THE MODE OF ACTION OF SODIUM BICARBONATE / ADDITIVE MIXTURES AGAINST CUCUMBER POWDERY MILDEW DISEASE

A thesis submitted in partial fulfilment of the requirements for the degree of
Master of Applied Science in Plant Pathology, Massey University.

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1997

ABSTRACT

The host-parasite-complex of cucumber (*Cucumis sativus* L.) and *Sphaerotheca fuliginea* (Schlechtendal ex Fr.) Pollacci, the causal agent of cucumber powdery mildew, was studied to determine whether stimulation of host defences was a mode of action of sodium bicarbonate/additive mixtures. Additives included shellspray mineral oil and sunlight dishwashing liquid. The roles in cucumber defence of phenolic compounds, enzymes: peroxidase (PO) and phenylalanine ammonia-lyase (PAL), and induced structural barriers, papillae, were investigated. Comparisons were made between the effects of bicarbonate/additive mixtures and the plant extract Milsana on these host defences, as related to three levels (cultivars) of host resistance to disease: susceptible, disease-tolerant and resistant.

Long and short duration glasshouse studies found disease incidence highest on less resistant cultivars and lowest on the most resistant cultivar, 'Slice King' ('SK'). Applied alone at 2 g/litre, sodium bicarbonate failed as a protectant. When combined with either additive, the fungicidal activity of bicarbonate was enhanced. Shellspray oil and bicarbonate-oil mixtures were the most effective treatments for less resistant cultivars, 'Lebanese' ('Leb') and 'Slicemaster' ('SM'). These treatments and Milsana provided comparable control against disease affecting 'SK'.

The role of phenolic compounds was investigated in thin-layer chromatogram bioassays for antifungal phenolics in leaf extracts. This revealed much variability in free state phenolic patterns between host resistance/treatment combinations, in the presence and absence of disease. Glycosidically-bound phenolics hydrolysed into their free form, aglycones, were more abundant than free phenolics. Because of their frequency and arbitrary concentrations, it was unclear whether stimulation of aglycone production had occurred in infected leaves, as a result of any specific treatment or level of host resistance. The presence of these fungitoxic substances could not solely account for differences in disease severity. Hence, data did not support the concept of stimulation of host materials by either mildew infection or the treatments applied.

The responses of PO and PAL to treatment and infection were assessed by spectrophotometric measurements of activity in leaf samples. Higher enzymic-activities in healthy leaves were coupled with increasing levels of host resistance to disease. During the 14 days post-treatment, significant increases were detected in healthy and inoculated leaves treated with bicarbonate and Milsana; levels varied between cultivars. General enzymic-activity was higher in inoculated than noninoculated 'Leb' and 'SM', and of a comparable level in 'SK' controls and leaves treated with materials other than shellspray oil and bicarbonate-oil mixtures. For these apparent "normal" increases in plants treated with other materials occurred during this time.

Fluorescence and bright-field microscopy revealed similar numbers of conidia germinated 24 h after inoculation, irrespective of treatment and cultivar, although, significantly fewer conidia germinated on leaves treated with oil and bicarbonate-oil mixtures. With increasing host resistance the number of multiple germ tubes produced 72 h post-infection was less, papillae deposited in epidermal cells had increased, and the number of haustoria less, 120 h post-infection. Shellspray oil and bicarbonate-oil mixtures provided the most significant reduction in multiple germ tubes and haustoria, and the least apparent stimulation of papillae production.

ACKNOWLEDGEMENTS

Sincere thanks to Dr. Peter Long and Dr. David Woolley for their encouragement and assistance with all aspects of this research. The friendship of Hugh Neilson and assistance given to me with all experimental procedures was cherished. I am indebted to Chris Rawlingson for his technical assistance and advice, and to Darryl Cook and Jonathon Dixon for help with laboratory procedures and for answering mundane questions. Thanks also to staff at the Plant Growth and Central Photographic Units at Massey University, and to Mr. S. Ardnt, Compo, Munst, Germany, for supplying the "free" sample of Milsana plant extract.

This work was supported by a Turners and Growers Research Grant, Helen E. Akers Scholarship, and the Massey University Graduate Research Fund.

Special thanks to Mum and Dad, and Nana and Pop, whose love, encouragement, generosity and continued support, were as welcome as they were necessary. I am grateful to the Boow family for caring, and for taking an interest in my work. Finally, thanks to Jared, who inspired and motivated me, especially during difficult times, and was a constant source of criticism and witticism which kept me on my toes. Without these people this research could not have been completed.

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1. INTRODUCTION

1.1 PATHOGEN BIOLOGY

1.1.1 Taxonomy And Host Range

Sphaerotheca fuliginea (Schlechtendal ex Fr.) Pollacci is an ascomycete fungus classified in the Family Erysiphaceae (Alexopoulos & Mims 1979), and has been known as the primary causal agent of powdery mildew disease on members of the plant family Cucurbitaceae Juss. (cucurbits) since the 1800's (Zitter et al. 1996). Powdery mildew is perhaps the major factor limiting field and glasshouse production of cucurbits in most areas of the world (Hammett 1977; Cohen et al. 1996; Reuveni et al. 1996). All cucurbits, including the cucumber (*Cucumis sativus* L.), are susceptible regardless of the growing environment.

1.1.2 Symptoms Of Disease

Given favourable environmental conditions, disease can occur under glass throughout the year (Hammett 1977). The first symptoms of infection appear as small, dusty-white, circular to irregular, somewhat superficial areas on stems (Kapoor 1967), petioles and abaxial (upper) surfaces of older leaves and shaded lower leaves (Sitterly 1978; Zitter et al. 1996). These lesions increase in number and coalesce, eventually covering entire leaves (Agrios 1988). Figures 1 and 2 contrast healthy with infected leaves. Severely infected young leaves may turn brown and become shrivelled. Localised areas of chlorosis that later become necrotic, and death of an organ, may ensue.

Small pin-head size fruiting bodies (cleistothecia), at first white, later brown, and finally black when mature, may be present in older areas of infection (Agrios 1988). Roots are not affected but flowers are often colonised. Cucumber fruit are usually free of visible infection (Hammett 1977), even when foliage is heavily infested. However, they are likely to be fewer in number, suffer sun damage (sunscald), or ripen prematurely or incompletely, due to premature senescence of infected leaves (McGrath 1996). Such fruit have lower soluble solids and consequently poor flavour (Dougherty 1980). Late developing fruit often fail to mature and appear small and misshapen (Sitterly 1978). In addition, powdery mildew

Figure 1.1 Healthy leaves of cucumber (*Cucumis sativus* L.) 'Lebanese'

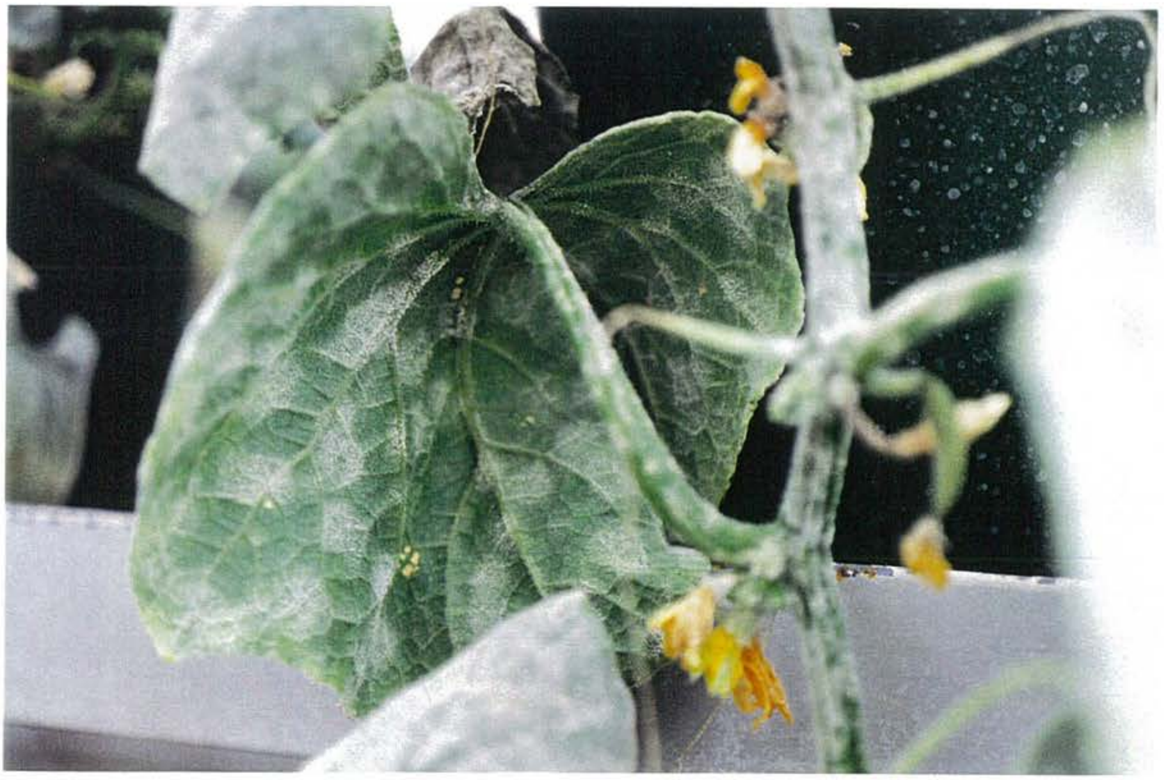


Figure 1.2 Lesions on leaves of cucumber 'Lebanese' characteristic of powdery mildew infection caused by *Sphaerotheca fuliginea* (Schlechtendal ex Fr.) Pollacci

infection predisposes plants to gummy stem blight (*Didymella bryoniae*) (Bergstrom et al. 1982). Thus infection can result in a reduction in yield and market quality, which appears to occur in proportion to timing and severity of disease development (Sitterly 1978).

1.1.3 Disease Cycle And Epidemiology

The primary source of inoculum consists of short-lived unicellular conidia, which mature in long chains on short euoidium-type conidiophores (Blumer 1967) (Figure 1.3). These arise at right angles from a network of mycelium on the leaf surface (Atkinson et al 1956), which is predominantly hyaline (translucent) in colour, darkening to brown with age (Kapoor 1967; Alexopoulos & Mims 1979) (Figure 1.4). Conidia are also hyaline, appearing smooth-surfaced (Hammett 1977), ellipsoid or barrel-shaped, and roughly 25-37 x 14-25 μm in size (Kapoor 1967). They contain an average of 15 distinct watery vacuoles and well-developed cell inclusions called fibrosin bodies (Hammett 1977), that can be clearly seen when fresh conidia are dusted onto clean glass slides or mounted in 3% aqueous KOH, and then viewed using polarised or transmitted light (Kable & Ballantyne 1963) respectively. Under conditions of low light intensity (Zitter et al. 1996), 95-97% relative humidity (Zaracovitis 1965), temperatures ranging from 22-31°C, and the absence of free water (Alexopoulos & Mims 1979), conidial germination commences within 2 h on a suitable host (Sitterly 1978). Conidia are self-sufficient in water and nutrients and can remain viable for 7-8 days (Zitter et al. 1996). If kept in contact with water on the plant surface they can be rendered nonviable, or may germinate abnormally and exhaust their food reserves without forming appressoria. The first or primary germ tube which emerges is short, and forms a convoluted appressorium which attaches to the epidermal cell 2-6 h post-inoculation. The appressorial germ tube matures at 8-12 h after inoculation and attempts penetration (Carver & Zeyen 1993).

Erysiphe cichoracearum was considered to be the primary causal organism of powdery mildew throughout most of the world before 1958 (Zitter et al. 1996). Today *S. fuliginea* is more commonly reported in glasshouses worldwide. It is thought that a shift in the predominance of these two fungi may have occurred, or that *S. fuliginea* was in the past misidentified on cucurbit hosts as *E. cichoracearum*, since criteria for identifying these

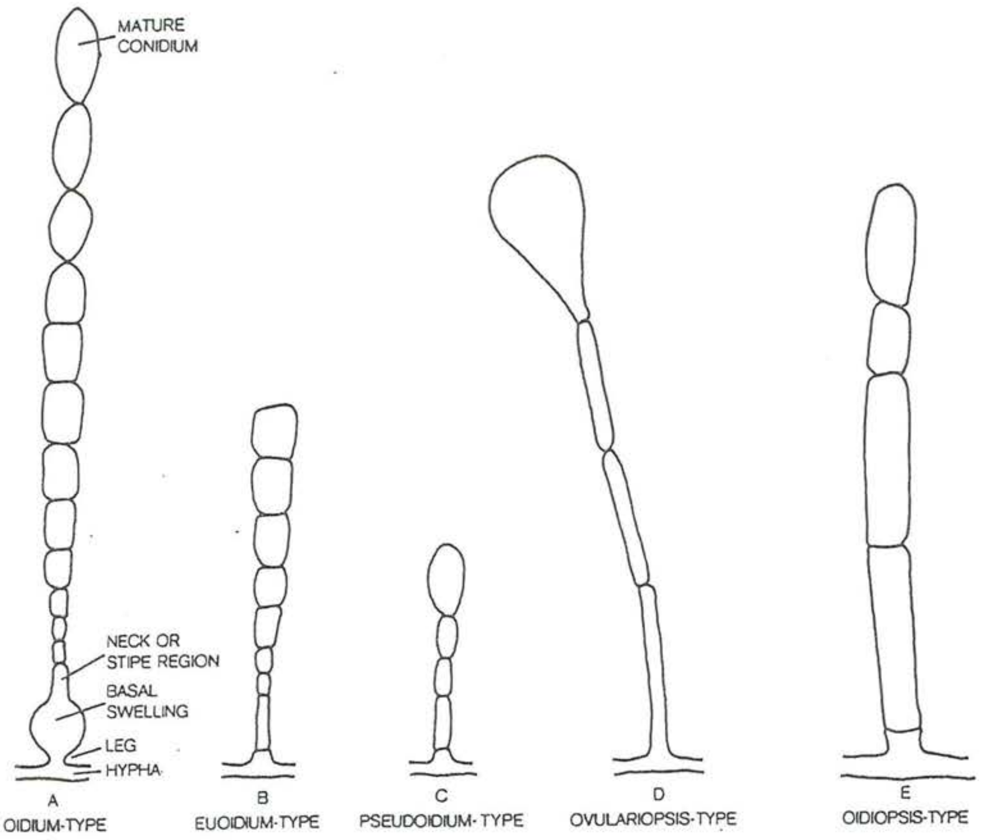


Figure 1.3 Morphology of Erysiphaceae (powdery mildew) conidial chains. Examples of each are A- *Erysiphe graminis* f.sp. *tritici*; B- Cucurbit powdery mildew (*Sphaerotheca fuliginea*); C-Grape powdery mildew (*Uncinula necator*); D-*Ovulariopsis* sp. (on *Vitex lucens*); E-*Oidium* sp. (on *Magnolia*) (Adapted from Hammett 1977)

Figure 1.4 Conidial chains of *S. fuliginea* arising from a leaf of cucumber 'Lebanese'

fungi from the conidial stage were not developed and published until the 1960's. Modern classification systems by Yarwood (1973) and Hammett (1977) distinguish the species on the basis of:

i. differences in climatic requirements:-*S. fuliginea* is more prevalent during the warmer months.

ii. morphological features (Blumer 1967):-edge lines produced by immature conidial chains are sinuate in *E. cichoracearum* and crenate in *S. fuliginea*
-fibrosin bodies are found only in *S. fuliginea*
-The germ tubes of *S. fuliginea* are short, thick and occasionally *forked* (Zaracovitis 1965; Hammett 1977), ending in convoluted appressoria (Sitterly 1978), whereas those of *Erysiphe* spp. are simpler in form (ie. straight and unlobed (Zitter et al. 1996)).

An infection peg forms beneath the appressorium, and penetrates through the host cuticle and epidermal cell wall, growing into the centre of the cell lumen. The plasma membrane invaginates, enlarges, and a specialised globular or club-shaped structure called an haustorium is formed in the cell lumen (Kapoor 1967; Clarkson 1992). This is separated from the neck by a septum and consists of a central body with many lobes embedded in an extrahaustorial matrix surrounded by an extrahaustorial membrane (Heintz & Blaich 1990). Due to the manner in which *S. fuliginea* obtains nutrition, the fungus is referred to as a biotrophic or obligate parasite, since growth and reproduction are entirely dependant on the fungus obtaining nutrition from a living host whose metabolism is still functional. Unlike necrotrophs which derive their nutrition from dead or dying cells (Clarkson 1992), powdery mildew fungi cannot utilise the breakdown products of host degradation. They rely on the assimilates produced by photosynthesis of the host plant, at least at the beginning of the infection cycle (Heisterüber et al. 1994).

It is through haustoria that mineral nutrients, water, amino acids, and carbohydrates (such as hexoses), are absorbed from the protoplast, in a complex interactive process between the cell cytoplasm and specialised tissue surrounding the body of the haustorium (Bushnell & Gay 1978). While the first haustorium is establishing, additional germ tubes are formed from other points on the same conidium, and hyphae grow out from the primary appressorium along the leaf surface (Sitterly 1978). Hyphal cells are thin-walled, vacuolate, and contain a single, large nucleus. Along the hypha are lateral swellings or lobed appressoria, one on each alternate hyphal cell (Sitterly 1978) which infect epidermal cells as before. This growing network of haustoria securely anchors the fungus to the host surface (Alexopoulos & Mims 1979). This non-ramification of fungal mycelium deeper than the epidermal cells results in ectoparasitism.

Physiological studies of infected cucumber leaves have shown increases in the rate of respiration, and impaired rates of photosynthesis (Abo-Foul et al. 1996). A temporary stimulation of the rate of photosynthesis, is often followed by sudden decline in both net and gross photosynthesis (Abo-Foul et al. 1996). Evidence provided by electron micrographs, associates this decline with marked deteriorations in the morphological organisation of chloroplast membranes, including damage to thylakoids, and loss of the stroma (and Calvin cycle enzymes through tears in the chloroplast envelope), with consequent increases in levels of uncoupled chlorophyll that can be utilised by haustoria. This reduction in light-harvesting capacity of the plant's primary photosynthetic organs, ultimately results in poor crop production.

The time between infection and appearance of the pathogen is usually short and 3-7 days after infection, many conidiophores have formed (Sitterly 1978; Zitter et al. 1996). A generative cell at the apex of each conidiophore produces conidia which are not abstricted, but cling together in chains. The actual method of abstriction for dispersal is unknown, but it is thought to follow a diurnal cycle (Yarwood 1977), and dispersal is almost exclusively by air currents in glasshouses (Blancard et al. 1994). One life cycle is estimated to be completed in 5-6 days (Yarwood 1957).

When environmental or nutritive conditions of the host become unfavourable (Sitterly 1978), and two sexually compatible mating types come into contact, the fungus may produce one or several asci inside a closed ascocarp, the cleistothecium (Sitterly 1978; Alexopoulos & Mims 1979; Agrios 1988). These overwintering fruiting bodies are 66-98 μm in diameter, globose, with a variable number of basal, mycelioid (resembling mycelium in being flaccid and indefinite (Alexopoulos & Mims 1979)) taxonomically characteristic appendages that are interwoven with the mycelium (Kapoor 1967). When ascospores are mature they are forcibly discharged as the cleistothecium ruptures under the strain of swelling asci. Increased genetic diversity resulting from sexual reproduction poses a threat to disease control for it could include new combinations of virulence genes and fungicide resistance (McGrath et al. 1996a).

Although the conidial (anamorphic) state of *S. fuliginea* is extremely common, cleistothecia (teleomorph) have rarely been reported on cucurbitaceous plants in fields or glasshouses around the world. McGrath et al. (1996b) recently charted their global location, and shows that to date, cleistothecia have only been observed in New Zealand in 1956 (Boeswinkel 1976) and in 1958 (Hammett 1977).

Three genetically distinct races (ie. mating groups within the species *S. fuliginea*) have been identified on cucurbits (Cohen et al. 1996; Zitter et al. 1996). They show pathological specialisation, attacking specific host genotypes within a species and also differ in their tolerance to fungicides (O'Brien & Weinert 1994). Race 1 and 2 are the most cosmopolitan, with race 3 being restricted at the moment to the United States (Floris & Alvarez 1996).

1.2 HOST BIOLOGY

1.2.1 Taxonomy And Growth Habit

The cucumber (*Cucumis sativus* L) is classified within the family of flowering plants known as the Cucurbitaceae Juss. It is indigenous to India, where it has been cultivated for over 3000 years (Sitterly 1978). A chromosome count of 14 and morphological features including angular stems, distinguish the cucumber from other members of the genus *Cucumis*, which have 24 chromosomes. Cucumbers are annuals, frost-sensitive, and have adopted a mesophytic requirement for water and humidity (Watson & Dallwitz 1996). Most grow as vines, but some modern cultivars produce compact, bushlike plants (Zitter et al. 1996).

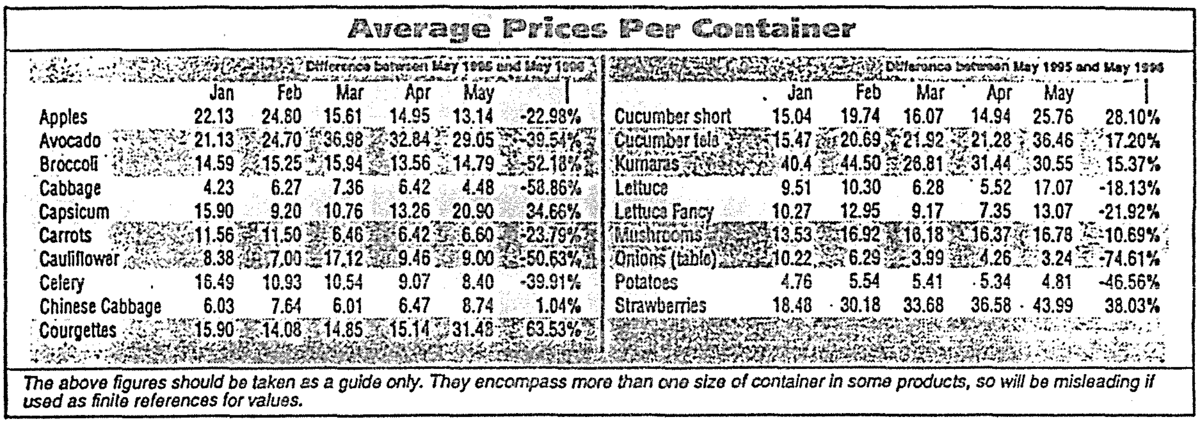
1.2.2 Floral And Fruit Morphology

Cucurbit flowers are solitary or aggregated in axillary inflorescences. They range in colour from green, white, yellow to orange, and are variable in size. Species have a gynoecious flowering habit ie. produce mainly female flowers and few male on the same plant. Fruits are usually fleshy and indehiscent (ie. do not split open to release seeds contained within) (Watson & Dallwitz 1996). Mature fruit are consumed fresh or cooked, and are valued for flavour and flesh texture qualities that make them ideal meat accompaniments, dessert and salad ingredients. Fruit possess a high water content and are relatively low in nutritional value, the most notable constituents being vitamins (A and C), and minerals (Zitter et al. 1996).

Seeds of several cultivars are important sources of oil and protein in parts of Africa, Asia and Latin America, and the skins have been utilised as material for baskets, insulation, oil filters, and as alternatives to pottery utensils (Sitterly 1978). The two major types of cucumbers are processing (pickling) and fresh (slicing). Varieties of the former are grown almost exclusively outdoors, while slicing varieties are grown outdoors and in glasshouses.

In New Zealand, cucumbers are grown solely for the local market. Estimates of crop production and the impact of powdery mildew disease with respect to this are sketchy due to a lack of complete survey data (M. Nicholls pers. comm.). Recent estimates place the total yield at 10 ha (c. 250 tons/ha) per growing season (M. Nicholls pers. comm.), with growers harvesting between 2-3 crops a season. Table 1.1 indicates the market value of cucumbers that make it an important speciality crop.

Table 1.1 Average prices ($) per container for New Zealand-grown cucumbers[a]



Average Prices Per Container

	Jan	Feb	Mar	Apr	May	Difference between May 1995 and May 1996		Jan	Feb	Mar	Apr	May	Difference between May 1995 and May 1996
Apples	22.13	24.80	15.61	14.95	13.14	-22.98%	Cucumber short	15.04	19.74	16.07	14.94	25.76	28.10%
Avocado	21.13	24.70	36.98	32.84	29.05	-39.54%	Cucumber tele	15.47	20.69	21.92	21.28	36.46	17.20%
Broccoli	14.59	15.25	15.94	13.56	14.79	-52.18%	Kumaras	40.4	44.50	28.81	31.44	30.55	15.37%
Cabbage	4.23	6.27	7.36	6.42	4.48	-58.86%	Lettuce	9.51	10.30	6.28	5.52	17.07	-18.13%
Capsicum	15.90	9.20	10.76	13.26	20.90	34.66%	Lettuce Fancy	10.27	12.95	9.17	7.35	13.07	-21.92%
Carrots	11.56	11.50	6.46	6.42	6.60	-23.79%	Mushrooms	13.53	16.92	18.18	16.37	16.78	-10.69%
Cauliflower	8.38	7.00	17.12	9.46	9.00	-50.63%	Onions (table)	10.22	6.29	3.99	4.26	3.24	-74.61%
Celery	16.49	10.93	10.54	9.07	8.40	-39.91%	Potatoes	4.76	5.54	5.41	5.34	4.81	-46.56%
Chinese Cabbage	6.03	7.64	6.01	6.47	8.74	1.04%	Strawberries	18.48	30.18	33.68	36.58	43.99	38.03%
Courgettes	15.90	14.08	14.85	15.14	31.48	63.53%							

The above figures should be taken as a guide only. They encompass more than one size of container in some products, so will be misleading if used as finite references for values.

[a] *Source of figures: Turners & Growers Wholesale Fruit And Veg Report, Horticulture News, July 1996.*

1.3 PLANT DEFENCE MECHANISMS

To survive the selection pressure imposed by herbivores, pests and pathogens during coevolution, plants have acquired effective structural features and biochemical mechanisms for disease resistance to defend themselves against attack (Keen 1990; Simms 1996). The mechanisms determining the resistance phenotype are unknown, but it is generally accepted that plants utilise a large arsenal of constitutive and inducible (active) defence mechanisms to prevent colonisation by pathogens (Kombrink et al. 1993). The same defence mechanisms are often utilised by a diversity of plant species against different types of pathogen: fungal, bacterial and viral, in both host and non-host resistance. Depending on the nature of invasion, products of the "same" biosynthetic pathway might accumulate, thus limiting the spread of a pathogen (El Ghaouth 1994). Examples of common defence reactions are given in Figure 1.5. These defences can be grouped into preformed and post-formed barriers to infection which may be structural (physical) or biochemical in nature.

The literature on plant defence mechanisms is extensive. The scope of this work does not allow for more than a brief overview of these. Several excellent reviews are available (Blanchette & Biggs 1992; Isaac 1992; Bennett & Wallsgrove 1994) and should be referred to for fuller comment. A thorough account of protein synthetic responses to environmental stresses, including pathogen challenge can be found in Artlip & Funkhouser (1995).

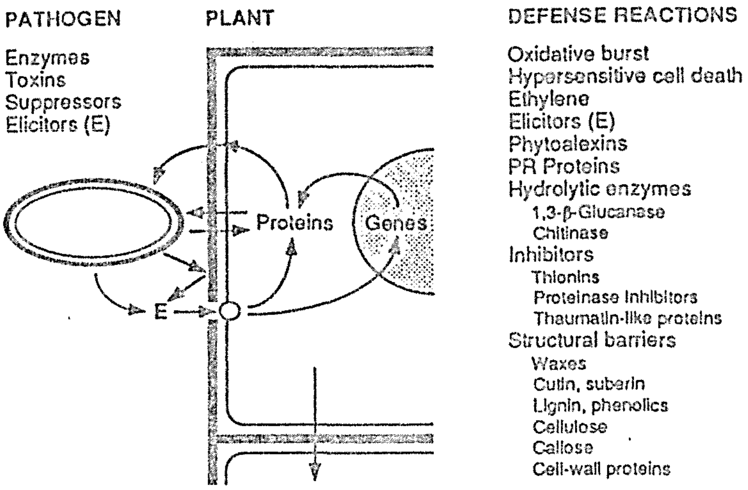


Figure 1.5 Schematic representation of plant-pathogen interactions and a list of typical plant defence responses (adapted from Kombrink et al. 1993).

1.3.1 Physical Barriers To Disease

Physical or mechanical impedance of hyphal growth is thought to be the first line of defence (Taylor 1987) against fungal pathogens. The first barriers to invasion are the morphological characteristics of the host's surface which influence the arrival and retention of inoculum (Royle 1976). Morphological features may operate in resistance in two ways:

i. passively- as preinfectional barriers characteristic of the unchallenged, healthy host, such as the thickness of the cuticle, trichome density, structure and composition of cell walls, timing of stomata opening.
ii. actively post-infection- to restrict pathogens and toxins from entering healthy tissue. Cork production, tylose formation in lumen of xylem vessels, extra lignification of cell walls and papillae, are examples of structures formed in response to the pathogen's

invasion (Royle 1976). The significance of papilla formation in host cells in response to fungal attack were discussed in depth by Aist (1976).

1.3.2 Biochemical Barriers To Disease

In most cases, infection by plant pathogenic microorganisms stimulates an active defence response in the metabolism of their target plants. Responses of plants vary, but depend ultimately on the interaction of genetic factors in both the host and pathogen (El Ghaouth 1994). These generally begin with molecular events:

i. alterations in protein synthesis overall and,
ii. shifts in levels of specific proteins (Artlip & Funkhouser 1995). Responses further depend on the nature, duration, and severity of the stress; and are frequently expressed as a combination of structural and biochemical changes (Matern & Kneusel 1988) which can be detected by modern microscopy, assaying and molecular techniques. Preinfectional biochemical barriers have included fungitoxic compounds such as phenolics inherent in the waxy cuticle (Adaskaveg 1992), cuticular exudations and leachates. Several phenolics have been observed in healthy cucumber leaves (Callebaut 1984; Daayf et al 1995) but their identity is unknown. Cyanogenic glycosides have also been detected in Cucurbitaceae, though most plants are noncyanogenic (Watson & Dallwitz 1996). There is still some debate as to their involvement in plant resistance to fungal pathogens.

The most frequently observed and best characterised biochemically active defence reactions are:

i. Rapid, localised cell death in and around the infected area (hypersensitive response:**HR**)- The hypersensitive reaction of plants to obligate parasites results in increased oxidative enzyme activity and a rapid enhancement of oxygen uptake. This is a sequence of events that occurs in the latter stages of a compatible host-pathogen interaction. The pattern is similar in both compatible (susceptible) and incompatible (resistant) host-pathogen interactions, however, when incompatible, the events proceed with greater rapidity. It is thought that the rapid development of the HR confers resistance upon the host plant (Artlip & Funkhouser 1995).

ii. Synthesis and deposition of phenolic compounds and proteins in the cell wall- phenolics are usually defined as substances that possess an aromatic ring bearing a hydroxyl group or its functional derivative (Metraux & Raskin 1994).

iii. Increases/decreases in an existing pool of proteins known as **PRs** (pathogenesis-related proteins) reported to have a role in defence against pathogen development (Punja & Raharjo 1996). This pool includes the oxidative enzymes peroxidase and polyphenoloxidase, the hydrolytic enzymes β-1,3-glucanase, hydroxyproline-rich glycoproteins and the chitinases. Two general groups of chitinase are known to occur in plants, acidic and basic. Acidic (extracellular) chitinases have been postulated to lyse hyphae of invading fungal pathogens during the early stages of pathogenesis and to release fungal cell wall fragments, which in turn may activate other defence-related mechanisms (Punja & Raharjo 1996). Several acidic chitinase isoforms have been induced in cucumber leaves susceptible to *S. fuliginea* in response to treatment with chitosan, that were constitutive in resistant cucumbers (Zhang & Punja 1994). The basic (vacuolar) forms may affect hyphae following plant cell collapse, when the vacuolar contents are released into the extracellular space.

iv. The *de novo* appearance and accumulation of protein(s)- this includes low molecular weight nonproteinaceous antimicrobial compounds, phytoalexins (Matern & Kneusel 1988), and structural materials like suberin and lignin after challenge by microorganisms and exposure to stresses. The presence of phytoalexins in cucumber has been claimed (Strange & Alavi 1981; Hammerschmidt & Kuc 1982) but there are also reports on the failure to detect them (Bailey & Mansfield 1982). Lignin reinforces cell walls at the site of attempted penetration. It is also an important component of papillae, structures deposited by the host cytoplasm, especially in response to biotrophic fungi, between the plasma membrane and cell wall, to likewise prevent penetration. Changes in the morphology of the host cell wall and plasmalemma, and also differences in the extent and rate of lignification, speed and quality (ie molecular composition) of papillae formation, are considered critical to the success of the expression of resistance against ectoparasites.

1.4 CURRENT METHODS OF CONTROLLING GLASSHOUSE DISEASES

1.4.1 Chemical Control

Glasshouse production forms the basis of high-value crop industries around the world (Elad et al. 1996). In general, commercial glasshouse operations aim at supplying high quality, high value products to specific markets (Menzies & Bélanger 1996). To achieve adequate economic returns, these crops are grown with intensive labour inputs and a high degree of advanced technology (Jarvis 1992). Disease management of glasshouse crops offers a particularly difficult challenge to growers because environmental conditions maintained at an optimum for plant growth are often conducive to disease development (ie. benefit the development and spread of pathogens) (Menzies & Bélanger 1996). Striking a balance between the two using whatever means necessary has invariably been the goal.

To avoid a loss of crop quality and reductions in yield (McGrath 1996), control strategies against *S. fuliginea* have relied heavily on two groups of active ingredients, azoles (dimethylation inhibitors: DMIs) and morpholines/morpholine-like compounds (Felsenstein et al. 1994). Both inhibit ergosterol biosynthesis of the fungus, but act on different steps in this pathway (Felsenstein et al 1994). Ergosterol plays an important role in the structure and function of fungal membranes. These synthetic materials also differ in their fungicidal modes of action, acting in either of two ways, as:

i. Protectant fungicides- Are most active when applied before inoculum arrival, persisting as a fungitoxic barrier on the plant surface to infection and re-infection. These are phytotoxic if they enter the plant in any appreciable quantities (Manners 1993). They usually affect several fungal metabolic processes (O'Brien et al. 1988), so are often referred to as 'broad spectrum' or 'non-specific' in activity. Application reduces sporulation and ultimately the amount of inoculum that is available in glasshouses to infect crops, and also the rate of increase of the pathogen ('r'- see below).

ii. Systemic fungicides- Have post-infection activity. These are translocated through the host to the site of infection where they act on specific pathways of fungal energy metabolism and nuclear processes to reduce sporulation and eradicate established infections. They are often referred to as 'site-specific inhibitors', and are applied less frequently than protectants, as they are not degraded by environmental forces such as wind and rain. New growth is protected but the strength of chemical activity becomes diluted as time passes due to loss of plant organs.

Inoculum pressure and the high crop value demand that frequent applications of the most effective fungicides be applied before the most significant part of an epidemic, the "logarithmic phase", to minimise the infection rate (Elad et al. 1996). Van der Plank (1968) suggested that the rate of disease development could be mathematically described at any time by the following equation:

$$\boxed{X=X_0e^{rt}}$$

where: X = Pathogen population (level of infection) at time 't'

X_0 = Pathogen population at time '0' (or the initial amount of inoculum)

r = rate of increase of pathogen

t = time period being considered

e = mathematical constant: 2.718

Any factor that reduces 'X_0', 'r' or 't' will reduce 'X', the amount of disease. A reduction in 't' is often more difficult to achieve in practice, especially with respect to glasshouse crops, so more emphasis is placed on reducing 'X_0'. Where 't' can be influenced, typical strategies employed could include a manipulation of planting or harvest dates, or fungicide usage to delay infection.

Of the few fungicides registered for the control of powdery mildew on glasshouse crops, several are rapidly losing their efficacy because of the emergence of resistant pathogen strains (Menzies & Bélanger 1996). Repeated usage of at least three popular systemic fungicides (benomyl, pyrazophos and triadimefon) over several growing seasons, has led to a partial or complete loss of sensitivity amongst races of *S. fuliginea*. This has occurred in production areas around the world, including the United States (as early as 1969) (Elad et al. 1989; Ziv & Zitter 1992), Europe, the Middle East and New Zealand. Their use in cucurbit production is now discouraged (Anon. 1996).

The success of resistant strains is in their ability to evolve new metabolic pathways to replace those targeted by site-specific fungicides, thus allowing them to function pathologically as normal (McGrath et al. 1996). Once they appear, they can survive for several years, so with continued use/long-term exposure of pathogens to previously effective fungicides, there is a high risk of promoting resistant populations of increased fitness in a short time (Reuveni et al. 1996).

This situation has resulted in renewed efforts to develop biologically-based control methods against powdery mildew. Various management strategies have been proposed for grower adoption to provide a balance between the effective control provided by systemic fungicides and the resistance problems that are associated with their over-use (McGrath 1996). Most are based on the alteration or mixture of site-specific with multi-site inhibitors, restricting their application to a specific use, or limiting the number of applications in glasshouses per year (O'Brien et al. 1988).

Reducing fungicide inputs necessary for profitable agriculture is also desirable, where feasible, because their application constitutes a considerable input cost (Warrington 1996), and local and international societies have expressed concern (Menzies & Bélanger 1996) about adverse effects of pesticides on:

i. The environment and ultimately the sustainability of the ecosystem- from air, water and soil contamination (Tzeng et al. 1996);

ii. Human (and animal) health- fungicides should not constitute a hazard to workers who apply agricultural chemicals, or to consumers of fresh produce as a result of residues detected on/in the final product (Anon. 1996). Consumers have become fearful of pesticide residues and their possible side-effects if ingested. Some have opted for organically grown produce but there are limits to the number of sulphur and copper sprays that can be applied in organic orchards without having detrimental effects on soil nutrients.

iii. Trade in primary produce- Fungicides are used extensively to eradicate diseases on horticultural produce in order to satisfy the quarantine requirements of importing countries (Anon 1996). Many countries now enforce strict regulations on levels and types of chemical residues permitted on domestic and imported produce destined for human consumption (Manners 1993). One example of this concerns produce treated with

Benlate™ (Du Pont) (McGrath 1996). The use of agricultural compounds should not result in breaches of domestic and overseas food standards.

iv. Non-target organisms, beneficial insects and natural enemies- especially those in glasshouses (the crop ecosystem) that biologically control insect pests.

1.4.2 Resistant Varieties

Genetic engineering of disease resistance is one of the main components of integrated plant protection systems (Simms 1996). Disease-tolerant hybrids of the long and short-type cucumbers grown in New Zealand glasshouses for example, 'Suprami F.1' and 'Maestro F.1' (Watkins New Zealand Ltd.), that display desirable characteristics and which are only moderately susceptible to powdery mildew, are available to growers to reduce 'X_t' (see above).

When developing resistant cultivars, attention must be given to the type of resistance bred for. In many cases, resistance is controlled by single dominant resistance genes in the host cultivars that match cognate dominant avirulence genes in the pathogen race (Kmecl et al. 1995). Although this type of resistance (also known as vertical or major gene resistance) can be very effective, problems arise concerning its stability or longevity and durability (Floris & Alvarez 1996). Durability is determined by:

i. the mutation rate of the pathogen,

ii. selection pressure placed upon mutant strains. Race-specific resistance is often short-lived because of the relatively frequent evolution of new mutant pathogen races that overcome this resistance (Kmecl et al. 1995). There are many instances where major gene resistance has been defeated by populations of *S. fuliginea* (McGrath et al. 1996a,b).

A more stable type of resistance is quantitative field resistance, which describes the degree of pathogen-resistance observed in the field. It may be the best approach to avoid the easy breakdown of resistance by the pathogen, and to reduce the rate of pathogen multiplication, if not entirely preventing infection. Briefly, new cultivars are bred with several different genes for resistance, with different mechanisms of action. The level of resistance observed may actually be the composite result of different resistances such as environmentally determined resistance, acquired resistance which can be triggered by biological and chemical inducers, resulting in an increase in nonspecific resistance to many different pathogens; and is usually inherited polygenically (Kmecl et al. 1995). However, at present it is not anticipated that genetic control will eliminate entirely the need for fungicides in glasshouses (McGrath et al. 1996). A thorough discussion of the genetics of plant (host) resistance and pathogen virulence can be found in an article by Simms (1996).

1.5 ALTERNATIVES TO CONVENTIONAL FUNGICIDES

Alternatives to existing synthetic compounds or at least methods for reducing the dependence on chemicals through integration with existing programs of pest and disease management together with resistant cultivars must be considered, thereby reducing injudicious pesticide use and the risk of resistance to all fungicide groups in the programme.

1.5.1 Biological Control

Research has demonstrated the potential of biological approaches for plant disease control, but there are only a few commercialised systems for the biocontrol of foliar diseases. This lack arises for several reasons (Elad et al. 1996):

i. availability of cheap and effective fungicides which can be easily applied;
ii. biotic and abiotic factors in glasshouses, including the microclimate and surface conditions of plant parts, do not favour survival and activity of introduced microorganisms;
iii. some systems perform less effectively than potent chemicals, and their effectiveness is not always consistent;
iv. the formulation and distribution of biocontrol preparations is regarded as far more difficult than for synthetic chemicals.

The literature on biological approaches to the suppression of powdery mildew diseases is extensive, and excellent overviews are provided by Elad et al. (1996) and Menzies & Bélanger (1996), which may be consulted for a thorough treatment of the subject. It appears that powdery mildew fungi are vulnerable to antagonistic microorganisms, due to the superficial development of all powdery mildew fungal parts except haustoria (Elad et al. 1996). Three natural antagonists have been tested beyond the laboratory and show promise for commercialisation (Menzies & Bélanger 1996):

i. The hyperparasitic coelomycete *Ampelomyces quisqualis* Ces. Schlecht. (Abo-Foul 1996) which inhibits conidial production and cleistothecial formation. Problems with high humidity requirements hampered the efficacy of early formulations of this antagonist (Menzies & Bélanger 1996). Recently however, the Ecogen company developed a formulation based on an isolate (AQ10) selected for its tolerance of lower humidities. AQ10 is currently being evaluated under glasshouse conditions for the control of *S. fuliginea* (Hofstein 1994; Menzies & Bélanger 1996).

ii. Several species belonging to the ballistospore-forming yeast genus *Tilletiopsis* (Hoch & Providenti 1979; Urquhart et al. 1994) have reduced the density of *S. fuliginea* conidia on glasshouse-grown cucumbers through the act of hyperparasitism

iii. Several species belonging to the yeast-like epiphytic genus *Sporothrix* (Bélanger et al. 1994) have also restricted the development of *S. fuliginea* on glasshouse-grown cucumbers through a different mode of action, antibiosis.

1.5.2 Cultural Control

Heated glasshouses, and restricted ventilation coupled with wet conditions, especially on the lower, shaded plant parts, often results in the glasshouse atmosphere remaining saturated for long periods of the day (Elad et al. 1996), and thus makes the environment very conducive to disease development. The temperature requirements of *S. fuliginea* for growth and reproduction overlap with conditions prevailing in glasshouses, up to a maximum of c. 30-35°C (Elad et al. 1996). Therefore modifications of the glasshouse environment based on climate control alone have so far had little effect against *S. fuliginea*. Sanitation which generally involves removal or destruction of all disease material is also practiced.

1.5.3 Biocompatible Fungicides

A Coating polymers-Antitranspirants, mineral oils (derived from highly refined petroleum), plant oils (eg. canola, safflower, soybean), and surfactants have been used in multiple-application programs as artificial barriers to infection on abaxial leaf surfaces. Research indicates that they have a broad spectrum of action against foliar pathogens on various host plants, including powdery mildew on cucurbits (Ziv & Zitter 1992), rose, hops, lilac, euonymous, apple, hydrangea; and powdery and downy mildews on grapevines (Northover & Schneider 1996). Extra benefits of these polymers include:

-a lack of phytotoxicity
-their biodegradability
-permeability to gases
-resistance to weathering in the short-term.

Surfactants are integral constituents of many pesticides because they favour absorption and cuticular penetration of the active ingredient (Mayr et al. 1994). There is some evidence that surfactants effect changes in photosynthetic and transpirational behaviour, and phenolic biosynthesis. Mayr et al. (1994) observed that Triton-X-100 induced phenol synthesis when applied to leaves and fruit of apple trees, and further, phenol accumulation after treatment was more pronounced in young than in mature leaves.

B Photoactivated pesticides-Control of powdery mildew on cucurbits and other crops, in recent field and glasshouse trials, has been achieved to levels equivalent or superior to conventional chemicals by weekly applications of a formulation developed from a methionine-riboflavin mixture (Tzeng et al. 1996). The observed morphological destructive effects on mildew colonies have suggested that the biocidal activity is one of eradication, through the action of various highly reactive oxygen radicals or active oxygen derivatives in this complex, which are generated by light. Particularly vulnerable are microbial cells hyaline in colour, such as the thalli of ectoparasitic powdery mildew fungi. The formulation is unique because it contains mainly food constituents and biodegradable ingredients in quantities considered too small to pose any danger to the environment.

C Simple inorganic salts eg. silicates, bicarbonates and phosphates in conjunction with surfactants- Chemicals with low mammalian and environmental toxicities (Horst et al. 1992) such as oxalate, bicarbonate, potassium and di- and tri-phosphate salts, alone and in combination with surfactants, have been investigated worldwide for their potential to control plant diseases. Sodium bicarbonate ($NaHCO_3$), commonly used by humans as a medicine, a toothpaste, an ingredient in baking powder and as a food additive for swelling bread and biscuit dough (Long et al. 1994), in quantities well above those which would be consumed from spray residues on fleshy plant organs and vegetative material, combined with mineral oil or liquid detergents has also shown considerable potential as a non-toxic disease control material.

Sodium bicarbonate is produced from the treatment of a strong NaCl solution with ammonia and CO_2 (Youngson 1994). The fungicidal properties of sodium bicarbonate against powdery mildew pathogens were first recognised by Currey (1924) as an effective way to control rose powdery mildew (*Sphaerotheca pannosa* var. *rosae* (Wallr. ex Fr.) Lev. In the late 1970's, research in Japan by Homma et al. showed that sodium bicarbonate inhibited powdery mildew on cucumber and in addition, the fungicidal effect was greatly enhanced when it was combined with surfactants (Homma et al. 1981a; Homma et al. 1981b; Homma et al. 1981c). Surfactants (surface-active agents) are materials usually chemically fairly inert, which are often added to agrichemicals to improve their adhesion and distribution on leaf surfaces, or their penetrating properties (Manners 1993) if a systemic. Many surfactant oils and emulsifiers are fungicidal in their own right (Amer et al 1993). Several researchers found that mixtures of fatty alcohols, methyl esters of fatty acids (Burchill et al 1979) and nitrogen-containing surfactants (Clifford et al 1981) eradicated apple powdery mildew (*Podosphaera leucotricha*) from infected buds. Hunter et al (1982) considered that the ability of a surfactant to control powdery mildew was independent of the electrical charge which its particles carried (Manners 1993): non-ionic, cationic, anionic or amphoteric, a property reputed to affect the efficacy of the material. Researchers in Israel recently found that soapy water in the form of a new detergent Zohar LQ-215, acted directly on developmental stages of white fly and on transmission of two viruses, ZYMV and CULTIVARYV in melons (Cohen et al. 1996).

Recent studies by researchers at Massey University showed that sodium bicarbonate/surfactant mixtures provided good control of powdery mildew on glasshouse cucumbers and roses (Weeds 1992), and that application to grapevines during field trials proved cost-effective compared with a standard fungicide spray programme (Wood et al. 1994). Sodium bicarbonate is a relatively inexpensive compound and research is continuing to determine effective doses for application on various plant hosts. Very little is known about the mode of action of these materials, though some degree of synergism is suggested. Synergism is defined as the joint action of two or more agrichemicals that is greater than the sum of their activity when used alone (Anon. 1996).

An alternative hypothesis is that application of chemical salts causes plants to generate "immunity" signals (Xiang et al. 1995), which in turn contributes to disease resistance and the level of control observed. This may be useful for plant protection in the short-term. These chemical inducers (CI) are considered to have the following advantages (adapted from Xiang et al. 1995):

i. Eliminate the potential problems associated with the introduction of plant pathogens as inducers.
ii. More readily produced, distributed, and stored than pathogens.
iii. Can be applied with the standard spraying equipment, thus eliminating the need for extra labour and expense.
iv. Induced resistance is generally nonspecific, being effective against a wide spectrum of fungal, bacterial, and viral diseases unlike systemic fungicides.
v. Main advantage over standard fungicides is that application of one inducer would protect plant against many diseases.
vi. Because CI appear to work through the activation of plant defence mechanisms, this type of protection should be durable, and since the mechanisms activated appear to be similar to those reported for resistant plants developed by breeders, induced resistance should be as safe as resistant plants in the environment.
vii. Since the mechanisms activated for defence are similar to those reported for resistant plants developed by breeders, induction with alternative chemicals could be as safe as resistant plants.

Two issues have still to be thoroughly addressed before CI can find routine application in agriculture: phytotoxicity and variability in efficacy:

Whether the fungitoxic activity of sodium bicarbonate/surfactant mixtures is associated with pH (Long et al. 1994): toxic ion concentrations that antagonise pathogen development, the triggering or enhancement of some aspect of host resistance, or some other way, their adoption should lead to a cheaper, more environmentally friendly material in the arsenal of disease control materials available to growers of a wide range of crops in New Zealand.

D Composts and plant extracts-Aqueous extracts of composts (especially animal manures) incorporated into the soil which contain a mixture of microorganisms, have controlled *S. fuliginea* on cucumbers and zucchini by up to 60% in recent studies (Tränkner 1991; Weltzein 1992; Elad et al. 1996). The mechanisms suggested for the underlying activity of these materials include effects on host resistance, and direct inhibition of the pathogens (Weltzein 1992). Similar levels of disease control have been achieved against *E. cichoracearum* by the high pressure spray application of a 5% emulsion of garlic extract (Qvarnstrom 1992).

Under semi-commercial conditions, weekly applications of Milsana (BASF, Germany), a 2% (v/v) commercial formulation prepared from aqueous plant extracts of the perennial giant knotweed (*Reynoutria sachalinensis* F. Schmidt (Nakai.) have significantly reduced disease incidence on Long English cucumber, and increased crop yield by 50% despite severe inoculum pressure (Herger et al. 1988). On treated plants, *S. fuliginea* colonies showed a lower hyphal density, and produced fewer conidiophores. Milsana reduced by more than half, the number of fungicide applications necessary for disease control (Dik & Van der Straay 1995).

The mode of action remains unclear, although it is known that it does not have any direct fungicidal properties (Menzies & Bélanger 1996). Biochemical studies of the treated plants revealed increased chlorophyll values, stimulation in the activities of several defence enzymes, such as peroxidase, polyphenyloxidase, phenylalanine ammonia-lyase (PAL) (Kowalewski & Herger 1992), β-1,3-glucanase, chitinase, and increased ethylene production (Herger & Klingauf 1990) *in planta*. A rapid accumulation of antifungal phenolic compounds, especially in infected cucumber leaves treated with Milsana, additional to those produced naturally in host tissues in response to pathogen attack, has also been observed using the technique of thin-layer chromatography (Daayf et al. 1995). To date, it appears that the extracts are only effective against diseases caused by obligate parasites (Menzies & Bélanger 1996), they offer only protective not curative activity, and the current price is a major deterrent as small-scale production keeps the cost of processing Milsana high. As such, high value crops for home gardens and glasshouses are the only markets that may be able to justify use of this product (Menzies & Bélanger 1996).

1.6 MODES OF ACTION OF PLANT DISEASE CONTROL MATERIALS

Very little is known about the mode of action of sodium bicarbonate/additive mixtures. There are three ways in which plant disease control materials act, and good basic information is required to decide whether the mode of action of sodium bicarbonate/additive mixtures is one of the following (adapted from Guest (1995)):

i. Indirect- the material acts as an elicitor which stimulates host defence mechanisms to produce antifungal compounds that are responsible for disease control.

ii. Direct- the fungicide acts on the pathogen alone. Control occurs through a number of physical and physiological processes such as interruption of fungal metabolism or membrane disruption. This mode is common to protectant and systemic fungicides.

iii. Complex Interaction- pathogen control occurs via a combination of indirect and direct effects.

A knowledge of the mode of action of a potential disease control material defines when the material can be used to greatest advantage in the disease cycle (in relation to the time of inoculation, infection, lesion appearance and sporulation). Consideration of additional effects on host resistance and residual activity of the material allows the development of disease prevention programs with application intervals defined accordingly.

1.7 RESEARCH OBJECTIVES

Based on a knowledge of the host, pathogen, fungicidal modes of action and plant defence mechanisms the primary aim of this work was to study the host-parasite-complex of *Cucumis sativus-Sphaerotheca fuliginea* to determine whether stimulation of host defences is a mode of action of bicarbonate/additive mixtures. Subsequent chapters deal with the four approaches taken to assess the role in defence of cucumbers against powdery mildew of;

1. Phenolic compounds
2. The enzyme-Peroxidase
3. The enzyme-Phenylalanine ammonia-lyase
4. Induced structural barriers