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LABORATORY AND FIELD EVALUATIONS OF PROPOLIS AS A PLANT PROTECTIVE AGENT

A thesis submitted in partial fulfilment
of the requirement for the degree of
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at Massey University

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ABSTRACT

Propolis is a plant derived resinous substance with known antibiotic properties. Laboratory and field trials were carried out in 1989/90 to evaluate propolis for control of insects and diseases in horticultural systems. Field trials were carried out in the organic block of Levin Horticultural Research Station. Ether extracts of propolis in agar (10, 100 1 000 and 10 000 ppm) were screened against 20 plant pathogenic fungi. Radial mycelial growth from fungal plugs were measured daily. Propolis inhibited the growth of all fungi tested although the sensitivity of fungi to propolis varied. The EC50 was between 100 and 10 000 ppm for all species with complete inhibition at 10 000 ppm in 16 species. Propolis collected from different geographic locations had different activity. There was less antifungal activity in water extracts than in ether extracts of propolis.

Ethanol, surfactant and ethanol extracts of propolis were sprayed on cucumber plants weekly in a glasshouse. Weekly estimates of powdery mildew cover (*Erysiphe cichoracearum*) for 5 weeks were analysed. Foliar spray applications of 1% propolis extract reduced powdery mildew cover from 84.5% in the untreated plants to 33.4% in the treated ones.

Eight treatments were tested on a 10 day spray calendar on zuccinis. Assessment for powdery mildew cover was made on four occassions. The number of harvested fruit from each plant were recorded. A 1% ethanol extract of propolis reduced powdery mildew only until the second assessment, 39% vs. 60% cover in the controls. The fruit number was not affected by treatments.

Late blight of tomatoes (*Phytophthora infestans*) in the field was not affected by foliar sprays of 1% propolis extract. Radish seeds treated with a seed dressing of 36% propolis extract were not protected against (*Pythium ultimum*) in agar petri plate trials

Laboratory screening of propolis against light brown apple moth (*Epiphyas postvittana*) and green peach aphid (*Myzus persicae*) did not indicate sufficient activity to be used in crop protection.

In conclusion propolis showed some antifungal activity in laboratory trials. Successful applications in the field using the methods evaluated here however would require concentrations of raw propolis that are both impractical and uneconomic. The potential for use of propolis in plant protection is likely to come from further chemical analysis, with identification of active components and their possible synthesis.

Key words: propolis, fungicide, insecticide, *Phytophthora infestans,* late blight of tomatoes, *Erysiphe cichoracearum*, powdery mildew of cucurbits, *Pythium ultimum*, damping off, light brown apple moth (*Epiphyas postvittana*), green peach aphid (*Myzus persicae*).

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CHAPTER 1

INTRODUCTION

Management of plant pests and diseases is necessary in most agroecosystems to obtain an acceptable quantity and quality of yield. The development of synthetic organic pesticides after World War II allowed for increased yields while reducing the apparent need for cultural and biological controls. The resulting dependence on pesticides for control has created technical problems of resistance, resurgence and secondary pest outbreaks, and cultural problems of environmental and health hazards. These problems have resulted in the term 'the pesticide crisis' and have caused an upsurge of interest in other control strategies including the use of naturally occurring pesticides (Perkin 1985). These include microbial, plant and animal derived products which are seen as less dangerous and generally more biodegradable than synthetic pesticides (Pimental 1985).

Research into natural products may produce a natural pesticide or a model on which to base synthetic pesticides. Natural pyrethrum and the synthetic pyrethroid analogues respectively exemplify both these cases. Pyrethroids have the desirable characteristics of being readily biodegradable, being selective in their action, requiring only low application rates and providing a quick knock down effect. These are the type of qualities sought in new pesticides (Todhunter 1985).

Other problems associated with the pesticide crisis can be minimised by integrated pest management where the application of any pesticide occurs only when necessary with respect to other control mechanisms.

Organic farming uses integrated pest management and promotes a holistic approach to agroecosystem management. However, fundamental to organic growing standards, is production without the use of chemically synthesised compounds except under exceptional circumstances. Naturally occurring pesticides may therefore be important for control in organic farming where pests are a problem.

This study investigated the potential of propolis as a pest control agent. Propolis, a product from beehives, has known antibiotic properties and in these trials was tested against plant pathogenic fungi in the laboratory and in the field and against insect pests in the laboratory.

CHAPTER 2

LITERATURE REVIEW

2.1 Review of pest and disease organisms

The pests and diseases studied in the outdoor trials of this research were those which are common in crops in the Levin area.

Diseases

For each plant disease studied in this trial the classification of the causal species, its epidemiology, etiology, symptoms and the methods used for control in horticultural systems are given.

2.1.1 Powdery mildew of cucurbits

Pathogen:

Class		Ascomycetes
Order		Perisporiales
Family		Erysiphaceae
Subfamily		Erysipheae
Species and genus	or	*Erysiphe cichoracearum* DC *Sphaerotheca fuliginea* (Schled. ex Fr.) Poll.

Etiology and Epidemiology

Outbreaks and development of powdery mildew in cucurbits are favoured by dry atmospheric conditions, moderate temperatures, low light intensity, fertile soil and succulent plant growth (Yarwood 1957). Glasshouses are consequently conducive to success of the pathogen particularly where continuous cropping occurs. Microclimate in the field also greatly affects the incidence and severity of disease with canopy structure being a key determinant.

The ascospores and conidia are spread by wind. They have a high water content and are therefore capable of germination in the absence of moisture at the plant surface and a relative humidity of less than 20%. Under suitable

conditions the spores germinate within two hours producing germ tubes and appresoria. After four days conidiophores are formed with the full life cycle taking 5-6 days (Stillerly 1956). *E. cichoracearum* is an ectoparasite as the haustorium absorbs food material from its host allowing growth of mycelium over the leaf surface (Brien and Dingley 1956).

Symptoms

The visible symptoms of powdery mildew on cucurbits progress from tiny, white round superficial spots to a white powdery covering over much of the leaf and stem surfaces. Young leaves and severely infected mature leaves and stems may become chlorotic and die. Fruit are generally free of visible infection and any reduction in yield is dependent on the time and severity of disease development. Late fruit may not mature and may be small and misshapen (Walker 1952).

Control

Prior to the 1950s, sulphur was the main material used for chemical control of powdery mildew. *E. cichoracearum* is vulnerable to elemental sulphur throughout its life cycle, except the cleistothecial stage. Application of 4-5 kg ha^{-1} of sulphur at 10-14 day intervals was a common field control strategy. The sulphur acts by selective toxicity with *E. cichoracearum* being more sensitive than the host plant. This however causes problems with sulphur sensitive plants such as a cantaloupe and cucumber. Consequently in the 1950s organic fungicides that could be used on sulphur sensitive species were developed. The nitrocompound dinocap, Karathane^{R} for example, was the first to be introduced and had both eradicant and protectant action. Now it is marketed only for its protectant property (O'Connor 1990).

Triadimefon and benomyl are more recent products that are both systemic and protectant in their action. Bayleton^{R} and Benlate^{R}, are trade preparations of these chemicals. Resistance build up by the plant pathogens against these chemicals is common. Their use should therefore be kept to a minimum by timing applications according to climatic conditions and by alternating with other products when frequent applications are required for protection.

Economic importance

Powdery mildew rarely directly affects fruit quality. It is only economically important when the disease reduces effective leaf area to an extent that it results in reduced yield.

2.1.2 Late blight of tomatoes

Pathogen:

Class	Phycomycete
Order	Pernosporales
Family	Pythiaceae (water moulds)
Genus	*Phytophthora*
Species	*infestans*

Etiology and Epidemiology

Germination of spores of *P. infestans* requires moisture so the disease is favoured in wet seasons and with cool nights and warm days when dew forms. The spores are killed with dry atmospheres and with temperatures greater than 27°C. The optimum temperature for germination is 10-15°C. After successful infection *P. infestans* produces copious mycelium which branches out through host tissue (characteristic of Pernosporales). Sporangia are produced on special branched mycelia (sporangiophores) that arise through stomatal openings on lower surfaces and margins of lesions. These sporangia produce zoospores which may then be liberated and dispersed to adjacent plants by rain splash, wind or insects. *P. infestans* may survive between crops in plant refuse in the soil (Brien and Dingley 1956).

Symptoms

The first symptoms appear as greenish/brown, irregular, water soaked patches on leaves and leaf stalks. As the mycelium moves through the host tissue these areas enlarge and darken. During wet weather sporulation occurs on these lesions and symptoms spread to the stems and fruit. The fruit lesions develop

from greenish brown to brown with definite margins. When conditions are warm and wet for prolonged periods at the plant surface the infected leaves are killed and within a few days the whole plant may die (Brien and Dingley 1956).

Control

Prior to the 1970s control of late blight of tomatoes and potatoes was achieved chemically using copper, principally Bordeaux mixtures ($CuSO_4$ and hydrated lime). This acts as a protectant on the surface of the plant by inhibiting germination of the spores. Various copper formulations have been developed, such as CuOCl, but with CuOH being the most common in usage now. New formulations, such as those marketed as Kocide^R and Champ^R, aim at reducing the particle size to minimise the amount of copper required to provide a protectant coating against the pathogen.

In 1977 metalaxyl was introduced for the control of late blight. With systemic action it was advantageous over the purely surface protectant character of copper. However resistant sporangia built up rapidly under blight favourable conditions with the resistant isolates being especially competitive over the susceptible ones (Davidse *et al* 1985). This initiated the implementation of new control strategies for foliar diseases based on formulated mixes (Staub *et al* 1984). Ridomil^R MZ 72WP is one such formulation mix which consists of metalaxyl and mancozeb (O'Connor 1990). Use of this systemic and protectant mix reduces the selection pressure from each chemical.

Chemical disease control in organic systems relies largely on the use of copper as a protectant. The copper must cover the plant surface whenever conditions are conducive to infection. However high levels of application may be harmful to the soil biota. There have been several reports of copper based materials causing a reduction in earthworm populations. Cluzeau and Fayolle (1988) for example compared vineyards that did and did not apply copper sprays. The sprayed orchards had a consequently higher level of soil copper and had virtually no earthworms whereas those with low soil copper levels had normal earthworm populations. Wei-chun Ma (1984) reported that additions of copper based materials to soil decreased cocoon production and may consequently seriously affect earthworm populations.

Management to minimise conditions that are optimal for *P. infestans* minimises the requirement for chemical control. The humidity and moisture at the plant surface can be reduced by encouraging good air flow within the crop. This is achieved by removing the lower leaves and pruning to produce an open canopy, training onto wires for example.

Tomatoes and potatoes should not follow potatoes in a rotation as the potato tuber and plant debris are common overwintering hosts for the pathogen. Likewise tomatoes should not be planted next to potatoes as tubers and the potato crop act as a source of inoculum for disease spread (C.M.I. 1985).

Economic importance

As a result of the direct effect on fruit quality and indirectly on the yield *P. infestans* has the potential to cause high economic losses.

2.1.3 Storage rot of kiwifruit

Pathogen:

Class	Fungi imperfecti
Order	Hyphomycetes
Family	Monilliales
Genus	*Botrytis*
Species	*cinerea*

Etiology and epidemiology

Botrytis cinerea causes kiwifruit to rot when stored at 0°C. Two stages of infection have been suggested by Pennycook (1984). At blossom time dying petals act as a food source for *B. cinerea* which can consequently grow and produce masses of spores. Inactive symptomless infections (quiescent infections) can become established in areas where the petals are attached to the newly formed fruit. About six months later these quiescent infections may be reactivated and rot symptoms develop.

The second suggested time of infection is at harvest when inoculum enters the fruit through the harvest wound. Beever *et al* (1984) suggest that this is the main source of infection.

Symptoms

The external symptoms of the storage rot are a conspicuous darkening and softening of the fruit starting at the calyx end. As the rot progresses through the fruit a faint pinkish fawn discolouration of the skin may develop. Later a thick white hyphal mass may appear with sporulation and nesting, or infection of neighbouring fruit may occur. Generally the first rots are visible after 3 weeks of storage after harvest, most are present after 4-6 weeks while some may not develop for 3-4 months.

Economic importance

Storage rot caused by *B. cinerea* was virtually unknown in kiwifruit in New Zealand before 1978, but by 1984 infection levels were commonly reaching 1% (Pennycook 1984). It is now considered a disease of major economic importance. The infection may spread to give 25% loss of fruit and any presence of storage rot can have a major detrimental effect on New Zealand's high quality export image.

Control

Control of the storage rot presently relies on chemical sprays at blossom and at harvest to reduce inoculum levels. A post harvest dip to inhibit entry of the pathogen through the harvest wound is not permitted because of residue restrictions on export fruit.

The dicarboximides vinclozolin and iprodione, marketed as Ronilan^{R} and Rovral^{R} are the two recommended fungicides to be sprayed at flowering and one day before harvest. They both have eradicant activity but as resistance may occur their use should be restricted to only the two sprays in the season (O'Connor 1990).

2.1.4 Damping off of seedlings

Pathogen:

Class	Phycomycete
Order	Pernosporales
Family	Pythiaceae (water moulds)
Genus	*Pythium*
Species	*ultimum*

Etiology and epidemiology

Like *Phytophthora infestans*, *P. ultimum* is a water mould and therefore requires water to complete its life cycle. *P. ultimum* survives as oospores and can probably subsist as a saprophyte or as a low grade parasite on fibrous roots (Walker 1952). *P. ultimum* can produce copious filamentous mycelia which ramify through cells of the plant. Asexual sporangia form and produce zoospores rapidly and hence given a food source the disease may quickly develop.

Symptoms and economic importance

The failure of seedlings to emerge is the most common symptom of *P. ultimum* attacking germinating seeds. This can cause major losses in seeds and is a particular problem in organic growing where standards require that seedlings are produced without the use of synthetic chemicals.

Control

In conventional seedling production *P. ultimum* is effectively controlled using fungicide seed dressings or slurries. Apron^{R}, for example, is a metalaxyl based systemic chemical that penetrates the seed coat and protects the emerging seedling from oomycete damping off pathogens (O'Connor 1990).

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Insect pests

Laboratory trials were carried out on two important horticultural pests, the light brown apple moth, *Epiphyas postvittana*, and the green peach aphid, *Myzus persicae*. Both these insects are major pests and the problems involved in their control are representative of those involved with many of New Zealand's insect pests. The following gives a background on their classification, life history, economic importance and control.

2.1.5 Light brown apple moth

Class	Lepidoptera
Family	Tortricidae
Genus	*Epiphyas*
Species	*postvittana*

Life history

E. postvittana overwinters as larvae on ground cover, mummified fruit or on evergreen host plants. The adults fly and lay eggs in early spring on leaves in fruit crops. The new larvae may colonise new leaves or new plants by suspending themselves on silk threads and moving in wind currents. Young larvae feed on the lower leaf surface and shelter themselves by spinning a web. Older larvae web leaves together or to fruit or roll the edge of leaves over (characteristic of leaf roller species). Pupation takes place on foliage or beneath the tree. Several generations occur each season depending on climate (Penman 1984).

Economic importance

E. postvittana is considered a major orchard pest in New Zealand because of the fruit and foliar damage it causes (Singh *et al* 1984) and its wide host range. Larvae remove the epidermal layer of fruit causing significant cosmetic

damage. This downgrading of fruit is amplified by secondary infections or by the fruit forming scar tissue over the feeding area, as in kiwifruit. Flowers, buds and growing tips may also be damaged by feeding larvae (Penman 1984).

Control

For many export markets there is nil tolerance for leaf roller damage in fruit. This emphasises the necessity for accurate timing of insecticide applications as only the young unsheltered larvae are exposed to chemicals. Use of sex pheromone traps for monitoring populations could improve the precision of application (Suckling *et al* 1984).

Leaf roller populations can be reduced by removing mummified fruit, grazing ground cover over winter and using shelter trees that are not favoured as host plants (Penman 1984).

Broad spectrum organophosphates are the main group of chemicals used for leafroller control, azinphos-methyl sold as Gusathion^{R} for example (O'Connor 1990). Their broad spectrum activity limits their use in integrated pest management and their general toxicity reduces the desirability of their use.

In organic systems, preparations based on *Bacillus thuringiensis*, (for example Thuricide^{R}) may be used for control of lepidopterous pests including leaf roller in apples. Thuricide^{R} does not harm ladybirds, lacewings, syrphid flies, bees, wasps or predatory mites and has only low mammalian toxicity (O'Connor 1990). However it is not systemic and must be ingested and therefore must be present as a deposit on the surface of the leaves where the larvae feed.

2.1.6 Green peach aphid

Class	Hemiptera
Family	Aphididae
Genus	*Myzus*
Species	*persicae*

Life History

M. persicae overwinters in cooler areas of New Zealand either as eggs on a primary woody host or as winged forms on a secondary host. The eggs hatch as

wingless forms in spring and as numbers increase winged forms are produced that migrate to secondary hosts. Winged and wingless forms are produced throughout summer by parthenogenetic reproduction (Penman 1984).

Economic importance

M. persicae, like other aphids may cause direct plant damage by foliar feeding resulting in yellowing, wilting and distortion. Its more economically damaging effect however is the transmission of many plant viruses (Fenemore 1984)

Control

Control of virus transmission is difficult as a result of the large numbers of aphids that may occur throughout the growing season and the short amount of feeding time required in some cases for virus transmission. Systemic insecticides provide some protection but continuous protection is required to inhibit virus transmission. As in leafroller control most of the chemicals used for aphid control are broad spectrum organophosphates, many with systemic action.

Aphid control in organic growing relies on biological control and natural substances such as derris dust, garlic, soap solutions, nettle, potassium permanganate, and natural pyrethrum sprays.

2.2 Propolis

2.2.1 Definition

Propolis is the material used by bees to seal hive walls and to strengthen the borders of their combs. The word is derived from the Greek :

pro- for or in defence
polis- the city, or the hive (Ghisalberti 1979).

2.2.2 Composition

Propolis is made by bees from the sticky plant substances on the surface of woody plants. The bee gathers these substances in its mandibles and may carry 10 mg in the pollen baskets on its hind legs back to its hive (König 1985). In the hive the plant substances are mixed with bees wax and the resulting propolis added to the hive. The plant substances collected may include different types of secretions, such as lipophilic substances, mucilage, gum, oil and possibly wax, and exudates, largely resin and latex (Walker and Crane 1987). Manufactured products such as paint, bitumen and mineral oils have been used by bees where plant sources were not available (König 1985).

Propolis generally consists of 55% balsams and resins, 30% waxes, 10% etheral oils and 5% pollen (Brown 1989). The balsams and resins are largely derivatives of flavins, vanillins, chrysin and allied compounds, with aromatic unsaturated compounds like caffeic and ferulic acids. 149 constituents of propolis have been reported of which 38 are flavonoids, 14 derivatives of cinnamic acid and 12 derivatives of benzoic acid. Eleven other groups have been listed including terpenes and sesqui terpenes, alcohols and hydrocarbons (Walker and Crane 1987).

The aromatic compounds found in propolis are also common in plant sources (Ghisalberti 1979). The roles of these secondary compounds in plants are still unclear although there is increasing evidence of their role in growth, development and particularly defence (Vickery and Vickery 1981). There are many examples of phenolic compounds inhibiting growth or spore germination of particular fungi and of compounds acting as deterrents, repellents or toxins to insects (Harborne 1988). Such compounds are found at the plant surface

where they may provide a chemical barrier to invading organisms. For example flavonoids isolated and identified from the leaf surface of *Helichrysum nitens* and *Erythrina berteroana* were found to have significant antifungal activity (Tomas-Barberan *et al* 1988). Other plant defence compounds, phytoalexins, are produced specifically in response to invasion of an organism and accumulate at the site of infection (Harborne 1987). Plant defence compounds are consequently relatively abundant at wound sites and at other points vulnerable to attack such as young buds and bud scales, the areas from which bees collect exudates for propolis.

Some of the compounds that have been identified in propolis such as quercetin flavone and cinnamic acids are toxic to insects and may act as a feeding deterrent (Eischen and Dietz 1987). Corsi (1981) found that the essential oils identified in propolis were typical of those from likely source plants in the area. For example vanillin, eugenol and borneol are typical essential oils of coniferous plants and juniper (*Juniperus* sp.) and were found in propolis collected from areas with this flora.

The compounds used in plant defence have a rapid turnover time as the plant breaks the compounds down or converts them to other secondary compounds. The concentration in tissues therefore varies greatly depending on age of the tissue, stage of life cycle, vulnerability to attack and time of year (Vickery and Vickery 1981). This will consequently affect the concentration and types of compounds found in propolis.

The existence or extent of transformation of the plant substances by the bee as it produces the hive propolis is unknown. By using gas chromatography - mass spectrometry analysis on *Populus* x *euroamericana* bud exudate and propolis Greenaway *et al* (1987) confirmed the bud exudate as a primary source of propolis. Other compounds identified were derived from wax secreted by the bees and materials such as sugars that may have been accidentally introduced by the bee during manufacture of propolis or during subsequent passage of the bees over the propolis. One glucoside was found in abundance in the bud exudate but not in any propolis sample. It was suggested that this compound underwent enzymic hydrolysis by the bees, either during collection from the poplar or during addition of beeswax to the propolis.

New compounds are still being identified in propolis and in possible propolis sources. Bankova *et al* (1989), for example, isolated and elucidated the structure of two esters of caffeic acid and two esters of ferulic acid with isomeric pentenyl alcohols from two species of poplar and from propolis.

2.2.3 Propolis in the bee hive

Bees make use of both the physical and antimicrobial properties of propolis. Bees wax gives the main physical support in the hive as it is estimated to be eight times stronger and seven times stiffer than propolis (Adey 1986). The elasticity and strength of propolis however is used to produce a thin layer on the internal walls of the hive or any cavity the bees may inhabit (Ghisalberti 1979). Propolis is used to block holes and cracks, to repair combs, to strengthen the thin borders of the comb and for making the hive entrance weather tight and easier to defend. Propolis is used with wax to cover hive invaders that are too big for the bees to remove from the hive, wax moths or snails for example. Antimicrobial properties may keep the hive free of fungi and bacteria which would be expected to thrive in the humid environment of the hive.

2.2.4 Plant sources

The range of plant species used for propolis contributes to its complex and variable composition (Eischen and Dietz 1987). In the northern temperate zone poplar, elm, birch , alder, beech, horse chestnut and conifer are accepted as the main sources of propolis (Ghisalberti 1979). Eucalypts and introduced poplars are important sources in Australia and collection from the native grass trees, *Xanthorrhea* sp. may also occur (König 1985).

The trigger for propolis production is unknown. In Italy the main collecting season is spring, in eastern and northern Europe mid summer and in USA late summer and autumn (Koenig 1985). Collection may be dependent on the availability and softness of plant exudates, most likely favoured by warmer conditions.

Yield of propolis varies between hives and between years. Ghisalberti (1979) suggested some colonies may produce 150-200 g/year.

2.2.5 Uses of propolis

Pharmaceutical uses

Records of the use of propolis date back to at least 300 BC when it was used for its resinous and glue like properties (Ghisalberti 1979). More recently its antibiotic properties were used in folk medicine. However it is only in the last 40 years that its composition, pharmacological properties and commercial uses have been researched. Much of this work has been carried out in Eastern European countries and therefore is of limited availability to western English speaking people.

There have been many reports on the antibiotic properties of propolis. Kivalkina (1948) produced the first published research on the activity of propolis on a range of bacteria including *Streptococcus aureus*, the typhoid bacillus. More recent research includes that of Mlagan and Sulimmovic (1982) who demonstrated the inhibitory effect of propolis on *Bacillus larvae in vitro* and Anastasiu (1978) the inhibitory effect on *Pseudomonas aeruginosa*. This latter work highlighted the greater susceptibility to propolis of gram positive than gram negative bacteria. Lindenfelser (1967) tested a range of propolis samples and found most demonstrated both antibacterial and antifungal activity.

The antibiotic, styptic, astringent, antiinflammatory and anaesthetic properties of propolis have been exploited for pharmacological uses. Examples include treatment of ear and respiratory infections, ulcers, wound healing and skin tissue regeneration (Ghisalberti 1979). Antiviral activity has also been reported for propolis. König and Dustmann (1985) suggest this may be attributed to caffeoylics, a family of antiviral active compounds found in propolis. These compounds have activity against avian *Herpes* viruses and research is continuing into viruses from mammalian hosts.

Propolis is generally regarded as harmless to humans. However cases of hypersensitivity have been reported causing severe allergic reactions of the skin. Esters of caffeic acid have recently been identified as the responsible contact allergens (Stüwe *et al* 1989).

Uses in agriculture

The above antibiotic properties of propolis suggests the possibility of the use of propolis as a plant protective agent. The range of fungi found to be sensitive to propolis by Lindenfelser (1967) included 25 phytopathogenic species. Garofolo (1987) reported propolis in combination with sulphur to be highly effective against a wide range of pests and diseases *in vivo* (Table 2.2). The propolis / sulphur treatment, treatment 1, and the propolis / sulphur followed by Thioram, treatment 2, both gave similarly good control of bacterial and fungal pathogens, with treatment 1 also having good control of the insect pests. Both the propolis treatments gave better control than the synthetic pesticides in all cases.

A comparison was made between the efficacy of these preparations on plants grown in an organic system to those grown in a conventional system. The plants grown in the organic system responded quickly with rapid elimination of the pest problems. In contrast the plants in the conventional system responded only slowly and eventually chemical control was required.

2.2.6 Extraction of propolis

Propolis is obtained from the hive by scraping the inner covers and top bars (Wright-Sunflower 1988). Various extractants have been used for obtaining the active ingredients from propolis. Meresta and Meresta (1982) found the best extraction method for activity against bacteria was a solvent mixture including methyl alcohol, ether, acetone and chloroform. Ethanol has often been used, but Meresta and Meresta found this to be less effective and more expensive than other organic solvents such as ethyl ether, ethyl acetate and methylene chloride.

Mlagan and Sulimanovic (1982) found both aqueous and ethanol extracts to be effective against *Bacillus larvae in vivo*, with the ethanol extract having a slightly greater activity.

Table 2.2 Activity of propolis plus sulphur against plant pests and diseases (Garofolo 1987)

Pest	Host plant	Percent efficiency Trt 1*	Trt 2*	Trt 3*
Bacteria				
Aplanobacter michiganensis	tomatoes (plant and fruit)	98.3	-	0
Micrococcus populi	poplar	87.9	-	0
Pseudomonas savastanoi	olives	98.8	-	0
Fungi				
Oomycetes				
Phytophthora infestans	potatoes and tomatoes	85.9	91.8	65.3
Plasmopara viticola	grapevines	87.7	92.5	77.4
Ascomycetes				
Taphrina deformans	peach (foliage)	89.9	95.7	75.8
Anntenaria elaeophila	olive (foliage)	91.2	97.6	69.9
Penicillium digitatum	citrus (fruit)	97.8	-	62.8
Sphaerotheca pannosa	peach and rose (foliage)	86.5	88.9	79.6
Uncinula necator	grapevines	89.3	91.7	78.9
Microsphaera lonicera		84.2	89.5	67.5
Basidiomycetes				
Puccinia sp.	tarragon (foliage)	88.6	90.8	69.8
Deuteromycetes				
Phyllosticta populina	poplar (foliage)	88.6	91.5	74.6
Botrytis cinerea	grapes	89.7	92.8	67.5
Cycloconium oleaginum	olive (foliage)	85.9	89.7	79.8
Insects				
Myzus persicae (peach aphid)	brassicas	5.8	-	79.7
Macrosiphum rosae (rose aphid)	roses	97.5	-	77.3
Eriosoma lanigerum (woolly apple aphid)	apple	96.7	-	65.9

*Treatment 1 : 150 grams hydro-alcoholic solution of propolis and 250 grams sulphur in 100 litres of water; applied at 10-15 day intervals around sunset.

*Treatment 2 : treatment 1 followed by 5-10 grams Thioram (a ramic acid-sulphur mix rich in micronutrients) at 25,50,100 $kgha^{-1}$ depending on the foliage development.

*Treatment 3 : application of dithiocarbamate or synthetic pesticide for pathogen or insect attack respectively, applied according to label instructions.

CHAPTER 3

LABORATORY EVALUATION OF BIOLOGICAL ACTIVITY OF PROPOLIS

Laboratory trials were carried out to determine and explore possible antifungal activity of propolis. There were three sources of propolis used throughout the trials, from an apiary in Hawke's Bay, MAF Invermay and Eltham apiary. The relevant source is stated at the start of each section.

3.1 DETECTION OF ANTIFUNGAL ACTIVITY OF PROPOLIS

AIMS

To : determine whether propolis has inhibitory activity against fungal growth.

: test ethanol and ether as extractants of propolis.

: determine if the solvent extract of propolis is a source of microbial contamination of agar plates.

MATERIALS AND METHODS

Source of propolis : Hawke's Bay.

This experiment was set up as a 5 * 3 factorial design with 3 replications. Treatments, which were added to cornmeal agar, were :

A. 1. 10,000 ppm propolis in ether
   2. 10,000 ppm propolis in ethanol
   3. ether
   4. ethanol
   5. no addition

The agar plates were inoculated with :

B. 1. *Botrytis cinerea* (a deuteromycete fungus)
   2. *Phytophthora cactorum* (an oomycete fungus)
   3. no inoculation

Two grams of propolis was finely chopped and placed in a measuring cylinder. A small quantity of solvent, about 5 ml, was added and shaken. The liquid with the dissolved and suspended propolis was poured off. This process was repeated until no more of the solid propolis could be removed by the solvent, as inferred by a lack of colour change of the added solvent. The propolis + solvent extracts were centrifuged at 5000 rpm for 15 minutes and the supernatant poured off and saved.

Treatments A1 and A2 were prepared by adding 20 ml of the 0.1 gml^{-1} extracts of propolis in solvent to molten Difco bacto cornmeal agar to make 200 ml. At 50°C ether evaporated rapidly and could therefore be added to the full 200 ml agar. Very little of the ethanol evaporated so it was added to 180 ml agar. The agar was prepared by mixing agar powder with water and autoclaving at 121°C (15 p.s.i.) for 15 minutes.

Treatments A3 and A4 were obtained by adding 20 ml of ether and ethanol to 200 ml and 180 ml of molten agar respectively.

Approximately 20 ml of molten agar was poured into each petri plate.

The cooled plates were inoculated with 5 mm diameter plugs cut from the margin of cultures of the test fungi. The plates were then incubated at 20°C for 5 days under Cromptons (code 31) fluorescent tube lighting on a 12 hour light/dark cycle.

Extent of fungal growth on the plates was marked 1,3 and 5 days after inoculation. Mean growths for the two day periods were calculated.

Results were analysed by ANOVA and LSD tests using the SAS statistical package.

RESULTS

Table 3.1 Effect of organic solvents and propolis on the growth of *Botrytis cinerea* and *Phytophthora cactorum* on agar

Treatment	*B. cinerea*	*P. cactorum*
	Growth (mm)	
control	14.1 a[z]	9.2 a
ether	15.0 a	8.3 b
ether / propolis	4.3 b	0.0 c
ethanol	1.3 b	0.0 c
ethanol / propolis	2.5 b	0.0 c

[z] Mean separation in columns by least significant difference, at 5% level.

1. A comparison of the ether and ether/propolis showed that propolis inhibited the growth of *B. cinerea* and *P. cactorum* on agar (Table 3.1).

2. Ethanol alone inhibited the pathogens' growth as much as either of the propolis extracts.

3. There was no microbial contamination of the plates by the propolis extract.

DISCUSSION

Ether is a suitable solvent for the extraction of propolis for agar plate trials. Unlike ethanol it did not affect the pathogens' growth probably because it evaporates rapidly when added to the molten agar. As there were different levels of activity against the two pathogens the need for further tests against a range of fungi was indicated to assess the spectrum of activity.

3.2 EVALUATION OF ANTIFUNGAL ACTIVITY OF PROPOLIS IN AGAR

AIM

To test the antifungal activity of propolis against a range of fungal plant pathogens.

MATERIALS AND METHODS

Source of propolis : Hawke's Bay.

This experiment was set up as a 6*20 factorial design with 3 replications. Treatments were :

1. cornmeal agar
2. ether
3. 10 ppm propolis in ether
4. 100 ppm propolis in ether
5. 1 000 ppm propolis in ether
6. 10 000 ppm propolis in ether

Propolis and ether were tested against the fungi in Table 3.2.1.

Fifteen grams of propolis was extracted in ether as in experiment 3.1. The four concentrations, 10, 100, 1 000 and 10 000 ppm solutions were prepared by adding the appropriate volume of the extracted propolis to molten agar.

Stocks of the fungi were cultured on agar and plugs taken for inoculation of the agar media as in experiment 3.1. Malt agar, as opposed to cornmeal, was used for treatments with fungi 1,6,7 and 8 above. The *Phytophthora* species were incubated in the dark at 24°C. The other fungi were incubated at 20°C under fluorescent tube lights as in experiment 3.1.

The plates were marked at the limit of fungal growth 24, 48 and 72 hours after inoculation. Mean daily growth was calculated and differences between treatments were analysed using ANOVA and LSD tests as in experiment 3.1.

Table 3.2.1 Pathogens and common names of associated diseases used for propolis screening

Pathogen		Common Name of Disease
Chondrostereum purpureum		Silver leaf of trees
Rhizoctonia sp.		Damping-off of seedlings
Alternaria alternata		Storage rot of apples
A. zinniae		Damping-off of seedlings
B. cinerea		Storage rot of kiwifruit
Colletotrichum acutatum	)	Anthracnose diseases of fruit
C. coccodes	)	and vegetables
Glomerella cingulata	)	
Fusarium oxysporum		Wilt diseases of vegetables
Pythium myriotylum	)	
P. sp.	)	Damping off of seedlings
P. ultimum	)	
Phytophthora cactorum (PL3)*	)	
P. cactorum (P149)*	)	Collar rot of apples
P. cactorum (P152)*	)	
P. citrophthora		Fruit rot in citrus
P. cinnamomi	)	Root rots in fruit and vegetables
P. dreschlera	)	
P. erythroseptica		Pink rot of potatoes
P. megasperma		Damping-off diseases of seedlings

* Three different isolates of *Phytophthora cactorum*

RESULTS

Propolis reduced growth of all fungi tested (Table 3.2.2). The R^2 values of >50% indicate that the amount of fungal growth was dependent on the concentration of propolis. Ten ppm propolis caused the least inhibition and 10000 ppm the greatest.

Table 3.2.2 Activity of propolis against plant pathogens in agar

Fungi	Control	Ether	PROPOLIS (ppm) 10	100	1 000	10 000	R^{2z}
	mm[x]	%[y]	%	%	%	%	%
Basidiomycetes							
Chondrostereum purpureum	61.37	106	96	96	78	0	71
Rhizoctonia sp.	16.80	122	138	117	84	0	72
Deuteromycetes							
Alternaria alternata	11.33	99	84	70	62	8	86
A. zinniae	8.97	87	97	72	52	0	89
Botrytis cinerea	20.77	102	100	98	75	-	61
Colletotrichum acutatum	6.20	101	88	66	37	27	55
C. coccodes	8.37	97	91	86	41	0	83
Fusarium oxysporum	11.55	100	98	95	73	0	73
Ascomycetes							
Glomerella cingulata	7.37	97	102	109	83	17	58
Oomycetes							
Phytophthora cactorum (PL3)*	6.25	94	90	46	0	0	93
P. cactorum (P149)*	10.35	92	94	83	22	0	88
P. cactorum (P152)*	48.33	102	103	64	22	0	96
P. cinnamomi	18.07	92	94	53	19	0	95
P. citrophthora	10.03	104	99	68	2	0	89
P. dreschlera	14.47	104	100	64	6	0	90
P. erythroseptica	14.47	100	97	72	12	0	90
P. megasperma	11.25	102	91	96	69	0	74
Pythium myriotylum	59.17	106	99	76	28	0	92
P. sp.	50.43	102	98	80	45	0	89
P. ultimum	62.93	95	91	76	22	0	92
LSD (0.05)					**20**		

[x] Mean daily growth of fungi in agar.

[y] Growth of fungi expressed as percentage of growth of fungi in control.

[z] Percentage of total variability accounted for by linear regression of fungal growth with log(propolis concentration).

* Isolates of *Phytophthora cactorum*.

The fungi varied in their sensitivity to propolis, with the LSD (5%) at 1000 ppm indicating significant differences between many species. The oomycetes appear to be the most sensitive taxonomic class, with less growth at 100 and 1000 ppm propolis (Table 3.2.3).

Table 3.2.3 Differences between taxonomic classes in antifungal activity of propolis at 100 and 1000 ppm

Taxonomic class	Mean % growth	
	100 ppm	1000 ppm
Oomycetes	71	23
Others	87 *	65 **

* Significant difference between means by student t test, at 5% level.

** Significant difference between means by student t test, at 0.1% level.

3.3 VARIABILITY IN ACTIVITY OF PROPOLIS

AIM

To determine whether :

1. consistent anti-microbial activity can be obtained from one source of propolis.
2. propolis collected at different times from one geographic location has different activity.
3. propolis collected from different geographic locations has different activity.
4. propolis collected from different parts of the hive has different activity.
5. washing propolis to clean it affects the anti-microbial activity.

MATERIALS AND METHODS

Source of propolis : as indicated.

In all experiments a factorial design was used with three replications. Propolis concentrations used were control (no propolis), 100 ppm, 1000 ppm and 10 000 ppm (dry weight) of propolis. Treatments for the other factors were tests for:

i). consistent activity from one source using two separate extractions of Hawke's Bay propolis and 3 separate extractions of the unwashed supply of Eltham propolis.

ii). seasonal variation using propolis collected in November and January from hives near Invermay.

iii). regional effects by analysing the results from 1 and 2 above to determine if differences occur between the anti-microbial activities of the Hawke's Bay, Eltham and Invermay propolis.

iv). effects due to location of propolis in the hive and effect of washing. Four classes of Eltham propolis were used:

   a. from the cut comb, which appeared red and shiny in comparison with the normal dull brown propolis scraped from between the hive trays
   b. untreated propolis scraped from the hive
   c. propolis scraped from the hive, washed in cold water and the visible dirt removed.
   d. propolis from b., washed in cold water in the laboratory and all floating material removed.

For each treatment approximately 3 grams of propolis was extracted in ether and the plates inoculated with *Pythium ultimum*, incubated and assessed as in experiment 3.1.

Mean daily growth was calculated and differences assessed using ANOVA and LSD tests as in experiment 3.1.

RESULTS

i). Variation in extraction procedure.

Two independent ether extractions of Hawke's Bay propolis did not differ significantly in their activity against *Pythium ultimum* (Table 3.3.1).

Table 3.3.1 Activities of two extractions of Hawke's Bay propolis against *Pythium ultimum*

Extraction of propolis	Growth % [y]		
	100	1 000	10 000 ppm [z]
Extraction 1	75	25	0
Extraction 2	74	20	0

[y] Fungal growth expressed as percent of control.

[z] Concentration of propolis.

ii). Seasonal variation.

The activity of propolis against *P. ultimum* differed between two samples collected at different times at Invermay (Table 3.3.2). There was a significant interaction between concentration and date of collection with the propolis collected in January having significantly greater activity at 100 ppm ($p<.01$).

Table 3.3.2 Effect of time of collection on propolis activity against *Pythium ultimum*.

Date of propolis collection	Growth %[x]	
	100	10 000 ppm[y]
November	90 a[z]	0 a
January	68 b	0 a

[x] Fungal growth expressed as percent of control.

[y] Concentration of propolis.

[z] Mean separation in columns by least significant difference, 1% level.

iii). Regional variation.

Propolis collected from different areas had significantly different ($p<0.05$) activities against *P.ultimum* (Table 3.3.3). The Hawke's Bay propolis had the greatest activity reducing fungal growth to 60% of the control at 100 ppm propolis and 15% at 1000 ppm. Eltham propolis had the least activity (85% and 43% respectively).

Table 3.3.3 Effects of geographic location on propolis activity against *P.ultimum*

Locality of propolis collection	Growth %[x] 100	1 000	10 000 ppm[y]
Hawke's Bay	60 a[z]	15 a	0 a
Invermay	78 b	18 a	0 a
Eltham	85 b	43 b	0 a

[x] Fungal growth expressed as percent of control.

[y] Concentration of propolis.

[z] Mean separation in columns by least significant difference, 5% level.

iv) Variation within the hive.

Propolis from the cut comb had significantly higher activity ($p<0.05$) against *P.ultimum* at 1000 ppm (Table 3.3.4).

Table 3.3.4 Effects of location of propolis within the hive on activity against *P. ultimum.*

Classes of propolis[x]	Growth %[w] 100	1 000	10 000 ppm[y]
Cut comb	92 a[z]	20 a	0 a
Between trays	85 a	43 b	0 a

[w] Fungal growth expressed as percent of control.

[x] See materials and methods for descriptions.

[y] Concentration of propolis.

[z] Mean separation in columns by least significant difference, at 5% level.

v). Variation in washing procedures.

Washing the untreated propolis resulted in a reduction in its anti-microbial activity at 1000 ppm (Table 3.3.5). The treated and untreated propolis were not significantly different at the 5% level.

Table 3.3.5 Effects of washing propolis on its activity against *P. ultimum.*

Classes of propolis[x]	Growth %[w] 100	1000	10 000 ppm[y]
Untreated	85 a[z]	43 a	0 a
Treated	82 a	46 a	0 a
Washed	83 a	60 b	0 a

[w] Fungal growth expressed as % of control.

[x] See materials and methods for descriptions.

[y] Concentration of propolis.

[z] Mean separation in columns by least significant difference, 5% level.

3.4 SOLVENTS FOR EXTRACTION OF PROPOLIS

AIM

To compare the activity of ether and water extracts of propolis.

MATERIALS AND METHODS

Source of propolis : Eltham.

Cold water extracts were prepared using the same method as organic solvent extracts in experiment 3.1 except the extracts were filtered through glass wool instead of centrifuging.

Hot water extracts were made by heating the crushed propolis in water and boiling for 1 minute. The extract was then filtered through glass wool.

Both aqueous extracts were added to the agar before autoclaving to avoid contamination of the plates.

Concentrations of 100, 500, 1 000, 10 000 and 40 000 ppm were made for cold water extracts and 100, 1 000, 10 000, 20 000 and 150 000 ppm were made for hot water extracts.

Results from experiment 3.2 were used for comparison with ether extracts.

Mean daily growth of fungi was calculated and differences expressed using ANOVA and LSD tests as in experiment 3.1.

RESULTS

All the extracts tested reduced growth of *P. ultimum* in agar, R^2 values for linear regression of 65.6% and 96.4% and 61.7% for cold and hot water and ether respectively The ether extract had the greatest activity, followed by hot and then cold water, Fig 3.4.1 and Table 3.4.1.

Table 3.4.1 Effect of extractant on activity of propolis against *P. ultimum*

Extractant	Growth %[y]
Ether	48.7 a[z]
Hot water	81.2 b
Cold water	95.7 c

[y] Fungal growth expressed as percent of control.

[z] Mean separation in columns by least significant difference, 5% level.

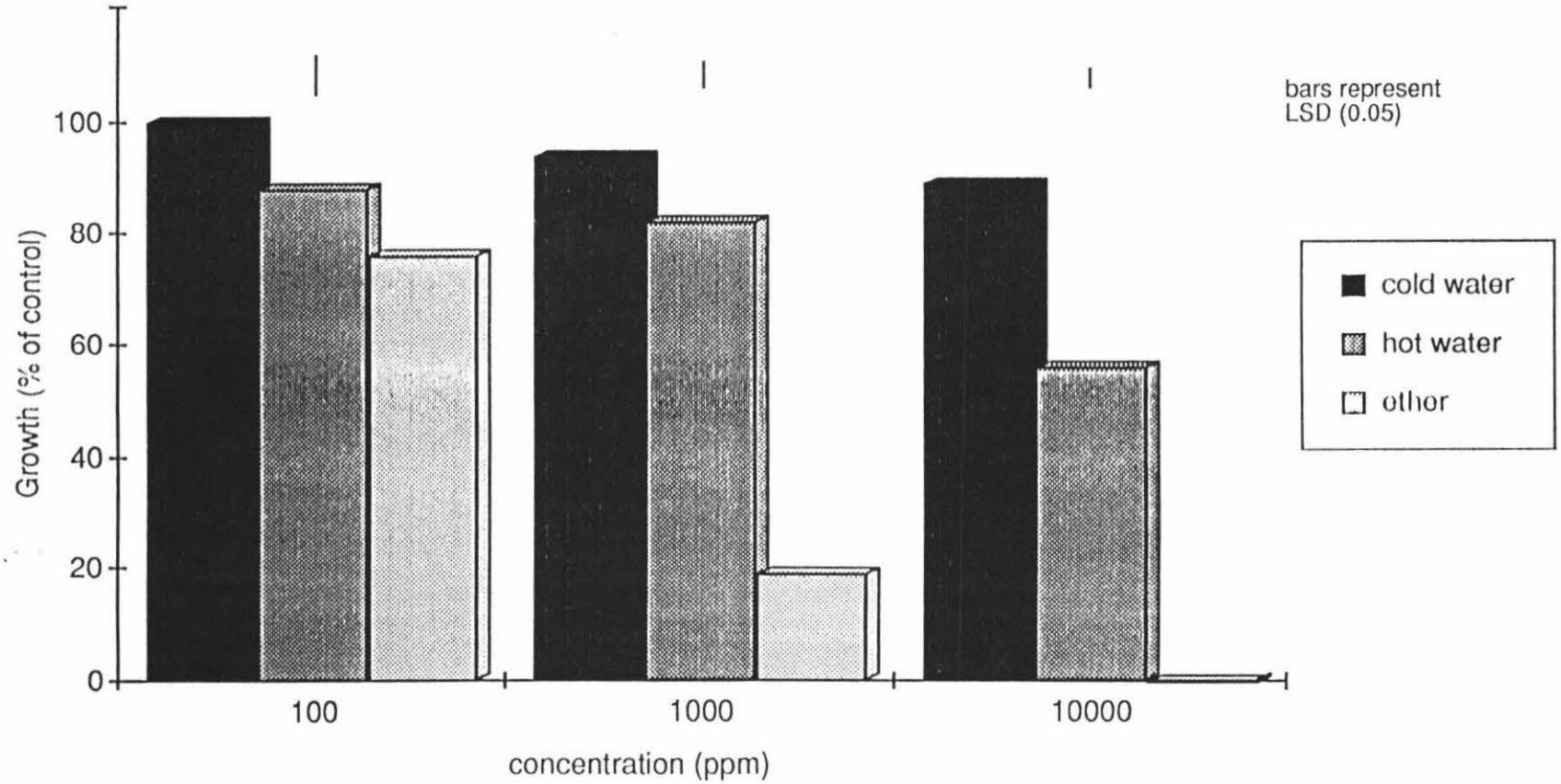

Fig 3.4 Antifungal activity of propolis extracted in cold and hot water and ether.

3.5 PROPOLIS AS A SEED DRESSING TRIAL

AIM

To evaluate the potential of propolis as a protectant seed dressing against soil plant pathogens.

3.5.1 PROTECTION OF COATED SEEDS IN A GROWING MEDIUM

AIM

To determine if seeds coated with propolis are protected against *Pythium ultimum* in a potting medium.

MATERIALS AND METHODS

The trial was set up as a 2*4 factorial design with 3 replicated treatments of :

A 1. untreated
2. *Pythium* inoculated growing mix

and

B 1. propolis 100 000 ppm (0.1 gml^{-1} solution of propolis (dry weight) extracted in 95% ethanol).
2. propolis 10 000 ppm (0.01 gml^{-1} solution of propolis (dry weight) extracted in 95% ethanol).
3. 95% ethanol
4. No seed dressing

Ninety five percent sand and 5% cornmeal by weight were mixed in a flask with sufficient water to make a stirrable mixture. This was then autoclaved and allowed to cool. Five millimetre diameter plugs of *P. ultimum* were added to the flasks. The mixture was kept at 20°C and stirred after 2 days to produce a uniform fungal inoculation source. The inoculum was added to a sterile peat pumice (1:1 with fertiliser) growing media at 5% by weight.

Cress seeds were immersed in the dressings, B1-4, for 2 minutes and air dried on filter paper in a laminar flow cabinet. Twenty seeds were sown per pot of growing mix A1 or A2.

Treatment differences in numbers of seedling emerged were analysed using Friedmann's non parametric test (Zar 1974).

RESULTS

Neither the propolis nor ethanol affected the emergence of seedlings in the sterile mix. *P. ultimum* was highly pathogenic and neither propolis nor ethanol gave any control of the disease (by Friedmann, 1% level).

TABLE 3.5.1 Number of cress seedlings emerged from a potting medium with and without *P. ultimum* 7 days after sowing

Treatments	Number of seedlings			
	mean	std error	mean	std error
	No *P.ultimum*		With *P. ultimum*	
Control	18.3	1.2	1.0	0.0
Ethanol	18.3	0.9	1.3	0.3
Propolis 0.01[y]	14.3	0.9	1.3	0.7
Propolis 0.1[z]	18.0	0.6	0.7	0.3

[y] 10 000 ppm propolis extract.

[z] 100 000 ppm propolis extract.

3.5.2 PROTECTION OF COATED SEEDS IN AGAR

AIM

To determine whether high concentrations of propolis are effective against *P. ultimum* when used as a seed dressing.

MATERIALS AND METHODS

A 3 * 3 factorial design with 3 replicates was set up. The treatments were :

A. seeds dipped for 2 minutes in :

1. propolis 360 000 ppm (0.36 gml^{-1} solution of propolis (dry weight) extracted in 95% ethanol).
2. propolis 180 000 ppm (O.18 gml^{-1} solution of propolis (dry weight) extracted in 95% ethanol).
3. 95% ethanol.

Each seed treatment was tested for its effect on seed germination both in the absence and presence of *P. ultimum*. Four seeds were placed on the uninoculated plates. Eight seeds were placed on the inoculated plates, four in the area covered by mycelial growth and four in the uncovered area.

The plates were kept under light at 20°C and the seeds assessed after 3 and after 6 days. The germinated seeds were counted and recorded as either having cotyledonary leaves or just germinated with no shoot growth.

Differences between treatments were analysed using Friedmann's non parametric test.

RESULTS

The results on both assessment dates were the same so only the six day assessment is presented in Table 3.5.2.

Use of propolis seed dressing did not affect seed germination (by Friedmann, at 1% level).

Propolis did not protect the seeds against *P. ultimum*.

TABLE 3.5.2 Germination of cress seedlings on agar with or without *P. ultimum* after 6 days.

Plate	Control		0.18 propolis[W]		0.36 propolis[X]	
	germ[Y]	cot[Z]	germ	cot	germ	cot
In *Pythium*						
mean	3.0	0	3.0	0	3.7	0
std error	0.6	0	0	0	0.3	0
Out *Pythium*						
mean	3	0	3	0	2	0
std error	0	0	0.6	0	0	0
Control						
mean	0	4	0	3.3	0	3.7
std error	0	0	0	0.3	0	0.3

[W] 180 000 ppm propolis.

[X] 360 000 ppm propolis.

[Y] Seeds germinated but no shoot growth.

[Z] Germinated seedlings with cotyledonary leaves showing.

CHAPTER 4

EVALUATION OF PROPOLIS ACTIVITY AGAINST POWDERY MILDEW

4.1 GLASSHOUSE GROWN CUCUMBERS

AIM

To determine whether propolis is active against powdery mildew (*Erysiphe cichoracerarum*) of cucumbers under glasshouse conditions.

E. cichoracerarum is an obligate pathogen and therefore propolis testing had to be carried out on the host plant.

MATERIALS AND METHODS

Source of propolis : Invermay.

A randomised block design was used with four treatments and 5 replications. The treatments were :

1. water only
2. water and surfactant*
3. 1 % ether in water and surfactant
4. 1 % propolis (0.1 gml^{-1} solution of propolis (dry weight) extracted in ether) in water and surfactant.

* surfactant used was Watkins Safer's^{R} Natural insecticide for fruit and vegetables (fatty acids in the form of a soluble concentrate) at 2 drops per 30 ml spray.

"Crystal apple" variety cucumber plants were grown in a sterile peat pumice (1:1 with fertiliser) potting mix in a 20°C +/- 5°C greenhouse. Four cucumber seeds were sown in each pot and on emergence thinned to three seedlings per pot with five replicate pots per treatment. Spraying commenced two weeks after sowing when the cotyledon leaves were fully opened.

The plants were sprayed until run off using a DeVilbiss mister.

Once powdery mildew appeared, at the fourth true leaf stage, the plants were scored weekly for the total leaf area and area of powdery mildew infection.

Assessment

The length and breadth of each leaf was measured. A conversion factor of *0.82 for cotyledon leaves and *0.71 for true leaves was used to estimate leaf area from these measurements. (This relationship was found by determining leaf area using graph paper outlines, and dividing by the length * breadth).

Results were analysed using ANOVA and LSD tests as in experiment 3.1.

RESULTS

Plants treated with propolis had less powdery mildew than control at all dates of monitoring, Fig 4.1. In the 5th week the propolis treated plants had 33.4% and the controls 84.5% powdery mildew leaf cover. At this date the total leaf area of the propolis treated plants were significantly greater than the controls, Table 4.1, Plate 4.1.

The ethanol and surfactant treatments also significantly reduced powdery mildew (at the 5% level), Fig 4.1. In the first two weeks the surfactant gave similar control to the propolis. In the latter weeks plants in the surfactant and ethanol treatments had less powdery mildew cover than those in the control treatment, but more than those in the propolis one.

TABLE 4.1 Effect of propolis on total leaf area of 9 week old cucumber plants infected with powdery mildew.

Treatment	Leaf area (mm^2)
Control	66.4 a[z]
Ethanol	76.4 ab
Surfactant	82.3 ab
Propolis	106.7 b

[z] Mean separation by least significant difference, 5% level.

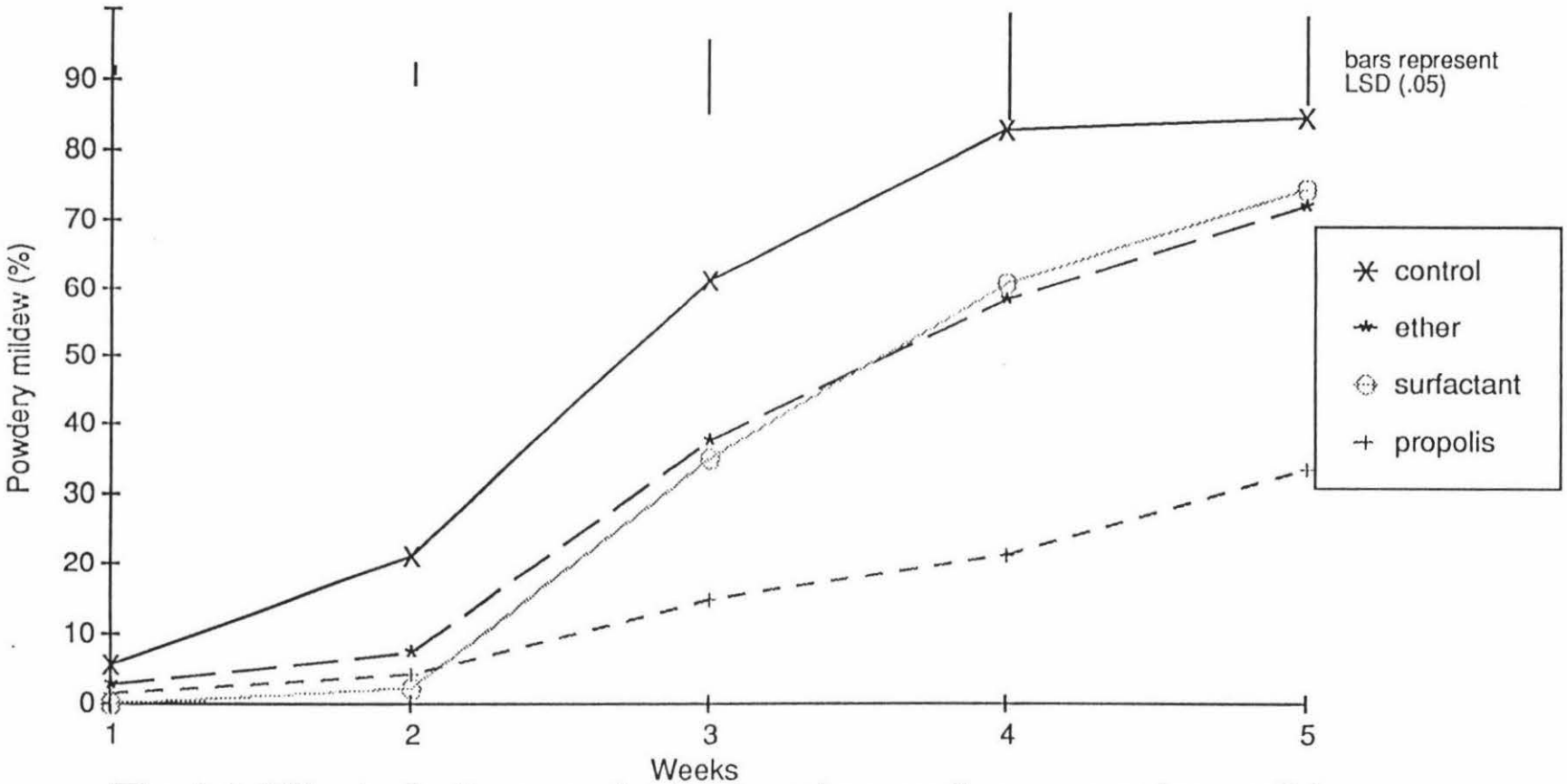

Fig 4.1 Effect of ether, surfactant and propolis on powdery mildew of glasshouse grown cucumbers

4.2 FIELD GROWN ZUCCINIS

The field trials were carried out at the Ministry of Agriculture and Fisheries' Levin Horticultural Research Station. The soil is a Levin silt loam, a deep well drained loamy soil with good moisture holding properties (DSIR 1954). The mean annual rainfall is 1122 mm with supplementary irrigation is only necessary in late summer.

A winter green crop of 'tama' rye was mowed and subsequently rotary hoed into the soil twice prior to sowing and planting the zuccinis and tomatoes.

AIM

To determine whether propolis is an effective control agent against powdery mildew (*Erisyphe cichoracearum*) of zuccinis under field conditions.

MATERIALS AND METHODS

Sources of propolis : Invermay and Eltham.

A randomised block design with 8 treatments and 4 blocks was set up (Plate 4. 2.1). The treatments were :

1. control (no spray).
2. ethanol (sprayed at 500 ml/plant, approximately 4% ethanol)*
3. propolis at 0.1% (sprayed at 500 ml/plant)*
4. propolis at 1% (sprayed at 500ml/plant)*
5. sulphur (Kumulus^{R} DF, an elemental sulphur formulation, at 160 g/100 litres water).
6. propolis at 1% mixed with sulphur at 1/2 the above rate, ie 80 g/100 litres.
7. Reysa** at 1%, extracted in water.
8. wetting agent used for the Reysa spray.

* Stock solutions of propolis extracted in 95% ethanol at 0.3 gml^{-1} were diluted to spray volume in water. The ethanol treatment was 95% ethanol diluted to give the same concentration of ethanol as in the 1% propolis treatment. Plants were sprayed until run off.

** Reysa is an extract of giant knotweed *(Reynoutria sachalinensis*, (F.Schmidt) Makai, Polygonaceae). The spray was prepared by adding tap water to the powdered extract to make a 1% w/v preparation. Surfactant was added at 0.5% v/v, left to stand for 1 hour and filtered through double cheese cloth.

Note : Sufficient coverage was achieved without using a surfactant with propolis extracted in ethanol.

Black Jack variety marrow seeds were sown on 9/12/89 at 1.0 metre spacing within the rows and 1.5 metre spacing between the rows. There were 4 plants per plot, with two guard plants between plots within the rows. There were four rows of plots with a guard row between each. The block was hand hoed for weed control. No other management was required.

A 10-14 day calendar spraying schedule began on 10/2/90 when the first powdery mildew appeared on the foliage.

The fruit were harvested 3 times a week from 2/2/90 until 2/4/90. The number harvested from each plant was recorded.

A visual assessment of powdery mildew was made weekly using the following original key.

Grade	Description
0	no powdery mildew on any leaf upper surface or any stalk.
1	few spots present on upper surface of lower leaves and/or stalk.
2	< 50% of lower leaves covered, spots on upper surface of upper leaves, ie < 25% total leaf area
3	Some lower leaves totally covered, others well spotted. Upper leaves, except newest, clearly spotted. ie < 50% total leaf area. Plant appears greyish.
4	most of lower leaf surfaces covered, upper leaves up to 50% covered. ie < 75% total leaf area.
5	total leaf area covered, plant grey, withered.

Total fruit numbers and powdery mildew assessment were analysed using ANOVA and LSD tests.

RESULTS

Propolis reduced the cover of powdery mildew on the first two monitoring dates, Fig 4.2, but gave no protection after that (Plate 4.2.2). There were no significant differences (at the 5% level) in number of fruit between any of the treatments.

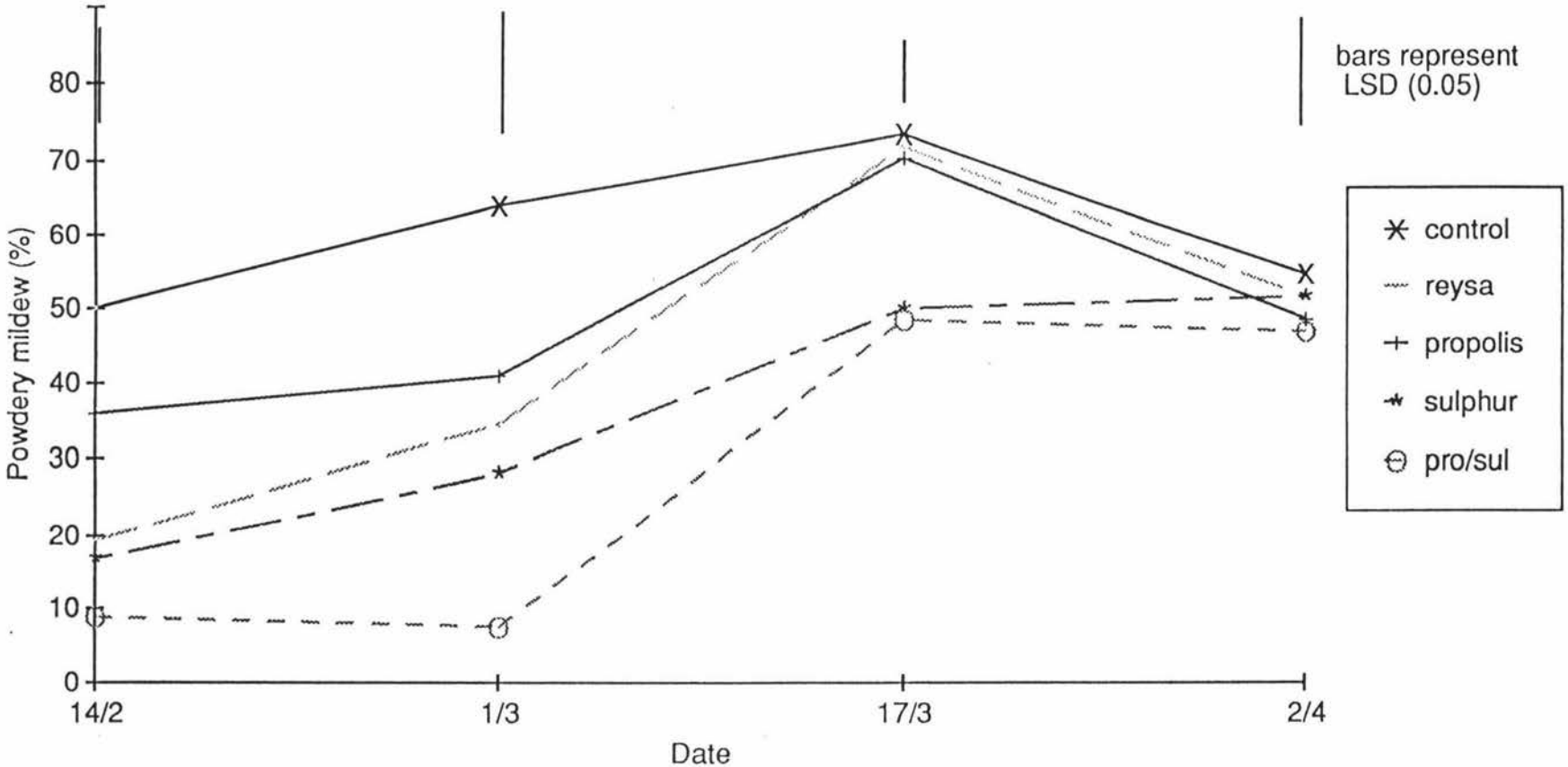

Fig 4.2 Effect of propolis, sulphur and reysa on powdery mildew of field grown zuccinis

Reysa also reduced powdery mildew only on the first two dates, and gave greater control than the propolis on the first date.

The best control was obtained with sulphur and the propolis/sulphur treatments with the latter giving greater control at the second monitoring date (Plate 4.2.3) By the end of the trial the powdery mildew had decreased in the control and similar cover of powdery mildew was found in all treatments.

Powdery mildew cover in the ethanol, wetting agent and propolis at 0.1% treatments did not differ significantly ($p<0.05$) from the control throughout the trial.

CHAPTER 5

EVALUATION OF PROPOLIS AGAINST LATE BLIGHT IN FIELD TOMATOES

5.1 CONTROL OF LATE BLIGHT

AIM

To evaluate propolis as a control agent against late blight (*Phythophthora infestans*) of tomatoes.

MATERIALS AND METHODS

Sources of propolis : Invermay and Eltham.

Details on the trial location and ground preparation are given in section 4.2.

A randomised block design with 6 treatments and 4 blocks was set up (Plate 5.1). The treatments were:

1. control
2. ethanol (sprayed at 500 ml / plant, approximately 4% ethanol).*
3. copper (Kocide^{R} 101, a cupric oxide formulation, at 250 g/100 litres water).
4. propolis 1% (sprayed at 500 ml/plant).*
5. copper (as above but spraying ceased at 1/2/90)
6. propolis (as above but spraying ceased at 1/2/90)

* Stock solutions of propolis extracted in 95% ethanol at 0.3 gml^{-1} were diluted to spray volume in water. The ethanol treatment was 95% ethanol diluted in water to give the same concentration of ethanol as in the propolis treatments.

Treatments 5 and 6 were included to investigate the duration of protection (if any) given by the copper and propolis treatments.

Extase variety of tomato was sown on 2/10/89. On 6/11/89 plants were transplanted to 9 rows with 0.45 metres within the rows and 1.5 metres between rows. The plants were pruned to two laterals and guided by two pairs parallel wires along the rows.

Spray applications on a 10 day calendar basis began on 7/12/89.

Six plants were treated in each plot with 2 guard plants between each treatment within the rows. There were 6 treatments per row. There were 4 treatment rows with 1 guard row between each.

Other pest and disease management.

The only major insect problem was tomato fruit worm (*Heliothis armigera conferta*). These were monitored by the placement of a DSIR pheromone trap in the tomato trial. Plants were sprayed with Thuricide^{R} at 0.75 g/litre when more than 20 adult moths were caught in a week (van Epenhuijsen *pers comm*). Sprays of Thuricide^{R} were applied on 3 dates (25/1/90, 6/2/90 and 27/2/90).

By 9/1/90 bacterial speck was common on the lower leaves of all treatments but to a lesser extent in the copper treatments. On 19/1/90 the lower leaves of all plants were removed to aid fruit ripening and this appeared to reduce the spread of bacterial speck.

The first harvest was made on 5/2/90. Fruit were then harvested twice a week. The first blight on fruit was observed on 12/2/90. A final strip pick was made on 19/3/90. The harvested fruit were sorted, counted and weighed as saleable fruit or according to presence of blight, insect damage, bird damage or splitting. Block and treatment fruit weights were analysed using ANOVA and LSD tests as in experiment 3.1.

Scoring of the plants for visual assessment of late blight was adapted from the British Mycological Society's key for late blight in potatoes. The values given are purely relative, not absolutes. The plants were scored weekly.

Grade	Description
0	: no disease observed.
0.1	: a few scattered plants blighted, not more than 1-2 spots per plot.
1.0	: up to 10 spots per plant, or a general light infection.
5.0	: about 50 spots per plant, up to 1 in 10 leaflets infected.
15	: up to 1 in 3 leaflets infected.
25	: nearly every leaflet infected, but plant retains normal form.
50	: Every plant affected and about 50% leaf area destroyed, plot appears green ,flecked with brown.
75	: about 75% of leaf area destroyed
95	: only a few leaves left on plants, but some stems still green.
100	: all leaves dead, stems dead or dying.

Late blight assessment figures were analysed using ANOVA and LSD tests.

RESULTS

Propolis did not control late blight on the tomato foliage or reduce the weight of blighted fruit (Fig 5.1 and 5.2, Plate 5.2).

Both copper treatments reduced blight. Copper 1 treatment reduced the weight of blighted fruit from 3.9 kg to 0.1 kg per plot and increased the saleable yield from 14.0 kg to 21.6 kg per plot (differences significant at the 5% level). In the final date of monitoring the visual estimates of blight damage were lower in the two copper treatments than the other treatments.

The yields of blighted and saleable fruit and the visual assessment of blight damage in the copper treatments where spraying stopped 6 weeks prior to harvest, were not significantly different from the copper 1 treatment where spraying was carried out until harvest.

There were no significant differences (LSD 0.05) between treatments in the weight of insect damaged, bird damaged or split fruit.

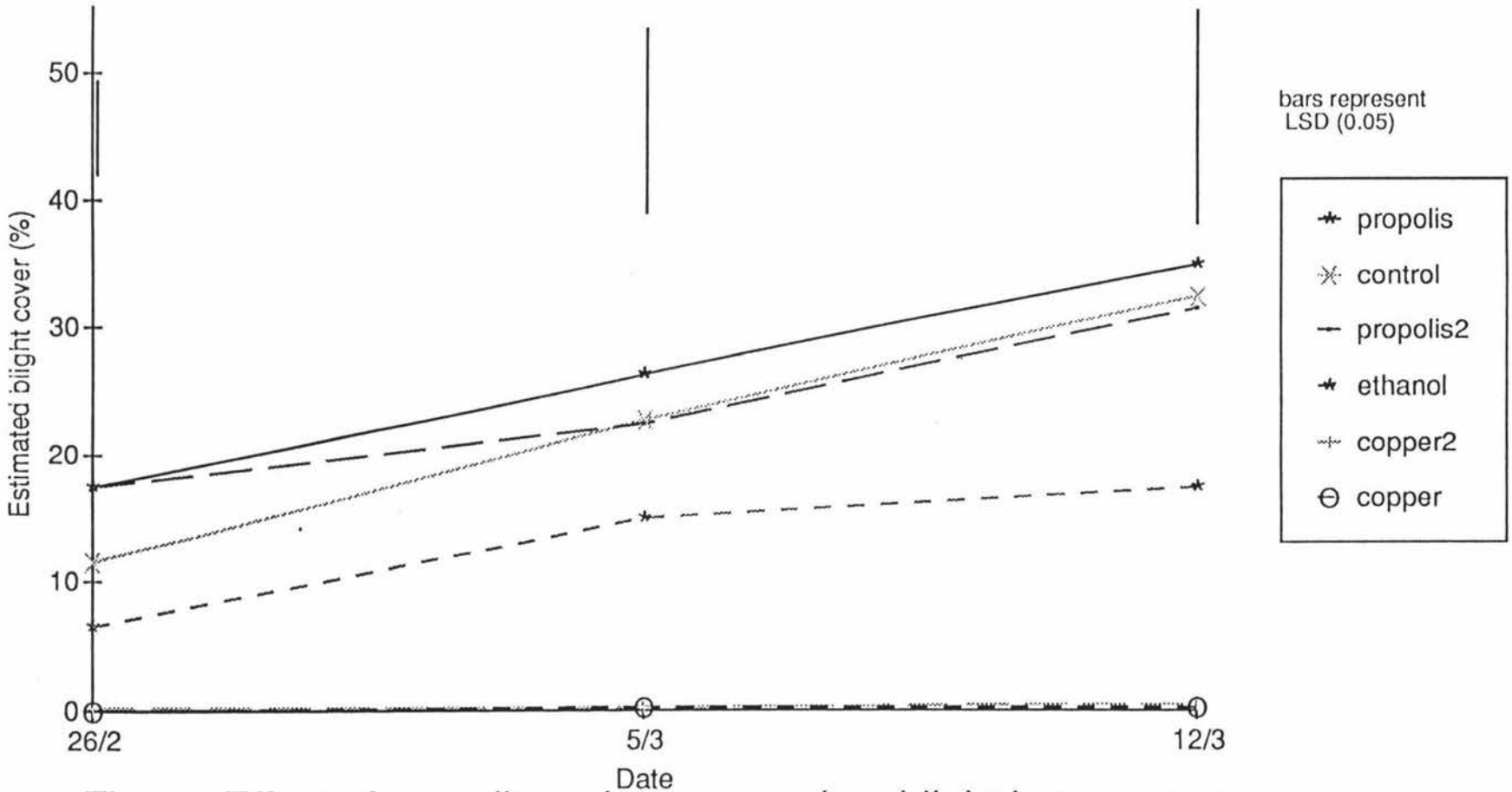

Fig 5.1 Effect of propolis and copper on late blight in tomatoes

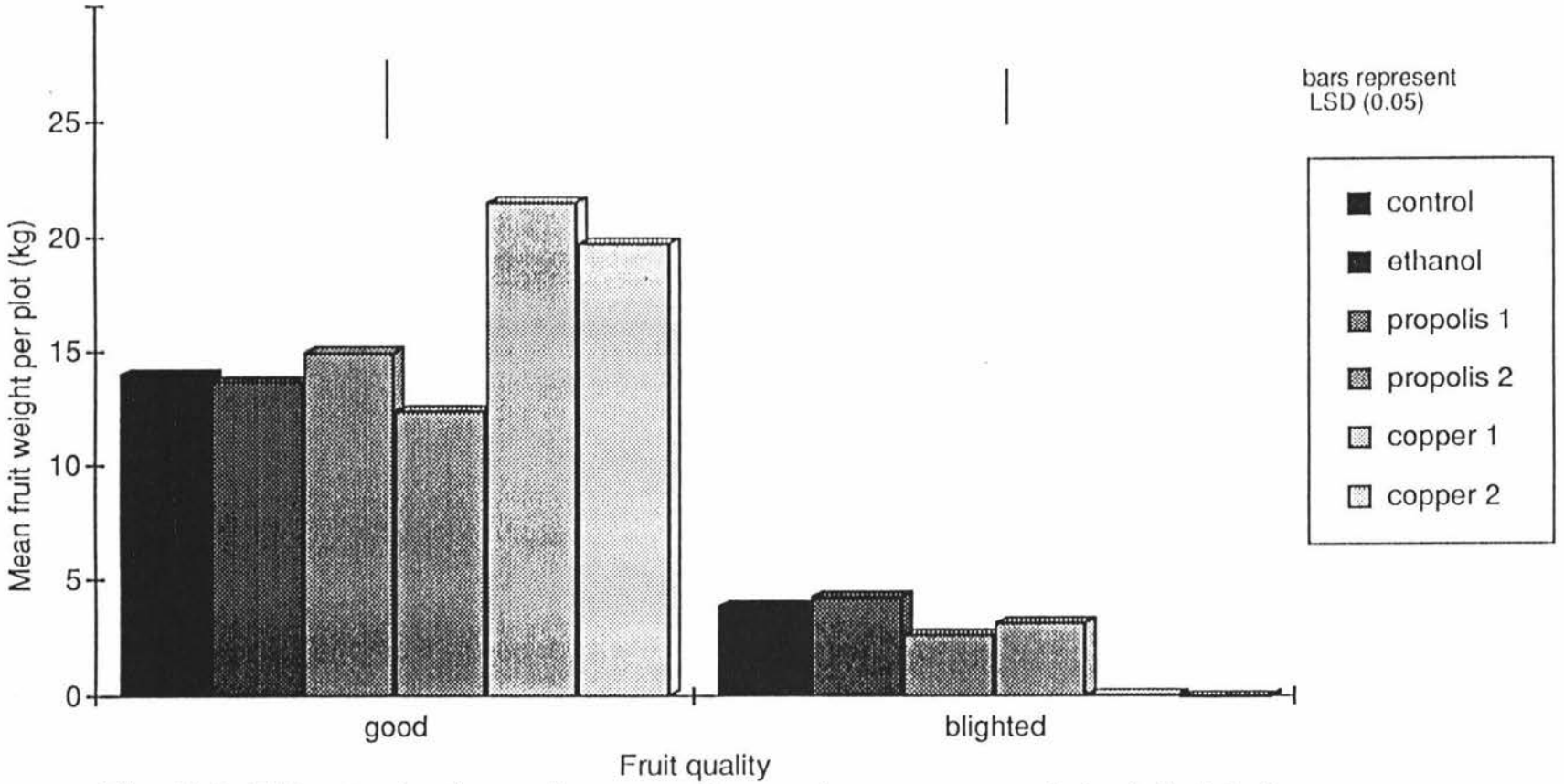

Fig 5.2 Effect of ethanol, propolis and copper on late blight damage to tomato fruit

5.2 EFFECT OF COPPER ON SOIL BIOLOGICAL ACTIVITY

AIM

To assess the effects of copper foliar spray treatments on soil biological, cellulolytic activity.

MATERIALS AND METHODS

The same tomato trial was used as in Part A.

One hundred and twenty 10 * 20 cm cotton strips were cut, washed and dried and the weights recorded for each strip. 5 cotton strips per plot were placed vertically in the soil beneath the tomato plants on 19/3/90. 3 weeks later these were recovered and air dried. The strips were then soaked individually in 50 gl^{-1} Calgon, to prevent further decomposition, and 7 gl^{-1} Na_2CO_3 overnight. Adhering soil was gently washed off in water. The strips were oven dried and the weights recorded.

Initial dry weight was subtracted from final dry weight to give an indication of the relative amount of decomposition. Results were analysed using ANOVA and LSD tests as in experiment 3.1.

RESULTS

There were no significant differences in weight loss of strips from any of the treatments (Table 5.2).

Table 5.2 Effect of copper, ethanol and propolis foliar sprays on decomposition of cotton strips placed in soil beneath tomato plants.

Treatment	Loss in weight (g)	
	Mean	Standard error
control	0.27	0.02
copper	0.25	0.09
copper 2	0.19	0.03
ethanol	0.41	0.17
propolis	0.23	0.06
propolis 2	0.30	0.03

CHAPTER 6

EVALUATION OF PROPOLIS ACTIVITY AGAINST STORAGE ROT IN KIWIFRUIT

AIM To test the efficacy of propolis as a control agent for storage rot (*Botrytis cinerea*) in kiwifruit.

MATERIALS AND METHODS

Sources of propolis : Invermay and Eltham.

A randomised block experiment was set up with 6 treatments and 4 replications. Plants in a four year old Hayward kiwifruit block were used. These were trained on a T bar system with 1 male : 3 female vines within the row. Each plot used three females, guarded by a male at each end. There were 4 plots per row and 6 rows. Three treatments were used in this experiment :

1. control (untreated).
2. ethanol (4 %).
3. propolis at 1% extracted in ethanol.

Treatments 2 and 3 were applied as a foliar spray (approximately 1 litre/plant) at blossom, in early December 1989.

The vines were pruned in January 1990. Weeds were controlled with a "Husquavana" weed eater. *B. cinerea* conidia, at $1.6 * 10^4$ conidia ml^{-1}, were sprayed on all vines on 25/5/90.

Treatments 2 and 3 were sprayed again on 28/5 and the fruit harvested on 29/5/90.

The fruit were harvested between 8.30 am and 11.00 am, and post harvest treatments applied between 1.00 pm and 4.00 pm. All fruit were passed over a grader for recording individual fruit weights. 100 fruit samples from each plot were randomly selected and graded according to export grading specifications. Results were analysed using ANOVA and LSD(0.05).

A further 200 fruit were taken for storage trials. Three trays of 33 pack size were packed and stored from the untreated, ethanol and propolis treatments. Fruit for a further three trays from treatment 2 were placed in a basket and immersed in 900 ml 95% ethanol in 9 litres of water for 2 minutes and three trays from treatment 3 in a corresponding dip containing 3.6% propolis. All dipped fruit were packed and left to dry in their open trays for 24 hours.

All fruit were stored at 0°C for 12 weeks and checked for *Botrytis* infections at 4 week intervals.

RESULTS

No stem end rot was found in any of the stored kiwifruit after 12 weeks. *B. cinerea* was found on wounds of 8 fruit, in a variety of treatments.

There were no significant treatment differences in total fruit weight or misshapen fruit. However there was a significantly higher percent of undersized fruit in the propolis treatment than the controls (30.3% and 15.8% respectively, LSD(0.05) 11.3%).

CHAPTER 7

EVALUATION OF ACTIVITY OF PROPOLIS AGAINST INSECTS

7.1 LEAFROLLERS

AIM

To detect any feeding inhibition or toxicity of propolis on light brown apple moth (*Epiphyas postvittana*).

MATERIALS AND METHODS

Source of propolis : Eltham.

The experiment was a completely randomised design of 2 treatments (10% propolis and control) and 10 replicates. Cubes, of approximately 30*20*20 mm, of the prepared diet (see appendix 2) were placed in plastic pottles. Ten first instar larvae were put into each pottle and the pottle sealed with gladwrap and a lid. Ten replicates (pottles) of 10% propolis diet and ten replicates of control diet, no propolis added, were prepared. The leafrollers were then left until adult emergence. Adult emergence was recorded over 3 weeks.

Differences between treatment means were analysed using the student t test.

RESULTS

Adult emergence was significantly lower in the propolis treatment up until the 17th day after initial emergence, Fig 7.1. The final mean percentages of adult emergence, of 54 and 41% for control and propolis respectively, however were not significantly different at the 5% level.

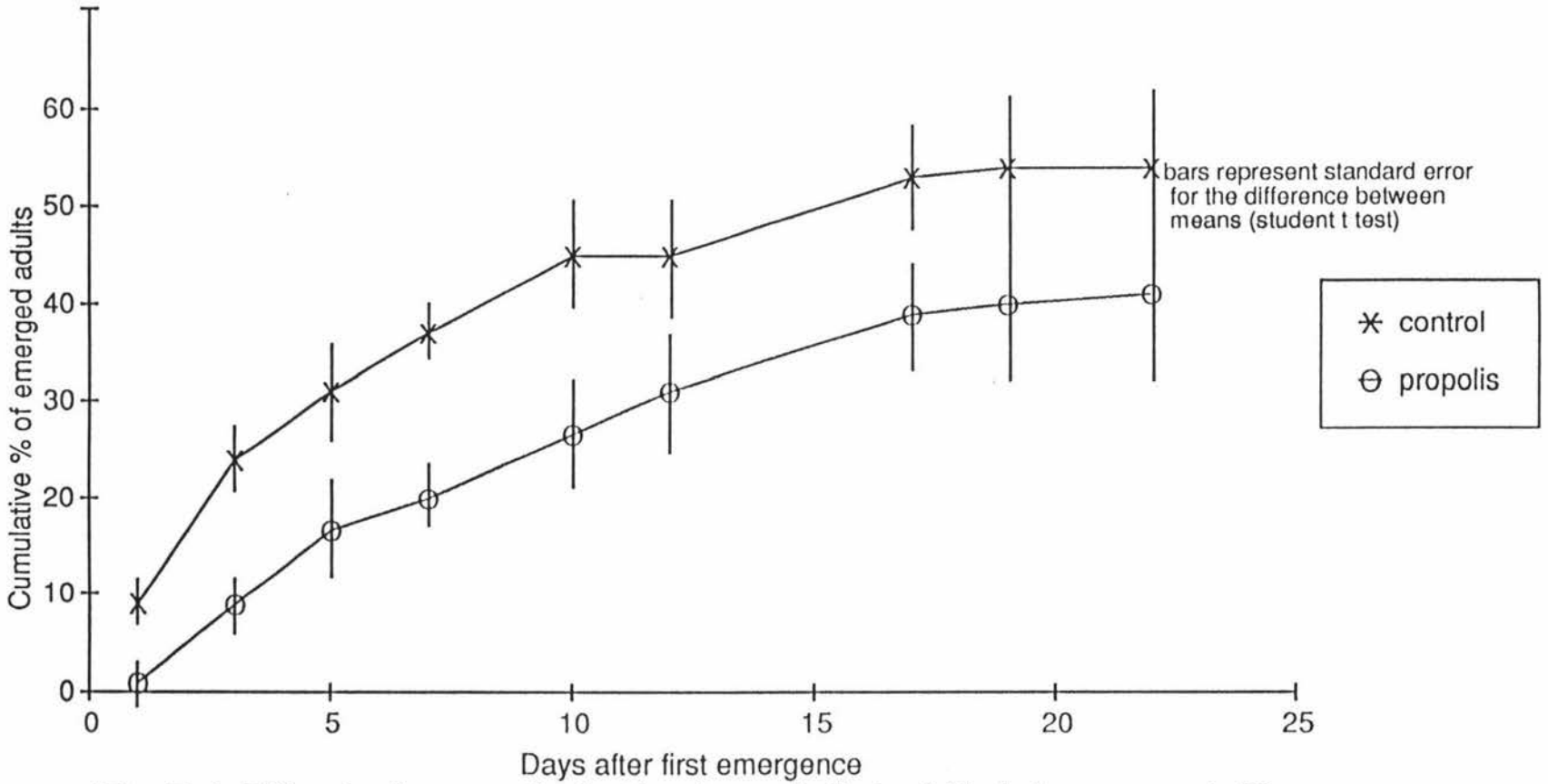

Fig 7.1 Effect of propolis in the larval diet of Epiphyas postvittana on adult emergence

7.2 APHIDS

AIM

To detect any feeding inhibition or toxicity of propolis on aphids

MATERIALS AND METHODS

Source of propolis : Eltham.

A completely randomised experiment with 3 treatments and 3 replicates was set up. The treatments applied as a foliar spray on cabbages were :

1. Control; water.
2. Surfactant; water and Tween 20.
3. Propolis; extracted in ethanol and sprayed at 1% dry weight, in water with Tween.

Approximately 20 green peach aphids (*Myzus persicae*) were counted onto one leaf of Flower of Spring variety cabbage plants using a fine point brush. Any leaves touching the aphid infested leaf were removed and the stalk of the trial leaf gummed with a thick paste to inhibit movement of aphids to or from the leaf. The leaves were sprayed till surface run off. Each plant represented one replicate.

The numbers of aphids were counted 2 days and 7 days after spraying.

Treatment differences were analysed using F and LSD tests.

RESULTS

Propolis did not significantly reduce the number of aphids at the 10% level, Fig 7.2.

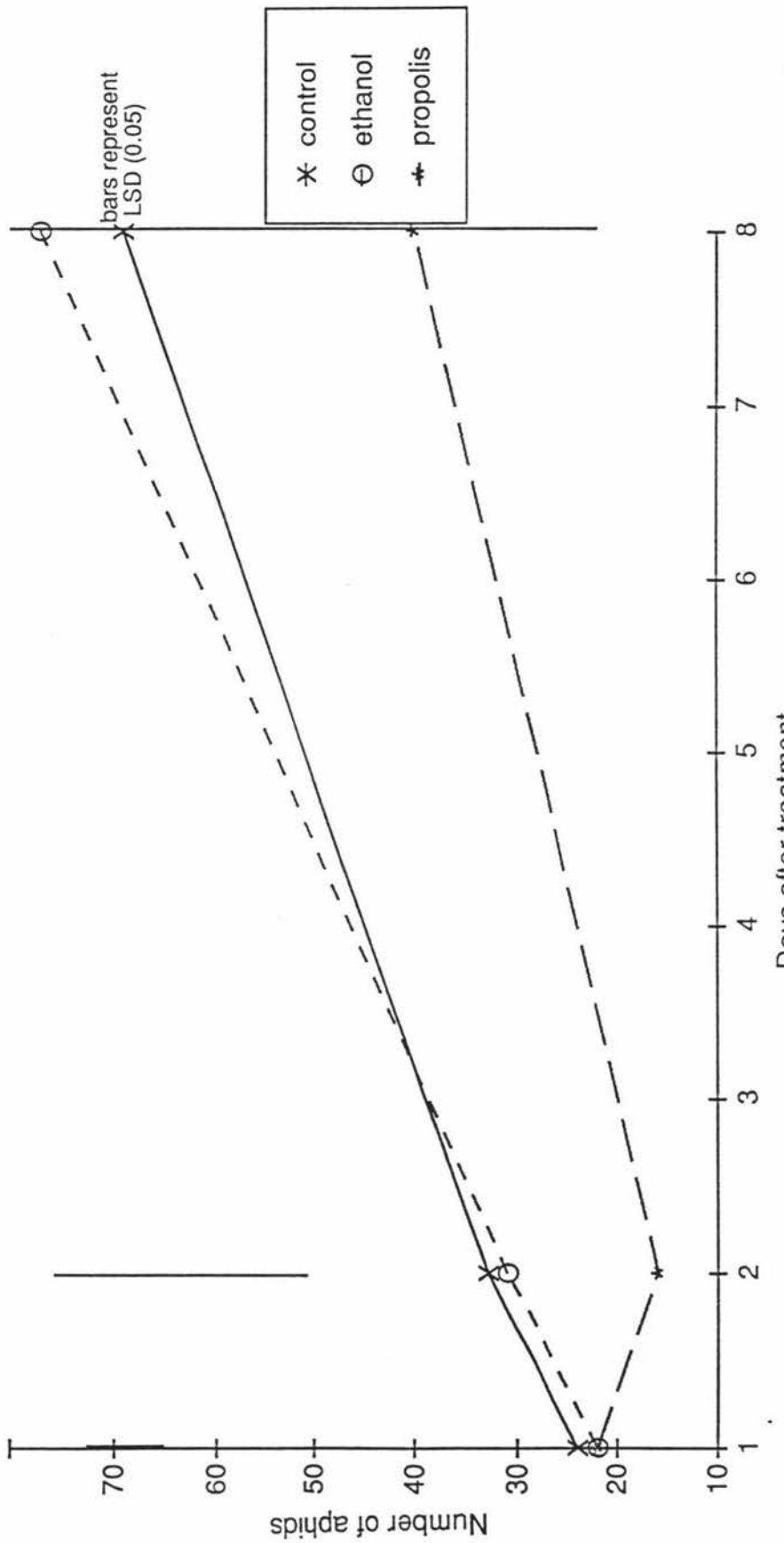

Fig 7.2 Effect of propolis on aphid numbers on cabbage leaves

CHAPTER 8

DISCUSSION

8.1 Antibiotic properties of propolis

Antibiotic properties of propolis against bacteria and fungi have often been reported (Ghisalberti 1979). These are attributed to biologically active plant defence compounds in the plant exudates collected by the bees.
Microorganisms vary in their sensitivity to such plant defence compounds and this partly determines the pathogenicity of an organism on the plant (Vickery and Vickery 1981). A range of sensitivity of microorganisms to propolis is therefore to be expected.

In this trial all fungi tested were sensitive to propolis. The growth of most fungi was totally inhibited on agar media by an extract of 1% of raw propolis. However the sensitivity of different fungi to propolis at concentrations less than 1% varied. The sensitivity to propolis appeared to be related to the taxonomic class of the fungi, with oomycetes the most sensitive. In testing propolis against a range of bacteria Lindenfelser (1967) found the gram positive acid fast species were the most sensitive. However Lindenfelser's results with a wide range of plant pathogenic and saprophytic fungi showed no apparent relationship between sensitivity to propolis and taxonomic group.

8.2 Variability in activity of propolis

The antifungal activity of propolis collected from different locations and at different times varied in these trials. The Hawke's Bay propolis had the greatest activity and the Eltham supply the lowest. No detail on the plant species from which the propolis was made is available but it seems likely the range in activity is at least partially attributable to the range of plants used as well as the physiological state of the plant.

Lindenfelser (1967) also found that the antifungal activity of propolis varied with location of collection. The same compounds were identified by paper chromatography in propolis samples from all areas, so the range in activity was

assumed to be due to varying quantities of the active compounds. Quantitative analysis of the chemical composition of propolis has shown regional differences (Ghisalberti 1979).

The relative amounts of waxes in the propolis samples could also have affected the relative activities in these trials. The concentrations used were based on the weight of raw propolis, not on the weight of material extracted (as in Lindenfelser 1967). Therefore propolis in which bees have incorporated more wax will have less plant compounds per unit weight, and will presumably be less active. This may have been the reason for the greater antifungal activity of the propolis from the cut comb in this trial. The cut comb propolis has a lower proportion of wax than propolis collected from between the trays.

8.3 Lack of in vivo activity of propolis

The antifungal activity of propolis was not so evident in the whole plant experiments compared to the laboratory assays. There was some protection of the cucumber plants against powdery mildew in the glass house trials but no protection against powdery mildew of zuccinis or late blight of tomatoes in the field, or protection of seeds against *P. ultimum*. Reasons for the lack of activity against the pathogens *in vivo* may relate to concentrations, spray coverage, interactions with the host plant and other organisms and the solvents used.

1 Concentration

The most likely explanation for the lack of field protection by the propolis is that insufficient concentrations of propolis were applied. In the field the spray is exposed to many environmental factors that may reduce its efficacy in comparison with the laboratory agar trials.

The limited control of powdery mildew of cucumbers that was obtained in the glasshouse, in comparison with no apparent control in the field, may indicate that the sheltered nature of the glasshouse environment protects the propolis from the effects of weathering found in the field. In the field the plant surface is exposed to more direct sunlight, wind and rain all of which may reduce the efficacy of any applied fungicide by removal from the plant surface or degradation of the active ingredient.

The requirement for higher application rates in the field is exemplified by the differences in EC 50 of plant pathogens on agar and the recommended field application rates for other fungicides. Iprodione, for example has an EC 50 (based on linear growth of mycelium in agar) of 0.3 and 0.7 ppm for *Monilinia fructicola* and *Botrytis cinerea* respectively (Elmer and Gaunt 1988, Beever and Byrde 1982). In the field, the recommended application rate for control of both of these pathogens is 300-700 ppm iprodione (O'Connor 1990), ie one thousand times greater.

In this trial, the EC 50s for *Phytophthora* spp. were between 100-1000 ppm dry weight of propolis. Using iprodione as a guide, this corresponds to a foliar spray application of between 10-40% propolis (40% was the maximum concentration of propolis in ethanol obtained in this trial). However, taking the tomato trial as an example, this is equivalent to approximately 250 grams of propolis per tomato plant per spray or a cost of $15 per plant per spray. Obviously at this quantity and cost the use of propolis for blight control in tomatoes is unfeasible.

2 Spray coverage

A better spray coverage may also have been obtained in the glass house. In the glass house the plants were sprayed with a small hand sprayer so close attention could be given to the coverage of each leaf. In the field the plants were bigger and a back pack sprayer was used so the same degree of attention could not be given to coverage. This may have reduced the active ingredient concentration on the plant surface and therefore reduced any protectant qualities of the propolis.

3 Host plant metabolism

Plants have a rapid turnover rate of compounds involved in plant defence, either breaking them down or converting them to other compounds (Van Etten *et al* 1982). It is possible that the host leaf, the tomato or zuccini, may leak enzymes and other compounds that could break down the active ingredients in the propolis on the leaf surface.

When seeds germinate there is often a reduction of phenols just prior to germination (Vickery and Vickery 1981). Enzymes produced to metabolise phenols could break down similar compounds in the propolis seed dressing.

This would therefore remove any protectant action of the propolis and make the seed vulnerable to *P. ultimum.*

4 Metabolism by other organisms

The presence of other organisms at the plant surface may also reduce the activity of the propolis against the target pathogens. As shown in this trial the sensitivity of microorganisms to propolis and its plant defence compounds varies. Some organisms' growth may be totally inhibited while others may be able to break down the active compounds. With the range of organisms likely to be present at the plant surface many of the compounds in propolis could be broken down. Consequently this would reduce any inhibitory effect of propolis on the target pathogen.

5 Solvent

The different extractants used in the laboratory and field trials may also have affected the results.

Washing the propolis in water prior to extraction in ether reduced its antifungal activity (Table 3.3.5). The antifungal activity of the water extracts (Table 3.4.1) confirmed that some of the biologically active compounds are soluble in water, the solubility increasing with temperature. The ether extracts had the greatest antifungal activity, presumably as a result of extracting more biologically active compounds.

This suggests that the activity of the propolis extract depends on the extractant used. Consequently there is likely to be a difference in activities of the ether extracts used in the (*in vitro*) agar trials and the ethanol extracts used in the *in vivo* trials. The activity of ethanol extracts could not be tested on agar as the ethanol appeared to be bound in an insoluble precipitate that was fungitoxic.

Meresta and Meresta (1982) found that ether extracts had higher antibacterial activity than ethanol extracts of propolis. The minimum concentrations of propolis required to inhibit bacterial growth were 90 and 120 ppm (dry weight of raw propolis) respectively. Assuming the extraction of antibacterial compounds is indicative of the antifungal compounds extracted, it would appear higher concentrations of ethanol extracts are required to give the same activity as the ether extracts. It may also be possible that some of the key antifungal compounds are extracted in ether but not ethanol.

Greater activity of ether extracts of propolis may also have contributed to the greater protection against powdery mildew of glass house cucumbers than powdery mildew of field grown zuccinis where ethanol extracts were used.

8.4 Control of powdery mildew in zuccinis

Propolis/Sulphur

The greater control over powdery mildew given by the propolis and sulphur mixture than by the separate sprays suggests a synergistic relationship. Garofolo (1987) reported control over a wide range of insects and diseases using a mix of sulphur and propolis (Table 2.2). Further experiments on control of powdery mildew with propolis using different combinations of propolis and sulphur would be worthwhile.

Reysa

Herger *et al* (1989) found that Reysa was capable of controlling powdery mildew infection in grape vines in Canterbury. In this trial Reysa initially reduced powdery mildew but did not give as much control as the sulphur or propolis/sulphur treatments.

As none of the treatments affected yield of zuccinis there would have been no economic benefit in applying any treatments for powdery mildew control. In commercial horticulture the economic benefit of fungicide application for powdery mildew control is dependent on the severity of the disease and the growth stage of the plants when infection occurs (K Fisher *pers comm.*).

Bitterness in zuccinis

Produce from all except the propolis and Reysa treatments were sold locally. One zuccini was returned because of its exceedingly bitter flavour that made the vegetable unpalatable. Bitterness in the cucurbits is generally attributed to the cucurbitacins, a group of oxygenated tetracyclic terpenes (Hutt and Herrington 1985), with physiological effects in mammals of respiratory paralysis, or in less acute cases gastro-intestinal irritations some with haemorrhagic enteritis. In zuccinis Hutt and Herrington (1985) confirmed that cucurbitacin E glycoside was the bitter principle and was present in concentrations of approximately 600 $mgkg^{-1}$. Taste panels could detect cucurbitacin E glycoside at 2 $mgkg^{-1}$ in zuccini pulp.

It is possible that the cucurbitacins provide protection for the zuccini against other pests including pathogens such as *Erysiphe cichoracearum*. In contrast however cucurbitacins are potent kairomones for a large group of phytophagous beetles, in which detection stimulates compulsive feeding. Ferguson and Metcalf (1985) reported that some species are able to sequester ingested cucurbitacins providing protection against predators.

Selective breeding has reduced or removed cucurbitacins in domestic cucurbits. However occurrences of bitter fruit have been reported. For example a single bitter water melon was discovered in a field of over 100 acres (Enslin *et al* 1954). The reasons for this rare occurrence are not clear but could represent occasional mutations in the appropriate genes.

8.5 Control of late blight of tomatoes

Copper fungicides are often used in organic systems for control of late blight in tomatoes and they gave good control in this trial. There was no indication of any reduction in soil biological cellulolytic activity as a result of use of foliar copper sprays. One of the main concerns associated with the use of copper is effects on soil organisms, such as reduction in earthworm populations recorded in areas where copper had been applied frequently over several years and had accumulated in the soil (Cluzeau and Fayolle 1988). The ground used for the tomatoes in this trial was previously in pasture, so accumulated copper in the soil from previous cropping activities was therefore unlikely.

Applying the minimum amount of copper for disease control will minimise the harmful effects on soil organisms. Discontinuing copper sprays for the last 6 weeks of the trial did not reduce the level of the disease control compared with the treatment that received sprays throughout the trial. This was despite windy, warm and wet weather that was favourable for removal of materials on the plant surface, and for late blight development. Spraying at 10-14 day intervals at the manufacturer's recommended rate is therefore likely to result in applying excess quantities of fungicide. Spraying based on weather conditions and on the estimated copper residues still present on the plant surface should reduce the amount of copper fungicide to the minimum required for control of the disease.

The initial occurrence and development of late blight was later than expected given the conducive conditions for disease. Training the plants on wires and removing the lower leaves would have allowed good air flow through the crop, thereby reducing relative humidity in the microclimate of the phyllosphere, and may therefore have assisted in delaying epidemic development of *P.infestans*.

8.6 Control of storage rot in kiwifruit

No results were obtained in this trial on the effects of propolis on *Botrytis cinerea in vivo* because of the low infection rate in the whole kiwifruit block. This has been a problem in other trials (Beever *et al* 1984). Choosing areas prone to high infection rates will increase the likelihood of obtaining results, although up to 20% of locally packed fruit has been thrown out because of storage rot (G. Cant *pers comm*). In this trial the initial screening of propolis could have been carried out by artificially inoculating individual fruit after harvest to ensure infection, followed by a dip in propolis. Propolis however is unlikely to be acceptable as a post harvest dip as dipping the fruit left a visible residue on the fruit surface.

There was a higher percentage of undersized fruit in the propolis treatment but the total yield was not reduced. This suggests the propolis spray at blossom reduced pollination and hence fruit size. This may have resulted from the propolis repelling bees, inhibiting pollen germination or having some other phytotoxic effect.

8.7 Activity of propolis against insects

The delayed development and perhaps mortality in the leaf roller larvae fed on the propolis diet may have been caused by secondary plant compounds. Quercetin, for example, is a flavonoid found in many plant species and has commonly been detected in propolis (Walker and Crane 1987). Its effect on insects varies. Lukefahr and Martin (1966) found that more than 70% of tobacco budworm, *Heliothis virescens* Lepidoptera, were killed with a 0.1% concentration of quercetin in their diet. However cotton bollworm, *H.zea*, had little mortality at concentrations less than 0.8%.

Many of the other compounds identified in propolis, reported by Walker and Crane (1987), affect insect growth, development, reproduction or metabolism (Beck and Reese 1976). As with the antifungal properties of propolis, activity

against insects will depend on the source of the propolis and the insect species tested.

8.8 User safety

Naturally occurring pesticides should be treated with the same caution as synthetic pesticides. During this trial I developed a rash on the hands and wrists which appeared to be a result of contact with propolis. The symptoms were typical of the hypersensitive skin response described by Stüwe *et al* (1989). Such complaints are apparently common amongst bee keepers in New Zealand when handling propolis (G. Rowe *pers comm*). Gloves should therefore be worn whenever handling propolis and work in the laboratory should be carried out in the fume cupboard. Full spray gear should be worn, gloves, spray suit and protective head gear, when mixing and spraying propolis in the field.

CHAPTER 9

CONCLUSIONS

1. Propolis inhibited the growth of plant pathogenic fungi *in vitro*. Most species tested were totally inhibited at concentrations of 1% propolis (weight of propolis before extraction).

2. The sensitivity of fungi to propolis varied, with species from the oomycetes showing most sensitivity.

3. The antifungal activity of propolis varied according to the date and location of collection from the hive and the extractant used. Ether extracts had greater activity than water extracts. It is suggested the above are related to concentration and types of secondary plant compounds in the propolis.

4. Propolis gave some protection against powdery mildew of glasshouse grown cucumbers but gave no apparent protection against late blight of tomatoes or powdery mildew of zuccinis in the field. The lack of field activity was attributed to : insufficient concentrations of propolis; spray coverage; host plant metabolism; metabolism of other organisms and the solvents used.

5. Propolis in the diet of *Epiphyas postvittana* larvae delayed the insects' development but propolis sprayed on cabbage leaves did not affect development or reproduction of *Myzus persicae*.

6. Allergic reactions to propolis highlighted the need to use protective gear when working with natural as well as synthetic pesticides.

7. Future research

Further information is required on the compounds associated with biological activity of propolis. Explanations should be sought for the variation in fungal sensitivities to propolis, variation in propolis activity according to its date and location of collection and extraction procedure, and the possible synergism between propolis and sulphur. Chemical analysis of propolis samples should be carried out alongside biological tests. Identification of active compounds could ensure the most efficient extraction is used to obtain the most active pesticide. Identification of plant species with high levels of active compounds could allow direct extraction from plants. This would remove the inefficiencies and

variability involved in propolis production, including collection by the bees from plants with low concentrations of active compounds, obtaining propolis from the hives and removal of bees wax.

There is an increasing understanding of the natural disease resistance mechanisms in plants (Harborne 1988). This should be utilised in the management of agroecosystems if only to suggest compounds for synthetic analogues which could be used to control pests. Long term management of agroecosystems must surely rely on understanding and manipulating natural mechanisms in the system. The system should therefore be designed to maximise interactions that minimise pest problems. Application of external pest control agents should only be applied when essential to protect the crop and to benefit long term management. The types of compounds used should have minimal detrimental effects on beneficial interactions and the environment as a whole.

REFERENCES

Adey, M. 1986. Bee products: the strength of propolis and beeswax. *Bee World* 68(1) : 7.

Anastasiu, R.I. 1978. Effect of propolis on *Pseudomonas aeruginosa. In vitro* experiments. *Proceedings of the International Symposium on Apitherapy :* 98-103.

Bankova, V.S., Popov, S.S. and Marekov, N.L. 1983. A study on flavonoids of propolis. *Journal of Natural Products* 46 (4) : 471-474.

Beck, S.D. and Reese, J.C. 1976. Insect-plant interactions: nutrition and metabolism. In Recent advances in phytochemistry 10: Biochemical Interactions between Plants and Insects (J.W. Wallace and R.L. Mansell eds). Plenum Press, New York : 41-92.

Beever, R.E. and Byrde, R.J.W. 1982. Resistance to the dicarboximide fungicides. In Fungicide Resistance in Crop Protection (J.Dekker and S.G. Georgopoulos eds.) Centre for Agricultural Publishing, Wageningen : 101-120.

Beever, D.J., M^{c}Grath, H.J.W., Clarke, D.L. and Todd, M. 1984. Field application and residues of fungicides for the control of botrytis storage rot of kiwifruit. *New Zealand Journal of Experimental Agriculture* 12 : 339-346.

Brien, R.M. and Dingley, J.M. 1956. Fungous diseases. In Plant Protection in New Zealand (DSIR publication). Govt. Printer Wellington : 3-63.

Brown, R. 1989. Hive products: pollen, propolis and royal jelly. *Bee World* 70(3) : 109-117.

Cluzeau, D. and Fayolle, L. 1988. Effects of earthworms of pesticide treatments, especially copper, in Champagne vineyards. *Comptes Rendus Hebdomadaires des Seances. Academie d'Agriculture de France* 74 (8) : 109-117.

C.M.I. 1985. Commonwealth Mycological Institute Descriptions of pathogenic fungi and bacteria, No. 838. Commonwealth Agricultural Bureaux Surrey.

Corsi, M. 1981. A contribution to knowledge about the essential oils of propolis. *Apiacta* 16 (4) : 153-154.

Cunningham, G.H. 1925. Fungous diseases of fruit trees in New Zealand and their remedial treatment. Brett Printing and Publishing Co. Auckland : 182-184.

Davidse, C.C., Donial, D.S. and van Westen, C.J. 1983. Resistance to metalaxyl in *Phytophthora infestans* in the Netherlands. *Netherlands Journal of Plant Pathology* 89 : 1-20.

DSIR 1954. Soil Bureau Bulletin No. 5 : General soil survey of the North Island.

Eischen, F.A. and Dietz, A. 1987. Growth and Survival of *Galleria mellonella* (Lepidoptera: Pyralidae) larvae fed diets containing honey bee - collected plant resins. *Annals. Entomological Society of America* 80 : 74-77.

Elmer, P.A.G. and Gaunt, R.E. 1988. Dicarboxymide resistance and disease control in brown rot of stonefruit. *Proceedings of the 41st New Zealand Weed and Pest Control Conference* : 271-274.

Enslin, P.R., Joubert, T.G. and Rehm, S. 1954. Bitter principles of the Cucurbitaceae. *Journal of the South African Chemical Institute* 7(2) : 131-138.

Fenemore, P.G. 1984. Plant pests and their control. Revised edition, Butterworth & co. London. 280pp.

Ferguson, J.E. and Metcalf, R.L. 1985. Cucurbitacins: Plant derived defence compounds for diabroticites (Coleoptera: Chrysomelidae). *Journal of Chemical Ecology* 11(3) : 311-318.

Garofolo, F. 1987. Il propoli nella difesa delle piante. Special publication Associazione Suolo e Salute.

Ghisalberti, E.L. 1979. Propolis : A review. *Bee World* 60 : 69-84.

Greenaway, W., Scaysbrook, T. and Whatley, F.R.S 1987. The analysis of bud exudate of *Populus* x euroamericana and of propolis, by gas chromatography-mass spectrometry. *Proceedings. Royal Society of London. Series B. Biological Sciences* 232 : 249-272.

Harborne, J.B. 1987. Natural fungitoxins. In Proceedings of the Phytochemical Society of Europe 27 Biologically Active Natural Products. (K.Hostettmann and P.J. Lea eds.) Oxford Sci. Publications : 195-211.

Harborne, J.B. 1988. Flavonoids in the environment: structure activity relationships. In Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular and Medicinal Properties (V. Cody, E. Middleton Jr, J.B. Harborne and A. Beretz eds) Alan R. Liss Inc. : 17-27.

Herger, G., Harvey I., Jenkins T. and Alexander R. 1989. Control of powdery mildew of grapes with plant extracts. *Proceedings. 42nd N.Z. Weed and Pest Control Conference* : 178-181.

Hutt, T.F. and Herrington, M.E. 1985. The determination of bitter principles of zuccinis. *Journal of the Science of Food and Agriculture* 36 : 1107-1112.

Jozwik, Z. and Trytek, J. 1985. The effect of propolis extracts containing flavonoid compounds on acid-resistant saprophytic bacilli. *Pszczelnicze Zeszyty Naukowe* 24 : 47-56.

Kandefer-Szersen, M., Jozwik, Z. and Trytek, J. 1985. Antiviral activity of propolis. *Annales Universitatis Mariae Curie-Sklodowska Sectio C. Biologia* 40 (1-25) : 25-30.

Kivalkina, B.P. 1948. *Pchelovodstvo* 10. Cited in Ghisalberti, E.L. 1979 *Beeworld* 60 : 69-84.

König, B. 1985. Plant Sources of Propolis. *Bee World* 66(4) : 136-139.

König, B. and Dustmann, J.H. 1985. The caffeoylics as a new family of natural antiviral compounds. *Naturwissenschaften* 72 : 659-661.

Lindenfelser, L.A. 1967. Antimicrobial activity of propolis. *American Bee Journal* 107 : 90-92, 130-131.

Lukefahr, M.J. and Martin, D.F. 1966. Cotton plant pigments as a source of resistance to the bollworm and tobacco budworm. *Journal of Economic Entomology* 59 : 176-179.

Meresta, L. and Meresta, T. 1982. Usefulness of various solvents for extracting biologically active substances from propolis. *Bulletin of the Veterinary Institute in Pulway* 25 (1-4) : 12-14.

Mlagan, V. and Sulimanovic, D. 1982. Action of propolis solutions on *Bacillus larvae*. *Apiacta* 17 (1) : 16-20.

O'Connor, B. (ed.) 1990. Agrichemical and Plant Protection Manual. N.Z. Agrichemical Manual Partnership Publication.

Penman, D.R. 1984. Deciduous tree fruit pests. In New Zealand Pest and Beneficial Insects (R.R. Scott editor). Lincoln University College of Agriculture, Canterbury : 33-50.

Pennycook, S.R. 1984. Spraying kiwifruit for control of *Botrytis* storage rot. *Orchardist* 57 : 33-34.

Perkin, J.H. 1985. Naturally occurring pesticides and the pesticide crisis. In CRC Handbook of Natural Pesticides: Methods. Vol 1 : Theory, Practice and Detection (N. Bhushan Mandova ed.) CRC Press Inc, Florida : 247-325.

Pimentel, D. 1985. Pests and their control. In CRC Handbook of Natural Pesticides: Methods. Vol 1 : Theory, Practice and Detection (N. Bhushan Mandova ed.) CRC Press Inc, Florida : 3-19.

Singh, P., Clare, G.K. and Ashby, M.D. 1984. *Epiphyas postvittana*. In Handbook of Insect Rearing, Vol 2. (Singh P. and Moore R.F. editors). Elsevier Science Publishers B.V., Amsterdam.

Staub, T. and Sozzi, D. 1984. Fungicide resistance : a continuing challenge. *Plant Disease* 68 : 1026-1031.

Stillerly, W.R. 1978. Cucurbits. In The Powdery Mildews (D.M. Spencer ed.): 632-643. Academic Press. London.

Stoessl, A. (1982). Biosynthesis of phytoalexins. In Phytoalexins J. Bailey and J.A. Mansfield eds. Blackie & Son Ltd, London : 133-180.

Stüwe, H.T., Bruhn, G., König W.A. and Hausen B.M. 1989. The synthesis of caffeic acid esters, a new group of naturally occurring contact allergens. *Naturwissenschaften* 76, 426-427.

Suckling, D.M., Penman, D.R., Chapman, R.B. and Wearing, C.H. 1985. Pheromone use in insecticide resistance surveys of light brown apple moths (Lepidoptera: Torticidae). *Journal of Economic Entomology* 78 : 204-207.

Todhunter, J.A. 1985. Foreword. In CRC Handbook of Natural Pesticides: Methods. Vol 1 : Theory, Practice and Detection (N. Bhushan Mandova ed.) CRC Press Inc, Florida.

Tomas-Barberan, F.A., Maillard, M. and Hostettmann, K. 1988. Antifungal flavonoids from the leaf surfaces of *Helichrysum nitens* and from the stem bark of *Erythrina berteroana*. In Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular, and Medicinal Properties (V. Cody, E. Middleton Jr., J.B. Harborne and P.J. Lea eds.) Alan R. Liss, Inc. : 61-65.

VanEtten, H.D., Matthews, D.E. and Smith, D.A. 1982. Metabolism of phytoalexins. In Phytoalexins J. Bailey and J.A. Mansfield eds. Blackie & Son Ltd, London : 181-217.

Vickery, M.L. and Vickery, B. 1981. Secondary plant metabolism. M^{c}Millan Press Ltd. London.

Walker, J.C. 1952. Diseases of vegetable crops. M^{c}Graw-Hill Book Company, New York.

Walker, P. and Crane, E. 1987. Constituents of propolis. *Apidologie* 18 (4) : 327-334.

Wei-chun, Ma 1984. Sublethal toxic effects of copper on growth, reproduction and litter breakdown activity in the earthworm *Lumbricus rubellus*, with observations on the influence of temperature and soil pH. *Environmental Pollution. Series A. Ecological and Biological.* 33 : 207-219.

Wilson, C.C. and Wisniewski, E. 1989. Biological control of postharvest diseases of fruit and vegetables : an emerging technology. *Annual Review of Plant Pathology* 27 : 425-441.

Wright-Sunflower, C. 1988. Panning for brown gold. *Gleanings in Bee culture* July 1988 : 414-416.

Yarwood, C.E. 1957. Powdery Mildews. *Botanical Review* 23 : 235-301.

Zar, J.H. 1974. Biostatistical Analysis. Prentice - Hall Publishers, New Jersey, 620 pp.

APPENDIX 1

4.1 Effect of propolis on powdery mildew in glasshouse grown cucumbers

4.2.1 Overview of zuccini trial

4.2.2 Powdery mildew damage on zuccini plant

4.2.3 Zuccini from propolis / sulphur treatment

5.1 Overview of tomato trial

5.2 Late blight damage in tomato plants

APPENDIX 2

A diet was made up using the following recipe :

Dry mix	30.50 g
water	108.00 ml
KOH	0.75 ml
vitamin mixture	3.00 g
sucrose	4.50 g
glucose	0.75 g
streptomycin	23.00 mg
penicillin	23.00 mg
water	29.00 ml
mould inhibitor	2.75 ml
propolis dry weight (extracted in ether)	4.30 g

The dry mix used, supplied by DSIR, was:

Artificial diet	%
Agar	12.32
casein	17.24
cellulose powder	49.25
Wesson's salt mix	4.93
wheatgerm	14.78
cholesterol	0.25
linoleic acid	1.23

dichloromethane (evaporates)

The dry mix, distilled water and KOH were heated together for 10 minutes. The vitamin mixture, sucrose, glucose, streptomycin, penicillin and water were thoroughly mixed together. This mixture, mould inhibitor and propolis in ether were added to the diet mix at 60°C. This made 183 g of diet. The diet was then cooled over night in the coolstore (approximately 1°C).