5% a.i. metalaxyl)

samples taken at the same time but at 20-25 cm depths at this site were less than 1 mg/kg of soil.

In some soils, relatively high levels of metalaxyl were detected in soils more than 30 weeks after application. In orchard A at Willunga, for example, 20 mg of metalaxyl/kg g soil were detected 46 weeks after the last application of 100 g of 'Ridomil' granules.

(ii) Soil leachates

Few sporangia were produced from mycelial disks flooded with tap water whereas production from similar disks was profuse when they were flooded with soil leachates. No sporangia formed on disks flooded with leachates prepared from untreated soils which were mixed with 'Ridomil' granules (Table 7.9).

Leachates from Lenswood soils treated 12 weeks previously with Ridomil, inhibited the production of sporangia completely in block E and by 96% in soils from block K. Leachates prepared from soil at Lenswood which had been taken from an area covered with plastic mulch over summer were included for comparison but there was no significant effect on the production of sporangia. Soil leachates from block A at Willunga, inhibited sporangia production by 93% and 90% in samples treated 31 weeks previously with 50 and 100 g of Ridomil per tree respectively. All leachates from fungicide and urea treated soils from orchard C at Willunga, inhibited the production of sporangia. Fosetyl-Al inhibited the production by 99% whereas that achieved with phosphorous acid and metalaxyl was 87% and 75% respectively. Soil amendments of urea but not lime were also inhibitory. At all sites no mycelial growth occurred from disks flooded with leachates of untreated soils where 'Ridomil' granules were added just before the samples were agitated

Table 7.9 Effect of soil leachates from 'Ridomil' treated soil on the production of *Phytophthora cambivora* sporangia.

Treatment	Weeks from last 'Ridomil'* application	No. sporangia per disk (±SE)	% inhibition
Lenswood - Block E			
Control - water	-	1.5 (±1.5)	-
Control - soil leachate	-	124.8 (±15.7)	-
Control + 'Ridomil'	0	0	100
'Ridomil' - 50 g/tree	12	0	100
Solarized soil	37	118.6 (±(35)	5
Lenswood - Block K			
Control - water	-	7.6 (±3.8)	-
Control - soil leachate	-	93.4 (±58)	-
Control + 'Ridomil'	0	0	100
'Ridomil' - 100 g/tree	12	3.6 (±5.1)	96
Willunga - Orchard A			
Control - water	-	0.6 (±1.2)	-
Control - soil leachate	-	37.4 (±43.1)	-
Control + 'Ridomil'	0	0	100
'Ridomil' - 50 g/tree	31	2.6 (±3.0)	93
'Ridomil' - 100 g/tree	31	3.7 (±4.8)	90
Willunga - Orchard C			
Control - soil leachate	-	146 (±18.1)	-
Control + metalaxyl	0	0	100
Fosetyl - Al 3,200 mg/m^2	21	0.1 (±0.3)	99
H_3PO_3 100 g/m^2	21	18.8 (±27)	87
'Ridomil' 25 g/m^2	21	36.0 (±35)	75
Urea 80 g/m^2	21	30.8 (±24)	79
Lime 500 g/m^2	21	130.63 (±63)	11

* (5% a.i. metalaxyl)

with water. Similarly no disk was viable after an incubation in leachates from the soil of block E Lenswood where 50 g of 'Ridomil' was applied 12 weeks before sampling. Leachates from other treatments at all sites were not inhibitory to mycelial growth.

(iii) Soil bioassay

Metalaxyl soil residues most inhibitory to mycelial growth were detected in the 0 to 5 cm depths of soil (Figure 7.6a) whereas less inhibitory levels were detected at lower depths of soil at all applied rates of 'Ridomil'. At the 0 to 5 cm depth, the degree of inhibition increased with increased rate of 'Ridomil' but a similar marked trend was not obvious in soils sampled at lower levels.

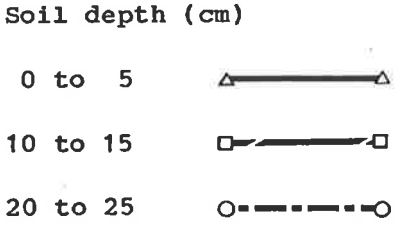
Determinations of metalaxyl levels by comparison with a standard curve showed concentrations less than 0.01 mg/l at the 20-25 cm depths of soil treated with either 25, 50 or 100 g of 'Ridomil' granules, (Figure 7.6b). At the 0-5 cm depth, higher metalaxyl concentrations were detected but these were less than 1 mg/l even for soils where 100 g of 'Ridomil' had been applied.

DISCUSSION

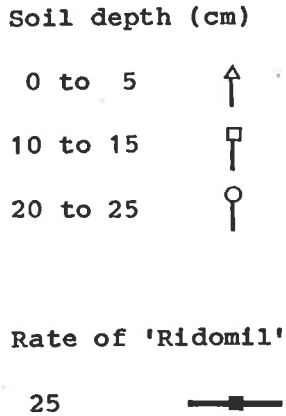
Metalaxyl applied to soil around tree trunks controlled *Phytophthora* crown rot of almond and cherry in some cases and not in others. For example metalaxyl applied to soil immediately after planting did not control *P. cambivora* on severely infected almond seedlings. On the other hand regular applications of metalaxyl in autumn and spring controlled *P. cambivora* in naturally infected Mahaleb cherry seedlings and mature almond trees. The experiments utilising previously infected almond seedlings may have been unrealistic as the plants were severely infected with extensive crown cankers

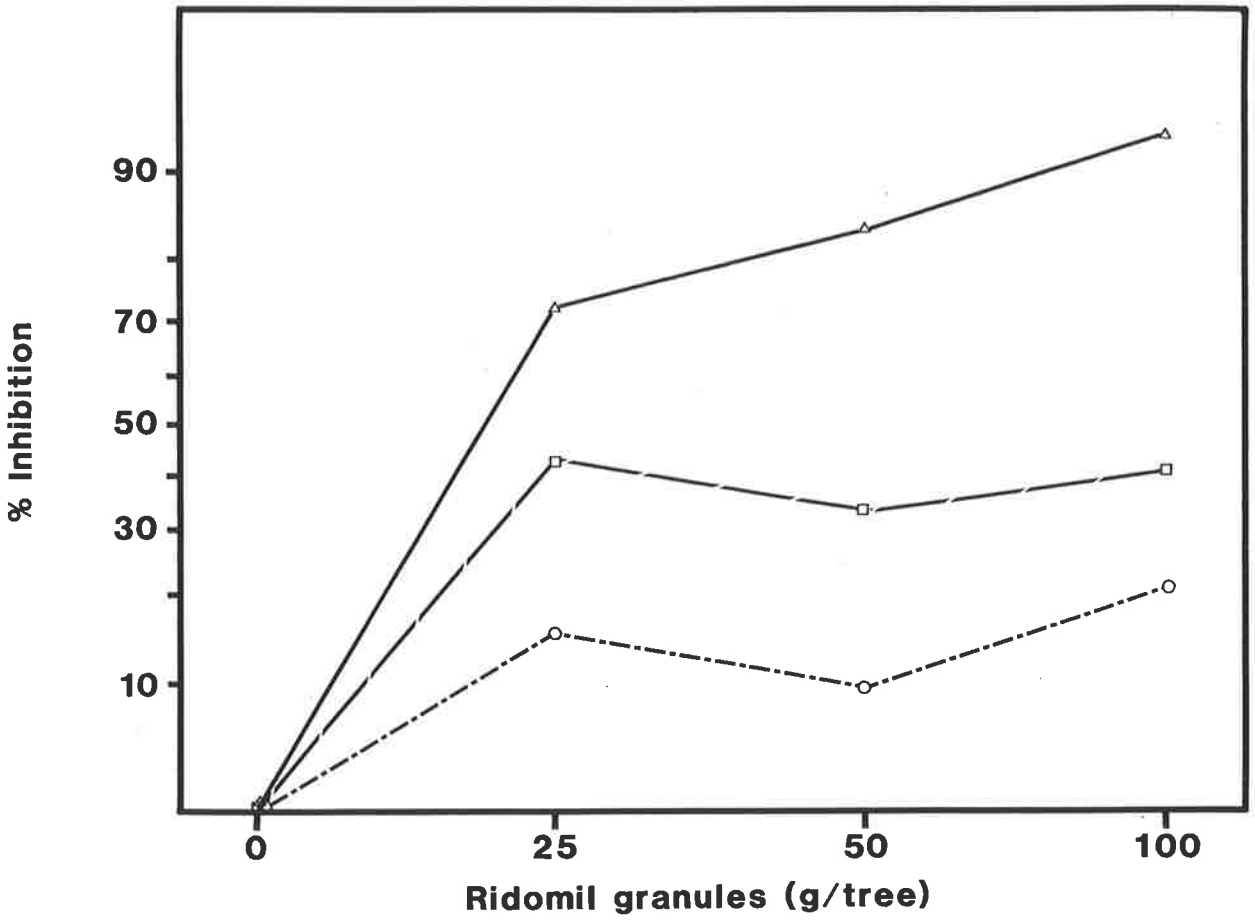
Figure 7.6(A and B) Bioassay of metalaxyl soil residues

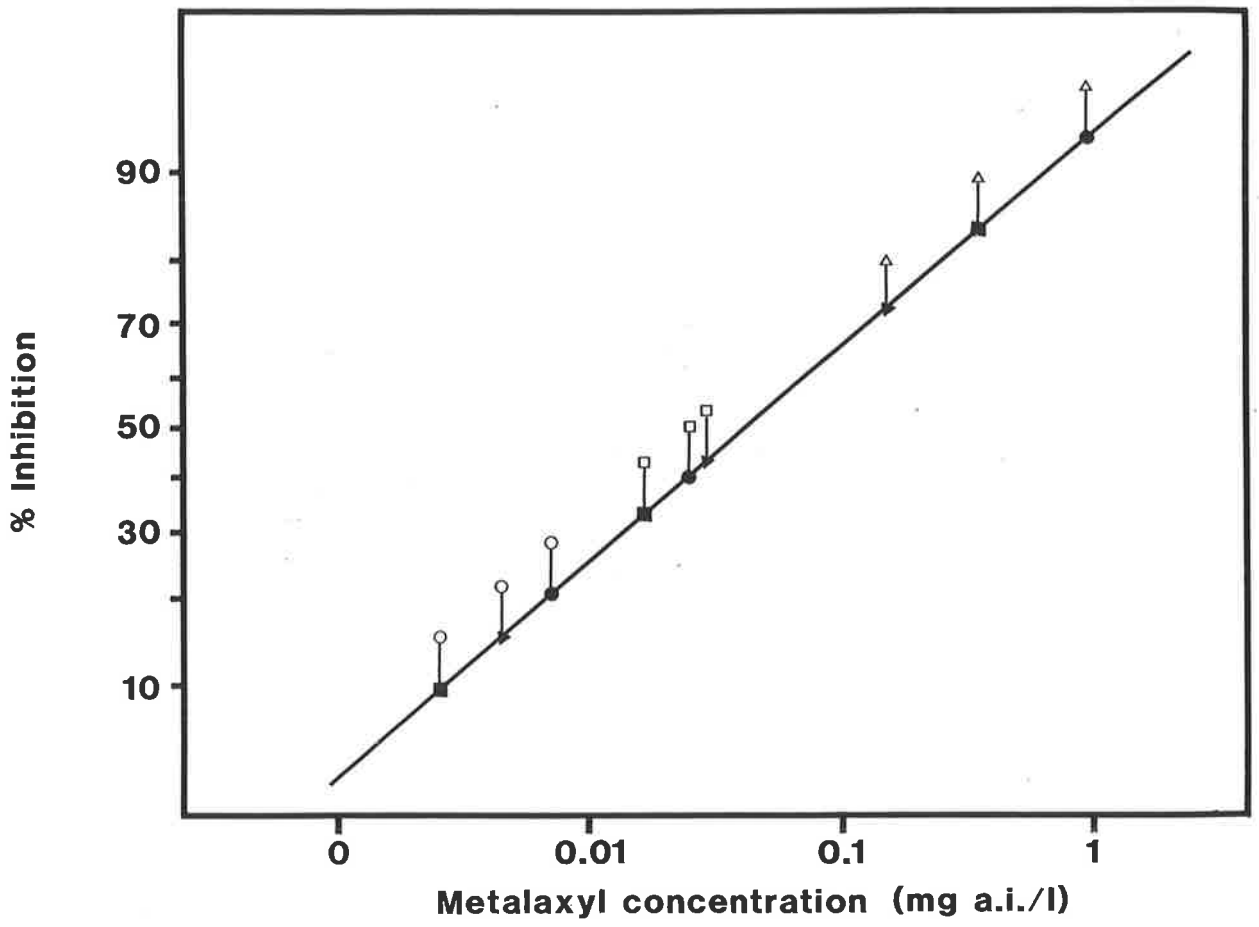
A Effect of metalaxyl soil residues on mycelial growth of *Phytophthora cambivora*



B Standard curve of probit percent inhibition of mycelial growth against log concentration of metalaxyl




% Inhibition
90
70
50
30
10
0
25
50
100
Ridomil granules (g/tree)


% Inhibition
90
70
50
30
10
0
0.01
0.1
1
Metalaxyl concentration (mg a.i./l)

and rotted roots at the time of treatment. In addition infected soil was placed beneath each seedling at planting. In normal situations it is unlikely that severely infected plants would be planted and that new plantings would be subject to similar high levels of inoculum. The failure of metalaxyl to rehabilitate severely infected plants in these experiments might have been due to insufficient levels of metalaxyl being leached into the soil to control the fungus. However, this is unlikely to be so particularly with the potted plants, as severely infected plants failed to respond to fungicide drenches that were applied throughout the root profile. The most probably reason is that the infected plants were so severely debilitated at the time of treatment that the normal uptake of metalaxyl was severely inhibited in the rotted roots and damaged stem.

Although pre-planting fungicide dips failed in the recent experiments further evaluation is justified considering the work of Jeffers and Wilcox (1986) who showed that apple root stocks naturally infected with *Phytophthora* species produced healthy plants after they were dipped in a solution of 1000 ppm metalaxyl. This treatment failed to eradicate *Phytophthora*, but similar plants dipped in water produced severe *Phytophthora* crown rot symptoms.

The failure of preplanting fungicide dipps to eradicate *Phytophthora* from naturally infected plants indicates that fungicide applications should continue after planting to prevent further development of disease.

These recent results have demonstrated the importance of controlling *P. cambivora* at or before planting for, not only is the fungus capable of killing plants when the crown infections girdle the stem, but also early

infections severely retard subsequent plant growth. In almonds one application of metalaxyl to soil around young plants did not prevent root infection or the development of crown cankers arising from natural infection although in one experiment the incidence of *P. cambivora* was less in plants treated with metalaxyl compared to those in the untreated. In the experiment where almonds were treated with 25 g of granules, measurements of metalaxyl concentrations in the soil indicated that the levels would have been too low to inhibit *P. cambivora* completely and prevent infection.

In contrast regular applications of metalaxyl around cherry seedlings prevented the development of crown cankers, but only so long as inhibitory levels of metalaxyl were maintained in the soil. Sharom and Edgington (1982) showed that metalaxyl is weakly adsorbed by soil and is readily leached from soils with low adsorption capacity. Thus cankers developed on cherry trees at Lenswood only after rainfall and irrigation had leached metalaxyl from soil resulting in concentrations of metalaxyl too low to inhibit *P. cambivora* in the soil or in cherry tissue.

Soil applications of metalaxyl did not rejuvenate or prevent to any great extent the further decline of trees that were infected at the time of application. This occurred in artificially inoculated almond and cherry trees and occasionally in naturally infected almond trees and confirms earlier studies that showed that cankers on stems of potted almond seedlings were not inhibited by metalaxyl applied after inoculation (Wicks and Lee 1985). Trees that were declining at the time of applying the soil fungicide may have succumbed to other pathogens not controlled by metalaxyl or they may have been less able to tolerate the waterlogged soil conditions that occurred frequently in the area.

Inoculating shoots of metalaxyl treated and untreated trees and comparing the development of cankers was shown to be a sensitive method of determining whether metalaxyl was taken up and translocated within a tree.

This method showed that metalaxyl was not taken up or at least not in sufficient quantities to inhibit the development of cankers following the treatment of mature trees at Pooraka and Lenswood. At Pooraka this was confirmed by soil residue analysis and bioassays of metalaxyl levels that showed metalaxyl to be concentrated in the top 5 cm of soil and that levels unlikely to be inhibitory to *P. cambivora* occurred at 10 to 25 cm depths. Almonds are deep rooted and in the sandy soils at Pooraka few feeder roots occurred in the upper 10 cm of soil indicating that the uptake of metalaxyl from those depths would have been unlikely.

The failure of metalaxyl to be taken up following repeated applications to soil around mature cherry trees at Lenswood is difficult to explain particularly when metalaxyl levels high enough to inhibit *P. cambivora* were detected in the 0-5 cm depths of soil where feeder roots were abundant. Microbial degradation of metalaxyl has been reported by Bailey and Coffey (1985) in some Californian soils that had received metalaxyl treatments and one soil that had not been exposed to the chemical. However enhanced bioedegradation is unlikely to be occurring in Lenswood soils as relatively high levels of metalaxyl were detected in these soils 34 weeks after metalaxyl was applied. In addition soil applications of metalaxyl controlled *P. cambivora* in young cherry trees on other sites of the orchard. One explanation could be that different rootstocks take up metalaxyl at varying rates. This aspect needs to be examined as in the present studies successful control of crown rot was achieved in both potted and field grown Mahaleb

rootstocks whereas metalaxyl failed when applied to cherries on Mazzard rootstocks.

Another explanation may be that the absorption and translocation metalaxyl is less efficient in old trees than young trees, although this is unlikely considering that cankers were controlled on both seedling and mature almond trees treated with metalaxyl.

Several workers have demonstrated that metalaxyl is absorbed directly through the bark of stems and translocated at concentrations sufficient to prevent the development of *Phytophthora* (Davis 1982, Matheron and Mircetich 1985c, Taylor and Washington 1984). This may be an important route of uptake of metalaxyl in some situations and may explain the lack of control at Pooraka and Lenswood. At Pooraka, metalaxyl was applied in a circular trench 30 cm from the trunk and at Lenswood metalaxyl was broadcast on the soil surface around the trunk. In both cases no applications were made directly on to the trunk so rainfall or irrigation is unlikely to have leached metalaxyl on to that area. On the other hand at Willunga where crown cankers were controlled, metalaxyl was applied in a trench close to the trunk, and rainfall was more likely to leach metalaxyl onto the trunk thus increasing the likelihood of uptake.

Whether the control achieved on these trees was a result of metalaxyl taken up through either the trunk, or feeder roots or both is unknown. In any case sufficient fungitoxic material was taken up and translocated at both the 100 and 200 g rates of 'Ridomil' granules to inhibit colonization for up to 96 days after application.

These results suggests that with deep rooted plants or in heavy clay soils where metalaxyl is unlikely to be leached to feeder roots metalaxyl should be applied directly or as close as possible in a band around the trunk. Such applications may not prevent root rotting of distal roots but should provide levels of metalaxyl both within the soil and trunk to prevent trunk infections and the development of cankers.

Similar conclusions were made by Ellis and Porpiglia (1984) who found that the uptake of metalaxyl by apple trees was not facilitated by broadcast treatments of metalaxyl under the tree canopy.

Phosphorous acid, the fungitoxic breakdown product of fosetyl-Al (Fenn and Coffey 1984) was shown in these studies to have potential for the control of *P. cambivora*; more so in cherries than in almonds as drenches were phytotoxic to almond leaves and shoots. Whether lower rates or different methods of application are capable of controlling natural infections without the accompanying phytotoxicity will need to be investigated further.

In the recent studies the mycelial growth of *P. cambivora* was sensitive to rates of phosphorous acid shown to be inhibitory to a number of other *Phytophthora* species (Coffey and Bower 1984b). The wide variation in the sensitivity to phosphorous acid between isolates of the same species is not unusual as Coffey and Bower (1984b) have also shown similar variations between A^1 and A^2 isolates of *P. cinnamomi*. Mycelial growth and the formation of sporangia of *P. cambivora* was shown in these studies to be inhibited by phosphorous acid but there are few other studies on the effect of this chemical on the various growth stages of *Phytophthora*. Coffey and Joseph (1985) showed that phosphorous acid inhibited the production of

sporangia, chlamydospores and oospores in *P. citricola* and *P. cinnamomi* and it is not unreasonable to expect that similar effects occur with *P. cambivora*. In the present studies and those reported by Matheron and Matejka (1986) phosphorous acid was as effective as metalaxyl in preventing the development of *Phytophthora* in artificially inoculated plants which suggests that phosphorous acid may be a useful alternative to metalaxyl. The use of trunk injections which have been successfully used with fosetyl-Al (Darvas *et al.*, 1984) and phosphorous acid (Pegg *et al.*, 1985) to control *P. cinnamomi* in avocado need to be evaluated as well as foliar sprays as a means of applying phosphorous acid to control *Phytophthora* diseases in both almond and cherry.

Foliar applications although less effective than soil applications in some cases (Coffey *et al.*, 1984b) warrant further evaluation as they may be the most economic means of control particularly if they can be tank mixed and applied with other pesticides.

One of the areas not resolved in these studies was the manner in which soil applied fungicides controls *P. cambivora*; whether this is by reducing the inoculum level in the vicinity of the trunk and thus reducing the likelihood of infection or by inhibiting further development of the fungus once it has invaded the trunk. The most likely explanation is that both methods probably function since leachates from fungicide treated soil were highly inhibitory to the production of sporangia and that metalaxyl applied before infection inhibited canker extension in inoculated stems or trunks.

Other modes of action are also possible as apart from mycelial growth and sporangia formation, chlamydospore formation and viability and oospore

production has been shown for a number of *Phytophthora* species to be sensitive to low concentrations of metalaxyl (Coffey *et al.*, 1984a; Ellis *et al.*, 1982; Farih *et al.*, 1981; Rana and Gupta 1984). Similar effects on the production of sporangia, chlamydospores and oospores have been shown in vitro with phosphorous acid (Coffey and Joseph 1985) but it is unknown whether these effects also operate in the soil.

Despite this the sole reliance of chemicals for the control of *Phytophthora* root and crown rots is unwise particularly in light of reports of several *Phytophthora* species being insensitive to metalaxyl (Hunger *et al.*, 1982; Joseph and Coffey 1984; Stack and Millar 1985) and phosphorous acid (Bower and Coffey 1984, Dolan and Coffey 1985) as well as cross resistance to related fungicides (Bruin and Edgington 1981).

Continued use of a fungicide or groups of fungicides with similar modes of action could result in the development of *Phytopthora* isolates insensitive to a specific fungicide group and lead ultimately to crop losses despite the use of fungicides. A means of preventing this is to use fungicide formulations containing mixtures of chemicals which have different modes of action. Whether the activity of metalaxyl and phosphorous acid is sufficiently different from each other to warrant their use together in mixtures is yet to be resolved.

However continued use of fungicides alone is unlikely to be the most economic means of control. There was no benefit in using fungicides on resistant rootstocks, and therefore the most effective control programme is more likely to be one that integrates the use of healthy nursery stock, resistant rootstocks and where possible site selection and preparation (that avoids or

reduces waterlogging) before using fungicides. Another method such as solarization which could be incorporated into an integrated programme and applied before planting is described in Chapter 8.

CHAPTER 8

EFFECT OF SOLARIZATION ON THE CONTROL OF PHYTOPHTHORA CAMBIVORA

INTRODUCTION

Solarization is described by Katan (1981) as the technique of heating field soil by covering the soil with transparent plastic sheeting. The upper layers of covered soil are heated by solar energy to temperatures about 10°C above ambient temperature. In some climates temperatures of 45° to 50°C can be reached to depths of 20 cm if the soil is covered for 4 to 6 weeks in mid summer. These temperatures are sufficient to kill most pathogens. Since the growth of most *Phytophthora* spp is inhibited at temperatures above 35°C the method seemed applicable to control this group of fungi. The technique has been mainly used to control vegetable diseases and has recently been used successfully in California to control *Verticillium* wilt without damaging trees in established pistachio orchards (Ashworth and Gaona 1982).

Solarization is an exciting development of plant disease control. It is simple, cheap, effective, non-hazardous to the user and does not involve the use of toxic chemicals that are usually used to control soil borne diseases. The technique is reported to have other advantages in that weeds are also controlled and an increased growth response, beyond disease control is frequently experienced (Katan 1981).

Solarization is a relatively recent development in disease control but its long-term effects in orchards are generally unknown.

Solarization has not been evaluated previously on tree crops in Australia although the technique seems ideally suited to the Australian environment and in particular for the control of diseases induced by *Phytophthora*. It was considered that solarization may be suitable for use in replant situations in established orchards where the use of soil fumigants is restricted because of their effect on surrounding trees. Several experiments were initiated to determine the effects of solarization on tree growth and the recovery of *P. cambivora* from both soil and infected plants. The experiments were also used to measure the temperatures at various depths in solarized soils in some South Australian orchards.

MATERIALS AND METHODS

At the same time that field experiments were undertaken, laboratory experiments were set up to determine what effect high temperatures similar to those reached in solarized soils would have on the viability and reproduction of *P. cambivora*.

(a) Laboratory experiments

(i) Effect of high temperatures on growth

In mid summer, soil temperatures beneath clear plastic sheeting are usually 8 to 10°C higher than the ambient levels. Measurements of soil temperatures at the Lenswood Research Centre showed that the maximum temperatures occurred after midday and that the high temperatures rarely persisted for more than 6 hours in any 24 hour period. To determine if *P. cambivora* could survive fluctuating periods of high temperature, 5 mm diameter disks of isolates P5, P9, P80 and P171 were incubated on CMA for two days before they were subjected to 35°, 40° or 45°C for six hours. After this period the

plates were removed and incubated at 20°C for 18 hours. A number of plates were returned to the same temperature for a further six hours before they were incubated again at 20°C. The procedure was repeated so colonies were exposed to either 1, 2, 3 or 4, six hour periods at each high temperature. The number of plates in which colonies continued to grow was recorded after all treatments were incubated at 20°C for a further 14 days. Six replicate plates for each of the two almond A^1 mating types and the cherry A^1 and A^2 mating types were used for each treatment.

(ii) Temperature effects on sporulation

An initial experiment was conducted with an almond A^1 (P5) mating type and a cherry A^2 (P9) mating type of *P. cambivora* to determine the optimum temperature for the production of sporangia.

Mycelial disks, 3 mm diameter, taken from the margins of colonies growing actively on LBA were flooded with soil extract and incubated at either 10°, 20°, 25°, 30° or 35°C for 3 days under fluorescent light. The mycelial disks were examined microscopically and the numbers of sporangia including those that had discharged were counted around the margin of the disks. Two replicate dishes each of four mycelial disks were used for each temperature and isolate.

Another experiment was conducted to determine the effect of fluctuating periods of high temperatures on the production of sporangia. The almond isolate P5 was the only isolate used in this experiment as it was shown previously to be the most tolerant to heat. Mycelial disks 3 mm diameter were removed from the margins of colonies grown on LBA. The disks were then flooded with a soil extract and incubated in the dark at either 35°, 40° or 45°C for six hours.

After this period the disks were removed from the high temperatures and incubated for a further 18 hours in light at 20°C. A number of disks were returned for further six hour periods at higher temperatures every 24 hours. This procedure was repeated over five days, disks were exposed to either 1, 2, 3, 4 or 5 six hour periods at either of the three high temperatures. All disks were incubated for a further three days at 20°C after which the sporangia produced around the margin of the disks were examined microscopically and counted. Eight replicates of mycelial disks were used for each treatment.

(b) Field experiments

(i) Almond

Solarization experiments in almonds were conducted in the Willunga orchard where *P. cambivora* was first recovered. In this experiment the effect of solarization on *Phytophthora* was determined by burying infected fodder beet seed at depths of 10 and 30 cm within and outside the solarized area. Beet seed was used in these experiments as preliminary studies had shown that *P. cambivora* could not be recovered from buried twigs that had previously been infected with the fungus. Beet seed was inoculated four weeks previously with an A^1 (P5) mating type of an almond isolate of *P. cambivora*. Flasks of 250 to 300 ml of beet seed were autoclaved twice before mycelial disks taken from actively growing colonies were added and mixed with the seed. During four weeks incubation at 25°C, the beet seed was shaken frequently to ensure that the inoculum was evenly dispersed and that all seeds were infected. One week before use, approximately 100 seeds were removed from each flask and half plated on to CMA and the other half onto $P_{10}VP^+$ selective medium. Only those samples were used in which all of the seeds tested were infected with *P. cambivora*.

Four solarized plots were covered with clear plastic 100 um thick. This was laid on moistened soil measuring 2 m wide and 4 m long across sites where old almond trees had been removed. The edges of the plastic sheet were buried to a depth of 10 to 15 cm. The row width was sufficiently wide to ensure that the solarized plots were not shaded by trees in adjacent rows.

Lots of approximately 500 seeds were each enclosed in nylon mesh bags, which were buried in January just before the plastic was laid. For each plot, a bag of seeds was buried in the middle of each solarized plot and another outside, but within 30 cm from the edge of the plot. Due to the unavailability of suitable recording equipment, soil temperatures were not measured in this experiment. Twelve weeks after burial, the seeds were dug up, washed in tap water to remove adhering soil, blotted dry and a subsample of approximately 100 seeds per bag plated on to $P_{10}VP^{+}$ selective medium. The seeds were placed individually on to the agar and separated from each other so that colony growth from individual seeds could be distinguished.

Immediately after the plastic sheet was removed 10 soil samples were collected from the solarized and unsolarized areas and baited with pears. Four weeks later, three week old 'Mission' almond seedlings were planted within and immediately adjacent to the solarized areas. Six seedlings were planted within each plot and six around the edge of each plot. All plants were dug up after four weeks, washed under running tap water and pieces of necrotic roots blotted dry and plated on to $P_{10}VP^{+}$ selective medium.

The solarized treatments were repeated the following season in the same orchard, in four different tree sites. The plastic sheet was laid in January and removed in May, just before the sites were planted with 'Chellaston', Hybrid and 'Nemaguard' seedlings previously described in Chapter 7. Similar

seedlings were planted in metalaxyl treated and untreated sites of the orchard where dead or declining trees had been removed the previous winter.

These seedlings, except for the 'Nemaguard' plants left in the centre of each plot and, on which butt diameters were measured, were dug up six months after planting and rotted root pieces plated on to $P_{10}VP^{+}$ selective medium.

(ii) Cherry

One year old Mahaleb cherry rootstocks were planted in spring 1983 at the Lenswood Research Centre and then the soil subjected to solarization for the following three summers. This experiment included metalaxyl treated and untreated plots for comparison. The trial design consisted of five rows each 3.5 m apart planted in a north-south direction. Plots of 5 trees with each tree 1.5 m apart were randomised and replicated twice in each row.

Clear plastic sheeting 100 μm thick was laid on the soil surface of the solarized plots for at least 12 weeks in mid summer, usually from late December or early January, in 1 m wide strips on either side of the plants. The plastic sheet around the edge of the plots was buried in a 10 to 15 cm deep furrow. The area at the centre of the row where the plastic sheets overlapped was sealed with a 5 cm wide strip of weather proof black tape. In the first season of solarization, small sections of plastic were left touching the trunk of the tree and this resulted in death of the underlying tissue. Consequently in subsequent seasons, a gap of at least 1 cm was left between the trunk and the plastic in the overlapping area. This was covered with sealing tape to prevent the plastic tearing from around the trunk. All trees in this experiment were irrigated with "low throw" sprinklers situated along the tree row. To ensure that all trees were watered during the solarization period, sprinkler outlets that were normally 30 cm high were

laid on the soil surface before they were covered with plastic sheeting. Tensiometers were installed in between trees in the row at a depth of 15 cm in each of four solarized and untreated plots. Measurements were recorded at weekly intervals throughout the solarization period.

Soil temperatures at depths of 10 cm and 30 cm within and outside the solarized area were recorded with thermistors connected to a "Honeywell" recorder in the first season and a "CR 21" Campbell micrologger in the later seasons.

Butt diameters 10 cm above the soil surface were measured on the middle three plants of each plot on four occasions within 32 months from planting. At various times before and after each solarization period a total of 130 soil samples from around the base of trees in different treatments were collected and baited with pears. These samples were collected at random from various treatments, but when trees began to decline or indicate the presence of crown cankers, soil samples from around these trees were also collected and baited.

In the final solarization treatment of this experiment, samples of either soil or vermiculite previously inoculated with the A^1 (P5) mating type of an almond isolate of *P. cambivora* and vermiculite inoculated with an A^2 (P9) mating type of a cherry isolate of the same species were buried at depths of 10 and 30 cm within and outside solarized areas. For each treatment four samples each of approximately 150 ml were placed in plastic containers and buried for 12 weeks. After this time each sample was dug up and baited with a pear. Two 150 ml samples of each inoculum type were also baited before burial.

(iii) Effect of solarisation on established infections

Two experiments were set up to determine the effect of solarisation on almond and cherry plants infected with *P. cambivora*. In the almond experiment, six month old 'Mission' seedlings growing in pots were inoculated with an A^1 (P5) mating type of an almond isolate. The plants were inoculated by wounding the stems and placing a mycelial plug in the wound made approximately 2 cm below the soil surface. The inoculated area of stem was re-covered with soil and three days later planted in summer at the Lenswood Research Centre. Each seedling was carefully planted so the inoculated area of stem was covered with at least 5 to 10 cm of soil. Plants were 30 cm apart in a east-west facing row with five plants per plot. Alternate solarized and unsolarized plots were each replicated three times. The clear plastic sheeting was laid at planting as previously described.

After eight weeks the plants were dug up, and the canker lengths and fresh shoot weight measured.

A similar experiment was conducted with Mahaleb cherry seedlings planted in the solarized and unsolarized areas. Six month old seedlings were inoculated with an A^2 (P9) mating type of a cherry isolate of *P. cambivora* as described above for the almond seedlings. These seedlings were planted in January in between the three year old Mahaleb trees planted in the main solarization experiment at Lenswood. Only five seedlings instead of the planned 15 were planted in the unsolarised plots. At least 15 seedlings were planted amongst the solarized plots. After 12 weeks exposure to solarization the plants were dug up and canker lengths measured.

RESULTS

(a) Laboratory experiments

(i) Growth at high temperatures

One or more six-hour periods of 45°C killed all isolates of *P. cambivora* (Figure 8.1). At 35°C and 40°C however there was considerable variation in the sensitivity of different isolates. For example the viability of the almond isolate P5 was not affected by any period at either 35°C or 40°C whereas the viability of other isolates were severely reduced after exposure to four periods at 40°C.

With the cherry isolates, the A2 mating type appeared the most sensitive to high temperatures since the viability was reduced after exposure to three periods at either 35°C or 40°C. On the other hand the viability of the A1 isolate was not inhibited by exposure at 35°C but was reduced by 50% after four periods at 45°C.

(ii) Sporangium production

Initial experiments showed that at constant temperatures the optimum production of sporangia in both almond and cherry isolates occurred between 20° to 25°C (Figure 8.2a). In this experiment sporangia were not produced at 35°C.

When high temperatures were maintained for six-hour periods, sporangia were produced at 35°C and 40°C but not 45°C. However when mycelial disks were exposed to either four periods at 35°C or three periods at 40°C the production of sporangia was inhibited completely. (Figure 8.2b).

Figure 8.1 The effect of six hour periods of high temperatures on the viability of *Phytophthora cambivora* cultures.

A^1 almond (P5)

A^1 almond (P171)

A^2 cherry (P9)

A^1 cherry (P80)

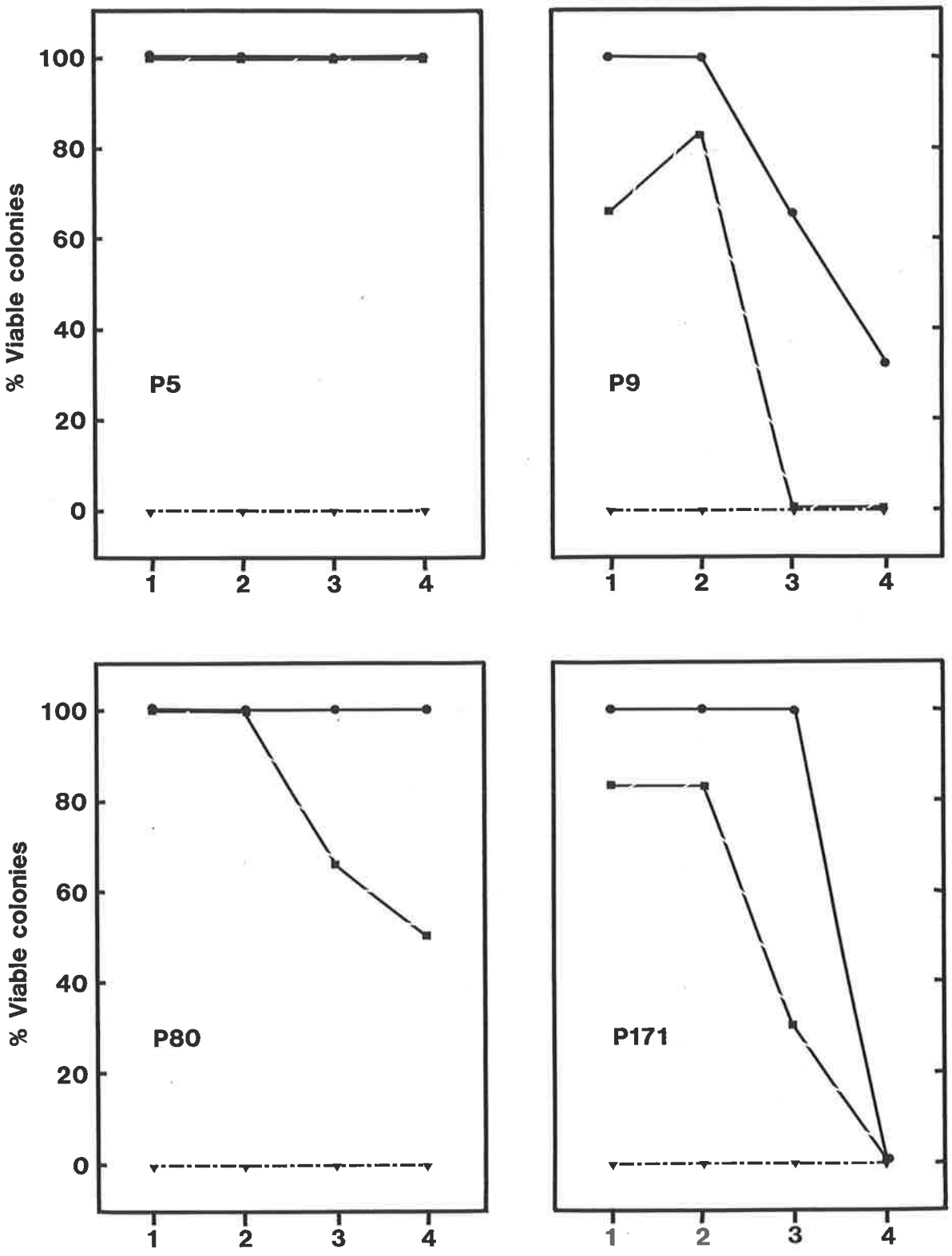
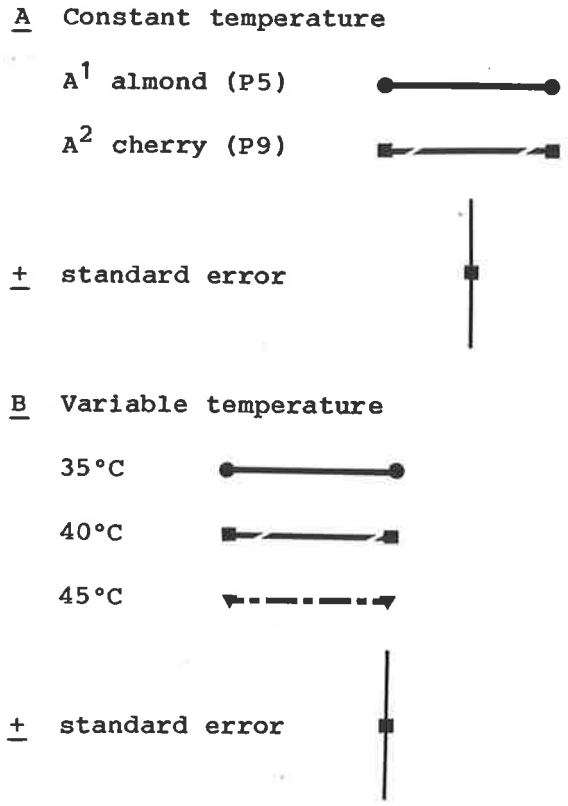
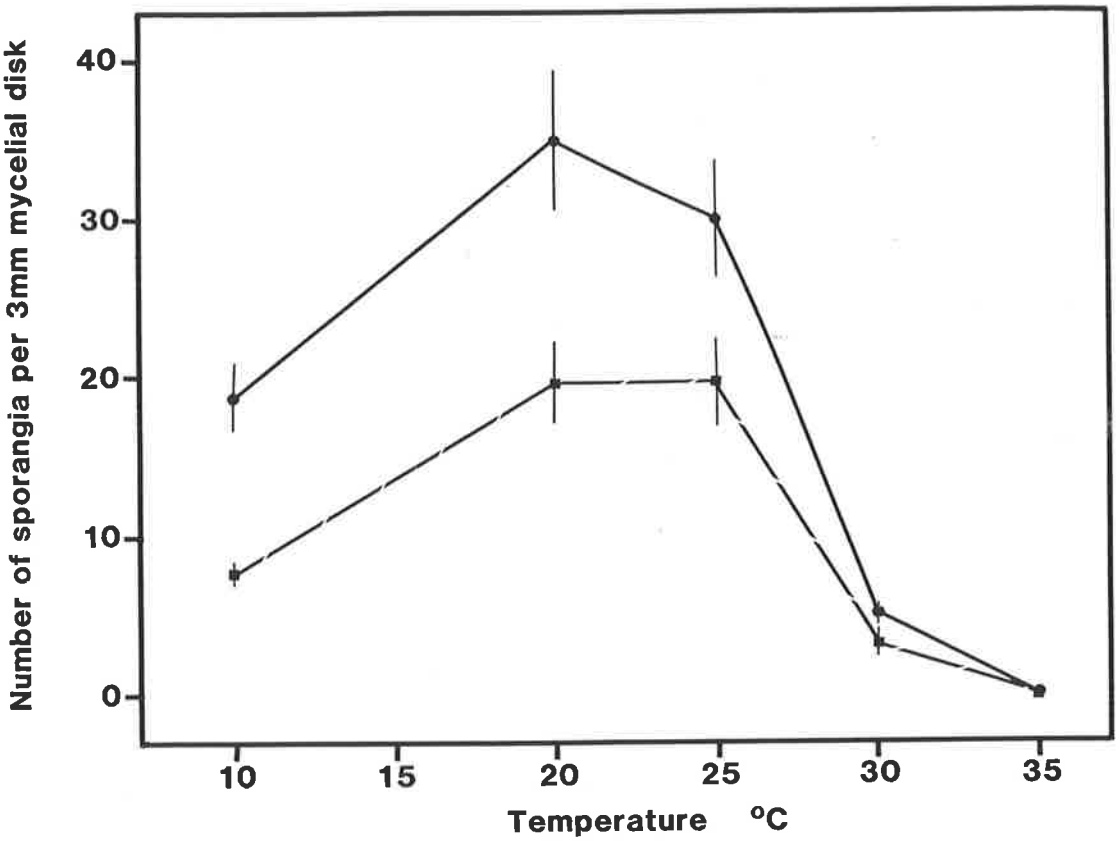
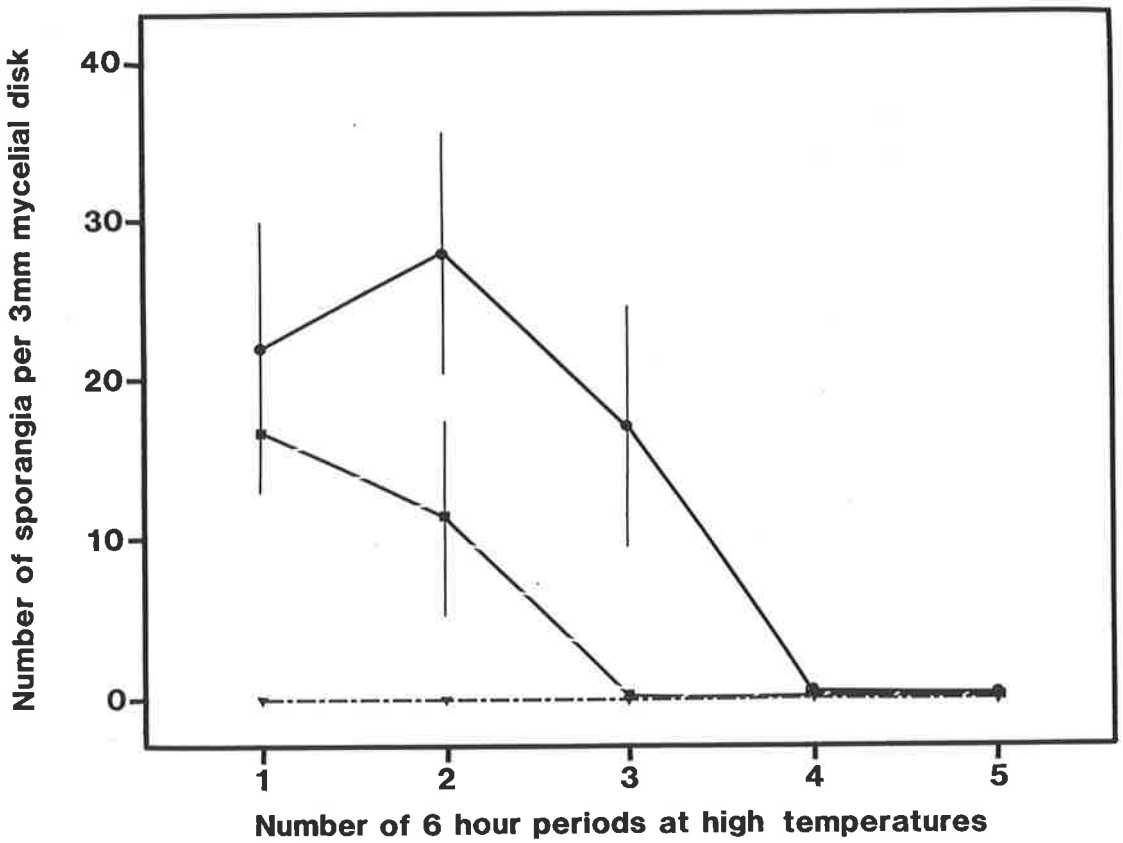

% Viable colonies
100
80
60
40
20
0
P5
P9
P80
P171
1
2
3
4
Number of 6 hour periods at high temperatures

Figure 8.2(A and B) Effect of temperature on the production of *Phytophthora cambivora* sporangia.




Number of sporangia per 3mm mycelial disk
40
30
20
10
0
10
15
20
25
30
35
Temperature °C


Number of sporangia per 3mm mycelial disk
40
30
20
10
0
1
2
3
4
5
Number of 6 hour periods at high temperatures

(b) Field experiments

(i) Almond

Although there were large differences in the recovery of *P. cambivora* from various replicates at the same depth, there was an overall trend for *P. cambivora* to be recovered less frequently from beet seeds buried in solarized soil compared to seeds from bare soil (Table 8.1). This was obvious only at the 10 cm depth however as the recovery from the 30 cm depths was overall similar in both solarized and unsolarized plots.

When soil from the plots were baited with pears, *Phytophthora* were detected in 30% and 40% of the 10 soil samples collected from each of the solarized and unsolarized plots respectively. Although the fine feeder roots were rotted and lesions on the main tap roots were obvious on most plants dug up from the solarized and unsolarized plots, *Phytophthora* was not recovered from necrotic roots plated on to $P_{10}VP^{+}$ selective medium.

In the experiment conducted the following season, *P. cambivora* and *P. megasperma* were isolated from 'Chellaston' and Hybrid, but not 'Nemaguard' seedlings dug up after six months growth in solarized soil (Table 7.5 - Chapter 7). Similar recoveries were made from plants removed from untreated soil with the exception of 'Nemaguard' where *P. cambivora* was isolated from roots. Butt measurements 12 months after planting showed no significant difference in the growth of Nemaguard plants in either solarized or untreated plots.

(ii) Cherry

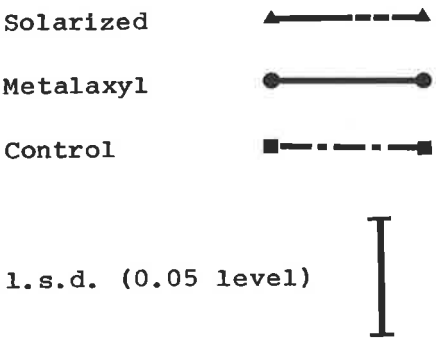
The summer of 1983/84 was mild and soil temperatures never reached 40°C, 10 cm beneath the plastic. Temperatures over 35°C were recorded at this

Table 8.1 Recovery of *Phytophthora cambivora* from infected beet seed buried in solarized and unsolarized soil

	% Recovery			
	Burial depth (cm)			
Replicate	Bare soil		Solarized	
	10	30	10	30
1	15	11	3	1
2	10	8	11	18
3	59	20	31	49
4	26	56	1	23
Mean	27.5	23.7	11.5	22.7

Figure 8.3 Maximum weekly soil temperatures beneath clear plastic sheeting, Lenswood Research Centre - 1985/86

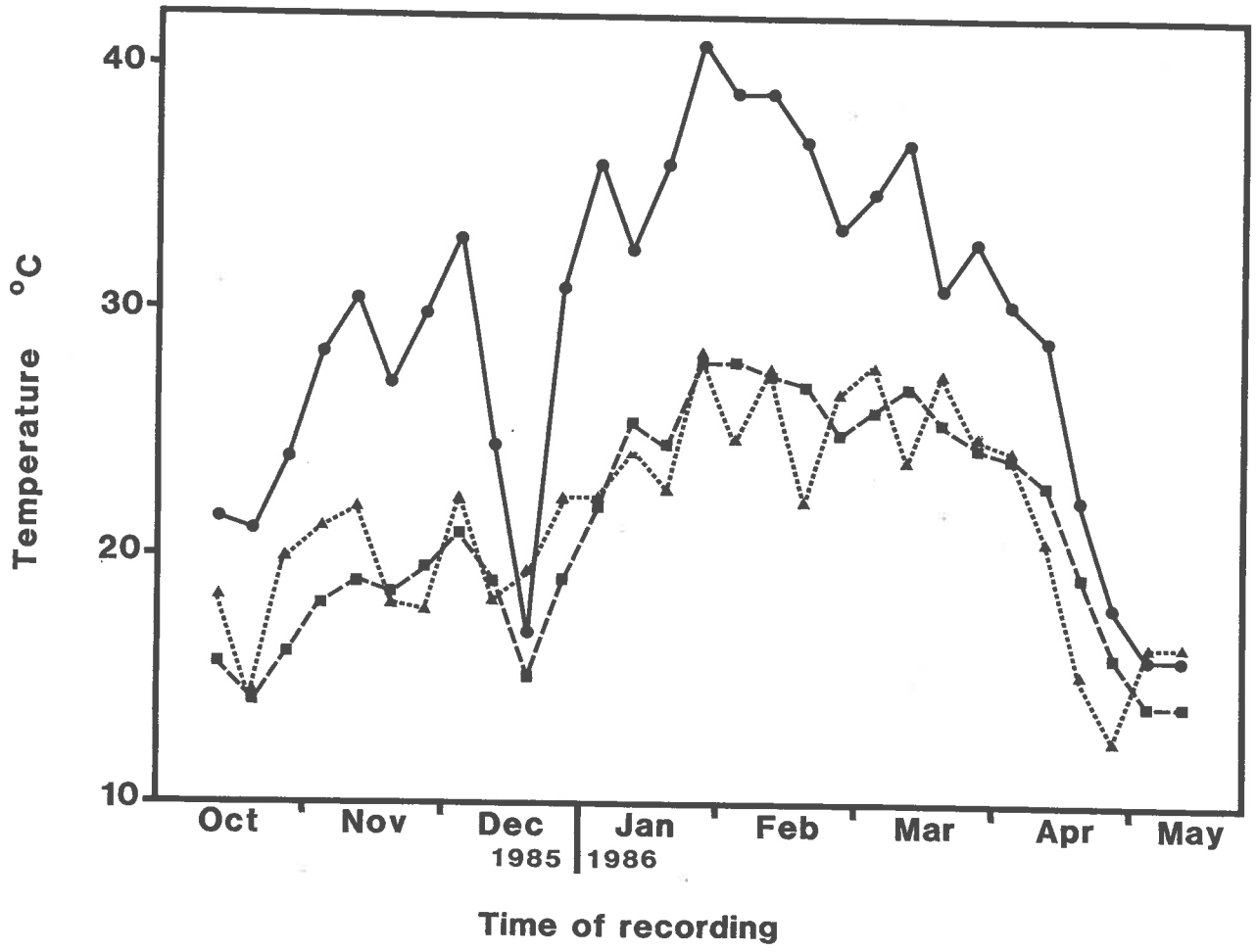
Figure 8.4 Butt diameters of Mahaleb cherry rootstocks grown in either solarized, metalaxyl treated or bare soil.

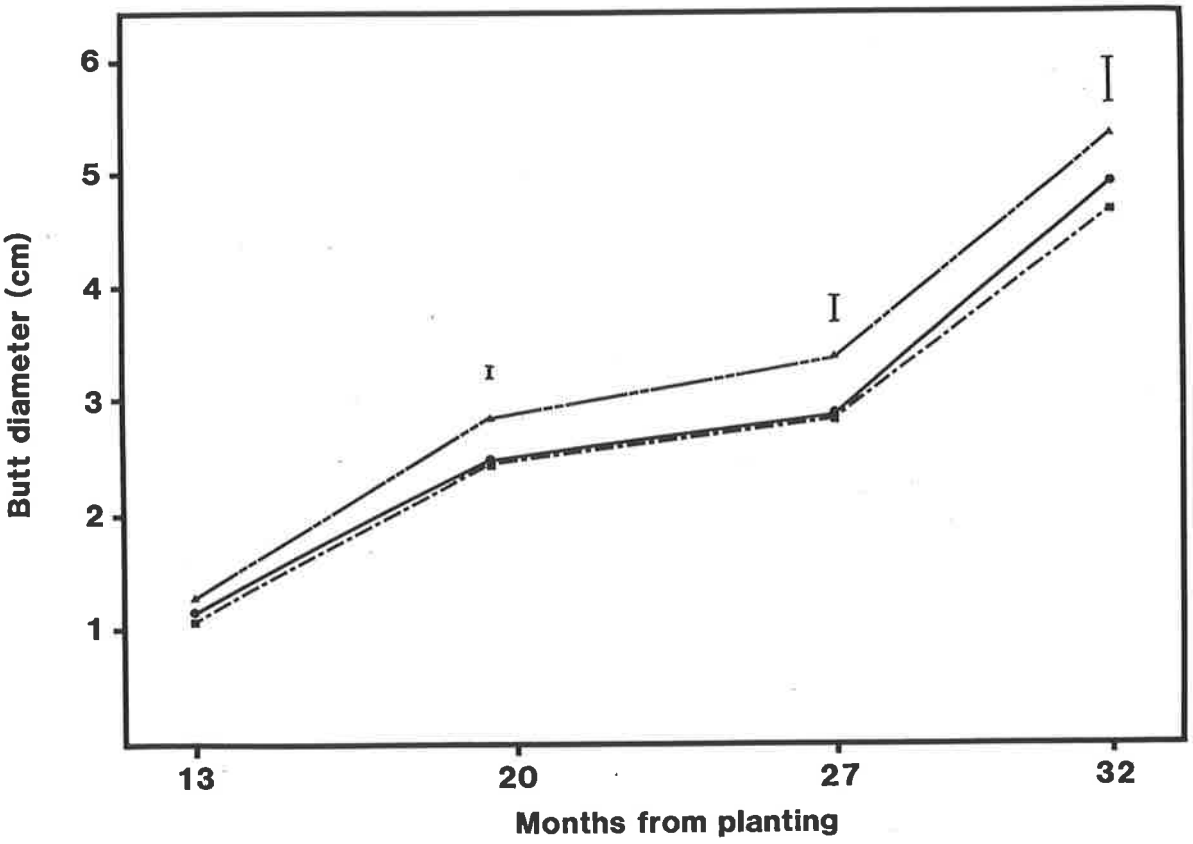


depth on 5 consecutive days on one occasion and on four separate days on other occasions. Temperatures at 30 cm below the plastic were generally 6° to 8°C less than those at 10 cm. In bare soil the maximum temperatures recorded at 10 cm was 33°C with the temperature remaining below 30°C on most occasions. At 30 cm, soil temperatures were mainly 2° to 5°C less than those at 10 cm. Summer temperatures were again mild in 1984/85 with temperatures over 40°C being recorded at 10 cm beneath the plastic on 3 consecutive days. During the solarization period, temperatures above 35°C were recorded at this depth on 35 days. Temperatures recorded at 30 cm under plastic and at other depths in bare soil showed similar trends as that recorded the previous summer. Similar temperatures were recorded in the summer of 1985/86 and maximum weekly recordings are shown in Figure 8.3. During that summer temperatures beneath the plastic reached 40°C on four days and above 35°C on 24 days. Temperatures at 10 cm beneath the plastic were at least 10°C higher than those at the 30 cm depth on most occasions during the solarization period.

Measurements of butt diameters showed that growth was significantly greater in trees grown in solarized plots compared to those in unsolarized or metalaxyl treated plots (Figure 8.4). Growth difference were detected 13 months after planting, but became most obvious after the second year. Tree growth in both the control and metalaxyl plots was similar at all times of measurement.

Although *P. cambivora* was recovered from soil in the trial site just before planting subsequent pear baiting did not detect the fungus until 18 months after planting. Twelve months later *P. cambivora* was recovered from soil, roots and cankers of declining trees in the untreated plots.


Temperature °C
40
30
20
10
Oct
Nov
Dec
1985
Jan
1986
Feb
Mar
Apr
May
Time of recording


Butt diameter (cm)
6
5
4
3
2
1
13
20
27
32
Months from planting

P. cambivora was not recovered from the solarized plots or those treated with metalaxyl until 43 months after planting.

Bullate oogonia formed when the recovered isolates were mated with A^2 isolates of *P. cambivora* and *P. cryptogea* indicating that isolates *P. cambivora* recovered from soil and infected plants were the A^1 mating type.

Measurements of soil moisture showed similar rates of extraction in both the bare plots and those covered with plastic sheeting (Fig 8.5). An assessment on the incidence of disease 43 months after planting showed crown cankers extending above ground on 36% of the plants in the untreated plots (Fig 8.6). Crown cankers were not found on plants in the solarized plots whereas cankers were detected on 12% of the plants in plots that had been treated 19 months previously with metalaxyl.

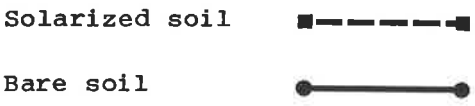
Solarization did not inhibit the recovery of *P. cambivora* from infested soil or vermiculite which was buried at 10 and 30 cm. The almond and cherry isolates were recovered at all depths from both solarized and bare soil (Table 8.2) as well as from samples baited before burial.

(iii) Established infections

Although the mean canker lengths were shorter in almond and cherry plants grown in solarized soil compared to those in bare soil, these differences were not significant at the P 0.05 level (Table 8.3).

Similarly differences in the growth of almond in either solarized or bare soil were not significant.

Figure 8.5 Soil drying patterns in solarized and bare soil.



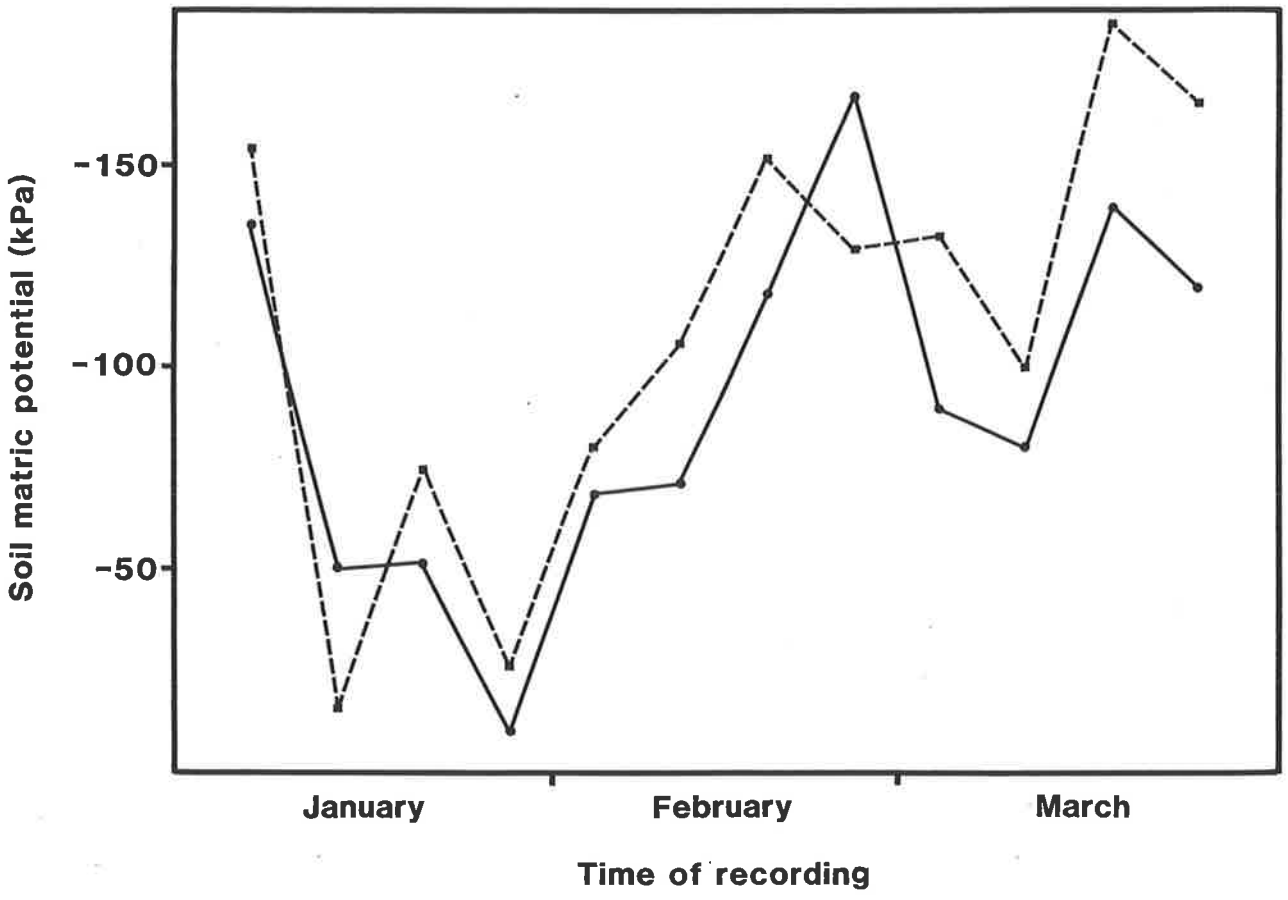
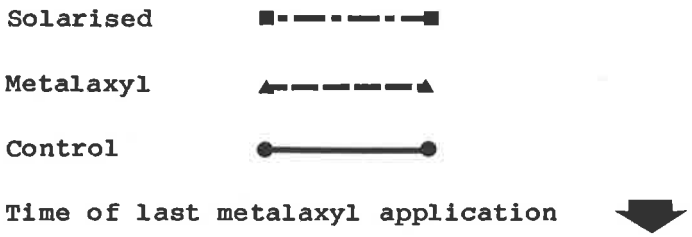

Soil matric potential (kPa)
-150
-100
-50
January
February
March
Time of recording

Figure 8.6 Effect of solarization on the incidence of crown cankers on Mahaleb cherry rootstocks naturally infected with *Phytophthora cambivora*.



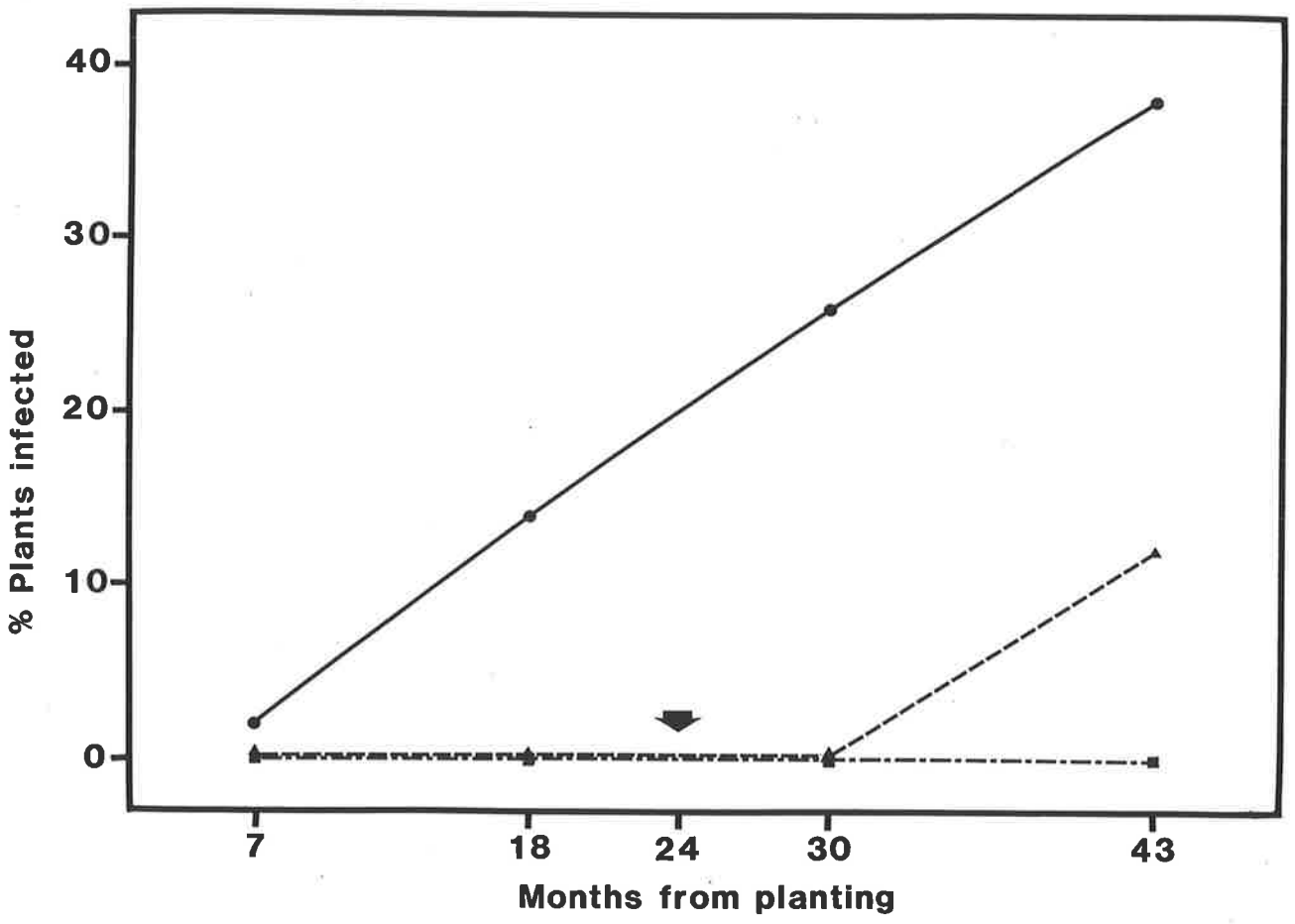

40
30
20
10
0
% Plants infected
7
18
24
30
43
Months from planting

Table 8.2 Recovery of *Phytophthora cambivora* from infested vermiculite and soil buried in solarised areas in 1986.

Inoculum	Recovery*			
	Bare soil		Solarised	
	10 cm	30 cm	10 cm	30 cm
Vermiculite infested with A[1] isolate (P5)	+++-	++--	++++	++--
Vermiculite infested with A[2] isolate (P9)	++--	+--	++--	+--
Soil infested with A[1] isolate (P5)	++--	++--	+++-	+++-

* four samples per treatment.

\+ = *Phytophthora* recovered.

\- = No *Phytophthora* recovered.

Table 8.3 Effect of solarization on the colonization of almond and cherry seedlings inoculated with *Phytophthora cambivora*

Treatment	Canker length (cm)		Shoot weight (g)	
	Almond	Cherry	Almond	Cherry
Solarized soil	2.3	6.6	5.2	not measured
Uncovered soil	2.8	7.5	6.6	not measured

DISCUSSION

These results show that solarization controls crown rot of cherry trees caused by *P. cambivora* and that in naturally infested orchards the disease is suppressed completely for more than 12 months after treatment. Mild summers occurred in the three years when solarization treatments were applied and resulted in soil temperatures that were less than those reported at equivalent depths in other solarization experiments conducted elsewhere in Australia (Porter and Merriman 1983, 1985; Kassaby 1985). While temperatures under plastic sheeting at soil depths of 1 to 5 cm were not measured in these recent experiments, recordings elsewhere indicated that they would have been greater than 45°C. These temperatures would have been lethal to *P. cambivora* and high enough to change the population density of microorganisms drastically in the upper 5 cm of soil (Stapleton and Devay 1982,1984). On the other hand temperatures at a depth of 30 cm would have had little effect on the viability of *P. cambivora* which was reflected in the recovery of the fungus from infested soil buried at that depth. *P. cambivora* was not eliminated from infested soil buried at 10 cms however, which was unexpected considering that the laboratory experiments indicated that the times of exposure at 35°C or more were sufficient to inhibit growth and reproduction of the fungus. Also similar experiments in South Africa eradicated *P. cinnamomi* from colonised wheat grain buried at 10 and 30 cm in solarized soil (Barbercheck and Von Broembsen 1986).

Direct effects of temperatures on the fungus do not explain why plants in the solarized plots appeared healthy throughout the experiment and for 12 months after the final solarization treatment, whereas surrounding plants in untreated and more recently metalaxyl treated plots developed crown cankers. Presumably some factor(s) in the solarized soil suppressed *P. cambivora* either directly or indirectly.

Pinkas *et al.*, (1984) reported that reinfestation of solarized soil by *P. cinnamomi* was inhibited and involved suppression of the fungus as expressed by slower hyphal growth and fewer chlamydospores. They also reported that the formation of sporangia was suppressed which is contrary to the recent studies with *P. cambivora* where sporangia were produced abundantly in soil leachates from solarized soil (table 7.9 Chapter 7).

The most likely explanation of the recent cherry experiment results is that temperatures in the solarized areas were high enough to change the microflora in the upper 5 cm of soil to those of thermophylic or thermotolerant organisms that were also antagonistic to *Phytophthora*. These organisms may have suppressed *P. cambivora* by either directly attacking hyphal or reproductive structures or indirectly by inhibiting sporangia stimulating bacteria. Other mechanisms may also be operating and these have been reviewed by Malajczuk (1983) who also lists the organisms that have shown antagonism towards *Phytophthora* species.

Solarization treatments conducted in almond soil were not successful as *Phytophthora* were recovered from both solarized and untreated soil after the plastic was removed, and there was no significant difference between the growth of Nemaguard seedlings in either solarized or unsolarized soils. A possible explanation is that soils in the area were difficult to wet when dry, resulting in uneven wetting of the treated areas before the soil was covered. To be effective solarized soils must be moist to allow the conduction of heat to lower depths of soil. At this site soils appeared wet on the surface, but may have been unevenly wetted or dry at lower depths. Thus the temperatures in the deeper and drier areas of solarized soil may have been too low to kill or suppress the growth of *P. cambivora* or markedly alter the population densities of other soil microorganisms. In

addition, temperatures in the very wet soil near the soil surface may have been too low to be effective since these wetter soils have a higher thermal capacity requiring more heat to raise the temperature than in drier soils. Furthermore the almond isolate of *P. cambivora* (P5) which was previously isolated from the orchard was the isolate most tolerant to exposure at high temperatures.

The comparison of the effect of solarization on the control of *Phytophthora* in the cherry (irrigated) and almond (non irrigated) sites demonstrates that only soils with wetted profiles should be solarized. This is most likely to be achieved in orchards with efficient irrigation systems and in particular where drip or low throw irrigation systems can also be incorporated beneath the plastic to ensure that the treated soil remains moist during the period of solarization.

Solarization was not effective in preventing the development of cankers on plants infected at the time of treatment. This suggests that solarization is unlikely to be a useful technique to eradicate *Phytophthora* from naturally infected nursery stock although imposing solarization at or soon after planting should prevent the development of *Phytophthora* related soil diseases.

Solarization was not detrimental to plant growth even though high soil temperatures were measured in the usual zone of root growth. In fact the increased growth response following solarization indicates that it may be a useful technique to ensure the rapid development of young trees (Stapleton and Devay (1985).

These studies have shown that there are several aspects of solarization that could have practical implications and warrant further investigation. In particular the mechanism that inhibits *Phytophthora* in solarized soils and the persistance of the solarization effect needs further study. In addition, fungitoxic gases trapped by tarping have also been implicated with solarization (Katan 1981) and this also needs investigating as it may be of use in mature orchards where temperatures inhibitory to *Phytophthora* are unlikely to be reached due to shading by the tree canopy.

GENERAL DISCUSSION

As a result of these studies, the following recommendations can be given to the almond industry.

(a) Plant 'Nemaguard' rootstocks in sites where trees have been killed by *Phytophthora* crown rot.

(b) Bud or graft 'Nemaguard' rootstocks at least 15 cm above the soil level to reduce the possibility of burying the scion and exposing the tissue to infection by *Phytophthora*.

(c) 'Deep rip' replant sites and treat the sites with lime or gypsum to improve soil drainage as 'Nemaguard' rootstocks as well as almond seedling rootstocks are intolerant of waterlogged soils.

(d) Plant seedlings on 10 to 20 cm high mounds to prevent water accumulating around the trunk.

(e) Avoid poorly drained soils for new plantings.

(f) Plant only healthy nursery rootstocks. Rootstocks should be obtained from nurseries where plants have been grown in recently fumigated soil.

(g) Apply up to 100 g of 'Ridomil' granules per tree to protect healthy trees adjacent to trees affected with crown rot.

(h) Apply 'Ridomil' in early spring in a shallow trench around and immediately adjacent to the trunk rather than broadcast onto the soil surface. Repeat applications may be required in autumn and winter.

(i) Do not apply 'Ridomil' to severely declining trees.

Similar recommendations apply to the cherry industry in relation to soil preparation and the use of healthy rootstocks and fungicides. With the use of cherry rootstocks however, the present studies suggest that the incidence

of disease is likely to be less if Mazzard rather than Mahaleb rootstocks are used in poorly drained soils.

With further studies it should be possible to refine and revise many of these recommendations that will ultimately result in efficient and effective control of *Phytophthora* crown rot.

Of immediate concern is to determine more precisely the most appropriate time to apply fungicides and to develop a simple and reliable method of monitoring fungicide levels in the plant or soil that will indicate the need to reapply fungicides. Unless such techniques are developed, fungicides are not likely to be used efficiently and trees are likely to be frequently under or over dosed. With soil applied fungicides the development of 'encapsulated' fungicide formulations that slowly release the active ingredient into the soil over an extended period of time may be of benefit to orchardists particularly if the applications were needed only once a year.

In the future however, chemical control of crown rot is more likely to be achieved with foliar applications of fungicides. This is evident from the work with phosphorous acid. Although the material was phytotoxic to almonds in the present studies, further investigations are required to determine the most appropriate means of application that do not give rise to phytotoxicity. Trunk injections of phosphorous acid solutions and other fungicide formulations need to be evaluated, but this technique is unlikely to be economic in crops such as almond and cherry. Frequent foliar applications of phosphorous acid at low rates also need to be evaluated as this may provide levels of phosphorous acid high enough to be inhibitory to *Phytophthora* in the trunk and roots without being phytotoxic.

Another technique may be to apply high rates of phosphorous acid after harvest, but before leaf fall. Although the application at this stage is likely to be phytotoxic, particularly to almonds, the effect may not be detrimental to next season's crop yield. This aspect needs to be investigated as pre leaf fall applications may provide sufficient phosphorous acid to be taken up, translocated and retained within the plant at levels inhibitory to *Phytophthora* the following spring. This technique also needs to be evaluated for use on nursery stock as foliar applications of 'systemic' fungicides in late summer may ensure that planting material is not infected with *Phytophthora*.

Because of the potential problems of enhanced biological degradation in soils regularly treated with fungicides as well as the possible development of strains of *Phytophthora* insensitive to soil fungicides, considerable effort is warranted in developing biological methods of control. More work is needed in breeding and selecting both almond and cherry rootstocks that are resistant to *Phytophthora*. However this work is long-term and involves evaluation of the rootstocks in relation to the compatibility with the scion, root production, effect on yield and many other horticulturally important factors not necessarily related to disease resistance. Determining the mechanism(s) of resistance in 'Nemaguard' rootstocks may provide information useful to a breeding and selection programme directed towards resistance to *Phytophthora*.

Another area that needs more research is the evaluation of microorganisms that could be used for biological control.

Bacteria and other microorganisms antagonistic to *Phytophthora* are not uncommon. Bacteria have controlled *P. cinnamomi* in artificially infested soil (Kellman and Coffey 1985) and *P. cactorum* in naturally infected soil (Utkhede 1986).

These bacteria as well as local isolates of organisms that show antagonism to *P. cambivora* in vitro should be evaluated as potential biological control agents. This could be done by dipping the roots of almond and cherry seedlings in a suspension of the organisms before the seedlings are planted in field soil naturally or artificially infested with *P. cambivora*.

It may also be profitable to evaluate chicken manure as a soil amendment for use around the tree trunk as well as mushroom compost and composted bark as a planting medium. If these are found to control *Phytophthora* in the field then isolations for organisms antagonistic to *P. cambivora* could be made from these materials.

Another approach worth evaluating is to protect plants from root and crown rot by inoculating them with non pathogenic isolates of *Phytophthora*. Dolan *et al.*, (1986) inoculated *Persea indica* seedlings with zoospores of *P. parasitica* and showed that this protected the plants against root rot caused by either *P. cinnamomi* or *P. citricola*.

Using this technique non pathogenic isolates of *P. megasperma* and other *Phytophthora* species isolated from orchard soils could be evaluated as protectants by inoculating almond and cherry seedlings with zoospores of

these fungi before the seedlings are planted in soil infested with *P. cambivora*.

Additional studies are also warranted on the mechanisms involved in the suppression of *Phytophthora* crown rot in solarized soil. It would be useful to demonstrate the presence of antagonistic microflora in the solarized soil and to determine if these attack the fungus either directly or indirectly by inhibiting bacteria that stimulate the production of sporangia. If antagonists are involved then their survival rate after solarization should be determined as well as the possibility of enhancing their survival by adding organic matter to the soil either before or after solarization.

Another factor to determine is whether the upper levels of solarized soil are recolonised by the pathogen growing from deeper levels of soil.

In summary the future direction of research that is most likely to result in the control of *Phytophthora* crown rot of almond and cherry can be separated into short-term studies involving the refinement of chemical methods of control and long-term studies associated with resistance breeding programmes and the development of biological control methods.

BIBLIOGRAPHY

Aldwinkle, H.S., Polach, F.J., Molin, W.T., and Pearson, R.C. (1975). Pathogenicity of *Phytophthora cactorum* isolates from New York apple trees and other sources. Phytopathology 65: 989-994.

Allen, R.N., Pegg, K.G., Forsberg, L.I., and Firth, D.J. (1980). Fungicidal control in pineapple and avocado of disease caused by *Phytophthora cinnamani*. Aust. J. Exp. Agric. Anim. Husb. 20: 119-124.

Ashworth, L.J. Jr., and Gaona, S.A. (1982). Evaluation of clear polyethylene mulch for controlling Verticillium wilt in established pistachio nut groves. Phytopathology 72: 243-246.

Baker, K.F. (1972). The U.C. system for producing healthy container grown plants. Calif. Agric. Exp. Stn. Man. 23: 68-86.

Bailey, A.M., and Coffey, M.D. (1984). A sensitive bioassay for quantification of metalaxyl in soils. Phytopathology 74: 667-669.

Bailey, A.M., and Coffey, M.D. (1985). Biodegradation of metalaxyl in avocado soils. Phytopathology 75: 135-137.

Baker, B.T., and Gathercole, F. (1977). Commercial almond growing. Dept. Agric. Fish., South Australia Bull. No. 9/77.

Barbercheck, M.E., and von Broembsen, S.L. (1986). Effects of soil solarization on plant-parasitic nematodes and *Phytophthora cinnamani* in South Africa. Plant Dis. 70: 945-950.

Blaker, N.S., and MacDonald, J.D. (1981). Predisposing effects of soil moisture extremes on the susceptibility of rhododendron to Phytophthora root and crown rot. Phytopathology 71: 831-834.

Borecki, Z., Ross, J.A., and Millikan, D.F. (1970). Endogenous factors in apple bark which stimulate and inhibit the growth of *Phytophthora cactorum*. Phytopathology 66: 173-174.

Bostock, R.M., and Doster, M.A. (1985). Association of *Phytophthora syringae* with pruning wound cankers of almond trees. Plant Dis. 69: 568-571.

Boughton, T.J., Malajczuk, N., and Robson, A.D. (1978). Suppression of infection of Jarrah roots by *Phytophthora cinnamomi* with application of calcium carbonate. Aust. J. Bot. 26: 611-615.

Bower, L.A., and Coffey, M.D. (1984). Development of resistance to phosphorous acid in *Phytophthora capsici*. (Abstr.) Phytopathology 74: 811.

Bruin, G.C.A., and Edgington, L.V. (1981). Adaptive resistance in Peronosporales to metalaxyl. Can. J. Plant Pathol. 3: 201-206.

Bumbieris, M., Wicks, T.J., and Windle, B.E. (1982). Phytophthora species in apple and cherry orchards in South Australia. Aust. Plant Pathol. 11: 28-29.

Byrt, P.N., Irving, H.R., and Grant, B.R. (1982). The effect of organic compounds on the encystment, viability and germination of zoospores of *Phytophthora cinnamomi*. J. Gen. Microbiol. 128: 2343-2351.

Campbell, A.I. (1969). The effect of some apple viruses on the susceptibility of two clonal rootstocks to collar rot caused by *Phytophthora cactorum*. J. Hort. Sc. 44: 69-73.

Coffey, M.D., and Bower, L.A. (1984a). In vitro variability among isolates of six *Phytophthora* species in response to metalaxyl. Phytopathology 74: 502-506.

Coffey, M.D., and Bower, L.A. (1984b). In vitro variability among isolates of eight *Phytophthora* species in response to phosphorous acid. Phytopathology 74: 738-742.

Coffey, M.D., and Joseph, M.C. (1985). Effects of phosphorous acid and fosetyl-Al on the life cycle of *Phytophthora cinnamomi* and *P. citricola*. Phytopathology 75: 1042-1046.

Coffey, M.D., Klure, L.J., and Bower, L.A. (1984a). Variability in sensitivity to metalaxyl of isolates of *Phytophthora cinnamomi* and *Phytophthora citricola*. Phytopathology 74: 417-422.

Coffey, M.D., Ohr, H.D., Campbell, S.D., and Guillemet, F.B. (1984b). Chemical control of *Phytophthora cinnamomi* on avocado rootstocks. Plant Dis. 68: 956-958.

Conway, W.S. (1982). Effect of post harvest calcium treatment on decay of Delicious apples. Plant Dis. 66: 402-403.

Cother, E.J. (1975). *Phytophthora drechsleri*: Pathogenicity testing and determination of effective host range. Aust. J. Bot. 23: 87-94.

Dakwa, J.T., and Sewell, G.W.F. (1981). Influence of rootstock type and time of inoculation on the resistance of five apple scion cultivars to collar rot caused by *Phytophthora cactorum*. J. Hort. Sc. 56: 357-362.

Darvas, J.M., Toerien, J.C., and Milne, D.L. (1984). Control of avocado root rot by trunk injection with phosethyl-Al. Plant Dis. 68: 691-693.

Davis, R.M. (1982). Control of Phytophthora root and foot rot of citrus with systemic fungicides metalaxyl and phosethyl-aluminum. Plant Dis. 66: 218-220.

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

Davison, E.M., and Tay, F.C.S. (1986a). The effect of aeration on colony diameter, sporangium production and zoospore germination of *Phytophthora cinnamomi*. New Phytol. 103: 735-744.

Davison, E.M., and Tay, F.C.S. (1986b). The effect of water logging on infection of *Eucalyptus marginarta*. seedlings by *Phytophthora cinnamomi*. New Phytol. 105: 585-594.

Dolan, T.E., and Coffey, M.D. (1985). Invitro and invivo assessment of *Phytophthora palmivora*. strains resistant to phosphorous acid. (Abstr.). Phytopathology 75: 1330.

Dolan, T.E., Cohen, Y., and Coffey, M.D. (1986). Protection of *Persea* species against *Phytophthora cinnamomi* and *P. citricola* by prior inoculation with a citrus isolate of *P. parasitica*. Phytopathology 76: 194-198.

Duniway, J.M. (1983). Role of physical factors in the development of *Phytophthora* diseases. In "Phytophthora. Its biology, taxonomy, ecology and pathology". Eds. D.C. Erwin, S. Bartnicki-Garcia and P.H. Tsao, pp. 175-187. (Am. Phytopathol. Soc : St Paul, MN.)

Ellis, M.A., Grove, G.G., and Ferree, D.C. (1982). Effects of metalaxyl on *Phytophthora cactorum* and collar rot of apple. Phytopathology 72: 1431-1434.

Ellis, M.A., and Porpiglia, P.J. (1984). Effects of application method on uptake of metalaxyl (Ridomil 2E) by apple trees. Ohio State Univ. Ohio Agric. Res. and Dev. Ctr. Res. Circ. 283: 5-8.

Farih, A., Tsao, P.H., and Menge, J.A. (1981). Fungitoxic activity of efosite aluminium on growth, sporulation and germination of *Phytophthora parasitica* and *P. citrophthora*. Phytopathology 71: 934-936.

Fatemi, J. (1980). The role of *Phytophthora* species in almond decline in Iran. Phytopath. Z., 99: 97-100.

Fenn, M.E., and Coffey, M.D. (1984). Studies on the in vitro and in vivo anti fungal activity of fosetyl-Al and phosphorous acid. Phytopathology 74: 606-611.

Fenn, M.E., and Coffey, M.D. (1985). Further evidence for the direct mode of action of fosetyl-Al and phosphorous acid. Phytopathology 75: 1064-1068.

Gates, J.E., and Millikan, D.F. (1972). Seasonal fluctuations in susceptibility of the inner bark tissues of apple to colonization by the collar rot fungus *Phytophthora cactorum*. Phytoprotection 53: 76-81.

Gerretson-Cornell, L. (1978). Notes on the morphology of an isolate of *Phytophthora cambivora*. (Petri) Buis. from Australia. Phyton. 36: 85-89.

Haasis, F.A., Marx, D.H., and Nelson, R.R. (1966). Morphological and physiological characteristics of mating types of *Phytophthora cambivora*. (Abstr.). J. Elisha Mitchell Sci. 82: 87.

Harris, D.C., and Bielenin, A. (1986). Evaluation of selective media and bait methods for estimating *Phytophthora cactorum* in apple orchards. Pl. Path. 35: 565-574.

Harris, D.C., and Tobutt, K.R. (1986). Factors influencing the mortality of apple seedlings inoculated with zoospores of *Phytophthora cactorum* (L.O.C.) Schroet. J. Hort. Sci. 61: 457-464.

Hartmann, H.T., and Kester, D.E. (1983). Plant propogation: principles and practices. 4th ed. Prentice-Hall, Englewood Cliffs, N.J.

Hayes, J.E., and Aldwinkle, H.S. (1983). Phytophthora root and crown rot of cherry in New York State. (Abstr.). Phytopathology 73: 366.

Helton, A.W., Dilbeck, R., and Thostenson, A.A. (1984). Phytophthora collar rot in Idaho orchards. (Abstr.). Phytopathology 74: 1138.

Hunger, R.M., Hamm, P.B., Horner, C.E., and Hansen, E.M. (1982). Tolerance of *Phytophthora megasperma* isolates to metalaxyl. Plant Dis. 66: 645-649.

Jeffers, S.N. (1985). Phytophthora crown rot of apple trees. Detection and occurrence of the pathogens and seasonal variation in colonization of two apple rootstocks. Ph.D. thesis Cornell Univ., N.Y. 165 pp.

Jeffers, S.N., and Aldwinkle, H.S. (1983). Use of apple seedlings to detect *Phytophthora cactorum* in apple orchards. (Abstr.). Phytopathology 73: 368.

Jeffers, S.N., and Aldwinkle, H.S. (1986). Seasonal variation in the extent of colonization of two apple rootstocks by five species of *Phytophthora*. Plant Dis. 70: 941-945.

Jeffers, S.N., Aldwinkle, H.S., Burr, T.J., and Arneson, P.A. (1981). Excised twig assays for the study of apple tree crown rot pathogens in vitro. Plant Dis. 65: 823-825.

Jeffers, S.N., and Wilcox, W.F. (1986). Preplant root dips for apple rootstocks naturally infested with *Phytophthora* species. (Abstr.). Phytopathology 76: 1105.

Jones, R.W. (1972). Titan, a seed source for F_1 Almond x Nemaguard Peach hybrids. Fruit Var. Hortic. Dig. 26: 18-20.

Joseph, M.C., and Coffey, M.D. (1984). Development of laboratory resistance to metalaxyl in *Phytophthora citricola*. Phytopathology 74: 1411-1414.

Julis, A.J., Clayton, C.N., and Sutton, T.B. (1978). Detection and distribution of *Phytophthora cactorum* and *P. cambivora* on apple rootstocks. Plant Dis. Rept. 62: 516-520.

Julis, A.J., Clayton, C.N., and Sutton, T.B. (1979). Occurrence of Phytophthora sp. in North Carolina apple orchard soils. Plant Dis. Rept. 63: 147-150.

Kassaby, F.Y. (1985). Solar-heating soil for control of damping-off diseases. Soil Biol. Biochem. 17:429-434.

Katan, J. (1981). Solar heating (solarization) of soil for control of soil borne pests. Ann. Rev. Phytopathol. 19: 211-236.

Kellan, M.K., and Coffey, M.D. (1985). Bacterial isolates control Phytophthora root rot of *Persea indica* and lupin. (Abstr.) Phytopathology 75: 1343.

Kester, D.E., and Asay, R.N. (1986). 'Hansen 2168' and 'Hansen 536': Two new *Prunus* rootstock clones. Hort. Science. 21: 331-332.

Kliejunas, J.T., and Ko, W.H. (1976). Dispersal of *Phytophthora cinnamomi* on the island of Hawaii. Phytopathology 66: 457-460.

Kouyeas, H., and Chitzanidis, A. (1968). Notes on Greek species of *Phytophthora*. Ann. Inst. Phytopathol. Benaki, N.S. 8. 175-192.

Kouyeas, H. (1971). On the apoplexy of stone fruit trees casued by *Phytophthora* spp. Ann. Inst. Phytopathol. Benaki, N.S. 10: 163-170.

Kuan, T.L., and Erwin, D.C. (1980). Predisposition effect of water saturation of soil on Phytophthora rootrot of alfalfa. Phytopathology 70: 981-986.

Lee, B.S., and Zentmyer, G.A. (1982). Influence of calcium nitrate and ammonium sulphate on Phytophthora root rot of *Persea indica*. Phytopathology 72: 1558-1564.

Lee, T.C., and Wicks, T. (1977). *Phytophthora cinnamomi* in native vegetation in South Australia. Aust. Plant Pathol. Soc. Newsl. 6: 22-23.

MacDonald, J.D. (1982). Effect of salinity stress on the development of Phytophthora root rot of chrysanthemum. Phytopathology 72: 214-219.

MacDonald, J.D. (1984). Salinity effects on the susceptibility of chrysanthemum roots to *Phytophthora cryptogea*. Phytopathology 74: 621-624.

MacDonald, J.D., and Shapiro, J.D. (1985). Influence of heat stress on root susceptibility to Phytophthora infection. (Abstr.) Phytopathology 75: 1306.

Mackay, A., Weste, G., and Sharpe, K. (1985). Survival of *Phytophthora cinnamomi* in buried *Eucalptus* roots. Phytopath. Z 144: 214-223.

Malajczuk, N. (1983). Microbial antagonism to *Phytophthora*. In "Phytophthora. Its biology, taxonomy, ecology and pathology". Eds. D.C. Erwin, S. Bartmicki-Garcia and P.H. Tsao, pp. 197-218 (Am. Phytopathol. Soc. St. Paul, M.N.)

Matheron, M.E., and Mircetich, S.M. (1985a). Seasonal variation in susceptibility of *Juglans hindsii* and Paradox rootstocks of English walnut trees to *Phytophthora citricola*. Phytopathology 75: 970-972.

Matheron, M.E., and Mircetich, S.M. (1985b). Pathogenicity and relative virulence of *Phytophthora* spp. from walnut and other plants to rootstocks of English walnut trees. Phytopathology 75: 977-981.

Matheron, M.E., and Mircetich, S.M. (1985c). Control of Phytophthora root and crown rot and trunk canker in walnut with metalaxyl and fosetyl-Al. Plant Dis. 69: 1042-1043.

Matheron, M.E., and Matejka, J.C. (1986). Efficacy of trunk applications of phosphorous acid and three other fungicides for control of Phytophthora gummosis on citrus. (Abstr.). Phytopathology 76: 1066.

McGuire, R.G., and Kelman, A. (1984). Reduced severity of Erwinia soft rot in potato tubers with increased calcium content. Phytopathology 74: 1250-1256.

McIntosh, D.L. (1964). Phytophthora spp. in soils of the Okanagan and Similkameen Valleys of British Columbia. Can. J. Bot. 42: 1411-1415.

McIntosh, D.L. (1966). The occurrence of Phytophthora spp. in irrigation systems in British Columbia. Can. J. Bot. 44: 1591-1596.

McIntosh, D.L., and MacSwan, I.C. (1966). The occurrence of collar rot caused by Phytophthora cactorum in planting of apple trees aged 1 to 7 years. Plant Dis. Rep. 50: 267-270.

Mircetich, S.R., Moller, W.J., and Chaney, D.H. (1974). Phytophthora crown rot and trunk canker of almond trees. (Abstr.). Phytopathology 64: 58.

Mircetich, S.M., and Matheron, M.E. (1976). Phytophthora root and crown rot of cherry trees. Phytopathology 66: 549-558.

Mirectich, S.M., and Matheron, M.E. (1981). Differential resistance of various cherry rootstocks to Phytophthora species. (Abstr.). Phytopathology 71: 243.

Moller, W.J., and Teviotdale, B.L. (1978). Diseases of almonds. In "Almond orchard management". Eds., W. Micke and D. Kester pp. 108-116. Divn. of Ag. Sc. Univ. Calf. Publn. 4092.

Newhook, F.J., Waterhouse, G.M., and Stamps, D.J. (1978). Tabular key to the species of *Phytophthora* de Bary. Mycol. Pap. 143: 1-20.

Old, K.M., Oros, J.M., and Malafant, K.W. (1984). Survival of *Phytophthora cinnamomi* in root fragments in Australian forest soils. Trans. Br. mycol. Soc. 82: 605-613.

Old, K.M., Moran, G.F., and Bell, J.C. (1984). Isozyme variability among *Phytophthora cinnamomi* from Australia and Papua New Guinea. Can. J. Bot. 62: 2016-2022.

Pegg, K.G., Whiley, A.W., Saranah, J.B., and Glass, R.J. (1985). Control of Phytophthora root rot of Avocado with phosphorous acid. Aust. Plant Pathol. 14: 25-29.

Pinkas, Y., Kariv, A., and Katan, J. (1984). Soil solarization for the control of *Phytophthora cinnamomi*: thermal and biological effects. (Abstr.). Phytopathology 74: 796.

Porter, I.J., and Merriman, P.R. (1983). Effects of solarization of soil on nematode and fungal pathogens at two sites in Victoria. Soil Biol. Biochem. 15: 39-44.

Porter, I.J., and Merriman, P.R. (1985). Evaluation of soil solarization for control of root diseases of row crops in Victoria. Pl. Path. 34: 104-108.

Rana, K.S., and Gupta, V.K. (1984). Effect of fungicides on the viability of *Phytophthora cactorum* propagules in the soil. Phytopath. Z. 110: 243-250.

Ribeiro, O.K. (1978). A source book of the genus *Phytophthora*. J. Cramer, Germany. 420 pp.

Rowe, R.N., and Beardsell, D.V. (1973). Waterlogging of fruit trees. Hort. Abstr. 43: 534-548.

Sewell, G.W.F., and Wilson, J.F. (1959). Resistance trials of some apple rootstock varieties to *Phytophthora cactorum* (L. & C.) Schroet. J. Hort. Sci. 34: 51-58.

Sewell, G.W.F., and Wilson, J.F. (1973a). Phytophthora collar rot of apples: seasonal effects on infection and disease development. Ann. appl. Biol. 74: 149-158.

Sewell, G.W.F., and Wilson, J.F. (1973b). Phytophthora collar rot of apple: influence of the rootstock on scion variety resistance. Ann. appl. Biol. 74: 159-169.

Sewell, G.W.F., and Wilson, J.F. (1974). Seasonal factors and *Phytophthora* collar rot. Rep. E. Malling Res. Stn. for 1973: 183-185.

Sewell, G.W.F., Wilson, J.F., and Dakwa, J.T. (1974). Seasonal variations in the activity in soil of *Phytophthora cactorum*, *P. syringae* and *P. citricola* in relation to collar root disease of apple. Ann. appl. Biol. 76: 179-186.

Sharom, M.S., and Edgington, L.V. (1982). The adsorption, mobility and persistence of metalaxyl in soil and aqueous systems. Can. J. Plant Pathol. 4: 334-340.

Shea, S.R., and Broadbent, Patricia (1983). Developments in cultural and biological control of *Phytophthora* diseases. In "Phytophthora. Its biology, taxonomy, ecology and pathology". Eds, D.C. Erwin, S. Bartnicki-Garcia and P.H. Tsao, pp. 335-350. (Am. Phytopathol. Soc.: St Paul, M N.)

Shea, S.R., Gillen, K.J. and Leppard, W.I. (1980). Seasonal variation in population levels of *Phytophthora cinnamomi* Rands in soil in diseased, freely-drained *Eucalyptus marginata* SM sites in the northern jarrah forests of south western Australia. Protect. Ecol. 2: 135-156.

Stack, J.P., and Millar, R.I. (1985). Isolation and characterization of metalaxyl-insensitive isolate of *Phytophthora-megasperma* f.sp *medicaginis*. Phytopathology 75: 1014-1019.

Stapleton, J.J., and Devay, J.E. (1982). Effect of soil solarization on populations of selected soil borne micro-organisms and growth of deciduous fruit tree seedlings. Phytopathology 72: 323-326.

Stapleton, J.J., and Devay, J.E. (1984). Thermal components of soil solarization as related to changes in soil and root microflora and increased plant growth response. Phytopathology 74: 255-259.

Stapleton, J.J., and Devay, J.E. (1985). Soil solarization as a post-plant treatment to increase growth of nursery trees. (Abstr.). Phytopathology 75: 1179.

Suzuki, T., and Hoshino, Y. (1979). Collar rot of apple caused by *Phytophthora cambivora* (Petri) Buism. Ann. Phytopath. Soc. Japan. 45: 344-352.

Taylor, P.A. (1977). *Phytophthora* spp. in irrigation water in the Goulburn Valley, Victoria. Aust. Plant Pathol. Soc. Newsl. 6: 41-42.

Taylor, P.A. (1980). Relative susceptibility of peach roots and trunks to *Phytophthora* rot (Abstr.). 4th Nat. Aust. Plant Pathol. Soc. Conf. Perth.

Taylor, P.A., and Washington, W.S. (1984). Curative treatments of *Phytophthora cactorum* in peach trees using metalaxyl and phosethyl-Al. Aust. Plant Pathol. 13: 31-33.

Tippett, J.T., and Hill, T.C. (1983). The relationship between bark moisture and invasion of *Eucalyptus marginarta* by *Phytophthora cinnamani*. Aust. Plant. Pathol. 12: 40-41.

Tippett, J.T., Shea, S.R., Hill, T.C., and Shearer, B.L. (1983). Development of lesions caused by *Phytophthora cinnamomi* in the secondary phloem of *Eucalyptus marginata*. Aust. J. Bot. 31: 197-210.

Tsao, P.H. (1960). A serial dilution end point method for estimating disease potentials of citrus Phytophthoras in soil. Phytopathology 50: 717-724.

Tsao, P.H., and Guy, S.O. (1977). Inhibition of Mortierella and Pythium in a Phytophthora-isolation medium containing hymexazol. Phytopathology 67: 796-801.

Utkhede, R.S. (1984a). Effects of nitrogen fertilisers and wood composts on the incidence of apple crown rot in British Columbia. Can. J. Plant Pathol. 6: 329-332.

Utkhede, R.S. (1984b). Effects of fungicides on apple crown rot caused by *Phytophthora cactorum*. Pestic. Sci. 15: 241-246.

Utkhede, R.S. (1986). Biological control of apple crown rot. (Abstr.) Phytopathology 76: 847.

Waterhouse, G.M. (1970). The genus *Phytophthora* de Bary (2nd ed.) Mycol. Pap. 122: 1-59.

Weste, G. (1975). Comparative pathogenicity of root parasites to wheat seedlings. Trans. Br. mycol. Soc. 64: 43-53.

Weste, G., and Ruppin, P. (1977). *Phytophthora cinnamomi*: Population densities in forest soils. Aust. J. Bot. 25: 461-475.

Weste, G., and Vithanage, K. (1978). Seasonal variation in numbers of chlamydospores in Victorian forest soils infected with *Phytophthora cinnamomi*. Aust. J. Bot. 26: 657-662.

Weste, G. (1983). Population dynamics and survival of *Phytophthora*. In "Phytophthora. Its biology, taxonomy, ecology and pathology". Eds., D.C. Erwin, S. Bartnicki-Garcia and P.H. Tsao, pp. 237-257. (Am. Phytopathol. Soc: St. Paul, M.N.).

Wicks, T., and Lee, T.C. (1985). Effects of flooding, rootstock and fungicides on Phytophthora crown rot of almonds. Aust. J. Expt. Agr. 25: 705-710.

Wicks, T., and Lee T.C. (1986). *Phytophthora* crown rot of almond trees. Aust. J. Ag. Res. 37: 277-287.

Wilcox, W.F., Jeffers, S.N., Hayes, J.E.K., and Aldwinkle, H.S. (1985). *Phytophthora* species causing root and crown rot of cherry trees in New York. (Abstr.). Phytopathology 75: 1347.

Wilcox, W.F., and Mircetich, S.M. (1982). Host non-specificity of *Phytophthora megasperma* isolates recovered from 14 different plant species (Abstr.). Phytopathology 72: 995.

Wilcox, W.F., and Mircetich, S.M. (1985a). Pathogenicity and relative virulence of seven *Phytophthora* spp. on Mahaleb and Mazzard cherry. Phytopathology 75: 221-226.

Wilcox, W.F., and Mircetich, S.M. (1985b). Influence of soil matrix potential on the development of Phytophthora root and crown rots of Mahaleb cherry. Phytopathology 75: 648-653.

Wilcox, W.F., and Mircetich, S.M. (1985c). Effects of flooding duration on the development of Phytophthora root rot and crown rots of cherry. Phytopathology 75: 1451-1455.

Zentmyer, G.A. (1980). *Phytophthora cinnamomi* and the disease it causes. Monogr. 10. Am. Phytopathol. Soc., St. Paul, M.N. 96pp.