Integrated management of greenhouse vegetable diseases: Development of microbial biocontrols, biorational chemical and cultural strategies

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NSW Department of Industry and Investment

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Final report of Horticulture Australia Ltd. project VG05084

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Purpose of the report
The purpose of this research has been to refine the roles of chemicals and assess potential new options for the integrated management of soil borne diseases in Australian greenhouse crops.

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Media Summary

The completion of this and a previous project (VG00069 "Integrated management of greenhouse cucumber and capsicum diseases") concludes seven years of research into the causes and management of wilt, stem and root rots of greenhouse vegetable crops. The major focus of this project has been directed at characterising the causal pathogens and developing management options to reduce the severe losses caused by Fusarium wilt and Pythium root rots of cucumbers. We showed that these diseases commonly occur together as a disease complex which increases the death rate of plants at all stages of development, often commencing soon after seedlings are transplanted. Major production areas in Australia were shown to have crops significantly affected by these diseases. Losses were estimated to be commonly at 30% but cases were recorded at 70-85% which is consistent with some overseas reports. In newer production areas such as peri-urban Brisbane and Coffs Harbour these diseases became established within 2-3 years of production commencing. In older production areas such as peri-urban Sydney and Adelaide these diseases, together with the withdrawal of the fumigant methyl bromide, has hastened the adoption of soilless production techniques; alas without much impact on disease incidence and severity. The major reason for this was determined to be poor crop and farm hygiene that enabled these pathogens to rapidly spread into new crops or fresh media from the surrounding environment where there was often crop waste and spent media stockpiled. As a result a new project was established (VG07118 "Greenhouse growers reducing crop losses with preventative disease management practices") to focus upon improving growers' awareness and compliance with preventative management using stricter hygiene and sanitation standards while this project evaluated a range of improved management options with microbial biocontrol and reduced-risk chemicals.

Within this project a range of commercially available biological control agents and fungicides were assessed for their ability to reduce disease losses. Unfortunately we were unable to demonstrate effective or consistent disease suppression with a range of microbial biocontrol bacteria (*Bacillus subtilis* and *Pseudomonas putida*) and fungi (*Trichoderma* spp.). Certain fungicide drench treatments also failed to reduce disease consistently over the life of a crop although we obtained some promising results in pot experiments which suggested that products containing the chemicals azoxystrobin (Amistar®) or prochloraz (Octave®) may be useful to reduce post-transplant losses. The most promising disease control observed came from treating the cucumber plants with the plant defence activator chemical, acibenzolar-S-methyl (Bion®), particularly in combination with potassium silicate. Seed treatments with the former product were effective and this use-pattern is preferred as only very small concentrations are required.

The most effective disease control was to use grafted cucumbers where the rootstock was tolerant or resistant to these pathogens. We demonstrated that this approach was very effective, although there is a significantly increased cost. We demonstrated that there is a simplified grafting method that can be utilised which would reduce labour costs over the currently used commercial grafting techniques that sometimes failed to develop into robust plants due to a poor graft union. Grafting is likely to be the best management strategy in situations or farms where these diseases are causing severe

losses. However, it would be prudent to evaluate different combinations of cucumber varieties and rootstocks in commercial-scale trials, including yield data, before wide-scale adoption. Isolates of the dominant strains of *Fusarium* have been sent to European plant breeders in an effort to identify new cultivars with resistance or tolerance to this pathogen. We look forward to seeing the fruits (or rather the seeds) of these endeavours.

This project also assessed some water disinfection treatments as these pathogens can survive and be spread in reused nutrient solutions. With increased nutrient costs, scarcity of water and recognition that waste nutrients can have negative environmental impacts, there is an imperative for increasing the reuse of waste nutrients. This work forms a foundation for further studies to identify affordable and effective water treatments.

All of the work described above addressed wilt, stem and root rot diseases in greenhouse cucumbers. One further component to this project was included after we obtained a voluntary contribution of funds from a consortium of companies concerned with severe outbreaks of Bacterial canker in greenhouse tomatoes. We screened isolates of the bacteria, *Clavibacter michiganensis* that causes this disease and showed that there are a several different strains affecting crops in Australia. This work forms a basis for further studies to improve detection and management of this important disease.

In summary, we have identified a number of strategies for the management of Fusarium wilt and Pythium root rots using cultural, chemical, biological and genetic strategies. In some cases, further evaluation and refinement will be required for their successful adoption, but this project has laid the foundations by developing a better understanding of their potential and limitations.

Technical Summary

Greenhouse production systems for cucumbers have become popular worldwide with an estimated 42 million metric tonnes produced in 2005 (FAO, 2006). Greenhouses protect crops from extremes in temperature and physical injury. Crop growth can be optimised in greenhouses with advanced technology by regulating light level, temperature, relative humidity and carbon dioxide concentration.

The potential advantages of greenhouse production systems may not be realised due to the prevalence of certain diseases. Diseases are particularly problematic in recirculated nutrient systems, in which substrates are re-used or where farm and crop hygiene practices are poor or lacking. Further factors of soilless production systems in greenhouses can make them conducive to disease development once plant pathogens enter. They include: stagnant water sitting around roots where there is poor drainage from media bags; a relatively small root volume that is densely confined; accumulation of excess mineral salts; and plant stress due to partitioning of assimilates to developing fruit. The two major soilborne pathogen needed to be controlled within the Australian greenhouse cucumber industry are *Fusarium oxysporum* and *Pythium* spp.

A keystone in integrated pest management in greenhouse production is crop hygiene. If plants are heavily infested with pests and diseases, control with biocontrol agents and soft chemicals is difficult and often ineffective. There are many basic practices which can be used to clean up pest and disease problems and to maintain a crop with lower pest and disease problems. Within this project we developed grower focus groups across four states to try to improve the management of greenhouse sanitation and hygiene.

Within Australian agriculture there has been a large push towards reusing water on-farm, particularly in hydroponic crops, however this poses significant problems for the spread of water borne plant pathogens. Disinfection is a vital requirement for water recirculation or reuse. Growers have access to a number of strategies that are commercially available but the choice of system can be confusing and costly. Within the scope of this project, we examined the effect of several commercially available water disinfestation units to reduce *Pythium, Fusarium* and *Thielaviopsis*, three model pathogens for the Australian glasshouse industry. The UV unit assessed appeared to be the most efficient at reducing disease levels, although there are limitations to using UV to disinfect water.

A range of chemicals and commercially available biological control agents were assessed for their ability to control diseases caused by *Fusarium* and *Pythium*. While the chemicals Amistar® and Octave® showed promise within the initial experiments, they showed no significance in controlling disease in on-farm experiments or in later experiments using infected cocopeat bags. Seed dressing and coating of chemicals were assessed as more biorational application methods. The most promising chemical disease control results came from the combination applications of Bion® and potassium silicate together. When examined over all experiments there is a significant interaction between Bion® and potassium silicate, and together they are more effective at reducing plant disease. Excellent disease control was observed through the use of

grafted cucumber plants. Alternative grafting methods have been evaluated at the Gosford Horticulture Institute with better unions formed between the rootstock and scion.

None of the commercial biological control options evaluated provided any statistical reduction in disease. Due to the failure of the tested commercial biological control organisms to reduce Fusarium disease severity, new potential biological control organisms should be isolated and assessed. Biocontrol bacterial characteristics such as endospore production, *in vitro* antibiosis, and the production of fluorescent siderophores are useful tools for reducing the number of different rhizobacteria that should be screened for biocontrol activity. Unless infinite funding is supplied, it is impossible to screen all bacteria and fungi isolated from suppressive soil sites. More than 300 different endophytic and rhizobacteria were isolated from cucumbers and initial screening was undertaken to assess their ability to suppress Fusarium wilt of greenhouse cucumbers.

Bacterial canker is a devastating disease of both field and greenhouse tomatoes, caused by the bacterium *Clavibacter michiganensis* subsp. m*ichiganensis* (Cmm). First found in Michigan, USA in 1909, Cmm has spread to all of the major tomato growing areas of the world including many of the main seed production regions. In Australia in recent years bacterial canker of tomato has emerged as a serious disease, which occurs in all major tomato growing areas. Interestingly the external symptoms of bacterial canker have altered over the past 10 years. Whether this is due to the use of newer tomato cultivars which react differently to infection by the bacterium or due to the introduction of new genetic strains of the bacteria is not known. It is possible that there are new strains present in Australia since Cmm can be seed borne and imported seed is generally untreated since the relaxation of Australian quarantine requirements in the early 1990s. The purpose of this work was to assess the diversity of Cmm in Australia.

The molecular results confirm the presence of multiple genotypes of Cmm found across Australia, indicating multiple incursions of Cmm into Australia, probably on contaminated seed batches, or plants. Pathogenicity testing also confirmed that the genetic variation affects the disease development. Further testing is required to compare the effect of the different genotypes against the different solanaceous host plants and to also compare disease development on solanaceous host plants grown under sprinkler irrigation, or in low grade glasshouse structures with frequent condensation drips, to determine why disease severities observed in field grown capsicums was not observed in the glasshouse experiment.

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Chapter 1. Background information

Greenhouse production systems for cucumbers have become popular worldwide with an estimated 42 million metric tonnes produced in 2005 (FAO, 2006). Plant breeding for hybrid cultivars with parthenocarpic fruit and all female flowers has assisted viability of year-round production of greenhouse crops in many regions of the world (McCreight, 1996). Greenhouses protect crops from extremes in temperature and physical injury. Crop growth can be optimised in greenhouses with advanced technology by regulating light level, temperature, relative humidity and carbon dioxide concentration. Soilless production in these protected environments with regulated supply of moisture and nutrients via trickle irrigation can result in more consistent yields and product quality than soil-based systems (Cockshull, 1985). The terms soilless or hydroponics have been defined as the production of crops isolated from the soil, either with or without a medium, with their total water and nutrient requirements supplied by the system (Jensen, 1999; Hanger, 1993).

Australian greenhouse cucumber production

There are inconsistent estimates of Australian greenhouse cucumber production. Biggs (2004) estimated production to be over 400 ha based on a consensus of industry representatives. The Australian Bureau of Statistics calculated 284 ha of production in 2006 (ABS document 71210DO003 Agricultural commodities, Australia, 2005-06). The true figure probably lies somewhere between these two estimates as ABS data is only gathered from registered businesses and Biggs included seed sales to project areas sown. New South Wales and South Australia dominate production (Biggs, 2004). In some cases, production is focussed around urban areas where there is ready access to markets such as in the peri-urban areas of the Sydney Basin, Brisbane, and Perth. Other production areas serve local and wider markets, such as Bundaberg, North Adelaide, Coffs Harbour, Mildura, Carnarvon and Darwin.

There are regional differences in the form of cucumbers grown (Biggs, 2004; author, unpublished). For example, the 'Lebanese' ('Middle Eastern' or 'Beit Alpha') cultivars are almost exclusively grown in the Sydney Basin. They have dark green slender fruit that are 10-20 cm long. 'Continental' ('Telegraph' or 'Long English') cultivars are predominant in Bundaberg and North Adelaide districts. They have a similar appearance to the 'Lebanese' type except fruit extend to 40 cm long. The Mildura district almost exclusively produces 'apple' cultivars. They have a pale skin with a blocky shape. Perth and Brisbane growers produce a mixture of the 'Lebanese' and 'Continental' cultivars. Almost all of Australia's production is consumed domestically (Biggs, 2004).

Soilless root substrates such as rock wool, coconut fibre as coir or cocopeat, and pine sawdust, are used in most of the Sydney, Bundaberg and Perth production areas (Biggs, 2004; authors, unpublished). Growers in North Adelaide, Darwin, Brisbane, Mildura, Coffs Harbour and Carnarvon mostly rely on soil-based production. Growers in North Adelaide and Mildura have been cropping for over thirty years and are largely dependent on fumigants or pesticide root drenching to control soil-borne fungal and nematode pathogens. Production has only commenced or expanded in the recent years in Brisbane, Coffs Harbour and Carnarvon and soil-borne disease problems are only now becoming a major constraint to yield (Tesoriero & Bertus,

2004). The recent restrictions in worldwide use of soil fumigants such as methyl bromide because of its ozone-depleting properties and detection of metham sodium in groundwater are factors hastening the move to soilless systems. Fumigation also removes disease-suppressive soil microflora and re-entering pathogens face little or no competition (Cook and Baker, 1983). Almost all of Australia's greenhouse cucumber production does not re-use wastewater (Biggs, 2004). A move to increasing efficiency of water use by recirculating nutrient solutions poses increased risks of disease spread, without disinfection (Jenkins & Averre, 1983; Jarvis, 1992).

Pathogens associated with wilt, root and stem rots on cucumbers

The potential advantages of soilless systems may not be realised due to the prevalence of certain diseases (Jarvis, 1992). Diseases are particularly problematic in recirculated nutrient systems (Gold and Stanghellini, 1985; Menzies *et al*., 1996), in which substrates are re-used (Postma *et al*., 2000) or where farm and crop hygiene practices are poor or lacking (Jarvis, 1992). Further factors of soilless production systems in greenhouses can make them conducive to disease development once plant pathogens enter. They include: stagnant water sitting around roots where there is poor drainage from media bags; a relatively small root volume that is densely confined; accumulation of excess mineral salts; and plant stress due to partitioning of assimilates to developing fruit (Jarvis, 1992).

Wilt, stem rot and root diseases; their causal pathogens and records from Australia are listed in Table 1.1.

Table 1.1 Major pathogens that cause wilts, stem rots and root diseases of cucumbers and their occurrence in Australia

Disease	Pathogen	Australian Records*
Acremonium hypocotyl rot	*Acremonium* sp.	-
Anthracnose	*Colletotrichum orbiculare*	+
Black root rot	*Phomopsis sclerotioides*	+
Botrytis blight	*Botrytis cinerea*	+
Charcoal rot	*Macrophomina phaseolina*	+
Fusarium wilt	*Fusarium oxysporum* f.sp. *cucumerinum*	+
Fusarium root & stem rot	*Fusarium oxysporum* f.sp. *radicis-cucumerinum*	-
Fusarium foot rot	*Fusarium solani* f.sp. *cucucrbitae*	+
Gummy stem blight	*Didymella bryoniae*	+
Monosporascus root rot	*Monosporascus cannonballus*	-
Penicillium Stem rot	*Penicillium oxalicum*	-
Phytophthora crown & root rots	*Phytophthora* spp.	+
Pythium damping-off & root rots	*Pythium* spp.	+
Sclerotinia rot	*Sclerotinia sclerotiorum, S. minor*	+

References: Tesoriero & Bertus, 2004; Australian Plant Pest Database, January, 2008: Martyn, R.D. 1996.

Surveys of Australian cucumber production in a previous research project (VG00069, Tesoriero & Bertus, 2004) revealed that wilt, stem and root rots were frequently associated with crop losses. *Pythium* and *Fusarium* were the most common pathogens isolated from affected plants. They were consistently isolated together from the same affected root tissue (authors, unpublished).

Destructive vascular wilt diseases caused by *Fusarium oxysporum* affect several members of the Cucurbitaceae family. Fusarium wilt of cucumber was first reported in 1925 but it was not until 1955 that it was described as causing commercial crop losses in Florida (Owen, 1955). The causal organism for Fusarium wilt disease of cucumber was thus named *F. oxysporum* f.sp. *cucumerinum*. This disease occurs in most countries of the world where cucumbers are grown, including Australia (Martyn, 1996; Wicks *et al*., 1978). Seven other f.spp. have been categorised on cucurbits: f.sp. *melonis* on rockmelon (Snyder and Hansen, 1940); f.sp. *niveum* on watermelon (Snyder and Hansen, 1940); f.sp. *luffae* on smooth loofah (Kawai *et al*., 1958); f.sp. *lagenariae* on bottle gourd (Matsuo & Yamamoto, 1967); f.sp. *momordicae* on bitter melon (Sun & Huang, 1983); f.sp. *benincasae* on Chinese winter melon (Gerlagh & Ester, 1985); and most recently, f.sp. *radicis-cucumerinum* on cucumber (Vakalounakis, 1996). This latter *forma specialis* was designated as causing a root and stem rot of greenhouse cucumbers rather than a wilt disease. However it is hard to see how this distinction was justified upon a critical review of the symptoms described. These included hypocotyl rot, root rot, plant stunting, yellowing and wilting of older leaves with brown vascular discolouration extending some 200 cm up the stem. Other similarities include the development of orange spore-masses (sporodochia) on stems under humid conditions, seedling damping-off in cool conditions, recovery from wilting during the evening, and accentuated disease development with heavy fruit loads (Figure1.1). The hypocotyl rot may be the only symptom that is not described for Fusarium wilt of cucumber. However, it is worth considering that classical symptoms described for Fusarium wilt of cucumber were based on field-grown plants as opposed to those in a greenhouse environment. Furthermore, Vakalounakis (1996) described both a slow ('within a few weeks') and 'sudden' death of affected plants 'without prior hypocotyl rotting'. Again these symptoms are difficult to distinguish from Fusarium wilt where wilting plants die within 3-5 days (Martyn, 1996).

Figure 1.1 A cucumber stem infected with *Fusarium oxysporum*. Note the orange colour comes from the sporodochia (spore masses).

Delineation of wilt-causing populations into f.spp. served a useful purpose for breeding resistant cultivars and rotating crop species. However, as more fungal isolates and plant genotypes were screened this distinction required further refinement. Isolates of *F. oxysporum* f.sp. *cucumerinum* from the USA, Israel and Japan were designated as Races 1, 2, and 3, respectively, based upon their differential reactions on a set of cucumber cultivars (Armstrong *et al*., 1978).

Reports dating back to the 1970s and earlier suggested that Fusarium wilt caused significant losses to cucumber production. In Australia, Wicks *et al*. (1978) noted that up to 80% of plants were killed by this disease in glasshouses on the northern Adelaide plains. The availability of cultivars resistant to Fusarium wilt greatly reduced economic losses in the USA and Europe (Martyn, 1996). Resistance had been developed to Races 1 and 2 of *F. oxysporum* f.sp. *cucumerinum* by incorporating a single dominant gene (*Foc*) into commercial cultivars (Netzer *et al*., 1977). Peterson *et al*. (1982) bred this gene into a cucumber cultivar (*Wisconsin-2757*) that was suitable for greenhouse production. Plant breeders have since incorporated this gene into many cultivars suitable for greenhouse production. This disease was not considered to be a problem in France (Blancard *et al*., 1994) but was still of importance in Japan (Namiki *et al*., 1994), Korea (Ahn *et al*., 1998) and China (Vakalounakis *et al*., 2004). In these Asian countries Fusarium wilt is one of the major limiting factors for cucumber production (Ahn *et al*., 1998). Presumably this is due to the prevalence of Race 3 forms of *F. oxysporum* f.sp. *cucumerinum* in Asia for which there is no robust resistance in cucumber cultivars. Although there are no specific records that *F. oxysporum* f.sp. *cucumerinum* is internally seed-borne there is evidence that it can be carried on the seed coat (Jenkins & Wehner, 1983).

Vakalounakis (1996) described *F. oxysporum* f.sp. *radicis-cucumerinum* causing a root and stem rot of greenhouse cucumbers in Crete. This pathogen has since been recorded in the Netherlands (Vakalounakis & Fragkiadakis, 1999), France (Reverchon *et al*., 2000), Canada (Punja & Parker, 2000), Spain (Moreno *et al*., 2001) and China (Vakalounakis *et al*., 2004). In Europe and North America there has been a resurgence in crop losses due to disease caused by this pathogen. Despite published descriptions of distinctive symptoms, temperature requirements and host ranges for this pathogen, it has been the result of genetic characterisation that has supported its delineation as a distinctly new entity. We will now examine these methods with specific reference to the two formae speciales of *F. oxysporum* causing diseases of cucumbers.

High root zone moisture levels, excess salts and small root volumes can lead to physiological stresses that particularly favour diseases caused by pathogenic Oomycetes such as *Pythium* species (Csinos & Hendrix, 1978; Jenkins & Averre, 1983). The low microbial populations and diversity found in soilless substrates is sometimes described as a 'biological vacuum' and one similar in this respect to fumigated soil. This is often associated with a lack of antagonistic microbes that 'buffer' the rhizosphere from plant pathogens (Postma *et al*., 2000). Soilless systems also favour the formation and dispersal of zoospores produced by several *Pythium* species (Stanghellini & Rasmussen, 1994). In the Netherlands, 15% of all chemicals applied to greenhouse cucumbers have been used for managing losses caused by *Pythium* spp. (Postma *et al*., 2000). This finding is in agreement with an earlier report by Moulin *et al*., (1994), that greenhouse cucumber growers in some countries of Western Europe have used the chemical, propamocarb every three weeks to control yield reduction caused by *Pythium* species (Figure 1.2).

Figure 1.2 A greenhouse with cucumbers suffering from Pythium root rot.

Several studies have shown *Pythium aphanidermatum* to be the most important *Pythium* species causing cucumber root disease. Jenkins and Averre (1983) reported it as the predominant species affecting greenhouse cucumbers in North Carolina. Stanghellini and Phillips (1975) and Al-Sa'di *et al*. (2007) recorded *P. aphanidermatum* causing damping-off of cucumbers in the Middle East with plant deaths estimated at 87% and up to 50%, respectively. Favrin *et al*. (1988) found *P. aphanidermatum* associated with severe root necrosis of greenhouse cucumbers in Canada. Blancard *et al*. (1992) and Moulin *et al*. (1994) found *P. aphanidermatum* was the dominant species associated with root rot of cucumbers in France. Moulin *et al*. (1994) noted that mature plants could be affected, causing wilting symptoms during the day but with no obvious necrotic root lesions. Isolates of *P. aphanidermatum* reduced yield by almost 20% in controlled experiments. Other species (*Pythium ultimum; Pythium intermedium; Pythium* Group HS*; Pythium sylvaticum; Pythium irregulare and Pythium* Group F) caused damping-off in seedling but had no effect on fruit yield. In the USA, Stanghellini *et al*. (1988) found *P. intermedium*, a zoosporic species, caused a root rot of hydroponically grown cucumbers. Herrero *et al*. (2003) studied the occurrence of *Pythium* in Norwegian greenhouse cucumbers growing in soilless systems. They identified twelve different *Pythium* species on cucumbers. In some cases they isolated several *Pythium* species from the same root sample. The most frequently isolated species were *P. irregulare* and *Pythium* Group F, while *P. aphanidermatum*, *Pythium paroecandrum* and *P. ultimum* isolates were the most aggressive in pathogenicity tests with cucumber. However, these authors found that *P. aphanidermatum* was the only species found associated with high mortality and yield reduction in commercial hydroponic cucumbers. Different isolates of *P. irregulare* demonstrated widely varying aggressiveness in these tests. Both *P. irregulare* and *P. ultimum* isolates were more aggressive when tests were conducted at 20^{o}C than at 25^{o}C. Other species identified were: *Pythium mamillatum*; *P. sylvaticum*; *P. intermedium*; *Pythium aquatile*; and *Pythium* Group HS. Two further taxa could not be identified. Of these, only *P. intermedium* and *P. aquatile* were shown to be non-pathogenic to cucumber seedlings. The non-pathogenic nature of *P. intermedium* in this study by Herrero *et al.* (2003) contrasts with the findings reported above by Blancard *et al*. (1992) and Stanghellini *et al*. (1988).

The interaction between plant disease and nutrition

The role of plant nutrition in disease is an interesting phenomenon. It is known that deficiencies in many nutrients can result in disease, although the exact nutrient and deficiency level is dependent on the host plant being studied (Marschner, 1995). An example of this is that potassium deficiency in cotton (*Gossipium hirsutum*) results in increased disease levels of Alternaria leaf spot (causal organism *Alternaria macrospora*)(Hillocks & Chinodya, 1989), where as increased levels of potassium in faba beans (*Vicia faba*) results in decreased disease levels of Alternaria leaf spot (causal organism *Alternaria alternata*) (Kumar, Upadhyay & Kumar, 2005). There are certain nutrients that have been well studied in plant disease interactions; examples of these are silicon (Si), phosphate (P), potassium (K), nitrogen (N), calcium (Ca), and aluminium (Al) (Engelhard, 1989). The molecular mechanism(s) underpinning the enhanced resistance or susceptibility of plants due to addition of most of these nutrients are unknown and only hypothesized.

Silicon and plant disease

The role of silicon as a potential supplement in crop production has in the last twenty years been the focus of a large amount of research in plant biology (Menzies & Belanger, 1996; Epstein, 1999). There is substantial evidence that silicon affects plant development, increasing plant growth and yield in many species and that silicon can modulate plant resistant reactions to multiple pathogens (Epstein, 1999; Ma, 2004). The most well examples of silicon disease suppression are of: powdery mildew on wheat (caused by *Blumeria graminis* f.sp. *tritici*) and cucumber (caused by *Sphaerotheca fuliginea*) (Leusch & Buchenauer, 1989; Fawe et al., 1998); rice blast disease caused by *Magnaporthe grisea* (Volk et al., 1958); rust on French beans caused by *Uromyces phaseoli* (Heath, 1981a); and crown and root rot of cucumber caused by *Pythium* species (Cherif & Belanger, 1992).

Initial research into silicon mediated disease suppression implicated silicon as a mechanical barrier against infection in plant leaves. This was due to silicon deposits being identified in the electron dense papillae areas, which developed around germinating spores, appressoria, haustoria, and in the immediate cellular area surrounding cells undergoing the hypersensitive response (Heath 1979; 1981b). However, further research into silicon mediated disease suppression showed that there was no accumulation of silicon in the electron-dense papillae regions formed around the infection sites of *Pythium ultimum* in cucumber roots (Cherif et al., 1992). Cherif et al. (1994) demonstrated that silicon application onto cucumber plants resulted in potentiation of chitinases, peroxidases and polyphenol oxidases when plants were later treated with *Pythium spp.* Recent research has suggested that silicon modulates a type of induced systemic resistance in a wheat (Remus-Borel et al., 2005), rice (Rodrigues et al., 2004) and pea (Dann & Muir, 2002), resulting in enhanced production of phytoalexins and pathogenesis-related (PR) proteins.

Chapter 2. Cultural control methods for reducing disease: On-farm hygiene and sanitation

Background

Soilless, greenhouse crop production by its contained, controlled nature should have less disease, however it is often observed that pest and disease levels may be as high or higher than field grown crops. A key factor in high disease levels is often poor farm hygiene.

Options for pest and disease control range from basic cultural practices such as good sanitation and hygiene, to biological and chemical control. Increasingly options for effective chemical control are becoming limited due to resistance in the pests and pathogens, the discovery of toxic effects of certain chemicals and residue accumulation and breakdown differing amongst field and hydroponic growing conditions. Many growers are now taking up integrated pest and disease management (IPM) strategies, which limit the use of harsh chemicals.

A keystone in IPM is crop hygiene. If plants are heavily infested with pests and diseases, control with biocontrol agents and soft chemicals is difficult and often ineffective. There are many basic practices which can be used to clean up pest and disease problems and to maintain a crop with lower pest and disease problems. In an ideal new farm situation the basics of implementing good farm hygiene practices are straight forward: use clean seedlings; use clean mix; use clean water; use netting to prevent insects where appropriate; and only allow "clean" people into the crop area. However eventually most growers do get pest and diseases, the question is then what do you do? In fast rotation crops like cucumber there are some basic measures which can be put into place to reduce the spread and severity of pests and disease within and between crops.

Good sanitation and hygiene practices and related methods of pest and disease exclusion include:

- Healthy plants
- Scouting
- Identifying pests and diseases
- Roguing
- Good working practices within a crop
- Footbaths
- Cleaning out structures between crops
- Sanitisation of water
- Clean media
- Clean seedlings
- Weed and algae control
- Screening
- Sealing surfaces
- Clean cutting implements
- Limiting visitors
- Successive plantings and plant free zones
- Controlling plant debris and finished crops

The purpose of this work was to assess the current status of good sanitation and hygiene practices on-farm, and to enhance grower knowledge and practice of good management.

Methods

Improving grower knowledge of good sanitation and hygiene practices should assist with preventing some disease outbreaks, and more importantly managing disease outbreaks so that they are less devastating.

Cucumbers were chosen as the target as it is the major levied greenhouse crop and can be severely affected by root rots and wilt diseases. Grower groups were engaged primarily in three states: New South Wales (groups in Coffs Harbour, Rossmore and Picton); South Australia (one group in Virginia); and Western Australia (one group in South Perth). Further farm and crop hygiene audits and grower group meetings have been held in Brisbane, Bundaberg, Mildura and Darwin. There was diversity in production systems and disease management practices across these production regions.

Survey forms were developed to assess knowledge, understanding and implementation of good hygiene and sanitation processes. Forms were also developed to assess pest and disease levels, and to determine any relationship between high levels and poor on-farm hygiene.

Results and Discussion

Initial auditing of properties in each of these regions revealed a number of issues that were likely to encourage the incidence and severity of diseases. These are summarised in Table 2.1 along with disease severity notes. The low disease incidence in Coffs Harbour, Brisbane and Darwin was mostly attributed to the fact that they were new to greenhouse cucumber production. This phenomenon has been described by the project chief investigator as the 'honeymoon' period. Reports and diagnostic samples from these areas have confirmed that root rot and wilt diseases are now causing significant losses.

Table 2.1 Summary of specific farm and crop hygiene problems associated with the different greenhouse growing areas of Australia.

Location	**Media**	**Farm/Crop Hygiene**	**Comments/Disease incidence & severity**
Perth, WA	Cocopeat	Clean farm, sealed outdoor areas & waste disposal system	Poor drainage & likely disease spread down rows. High levels of Fusarium wilt & Pythium rot rots
Virginia, SA	Soil (fumigated)	Unsealed areas around greenhouses. Crop residues and pruning's left on ground. Weeds around farm perimeter.	Low disease incidence in young crop but high level in older crops (plants left unrouged). Reliance on soil fumigation.
Rossmore, NSW	Cocopeat, compost	Unsealed areas around greenhouses. Crop waste	Poor drainage & likely disease spread down rows

Location	Media	Farm/Crop Hygiene	Comments/Disease incidence & severity
	mix	and used media in piles outside greenhouses. Weeds around houses and farm perimeter. Poor regard to crop hygiene by workers. Pruning's left on ground. Poor segregation of new media and seedlings.	in some areas of greenhouses. Fungus gnats (fungal vectors) abundant. High levels of Fusarium Wilt & Pythium Rot Rots (both in new transplants and mature crops).
Picton, NSW	Cocopeat, compost mix	As for Rossmore	As for Rossmore
Coffs Harbour, NSW	Cocopeat	Clean farm, grassed outdoor areas & waste disposal system.	First crop, low disease incidence.
Mildura, Victoria	Soil (fumigated)	Clean farm. Unsealed areas around greenhouses.	Low disease incidence. Pythium root rots in some areas (poor drainage). Reliance on soil fumigation.
Bundaberg, Qld	Sawdust	Clean farm, grassed outdoor areas & waste disposal system	Low disease incidence except for Pythium root rot (possible water source)
Brisbane, Qld	Soil	Clean farm. Unsealed areas around greenhouses.	First crop, low disease incidence. Fungus gnats observed.
Darwin, NT	Soil	Unsealed areas around greenhouses	First crop, low disease incidence.

An initial survey form assessing the knowledge and practice of good on-farm hygiene and sanitation was developed (Appendix 1). Growers were assessed on a scale of 1-10 for undertaking certain good practice activities (e.g. maintaining a weed free zone around the greenhouse). Scores for all practices were tallied and a grower who achieved a score of 290 or higher was assessed as practicing good hygiene. This form was used only for initial surveys of farms. A secondary form assessing on-going pest and disease issues and relating them to hygiene issues were also developed (Appendix 2).

Reusing media substrates by growers in the Rossmore and Picton areas of NSW was shown to strongly correlate with a higher disease incidence. Poor drainage was observed at these same locations along with Perth producers. Growers were encouraged to lift media off the floor and many have now achieved this by building raised reinforcement grating. Despite these improvements there remain several risk factors that have not been addressed by some growers in the Rossmore and Picton districts. Consequently disease severity and crop losses remain a significant problem on these farms. Recent audits in these districts have revealed the following factors are poorly or only sporadically managed: crop wastes, fungus gnats, worker hygiene, weeds and unsealed areas around greenhouse openings (Figure 2.1). Based upon these

observations and results from the surveying a list of the most important farm and crop hygiene risk factors was developed, with each risk factor grouped with an appropriate management strategy (Table 2.2).

Figure 2.1 Crop waste is often left directly outside the open doors of greenhouses, allowing plant pathogens and insects to move directly into a new crop.

Table 2.2 A description of various farming practices and their implications on on-farm hygiene and management strategies which can be implemented to reduce risk.

Farm Practice	**Hygiene Risk Factor**	**Management strategy**
Vehicle, visitor & worker access	Vehicle tyres	Tyre dips containing disinfectant prevent pathogens such as *Fusarium oxysporum*[1] from being spread.
	Clothing	Laundered worker uniforms and disposable overalls preferred;
	Footwear	Dedicated footwear, over-shoes or footbaths
Farm perimeter and general appearance	Wind-spread pathogens & pests	Windbreaks. Seal, mulch, gravel or plant unsealed areas with appropriate landscape vegetation. Covered and hygienic waste disposal system Agreement with neighbours (area-wide approach).
	Weeds	Chemical and mechanical controls. Seal, mulch, gravel or plant appropriate landscape vegetation for unsealed areas. Agreement with neighbours (area-wide approach).
	Water-borne pathogens	Drainage and wastewater systems that minimise surface water puddles or flows into greenhouses. Disinfection of source water (where required[2]). Disinfection of recycled water.

Seedlings, media & water	Seedling infections	Purchase or produce disease-free seedlings. Inspect consignments upon delivery and reject or discard unhealthy plants. Place seedlings on raised benches or storage racks in a clean and insect-screened area that is separate from older plants. Chemical or biological controls
	Contaminated media	Sealed and covered media preparation and storage areas that are protected from cross-contamination with dust, surface water and infected crop waste
	Water-borne pathogens	Disinfection of source water (where required). Disinfection of recycled water.
Greenhouse fittings	Contaminated surfaces	Avoid plant material coming into contact with the floor Clean and disinfect
	Contaminated irrigation lines and drippers	Maintain free of algal build-up Disinfect between crops
	Contaminated drainage troughs	Ensure drainage does not allow water pooling. Clean and disinfect between crops
	Contaminated twine & hangers	Replace or disinfect between crops
	Trolley, bins & machinery	Disinfect regularly
Worker practices	Contaminated tools	Disinfect (70% alcohol or other)
	Manual spread	Monitor & flag diseased plants with coloured plastic tape
		Quarantine workers to/from infected plants/sections of crop
		Work on younger (healthier) crops before older ones

1. A related strain of this fungus affecting cotton was detected in soil adhering to tyre treads.
2. Laboratory testing can confirm the risks of water-borne pathogens

Reauditing farms for hygiene standards showed that there was little impact from project activities to date, excepting a few enterprises. Audits revealed systemic management problems with disposal of waste material, fungus gnat infestations, weeds and reusing media (Figure 2.2). Many growers have improved drainage of plant substrates by suspending bags above drains. This has reduced disease losses. Excess irrigation, poor drainage, temperature extremes and fungus gnats (particularly infestations at the seedling stage) were all shown to correlate with increased disease incidence and severity.

A supplementary HAL project was funded VG07118 “Greenhouse growers reducing crop losses with preventative disease management practices”. This project was dedicated to developing an on-farm hygiene manual, setting up grower groups and improving on-farm hygiene. Len Tesoriero and Leanne Forsyth assisted with the writing and development of this project and the book produced by Jeremy Badgery-

Parker: “Keep it clean. Reducing costs and losses in the management of pests and diseases in the greenhouse.”

Figure 2.2 An example of severe disease losses caused by reusing growing media. On the left are tomatoes grown in new media, on the right are tomatoes grown in reused media with Fusarium wilt.

Chapter 3. Water disinfestation strategies and their role in reducing disease

Background

Within Australian agriculture there has been a large push towards reusing water on-farm, particularly in hydroponic crops, however this poses significant problems for the spread of water borne plant pathogens. Disinfection is a vital requirement for water recirculation or reuse. Growers have access to a number of strategies that are commercially available but the choice of system can be confusing and costly. Some growers have spent considerable amounts of money on a disinfection system that doesn't perform or has negative side effects.

To date most greenhouse vegetable growers in Australia use run-to-waste irrigation/fertigation systems. Recently there has been an increased interest in water recycling. There are several reasons: increasing fertiliser and water costs, lower water availability associated with drought; and a desire to reduce environmental effects from nutrient-rich waste water that may contain traces of pesticides.

There are many different plant pathogens that can be spread with water. The choice of disinfection type or application method depends on eliminating or reducing the risks associated with spread of (at least) the major pathogens. Cucumber growers need to manage the fungi *Fusarium oxysporum* and *Phoma cucurbitacearum* as well as any of several species of the water mould, *Pythium*. Growers of solanaceous crops have to manage these pathogens plus further and sometimes more resilient organisms. For example bacteria, particularly *Clavibacter michiganensis*, the cause of bacterial canker is very resistant to chemical disinfestation. Other fungal, water mould and viral pathogens must also be controlled in solanaceous crops. Examples are: *Verticillium dahliae*, *Phytophthora* spp. and Tomato mosaic virus.

This report contains results of water disinfection tests. Some tests were done in conjunction with project VG04012 (Effective management for root diseases of hydroponic lettuce). Chemical disinfectant efficacy tests are reported in that document. There are far too many combinations of products, pathogens and use-patterns to be considered here in full. Assessments also need to take into consideration quenching effects of dirt-loads and organic matter upon efficacy. In some cases pathogens can be protected by being encased inside plant tissue and thereby survive chemical disinfectants and UV treatments. Finally, costs can vary greatly for the different disinfection strategies that make the decision even more difficult. Growers who wish to recirculate water also need to monitor nutrient status of wastewater and determine a mixing ratio with fresh water. These are likely to change with crop age and weather conditions.

Methods

For the experiments conducted within this chapter three indicative organisms were used: *Pythium aphanidermatum*, *Fusarium oxysporum* and *Thielaviopsis basicicola*. These organisms all have different infective structures, all can readily be spread by water, and all are relatively easy to detect. Pythium was used as an indicative oomycota water mould species as it produces hyphae with few cross walls, thicker

more resilient oospores and sporangia. *Fusarium oxysporum* was chosen as a typical fungi, it produces macro and micro conidia (spores) as well as longer lasting resilient chlamydospores and hyphae with regular cross walls. *Thielaviopsis* was used as a slightly more chemically resilient fungus, capable of producing masses of chlamydospores with thick cell walls.

Experiment 1. Water disinfection with UV light
Samples of water (1-2L), pre- and post-UV treatment were assayed for the presence of plant pathogens. The sampling and testing period was over 3 months between August and November 2006. The first sets of water samples were used to refine procedures for sampling and laboratory detection. *Pythium* and *Phytophthora* were detected using a baiting technique with semi-selective agar media. *Fusarium* was detected from a concentrated filtrate of 1L of water samples that was then placed on selective and general-purpose agar media. These methods are largely qualitative, but comparing recovery rates gives a crude estimate of relative efficacy of the UV treatments. Quantitative estimates of *Pythium* and *Fusarium* in water samples were also made using dilution end-point assays.

During November 2006 an experiment was conducted to quantify the efficacy of the UV unit to *Fusarium* and *Pythium*. It was decided to inoculate the water holding tank with spores of the *Fusarium* fungus since endogenous levels were below detectable limits in previous trials. To inoculate *Fusarium* into the tank, 25 cucumber-infected stems were selected that were covered with sporodochia (fungal structures producing orange-coloured spore masses). After the stems were washed to release the *Fusarium* spores, the water containing those spores was added to the tank and mixed in with a plastic pipe (Figure 3.1).

Figure 3.1 Inoculating water holding tank with Fusarium

Water samples were taken soon after fungal spores were added to the tank. Another two water samples were taken before the UV light was turned on (Figure 3.2). Once the UV light was turned on water samples were taken after 10 and 20 mins. The UV light was then turned off, and a water sample was taken after 10 mins. The UV light was again turned on and samples were taken after 10 and 20 mins. A final water sample was taken 10 mins after the UV unit was turned off for the final time. The purpose of the additional water samples was to see if the *Fusarium* and *Pythium* levels had changed following the tank water flow.

A further set of water samples was taken from the same tank a week later (14/11/06). On the 22/11/06 more water samples were taken and, to reduce the amount of *Fusarium* present in the tank, half the water was drained and refilled with runoff water. Water samples were taken 5 and 10 mins before the UV unit was turned on and then 10 mins after the UV unit had been on.

Figure 3.2 Collecting water samples following UV treatment

Experiment 2. Water disinfestation with an ultrasonic and ultra-violet disinfection unit to fungal plant pathogens

A 50 L plastic bin was filled with reverse osmosis (RO) -treated water and a submersible pump used to deliver spore suspensions of plant pathogens to the Omni Unit at a flow rate to 60 mL/sec (3.6 L/minute). The unit is designed so that the water first enters a sonication chamber and is then sent through the UV-lamp

The sonicator was primed with RO water. Both the UV and sonicator were switched off, inoculum was added to the tank and allowed to circulate for 2 minutes and a 600 mL water sample was collected to determine base line levels of inoculum. The units were switched on and allowed to run for 50 seconds before a 600mL sample was taken. The unit was switched off and on six times and similar samples were taken to replicate the combined sonicator + UV treatment. This process was repeated with each of the two pathogens.

Disinfection efficacy was interpreted from estimates of the viable colony forming units (cfu) on defined agar media. Treated water samples were diluted four-fold in 0.05% water agar to $1/4^6$ and eight (10µL) drops were transferred onto either ¼-strength Potato Dextrose Agar + novobiocin (for *Fusarium oxysporum*) or Potato Carrot Agar + Pimaricin & Rifampicin (for *Pythium* spp). Plates were incubated in the

dark for 24-48 hours and each drop was scored for presence or absence of the target pathogen. Data was analysed by the most probable number (MPN) technique to estimate concentration of viable colony forming units.

Experiment 3. Water disinfestation with an ultrasonic and ultra-violet disinfection unit to fungal plant pathogens

This experiment was conducted as described above in experiment 2 except for the following:

- A larger pump was used with a flow rate of 300mL/second (~18L/min).
- Three fungal pathogens were *Fusarium oxysporum, Thielaviopsis basicola* and *Pythium aphanidermatum*.
- Only three replicate samples were taken due to the higher flow rate and the limiting inoculum volume.

Results

Experiment 1. Water disinfection with UV light

The results of all the preliminary assays are presented in Table 3.1. *Pythium* was consistently recovered from untreated water in the holding tank. The recovery rate from these water samples was highly variable, averaging about half that of water taken from the waste sump. The UV treatment effectively eliminated *Pythium* on all six sampling dates.

Table 3.1 Recovery of plant pathogens from water samples

Sample Date (PHDS#)	Sample origin /treatment	*Pythium*	*Phytophthora*	Other fungi*
23/08/06 (06/637)	Holding tank	3/10	nd	nt
	UV	0/10	nd	nt
5/09/06 (06/678)	Waste sump	10/10	nd	nt
	Holding tank	10/10	nd	nt
	UV	0/10	nd	nt
8/09/06 (06/686)	Holding tank	7/10	nd	>100 cfu
	UV	0/10	nd	46 cfu
	ClO_2	7/10	nd	nt
13/09/06 (06/699)	Holding tank	8/10	nd	>10 cfu
	UV	0/10	nd	>10 cfu
14/09/06 (06/703)	Holding tank	8/10	nd	nt
	ClO_2 (0.3ppm)	8/10	nd	nt
20/09/06 (06/721)	Waste sump	10/10	nd	>10 cfu
	Holding tank (top)	10/10	nd	>10 cfu
	Holding tank (bottom)	10/10	nd	>10 cfu
	UV@ 10min	0/10	nd	2 cfu
	UV@ 20min	0/10	nd	2 cfu
4/10/06 (06/760)	Holding tank	2/10	nd	>10 cfu
	UV	0/10	nd	5 cfu
2/11/06	Waste Sump	10/10	nd	nd
	Holding tank	1/10	nd	nd

nd = not detected; nt = not tested; cfu = colony forming units/plate

Only trace levels of *Fusarium* were confirmed from these assays, and only from the waste sump and holding tank. Most fungal isolates counted were identified as the saprophyte, *Geotrichum*.

The data from the *Fusarium* inoculation trial is presented in Tables 3.2, 3.3 and 3.4.

Table 3.2 Recovery of *Fusarium* and *Pythium* from water samples taken on the day of inoculation

Water sample	Pathogen	
	Fusarium	*Pythium*
Pre UV ~3 mins post inoculation of tank	+++	+
Pre UV ~5 mins post inoculation of tank	+++	+
Pre UV ~10 mins post inoculation of tank	+++	+
UV on for 10 mins (first run)	++	nd
UV on for 20 mins (first run)	++	nd
UV off water has run for 10 mins	+++	+
UV on for 10 mins (second run)	+	nd
UV on for 20 mins (second run)	+	nd
UV off for 10 mins	+++	+

+++ = high level of pathogen; ++ = moderate level of pathogen; + = low level of pathogen; nd = not detected

The UV treatment reduced *Pythium* to undetectable levels and significantly reduced *Fusarium* levels. The filter paper used in this assay turned pink with the development of *Fusarium* much more quickly from untreated water samples. The level of inoculum put into the tank was possibly too high.

Table 3.3 Recovery of *Fusarium* and *Pythium* from water samples

Water sample	Pathogen	
	Fusarium	*Pythium*
Pre-run 5 mins	+	+
Pre-run 10 mins	5120 cfu/mL	+
UV on for 10 mins	80 cfu/mL	nd

cfu = colony forming units; nd = not detected; + = detected but unquantifiable

When the water was re-sampled six days later the UV unit eradicated the *Pythium* from the water as previously. The UV treatment reduced the *Fusarium* levels by 98%, however it did not eradicate it (Table 3.3). *Fusarium* levels were again very high (>5,000 cfu/mL) and unlikely to be seen in a natural glasshouse recycled water situation.

After the tank water had been diluted *Fusarium* was no longer detectable from the UV treated water samples (Table 3.4). *Fusarium* levels in untreated water were an order of magnitude less than was detected a week earlier, but still over 500 cfu/mL.

Table 3.4 Recovery of *Fusarium* and from water samples

Water sample	*Fusarium* level
Pre-run 10 mins	~640 cfu/mL
UV on for 10 mins	nd
Post run UV off 10 mins	~640 cfu/mL

cfu = colony forming units; nd = not detected

Experiment 2. Water disinfestation with an ultrasonic and ultra-violet disinfection unit to fungal plant pathogens

All water samples treated with the combined UV + sonicator had no recoverable *Pythium* and *Fusarium*. This suggests that the unit was 100% effective under these running conditions. The pre-treatment sample indicated that a level of *Fusarium* inoculum was present (2 x 10^5 cfu / mL). In contrast, there was only very low recovery of the *Pythium* inoculum (<100 cfu/mL). This suggests a problem with the inoculum preparation.

Experiment 3. Water disinfestation with an ultrasonic and ultra-violet disinfection unit to fungal plant pathogens

The UV-sonicator unit reduced the levels of *Thielaviopsis* from 6.19 x 10^3 to 171 cfu/mL (Table 3.5). This represents a 97% reduction in the inoculum concentration.

The UV-sonicator unit reduced the levels of Fusarium from 1.20 x 10^5 to low numbers, too low for the MPN calculation (<100 cfu/mL).

There was a low recovery *Pythium* in the untreated water samples but the treated had none detectable in the treated water.

Table 3.5. Recovery of Plant Pathogens with the Omni Enviro Sonicator and UV lamp Unit with a flow rate of 300 mL/s

Pathogen	Treatment	cfu/mL[1]
Thielaviopsis	Pre-treated	6.19 x 10^3
	Treated	171
	Untreated post-test	2.78 x 10^3
Fusarium	Pre-treated	1.20 x 10^5
	Treated	Trace (<100)
	Untreated post-test	2.40 x 10^5
Pythium	Pre-treated	Trace (<100)
	Treated	0
	Untreated post-test	Trace (<100)

1. cfu = Colony forming units, mean of three replicates.

Discussion

Experiment 1. Water disinfection with UV light

The UV unit is sufficient for eradicating *Pythium* from the tank water. The UV unit significantly reduces *Fusarium* in the water, and at lower initial concentrations, to below detectable limits. It would appear that the *Phytophthora* detected in the wastewater sump did not survive in the holding tank. Similarly, *Fusarium* was only found at trace levels in the waste sump and holding tank.

Overall there appears to be less *Phytophthora* and *Fusarium* in the waste sump than there was when we previously tested the water. It was not clear whether this was due to residual chlorine dioxide (subsequently installed on the town water supply) or to other undetermined factors. There was a noticeable decrease in the number and

severity of *Fusarium* infected plants on farm compared with previous years. It was also noted that the grower heated the greenhouses that winter to higher temperatures than previously. This could explain this lower disease incidence and severity independently of the chlorine dioxide use. Alternatively, chlorine dioxide treated recycled water (measured at the dripper as 0.3ppm) had similar *Pythium* recovery levels to the untreated tank water (Table 3.1).

Experiments 2 and 3.
The Omni Enviro-Unit, with combined UV and sonication treatments, was demonstrated to be highly efficacious at a slower flow rate (60 mL/s) for disinfecting water suspensions of the plant pathogens, *Fusarium oxysporum* and *Pythium aphanidermatum.* At the higher flow rate (300 mL/s), the unit was equally efficacious for *Fusarium* and *Pythium* as it was at the lower flow rate. The fungus, *Thielaviopsis* was included in the second experiment because it forms highly resilient chlamydospores. The unit reduced the spore concentration by 97% at this flow rate.

Conclusions

Within the scope of this project, we examined the effect of several commercially available water disinfestation units to reduce *Pythium, Fusarium* and *Thielaviopsis*, three model pathogens for the Australian glasshouse industry. The UV unit assessed appeared to be the most efficient at reducing disease levels, although there are limitations to using UV to disinfect water. There are a number of potential problems to consider when using UV light to disinfect water including: the UV light will age over time and the intensity of the light may be reduced; the tube where the UV light is based may become coated with a biofilm or nutrient deposits over time, which will reduce the effectiveness; the turbidity of the water being disinfected needs to be assessed, as the more turbid the water the less the UV light will penetrate the water; the flow rate of the water may also become a factor over time, as the faster the water flows the less time the pathogen propagules in the water are exposed to the light; and finally the most important consideration with using a UV light disinfestation system is cost. The costs of water disinfestation systems can vary from $15 000 - $250 000. At the higher cost end of the disinfestation system spectrum, the UV units are self cleaning, reducing the effect of biofilm and nutrient coating, and the UV lights are also larger with internal checking to ensure that the appropriate intensity of light penetrates across the tubing.

Internationally there are other pathogens such as Cucumber green mottle mosaic virus, which can severely affect cucumber crops and are capable of being dispersed through reused nutrients. Virus' are more resilient to UV light, chemicals and sonication than other pathogens. As a result in the Netherlands, nutrient water is often heated to 94^{o}C to denature the virus'. If there is an incursion of Cucumber green mottle mosaic virus into Australia, water disinfestation will need to be heavily re-examined.

Chapter 4. Screening of microbial biocontrols and biorational chemical usage for the control of Fusarium wilt of cucumber

Background

Fusarium and Pythium root rot and wilts of cucumber are severe diseases worldwide. In previous research Len Tesoriero identified *Pythium* and *Fusarium* as causing diseases in greenhouse grown cucumbers from all the major production regions including: Sydney basin, Bundaberg, Virginia and Perth. Plants from the new cucumber growing regions of Brisbane, Coffs Harbour and Carnarvon have been sent the diagnostic laboratory and *Fusarium oxysporum* and *Pythium spp.* have been identified as the main cause of disease.

Internationally there has been much research undertaken to assess options to reduce the impact of these diseases. The main methods identified have been: variety selection, grafting, biological control and chemical control. In an earlier project (VG00069 "Integrated management of greenhouse cucumber and capsicum diseases") we demonstrated that regular applications of microbial suspensions containing a mixture of both the bacterium, *Bacillus subtilis* and the fungi, *Trichoderma spp*. halved plant death rates in commercial crops. Within Australia there are no chemical or biological products registered or under permit to treat cucumbers with Fusarium or Pythium disease.

Methods

Experiment 1. Evaluating chemical drenching and biological control options for reducing Fusarium wilt of cucumber

Initial screening for Fusarium wilt control assessed current commercially formulated microbial biocontrol products: Fulzyme™Plus (*B. subtilis*), Tri-D25 (*Trichoderma harzianum* & *T. koningii*), and Effective Microorganisms (3 products: EM1, EM2 and EM3). Also screened were the fungicides Benlate® (benomyl) (previously shown to suppress Fusarium wilt), Octave® (prochloraz), DPX-LEM17-053 (Du Pont chemical) and Amistar® (azoxystrobin). These chemicals were initially assessed as drench treatments. A mineral Silicon solution (potassium silicate [K_2SiO_4]) was also assessed and applied before the *Fusarium* inoculum.

Cucumber seeds (cv. Deena) were sown into pots containing sterile UC mix (Baker, 1957). Seeds were sown at 12 seeds per pot and later thinned to being 10 plants per pot. Plants were fertilized using Thrive™ soluble plant fertilizer once a week. Plants were treated with chemicals and biological products one day prior to inoculation with *Fusarium oxysporum*. Plants were inoculated with *Fusarium* upon the emergence of the first true leaf. The experiment contained seven replicates for each treatment. Treatments were randomised using random number generators.

Fusarium inoculum was prepared by growing Fus #53 (DAM number 821) on ¼ strength potato dextrose agar for 1-2 weeks. Colonised agar plates were blended into a slurry in sterile water and pots were inoculated with approximately one plate of agar per pot. Control treatments used uncolonised agar plates.

Disease levels were assessed every week, and dying plants removed. The roots and lower stems of dying plants were plated on selective agar to determine whether *Fusarium* was likely to be the primary cause of death. Plants were grown for six to nine weeks.

Experiment 2. Evaluating levels of chemical drenching and other biological control options.

In this experiment further assessments of commercial fungicides were undertaken and a different microbial biocontrol MicroPlus™, which contains the actinomycete bacterium, *Streptomyces lydicus*. As Octave® and Amistar® showed good levels of control in the initial screening, a further assessment was required, including the use of lower rates and other strobilurin fungicides such as Flint®. As with the previous experiment, plants were drenched with chemical treatments. In this experiment the rates of Octave® were halved and quartered to study the dose response effect on disease control and phytotoxicity.

Plants and inoculum were grown and prepared as in experiment 1. Disease was assessed as in experiment 1. The experiment contained nine replicates for each treatment.

Experiment 3. The effect of fungicide drenchings on the combinations of Fusaruim and Pythium.

A third experiment further assessed chemical drenches to control a combination of *Fusarium* and *Pythium*. They are frequently isolated together from diseased farm plants, and experiments have previously shown that the combination of both pathogens together results in more severe disease (Tesoriero pers. com). As a result we also wished to further test the effectiveness of the chemical Octave®, Amistar® and Flint®. Two different *Pythium* species were assessed: *Pythium aphanidermatum* (Pa) and *Pythium spinosum* (Ps).

Plants and *Fusarium* inoculum were grown and prepared as in experiment 1. Disease was assessed as in experiment 1. *Pythium* inoculum was prepared as per *Fusarium* inoculum except that potato carrot agar plates were used to stimulate the production of oospores and sporangia. The experiment contained five replicates for each treatment.

Experiment 4. Seed dressing and soaking preliminary trial

Dressing seed with chemicals is desirable because it can be applied prior to seed sale and thereby reduce occupational health and safety risks and potential for undesirable food resides. Chemical fungicides were also assessed: Amistar®, Tecto® (thiabendazole), Dynasty®-CST (formulation of metalaxyl, azoxystrobin and fludioxanil), Dynasty®100 (metalaxyl & azoxystrobin) and Maxim® (fludioxanil) were assessed as was the plant defence inducer, Bion® (Acibenzolar-S-methyl). Two different application methods were used: imbuing (soaking) the seeds in the wet chemical or dressing seeds with the chemical and talc powder in a slurry.

Plants and inoculum were grown and prepared as in experiment 1. Disease was assessed as in experiment 1. The experiment contained five replicates for each treatment.

Experiment 5. Seed dressing and soaking into clean mix
Further assessments were made of Tecto® and Bion® as seed coats both by dressing and soaking. The first trial sowed treated seed into clean potting mix and plants were later challenged with *Fusarium*.

Plants and inoculum were grown and prepared as in experiment 1. Disease was assessed as in experiment 1. The experiment contained five replicates for each treatment.

Experiment 6. Seed dressing and soaking into infected mix
Further assessments were made of Tecto® and Bion® as seed coats both by dressing and soaking. In this second experiment l seed was directly sowed into mix already contaminated with *Fusarium*.

Plants and inoculum were grown and prepared as in experiment 1. Disease was assessed as in experiment 1. The experiment contained five replicates for each treatment.

Experiment 7. On farm assessment of disease control.
As a result of previous experiments it was decided to do an on-farm experiment to assess the efficacy of chemical drenching at reducing plant losses and the effect of the chemical treatments on the number of cucumbers harvested. These trials would also be used to begin the accumulation of residue data with the long term view of applying for permits in collaboration with AgAware Consulting and HAL.

Cucumber plants in cocopeat bags were treated by drenching with the chemicals Octave®, Amistar® and Flint®. Two different levels of Octave® and Amistar® were used. Plants were treated with the chemicals every two weeks. Plants were approximately four weeks old at the start of the trial. Cocopeat bags used in the experiment were marked with spray paint to designate treatment. Four rows of the growers greenhouse was taken up by the trial. At the end of each row three cocopeat bags with cucumbers were left untreated as buffer zones. Within each row each treatment was applied as a block of ten bags. Each bags contained three cucumber plants. The order of each treatment block within the row was determined by randomly picking numbers from a hat. Cucumber fruit of a marketable size was picked into a marked tub every two to three days, from each treatment block. Fruit numbers were counted and recorded. Disease levels were assessed on a weekly basis, with plants rated from 0-3, with 0 indicating no disease; 1 indicating low disease symptoms of water soaking at the base of the cucumber stem; 2 indicating Fusarium sporodocia lesions on the stem; and 3 indicating a fatal wilt of the cucumber plant and/or plant death.

The plants were grown under commercial conditions with commercial fertilizer applied through drippers and appropriate disease control methods for other diseases applied e.g. applying a chemical spray for powdery mildew.

Experiment 8. On farm assessment of disease control #2
In an attempt to integrate improved hygiene and on-farm sanitation with reduced disease levels the second on-farm experiment was split into two sub-experiments with half the trial area cleaned by the grower as he would typically clean the greenhouse

between crops and the other half cleaned and sanitised using previously identified good hygiene practices.

The good hygiene practices undertaken were as follows: thorough cleaning of all surfaces within the greenhouse with a high pressure water gurney mixed with Virkon™ detergent; scrubbing of floor and drainage channels to remove all caked on soil and plant debris; weeds wacked down using a whipper-snipper before being sprayed out using a herbicide; clean new weed matting put down; clean new drippers installed; and clean new cocopeat bags used. The changing of drippers, weed matting and cocopeat bags were also undertaken in the grower cleaned half of the trial.

The trial was composed of six rows of cucumber plants with 60 bags of cucumbers within each row used within the trial. There were approximately six buffer bags of cucumber plants on either side of the treated plants. Cucumber seedlings were first treated with the appropriate chemical within one day of being planted into the greenhouse. The chemicals assessed in this trial were Octave®, Amistar® and a mix of Bion®, potassium silicate, Fulzyme™Plus (*B. subtilis*), Tri-D25 (*Trichoderma harzianum* & *T. koningii*), and two different strains of putative *Psuedomonas fluorescens* biocontrol bacteria isolated within this project (chapter 5). Two different levels of Octave® and Amistar® were again used. Plants were treated with the chemicals every two weeks.

Cucumber fruit of a marketable size was picked into a marked tub every two – three days, from each treatment block. Fruit numbers were counted and recorded. Disease levels were assessed on a weekly basis, with plants rated from 0-3, with 0 indicating no disease; 1 indicating low disease symptoms of water soaking at the base of the cucumber stem; 2 indicating Fusarium sporodochia lesions on the stem; and 3 indicating a fatal wilt of the cucumber plant and/or plant death.

The plants were grown under commercial conditions with fertilizer applied through drippers and appropriate disease control methods for other diseases applied e.g. applying a chemical spray for powdery mildew.

Experiment 9. Preliminary combinations of silicon and Bion® and other biologicals in cocopeat bags

As a result of the trend observed in experiment 8, where disease levels appeared lower in plants treated with the mix (Bion®, potassium silicate, Fulzyme™Plus (*B. subtilis*), Tri-D25 (*Trichoderma harzianum* & *T. koningii*), and two different strains of putative *Psuedomonas fluorescens* biocontrol bacteria isolated within this project) it was decided to assess different combinations of these organisms and compounds in a controlled greenhouse trial. The chemicals and combinations assessed were: Bion® alone; potassium silicate alone; Fulzyme™Plus alone; Tri-D alone; the putative *Psuedomonas fluorescens* biocontrol bacteria alone; Bion® and potassium silicate together; Bion®, potassium silicate and the putative *Psuedomonas fluorescens* biocontrol bacteria; Bion®, potassium silicate and Fulzyme™Plus; Bion®, potassium silicate and Tri-D; all of the treatments together; and Amistar® at a high rate. Plants were treated every two weeks with the appropriate chemical/biological combination.

Instead of using UC mix, cocopeat bags collected from diseased plants on farm were used. The cocopeat itself may be playing a role in the germination of the biological control agents or the chemical availability and uptake of potassium silicate.

To ensure consistency between cocopeat bags, all the cocopeat was removed and mixed in large 100L containers. To ensure that appropriate controls were in place within the experiment bags of cocopeat were sterilised using the autoclave to remove the pathogens. The cocopeat was tested for *Fusarium* and *Pythium* prior to use and levels were consistently high in untreated bags, while the pathogens were undetectable in the sterilised cocopeat.

Cucumber seeds (cv. Deena) were germinated in seedling trays, and planted into the appropriately labelled cocopeat bag once all seedlings had reached the developmental stage of first true leaf emergence.

Disease was assessed as in experiment 1. The experiment contained nine replicates for each treatment.

Experiment 10. Potassium silicate and Bion® in cocopeat bags #2
As the results observed in experiment 9 were not consistent with observations from experiment 8, it was decided to repeat the experiment. Chemical and biological treatments were as per experiment 9. Plants were treated immediately after planting into the cocopeat bags and then every two weeks with the appropriate chemical/biological combination. Plants were fertilized using a commercial formulation of A and B fertilizer.

Disease was assessed as in experiment 1. The experiment contained nine replicates for each treatment.

Experiment 11. Potassium silicate and Bion in cocopeat bags #3
As a result of the positive disease control observed in experiment 10, it was decided to repeat several of the treatments. The used cocopeat bags were reused, but first remixed together to ensure consistency.

Chemical and biological treatments used in this experiment were: Bion®; Bion® and potassium silicate; Bion®, potassium silicate, Tri D and Fulzyme™Plus together; Bion®, potassium silicate, Ridomil® and Amistar®; Bion®, potassium silicate, Ridomil®, Amistar® and Octave®; Bion®, potassium silicate and Ridomil®; Ridomil® alone; Ridomil® and Amistar®; and Ridomil®, Amistar® and Octave®. The Ridomil® treatments were included due to the increasing disease pressure of Pythium root rots.

Plants were grown and treated as described in experiment 10. Disease was assessed as in experiment 1. The experiment contained nine replicates for each treatment.

Experiment 12. Potassium silicate and Bion in cocopeat bags #4
As a result of the positive disease control data observed in experiments 10 and 11 it was decided to repeat experiment 11 again.

Plants were grown and treated as described in experiment 11. Disease was assessed as in experiment 1. The experiment contained nine replicates for each treatment.

Experiment 13. Grafted cucumbers in potting mix to control Fusarium and Pythium.
Grafted cucumber seedlings were bought from a commercial supplier. Two different rootstocks were grafted to the cucumber variety Deena (64-50 and Ferro). The grafted seedlings were compared with non-grafted cucumbers (cv. Deena). Seedlings were transplanted into pots containing UC mix soil, at a rate of 5 seedlings per pot. The pots were then inoculated with either *Fusarium oxysporum* or *Pythium aphanidermatum*. The inoculum was produced as previously described. Disease was assessed as in experiment 1. The experiment contained nine replicates for each treatment.

Experiment 14. Grafted cucumbers in cocopeat bags.
Grafted cucumber seedlings were bought from a commercial supplier. Two different rootstocks were grafted to the cucumber variety Deena (64-50 and Ferro). The grafted seedlings were compared with non-grafted cucumbers (cv. Deena). Seedlings were transplanted into bags containing contaminated cocopeat, at a rate of 3 seedlings per bag. The used cocopeat bags were reused, but first remixed together to ensure consistency.

Disease was assessed as in experiment 1. The experiment contained nine replicates for each treatment.

Results and Discussion

Experiment 1. Evaluating chemical drenching and biological control options for reducing Fusarium wilt of cucumber
Statistically only the chemical applications of Benlate®, Octave® and Amistar® reduced mean cumulative death rates (Figure 4.1). Interestingly of the ~30% plants which died in the Octave® treatment, no Fusarium was re-isolated from roots and stems possibly indicating another cause for the plant death. Plants treated with Octave® had stunted root systems suggesting a phytotoxicity. While not shown in the final counts of dead versus live plants potassium silicate did show some promise as a control option. The majority of the plants that died, did so in the final week of the trial, possibly indicating that the silicate needed reapplication. None of the microbial biocontrols decreased cumulative death rates.

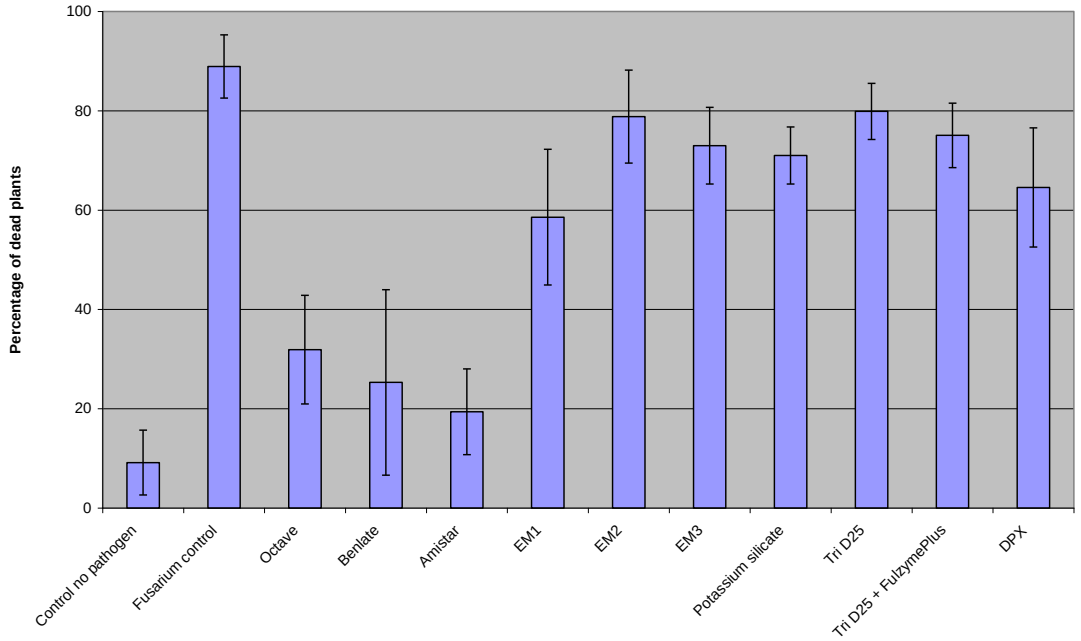

Figure 4.1 Mean cumulative death rate of cucumber plants treated with microbial and chemical products in experiment 1.

Experiment 2. Evaluating levels of chemical drenching and other biological control options

Disease levels were significantly reduced by all treatments (Figure 4.2). Disease control obtained by using any of the chemicals was statistically better than that from the *Streptomyces lydicus*. The lower levels of Octave® and Amistar® did not reduce their effectiveness at controlling Fusarium wilt under these conditions (UC mix soil, only pathogenic *Fusarium* present).

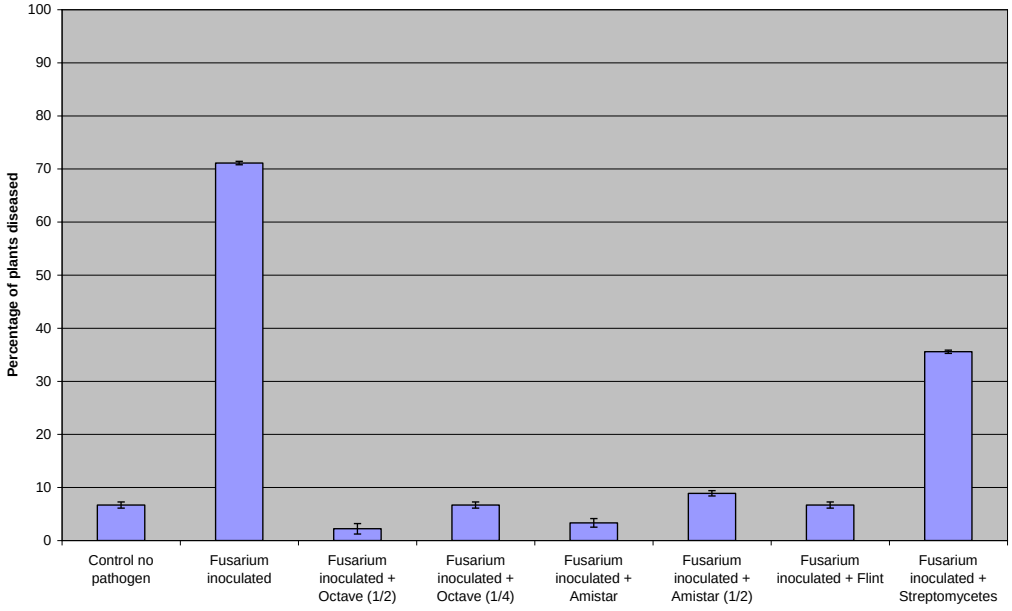

Figure 4.2 The effect of chemical treatments and *Streptomyces* on the percentage of diseased plants in experiment 2.

Experiment 3. The effect of fungicide drenching on Fusaruim and Pythium.
The results of this experiment contradict previous experiments in that Octave® failed to control *Fusarium* or the *Fusarium-Pythium* combinations (Figure 4.3). Interestingly there appears to be a difference between the two strobilurin fungicides in that Amistar® controlled *Fusarium* and the *Fusarium-Pythium* combinations while Flint® only controlled *Fusarium* alone.

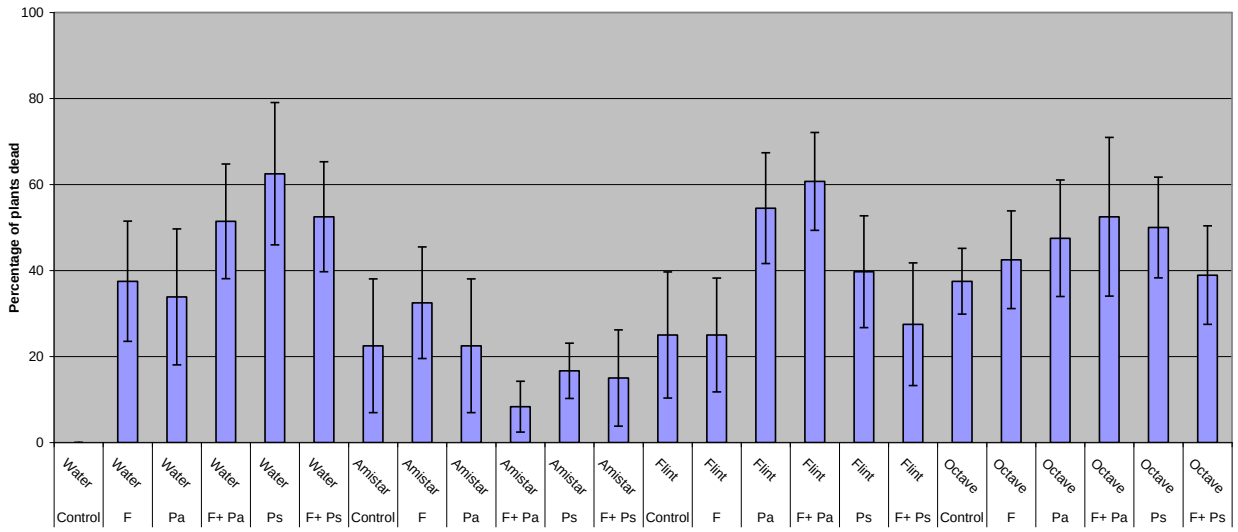

Figure 4.3 The mean cumulative death rate of fungicide drenches to Fusarium and Pythium observed in experiment 3. F = *Fusarium oxysporum*; Pa = *Pythium aphanidermatum*; Ps = *Pythium spinosum*

Experiment 4. Seed dressing and soaking preliminary trial
Due to a high variation in levels of disease between pots within treatments there was no strong statistical evidence for disease control, although there appeared to be a trend of Tecto® and Bion® reducing disease (Figure 4.4).

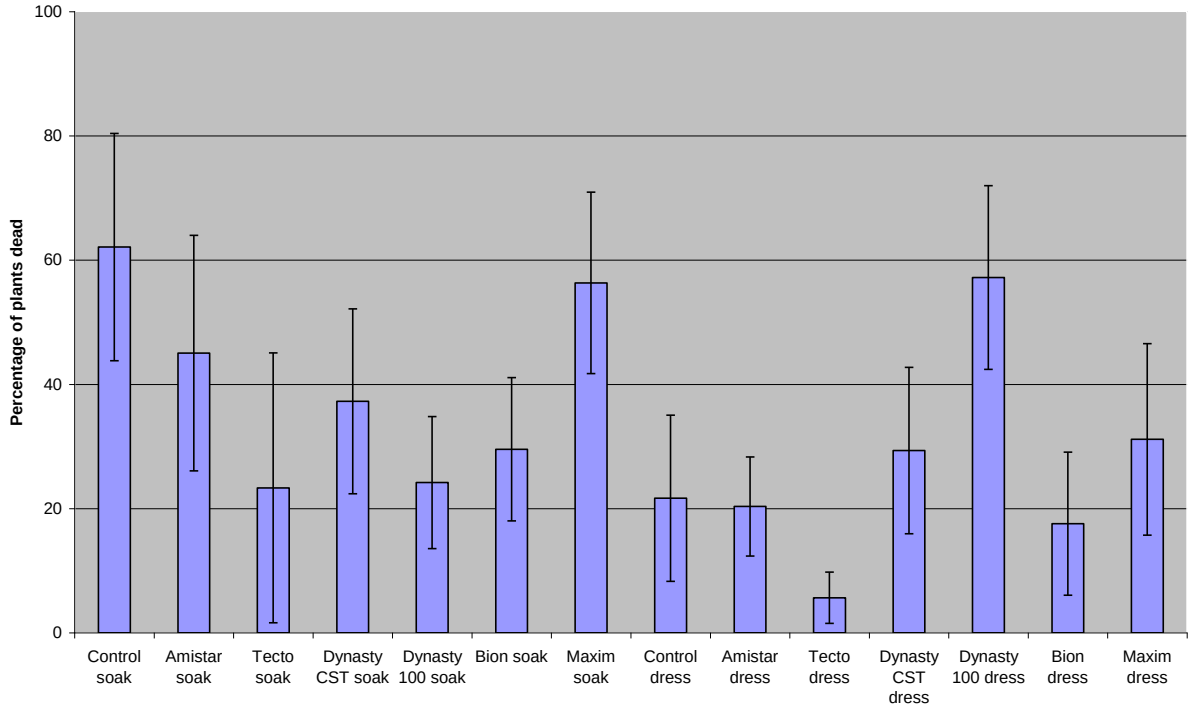

Figure 4.4 Effect of cucumber seed soaking and dressing on reducing the effect of Fusarium wilt.

Experiment 5. Seed dressing and soaking into clean mix

In this initial experiment where seeds were sown into a clean potting mix, levels of disease were low, with few differences between the plants that were dressed with chemical and the control plants (Figure 4.5).

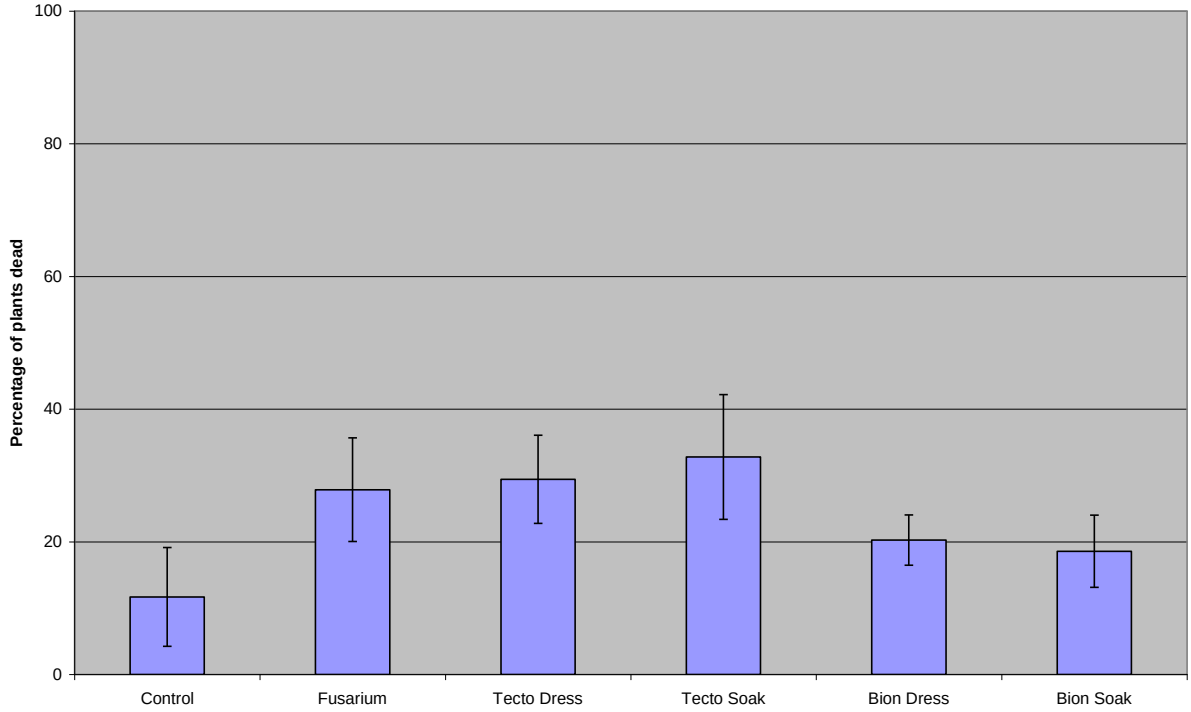

Figure 4.5 The effect of chemical seed treatments on Fusarium disease of cucumbers (seed sown into clean soil).

Experiment 6. Seed dressing and soaking into infected mix
In this second experiment where seeds were sown into a contaminated potting mix there was strong expression of disease, and good disease control shown by both the Bion® dressing and the Bion® soaking treatments (Figure 4.6).

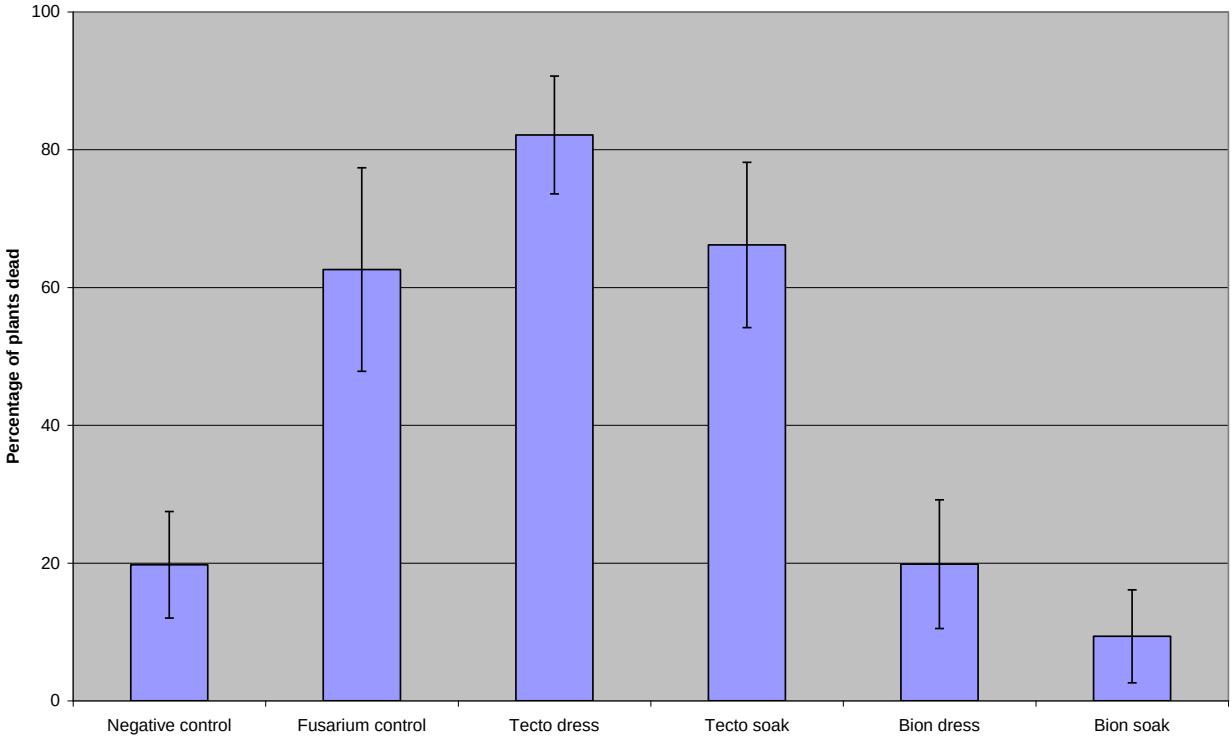

Figure 4.6 The effect of chemical seed treatments on Fusarium disease of cucumbers (seed sown into infected soil).

Experiment 7. On farm assessment of disease control
Statistically there was no difference in either the level of disease observed or the numbers of fruit picked that could be contributed to any of the chemical treatments. Approximately 11,400 cucumbers were picked within the trial with an approximate weight of 1,700kg. There did appear to be a trend in the levels of healthy plants observed within the Amistar® high treatment (Figure 4.7), however at the 95% level this was not significant.

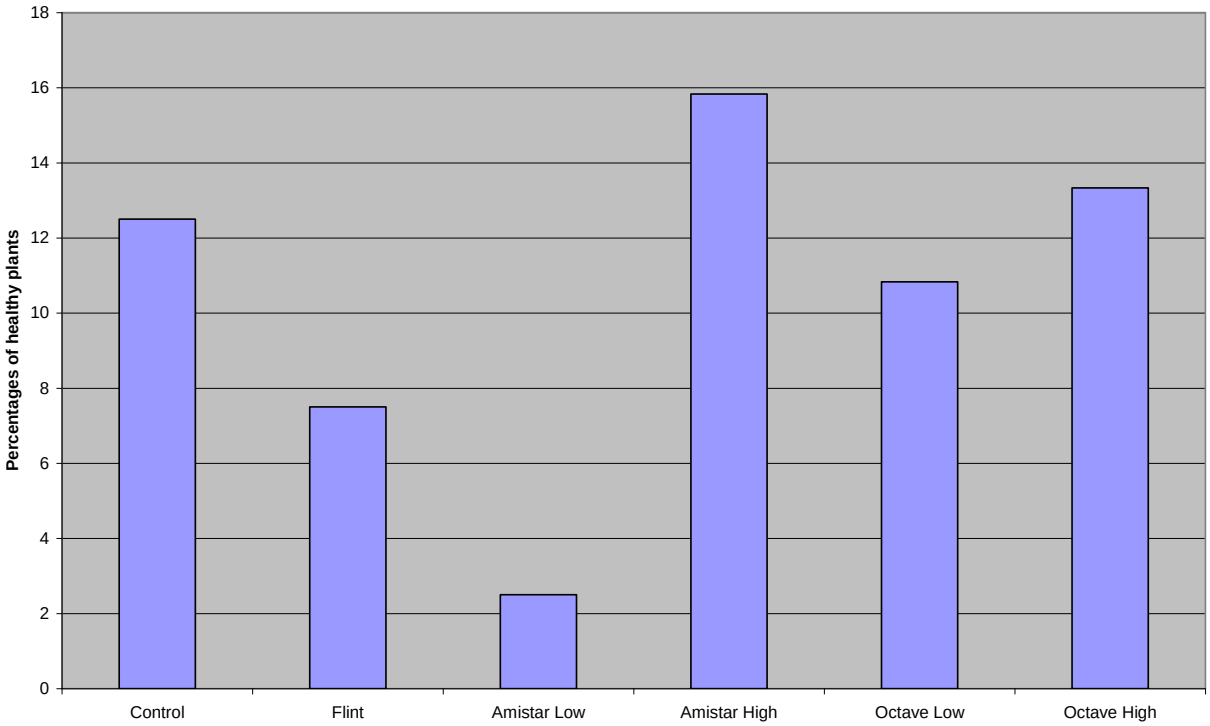

Figure 4.7 The effect of drenching on the numbers of healthy cucumber plants two months into the commercial farm experiment.

Experiment 8. On farm assessment of disease control #2

Statistically there was no difference in either the level of disease observed or the numbers of fruit picked that could be contributed to any of the chemical treatments. Approximately 27,970 cucumbers were picked within the trial with an approximate weight of 4,200kg. There did appear to be a trend in that both the high Amistar®, the high Octave® and the mixture (biologicals, potassium silicate and Bion®) appear to have more healthy plants at the end of the experiment (Figure 4.8). Due to the large edge effect, differences could not be drawn between the areas cleaned by the farmer and those cleaned by DPI staff (Figure 4.9). Disease progress through the experimental area was heavily biased towards the side of the greenhouse where the grower had reused cocopeat bags and had nearly 100% disease loss. Disease level within and between the rows was not consistent as inoculum was not applied, it was only allowed naturally to enter the system.

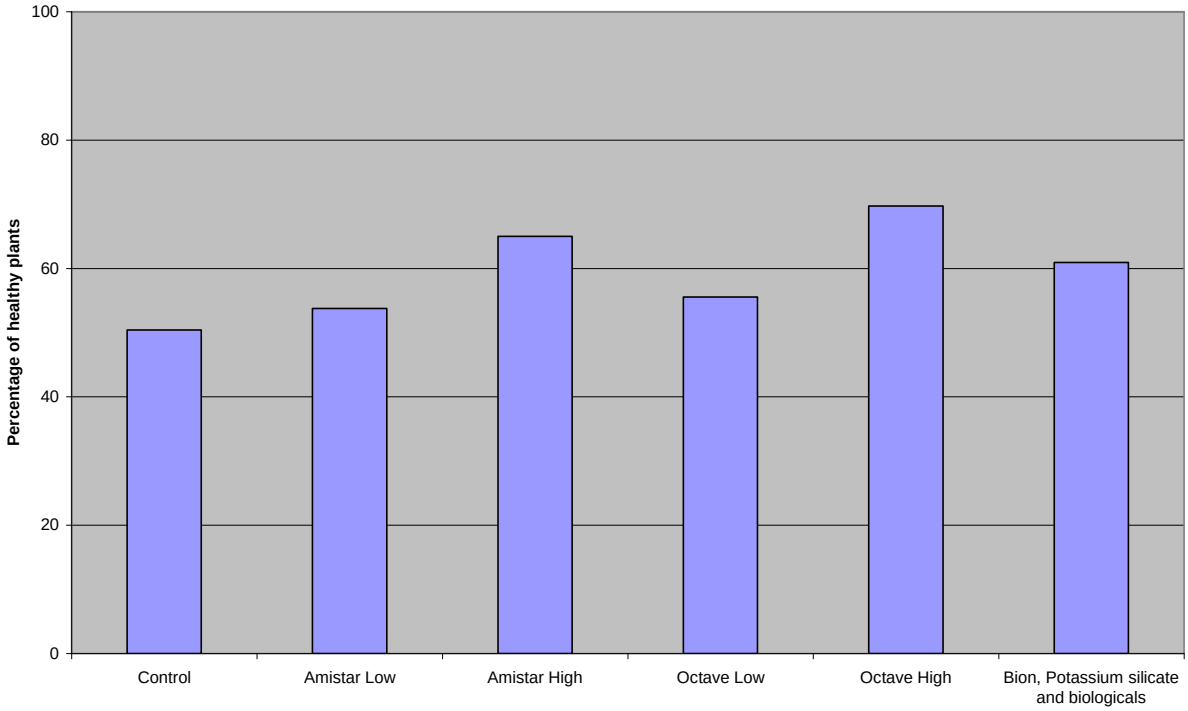

Figure 4.8 The effect of chemical drenching on the numbers of healthy cucumber plants at the conclusion of the second commercial scale on-farm experiment

Figure 4.9 Cucumber plants growing in the on-farm experiment

Experiment 9. Preliminary combinations of potassium silicate, Bion® and other biologicals

None of the treatments statistically reduced plant death (Figure 4.10). The two combination treatments of Bion®, potassium silicate and the putative *P. fluorescens*

bacteria and combination treatment of all of the biological controls together with potassium silicate and Bion®, may have reduced the level of plant death at a 10% level.

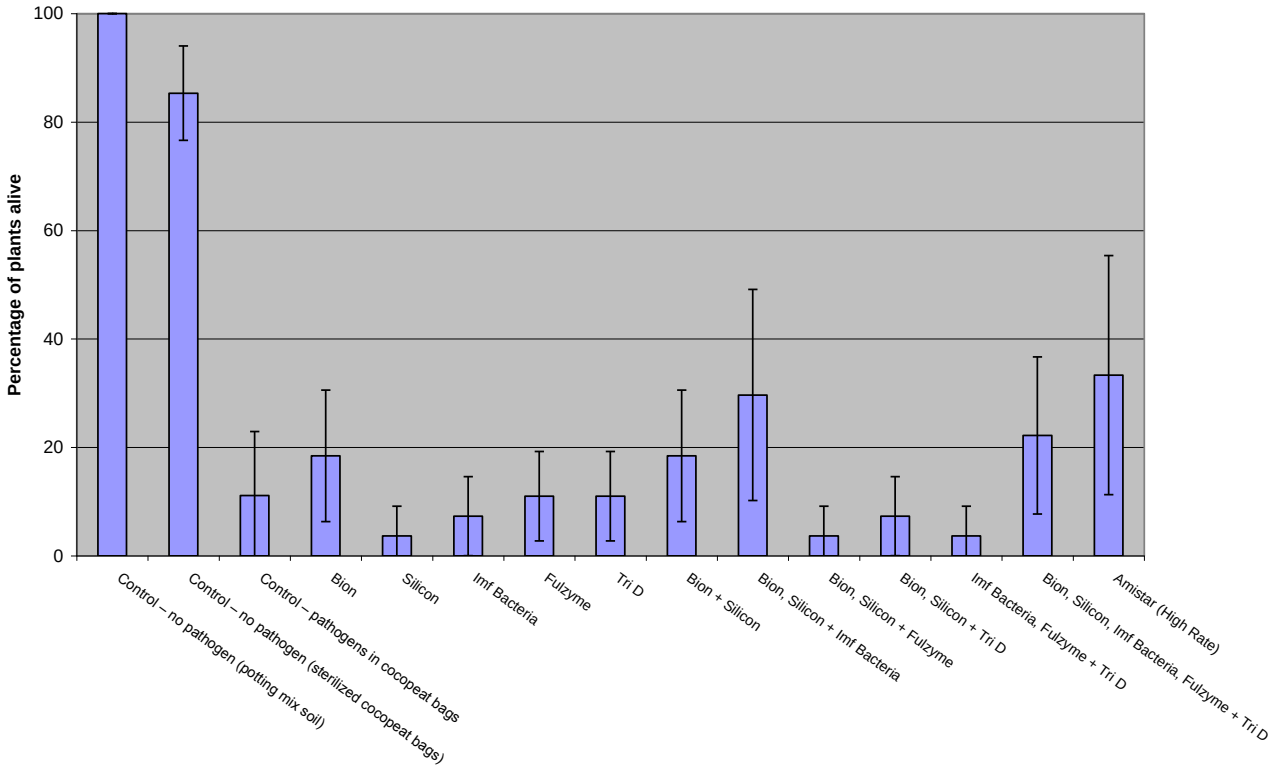

Figure 4.10 The effect of potassium silicate, Bion® and biological control options at reducing disease levels.

Experiment 10. Potassium silicate and Bion in cocopeat bags #2
In this experiment there were a number of treatments which statistically reduced disease levels and increased the number of plants alive at the end of the experimental period (Figure 4.11). Both Bion® and potassium silicate reduced disease levels, and when combined together the level of disease control was enhanced. The further combination of Bion®, potassium silicate and any one of the biological controls did not appear to add any benefit except when all of the biological controls were added together. Interestingly the combination of all the biological control products together did not enhance disease control without Bion® and potassium silicate. The effect of Amistar® at reducing disease levels was not significant. Isolation of plant pathogens from plants dying within this experiment revealed that in addition to *Fusarium*, there were a number of *Pythium* species present, and some *Rhizoctonia*.

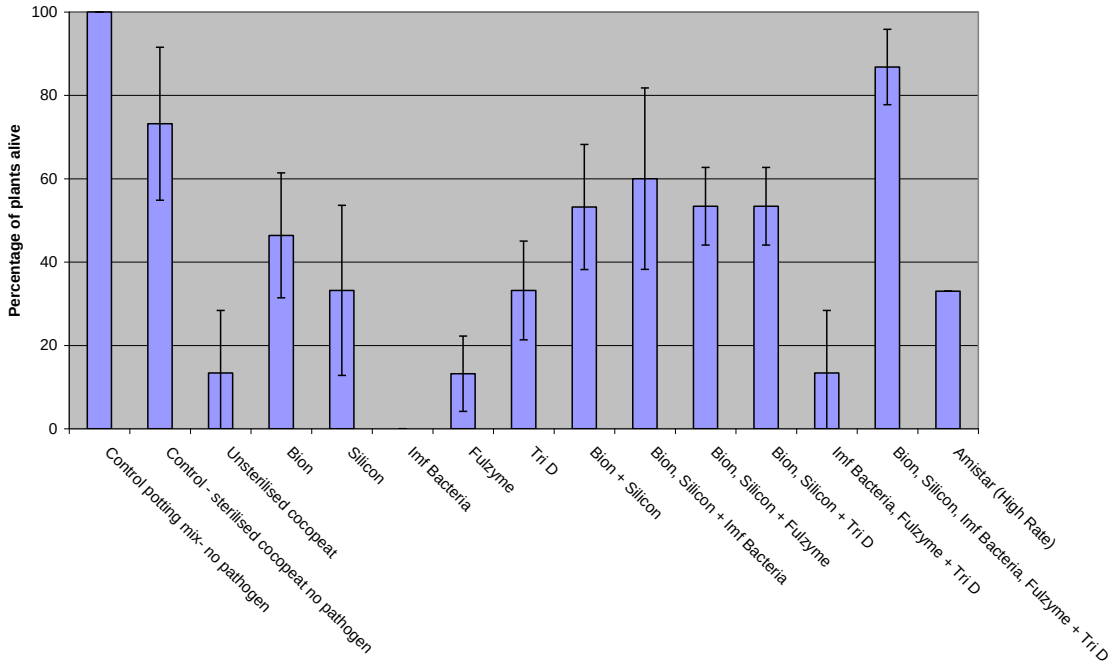

Figure 4.11 The effect of potassium silicate, Bion® and biological control options at reducing Fusarium wilt in cocopeat bags (note there was no standard deviation on the Amistar® percentage of plants alive as it was consistently 35% across all replicates).

Experiment 11. Potassium silicate and Bion in cocopeat bags #3

In this experiment there were a number of treatments which statistically altered disease levels and increased the number of plants alive at the end of the experimental period (Figure 4.12). Bion® alone and in combination with potassium silicate were the best treatments at the end of the trial. Interestingly, Ridomil® statistically reduced the numbers of healthy plants, regardless of what it was applied with.

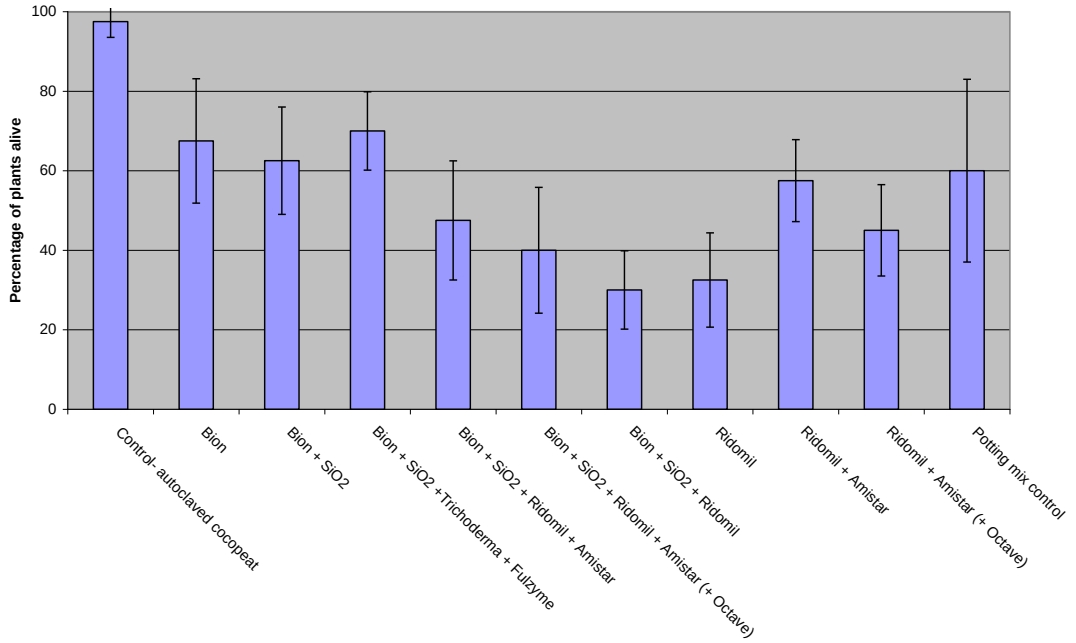

Figure 4.12 The effect of Bion® and potassium silicate on the number of plants alive

Experiment 12. Potassium silicate and Bion in cocopeat bags #4
In this experiment there were a number of treatments which statistically altered disease levels and increased the number of plants alive at the end of the experimental period (Figure 4.13). Bion® alone and in combination with potassium silicate significantly reduced the plant mortality. Once again the addition of Ridomil® statistically increased plant disease, regardless of what it was in combination with. Within this experiment an infestation of fungus gnats appeared within the glasshouse, possibly transferring pathogens into the control no-pathogen bags.

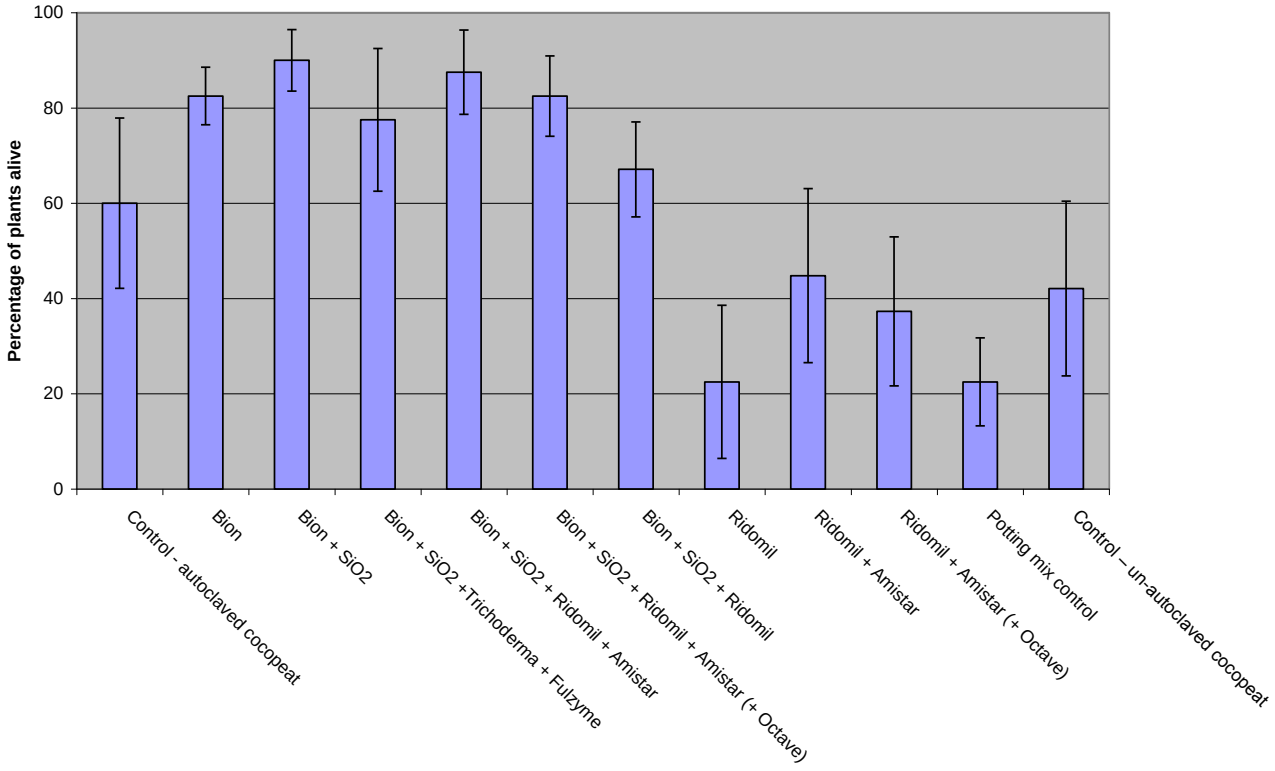

Figure 4.13 The effect of Bion and potassium silicate on the number of plants alive

Experiment 13. Grafted cucumbers in potting mix against Fusarium and Pythium
The grafting combination of Deena on the rootstock of Ferro gave the best disease control against both *Pythium aphanidermatum* and *Fusarium* (Figure 4.14). There were some limitations to the grafted plants, with the graft itself possibly not being appropriate for cucumbers as there appeared to be some nutrient deficiency symptoms in the plants despite being fertilized on a regular basis (ungrafted plants did not show nutrient deficiency symptoms).

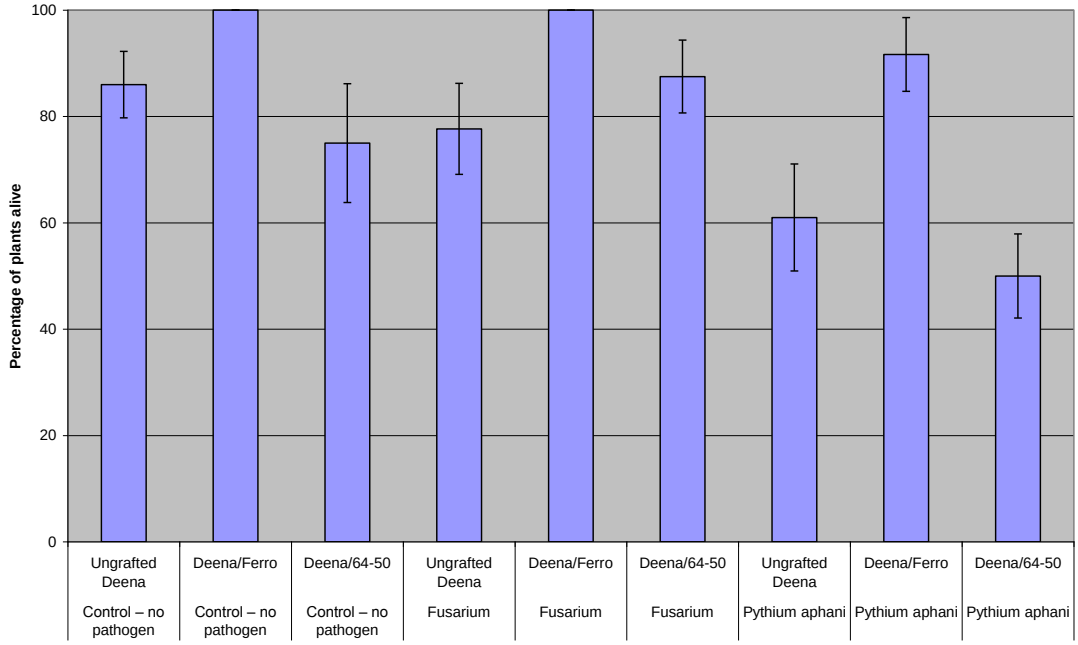

Figure 4.14 The effect of grafted cucumbers on the number of plants alive after being treated with either *Fusarium, Pythium aphanidermatum* or water.

Experiment 14. Grafted cucumbers in cocopeat bags.
Both the grafted cucumber plant combinations (rootstocks Ferro and 64-50) were significantly had less plant death than ungrafted cucumbers when grown in contaminated cocopeat (Figure 4.15). Within this experiment an infestation of fungus gnats appeared within the glasshouse, possibly transferring pathogens into the control no-pathogen bags.

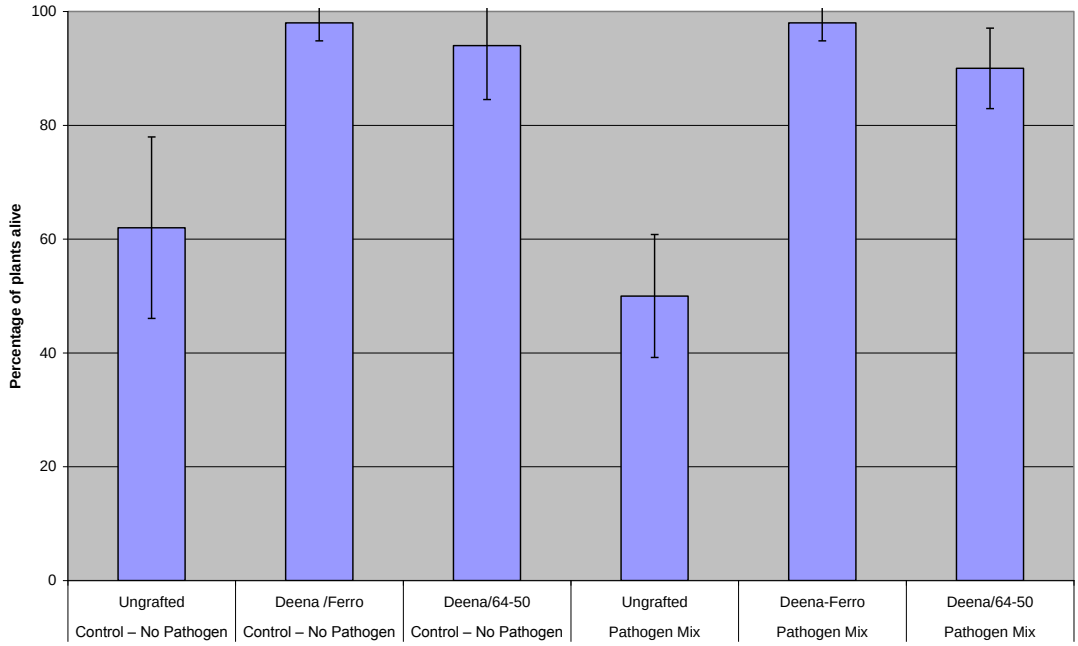

Figure 4.15 The effect of grafted cucumbers on the number of plants alive when placed in infected cocopeat mix.

Conclusions

While the chemicals Amistar® and Octave® showed promise within the initial experiments, they showed no significance in controlling disease in on-farm experiments or in later experiments using infected cocopeat bags. This difference in efficacy may relate to the on-farm experiments being varied due to a natural variability in inoculum throughout all treatments, another possible explanation maybe that in the cocopeat bags there are microflora capable of degrading the fungicides, or there could be a resistance/tolerance build up.

None of the commercial biological control options evaluated provided any statistical reduction in disease. Due to the failure of the tested commercial biological control organisms to reduce Fusarium disease severity, new potential biological control organisms should be isolated and assessed.

The use of grafted cucumber plants should be encouraged as the best overall disease control observed though out the project was by the grafted cucumbers in the cocopeat bags (Figure 4.14). As previously discussed the grafted plants displayed some nutrient deficiencies possibly relating to the graft junction not being appropriate for cucurbits. The angle graft used by a commercial supplier was used for ease within their processes of grafting tomatoes. Alternative grafting methods have been evaluated at the Gosford Horticulture Institute with better unions formed between the rootstock and scion.

The most promising chemical disease control results came from the combination applications of Bion® and potassium silicate together. When examined over all four experiments (9-12) there is a significant interaction between Bion® and potassium silicate, and together they are more effective at reducing plant disease. Bion® is a synthetic analogue of the plant hormone salicylic acid, capable of inducing a range of plant defence responses. It has been published that salicylic acid also enhances the expression of hydroxylproline rich proteins, which are plant cell wall bound proteins

capable of binding mineral elements such as calcium and silicon (Kauss *et al.*, 2003). Currently further research is underway to determine whether the levels of silicon within cucumber plants treated with Bion® is higher than untreated plants, as the extra deposition of silicon in the cell walls could act as a physical barrier to the systemic infection of *Fusarium* and *Pythium* within cucumber roots and stems. Alternatively potassium silicate can act as a plant defence inducer, potentiating a number of defence enzymes in a wide range of crop species (Ma, 2005). The combination of Bion® and potassium silicate may give additional enhancement to the cucumber defence response. To determine whether this occurs a final experiment quantifying levels of cucumber plant defence enzymes is being undertaken. It is important to understand if the Bion® and potassium silicate combination is working via the physical deposition of silicon or the defence response, as understanding of how it works will assist with understanding when it won't work and the physiological costs to the cucumber plant associated with the interaction, e.g. if the additional control is through the additional deposition of silicon within the cell walls, silicon may only need to be applied once or twice at the early seedling stage.

Chapter 5. Isolation, characterisation and screening of new biological control organisms

Background

Within this project to date none of the commercial biological control options evaluated provided any statistical reduction in disease. Due to the failure of the tested commercial biological control organisms to reduce Fusarium disease severity, new potential biological control organisms should be isolated and assessed.

Rhizosphere bacteria have been found to reduce the severity of Fusarium diseases in various crops by: competing for nutrients; colonizing root sites instead of pathogens such as *F. oxysporum*; releasing substances that are toxic to pathogens; or promoting the plant's own defence mechanisms (Whipps, 2001). A number of isolated rhizobacteria have been identified to promote plant growth and yield, either directly by plant growth promoting effects, or indirectly by influencing the rhizosphere environment (Kloepper *et al*., 1980; Glick *et al*., 1995). In both cases they are referred to as plant growth promoting rhizobacteria (PGPR) (Kloepper & Schroth, 1978). Several groups of soil microorganisms are considered to be PGPR. Most PGPR belong to the genus *Pseudomonas* (Pieterse *et al*., 2001). Fluorescent pseudomonads are thought to be good candidates for biocontrol organisms because they possess a versatile metabolism, enabling them to utilize various substrates released by the roots (Lynch & Whipps, 1991). They also have: short generation times; mobility within soil; the capability to colonize roots; and can produce a wide range of secondary metabolites including plant growth regulating substances and antibiotic compounds (Fravel, 1988; Levy *et al*., 1992; Duffy & Defago, 1997). Indeed, fluorescent bacteria predominate the few commercialized biocontrol organisms available (Compant *et al.*, 2005).

One of the few commercialized biocontrol bacteria, *Psuedomonas fluorescens* strain WCS417r, has been shown to suppress Fusarium wilt in a number of different crop plants including radish, tomato and carnation (Van Peer *et al.,* 1991; Leeman *et al.*, 1995; Duijff *et al.,* 1998), as well as in the model plant species *Arabidopsis thaliana* (Pieterse *et al.,* 1996). Analysis of the mechanisms behind the disease suppression due to strain WCS417r and other PGPR has identified a key role of induced systemic resistance (ISR) in disease suppression (Kloepper *et al*., 1992; Pieterse *et al*., 1996). Induced resistance can result from two events: the microbe sensitizes the plant, and/or the microbe induces the plant's defence responses creating a highly antimicrobial environment inside the plant tissue (Pieterse *et al.,* 1998).

ISR is phenotypically similar to systemic acquired resistance (SAR), as there is a systemic increase of plant resistance to a broad range of pathogens (van Loon *et al.*, 1998). SAR is a well-studied phenomenon in plants that occurs after an incompatible reaction, resulting in aquired resistance to a broad range of pathogens (Ross, 1961; 1966). SAR may be due to increased pathogenesis-related (PR) gene expression, enhanced phytoalexin accumulation, thicker plant cell walls, or tissue potentiation, which is a decrease in the lag time between any subsequent pathogen inoculation and the activation of the plant defence responses (Dixon *et al*., 1994; Hammershmidt, 1999). The most consistent feature of ISR is the induction by non-pathogenic organisms (Hoffland *et al*., 1995; van Loon *et al.,* 1998). Systemic synthesis of PR

proteins and phytoalexins does not always occur in ISR, although tissue sensitization appears to always occur (van Wees *et al.,* 1999). In studies on *A. thaliana*, the underlying signalling pathways between SAR and ISR have been shown to differ, although there are points of commonality between the two pathways (Pieterse *et al.*, 1998; Thomma *et al*., 2001). To enhance overall plant resistance for disease control, ISR is preferred over SAR (Han *et al*., 2000), as ISR has little negative effects on plant growth while SAR requires necrosis, which can be detrimental to plant growth (Hoffland *et al*., 1996).

Various researchers have shown that rhizosphere bacteria with the ability to provide biological control appear to comprize less than 10% of the total population of bacteria in the rhizosphere (Schroth & Hancock, 1981; Schroth & Hancock, 1982; Suslow & Schroth, 1982; Weller & Cook 1986). The chance of selecting effective strains for disease control should first be improved by isolating bacteria from the same environment in which they will be used (Glick *et al*., 1995). For example, bacteria selected from a cucumber rhizosphere could be used to target Foc, which inhabits cucumber soil. Isolating bacteria from soils known to be suppressive to the target pathogen (Schneider, 1982) may further increase the chances of finding effective strains (Cook & Baker, 1983); biotic factors such as rhizosphere bacteria are often involved in suppressing disease in suppressive soils (Dunleavy, 1955; Broadbent et al., 1971; Schippers *et al.,* 1987).

The aim of this research was to assess rhizosphere bacteria for the control of Fusarium wilt of cucumber. To achieve this end, rhizosphere bacteria were isolated from disease suppressive pots and characterized for typical biocontrol characteristics.

Methods

Isolation of bacteria

Bacteria were isolated from the stems, roots and crowns regions of cucumber plants, which were surviving within a grower's glasshouse. The plants were selected as they showed minimal disease despite high levels of disease being present in the surrounding cocopeat bags.

Plant sections were surface sterilizing the plant sections by a short 10 second soak in 1% sodium hypochlorite before being rinsed in sterile deionised water. The plant sections were then ground with mortars and pestles with 5ml sterile water. The resulting slurry then underwent ten fold dilutions which were plated out onto Kings B medium (KB) agar plates and incubated at 24^{o}C for 24-48 hours. Bacterial colonies were picked off the plates and purified by three successive rounds of 16 streaking to isolate single colonies. Once a pure culture was obtained the bacterium was stored in sterile water for short term usage and in glycerol at -80^{o}C for longer term use.

Assessments of bacterial characteristics

Bacteria were grown on KB agar for 24 hours prior to expose to UV light and assessment of fluorescence. Fluorescence observed was described as either, blue, green or white and rough estimates of intensity were determined by eye. Bacterial colonies were described based upon colour and morphology.

In-vitro antagonism assays

Bacteria were streaked across the centre of ¼ strength potato dextrose agar plates, and plugs of agar colonised by *Fusarium oxysporum* f.sp. *cucumerinum* placed on either side. Two different isolates of Fusarium were used. The isolates were selected based upon differences in their genetic fingerprint and ability to cause disease on cucumbers. The paired antagonism plates were assessed four days after the Fusarium was placed on the agar. The bacteria were assessed as being antagonistic *in-vitro* if growth of the fungal mycelia was inhibited. When inhibition was observed, the experiment was replicated three times.

Plant screening

Cucumber seeds (cv. Deena) were sown into pots containing sterile UC mix (Baker, 1957). Seeds were sown at 12 seeds per pot and later thinned to 10 plants per pot. Plants were fertilized using Thrive^{TM} once a week. Plants were treated with the bacteria one day prior to inoculation with Fusarium, and again two weeks later. Plants were inoculated with Fusarium upon the emergence of the first true leaf. The experiment contained 6 replicates for each treatment. Treatments were randomised using random number generators.

Bacterial inoculum was prepared by inoculating sterile flasks containing potato dextrose broth, flasks were shaken at 24^{o}C for 2 days before being diluted and applied to the cucumber seedlings.

Fusarium inoculum was prepared by growing Fus #53 (DAM number 821) on ¼ strength potato dextrose agar plates for 1-2 weeks. Colonised agar plates were blended into a slurry in sterile water and pots were inoculated with approximately one plate of agar per pot. Control treatments used uncolonised agar plates. Plants were grown and disease assessed as previously described in chapter 4.

Results and Discussion

More than 308 bacteria were isolated, purified and characterised. In general three different colony morphologies were observed: opaque with a clearly defined circular edge, pale coloured with a clearly defined circular edge, and opaque with an ill defined irregular edge. The occasional non-circular yeast like actinomycete growth was also observed on occasion. Of the bacteria isolated 54% were fluorescent under UV light and only 7% consistently inhibited the growth of Fusarium *in vitro* (Figure 5.1).

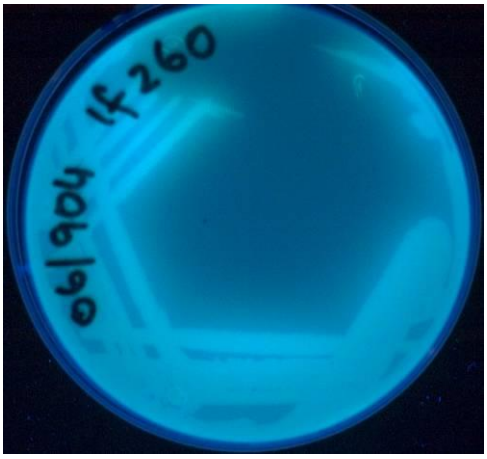

Figure 5.1 Bacterial strain lf260, isolated from a cucumber plant fluorescing *in vitro*.

Three individual experiments were setup to examine the effect of bacterial inoculation on the reduction of disease, examining 20 different isolates. Typical results were no significant reduction of disease. There were two isolates lmf293 and lmf263 have slightly reduced the severity of Fusarium wilt (Figure 5.2). Overall there is still a lot of work to be undertaken to further characterise these bacteria.

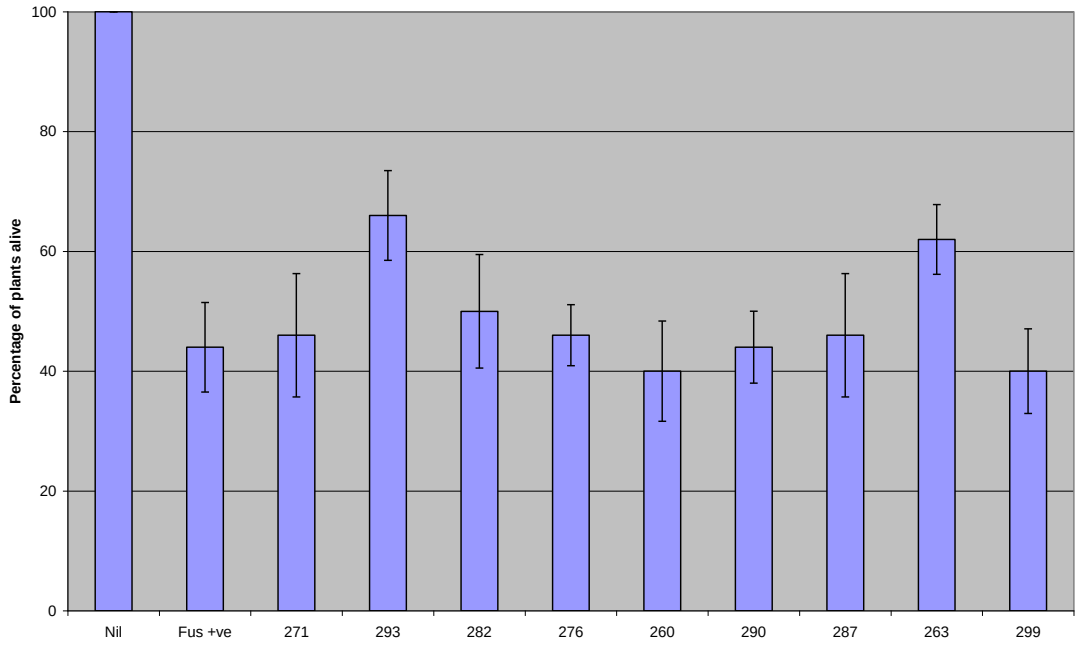

Figure 5.2 The effect of bacterial inoculation on the percentage of plants alive at the conclusion of a Fusarium wilt trial. Numbers represent bacterial isolates used.

Conclusions

Biocontrol bacterial characteristics such as endospore production, *in vitro* antibiosis, and the production of fluorescent siderophores are useful tools for reducing the number of different rhizobacteria that should be screened for biocontrol activity. Unless infinite funding is supplied, it is impossible to screen all bacteria and fungi isolated from suppressive soil sites. Features such as the ability to produce endospores should be further focused upon as it allows bacterial strains to be resistant to desiccation, heat treatment and UV light, making formulation of the biocontrol organisms at the commercialization stage easier (Rhodes, 1990. The ability to produce fluorescent siderophores has been shown to be involved in ISR (Maurhofer *et al.*, 1994), as well as iron chelation, which is involved in reducing the germination of conidia of *Fo* in soils (Scher & Baker, 1982; Leeman *et al.*, 1996). The ability to produce antibiotics *in vitro* is a useful character for a biocontrol organism to have but many researchers have shown that *in vitro* and *in planta* secretion of antibiotics are not correlated (Whipps, 2001). Although a number of bacterial strains showed antibiosis against *Foc* on PDA, few were effective at reducing disease.

Chapter 6. Characterisation of isolates of Clavibacter michiganensis in Australia

Background

Bacterial canker is a devastating disease of both field and greenhouse tomatoes, caused by the bacterium *Clavibacter michiganensis* subsp. m*ichiganensis* (Cmm). First found in Michigan, USA in 1909, Cmm has spread to all of the major tomato growing areas of the world including many of the main seed production regions (Smith, 1910; Gleason *et al*., 1993). Bacterial canker of tomato is the number one disease affecting tomato production in the Australian greenhouse industry.

Symptoms of bacterial canker infection in tomato can range from mild marbling on fruit through to complete plant death, depending on the tomato variety, environmental conditions and nutrient management (Walker and Kendrick, 1948, and Strider, 1970). Early symptoms which typically appear are scorching marks on the leaves similar to the nutritional disorders potassium deficiency or phosphorous toxicity (Weir and Cresswell, 1993). Later symptoms can include lesions on stems, wilting of plant, birds-eye cankers on the fruit, and the internal symptom of vascular browning (Gleason *et al*., 1993). Depending on the tomato cultivar and environmental conditions it can take up to 90 days for symptoms to develop (Gitaitis *et al*., 1991). Canker infection can result in yield reductions of up to 100% (Gleason *et al*., 1993).

The bacteria which causes canker can be spread in a number of ways. Initially it can be introduced onto a farm by infected soil/media, seed, seedlings, visitors, infected cutting implements from other farms, and potentially by contaminated water (Gleason *et al.,* 1993). Cmm can spread throughout a farm quickly, with devastating results if appropriate quarantine and hygiene actions are not undertaken appropriately in the early stages of the disease (Werner *et al.,* 2002). For example, if an infected plant is unnoticed, the bacteria can move through the hydroponic nutrient channels, it can move with workers cutting implements, on hands etc. infecting other plants. Unfortunately with the potential for a long time delay before disease symptoms are expressed, bacterial canker can be spread unknowingly throughout a greenhouse (Gitaitis *et al.,* 1991).

Once Cmm is found on-farm it is particularly hard to eradicate, with reports of the pathogen lasting more than 4 months between tomato crops (Fatmi and Schaad, 2002). Canker bacteria can reside on plastic surfaces, in soil/potting media, in water/nutrient solution, on cutting implements and in other alternative host plants (Chang *et al.,* 1992). Cmm can reside in a number of alternative host plants including weeds and some solanaceous crop plants such as capsicum (Chang *et al.,* 1992). This means that to effectively remove Cmm from a greenhouse all material inside the greenhouse must be sanitised, and all material which will re-enter the greenhouse should be carefully cleaned and disinfected before use.

Currently there are no registered, effective controls for canker; the only effective methods are the quarantine and eradication of infected material. Internationally there has been published research indicating that chemicals such as copper and some antibiotics may be effective at limiting the spread of the bacteria. However antibiotics

are banned for use on food products within Australia, and have been shown to only be a short term solution as resistance to antibiotics occurs quickly. Research on Australian strains of Cmm has already shown that there is resistance to antibiotics, so the use of them would not only be illegal, it would be ineffective.

In Australia in recent years bacterial canker of tomato has emerged as a serious disease, which occurs in all major tomato growing areas. Interestingly the external symptoms of bacterial canker have altered over the past 10 years. Whether this is due to the use of newer tomato cultivars which react differently to infection by the bacterium or due to the introduction of new genetic strains of the bacteria is not known. It is possible that there are new strains present in Australia since Cmm can be seed borne and imported seed is generally untreated since the relaxation of Australian quarantine requirements in the early 1990s. The purpose of this work is to assess the diversity of Cmm in Australia.

Methods

Isolate collection

A general request was put out through grower magazines, seed company representatives, consultants and researchers for samples of tomato plants suspected of having bacterial canker. Tomato plant samples were sent into the laboratory and when bacterial canker was identified as the cause of the disease, attempts were made to isolate the bacteria onto Kings B agar (KB). If the resulting cultures were too contaminated with other bacterium that identification of Cmm was difficult, the bacteria were subsequently cultured on semi-selective KB plates amended with polymixin sulphate. Bacterial colonies were picked off the plates and purified by three successive rounds of 16 streaking to isolate single colonies. Once a pure culture was obtained the bacterium was stored in glycerol at -80^{o}C for longer term use and on beads using the Protect bead system (Technical services, Lancashire, England).

Isolates of Cmm were also obtained from historical bacterial collections in Orange, NSW (DAR collection); Brisbane, QLD (BRIP collection); and the Belgium culture collection for international isolates. All international isolates were brought into Australia under strict quarantine conditions with an AQIS approved importation permit.

DNA extraction and genotyping

DNA was extracted from the purified bacterial cultures using the Qiagen DNeasy® Blood and tissue extraction kit as per the manufacturers instructions. DNA was quantified using a NanoDrop™ spectrophotometer (NanoDrop Technologies, Wilmington, USA).

The ITS rDNA regions of the bacterium were amplified using the Fegan *et al.* (1998) primers. The presence of a single band was used to determine whether there were any veiled cultural contaminants. Samples which had doublets were discarded and the bacteria reisolated, repurified and re-extracted.

Arbitrarily primed PCR was undertaken using the ERIC, BOX and REP primers (de Leon *et al*., 2009). These primers amplify repetitive element regions of the bacterial genome. PCR products were visualised on 1% agarose gels stained with ethidium bromide and viewed under UV light. Bands were scored as present or absent by eye.

Pathogenicity experiment 1. Preliminary screening
Tomato seeds (cv. Gros Lissie) were germinated in seedling trays containing UC Mix. Once all seedlings had reached the developmental stage of first true leaf emergence, they were planted into appropriately labelled pots at a rate of four tomato plants per pot. Plants were grown in a plant growth cabinet with a daylength of 12 hours, temperature setting of 20-26^{o}C with the humidity set at 60%.

Plants were inoculated with Cmm isolates DAM 2019, 2023, 2025, and 2035 two different ways: bacterial suspension was injected into the stem; and in the second treatment the bacterial suspension was drenched into the soil around the based of the tomato stems. Plants were inoculated one day after seedling transplant.

Plants were fertilized using Thrive^{TM} once a week. The experiment contained three replicates for each treatment. Treatments were randomised using random number generators. Disease progress was assessed on a weekly basis. When severe disease was observed (wilting, necrosis of plant) the vasculature of the tomato plant was examined for bacterial ooze, which when observed was tested as being Cmm by immunostrip.

Pathogenicity experiment 2. Assessment of the effect of tomato cultivars on disease symptoms
Tomato seeds of a range of varieties were planted directly into small 200mm pots containing UC mix. Seeds were planted at a rate of 6 seeds per pot. The varieties used were: Clarence, Merecedes, Cherelino, Katalina, Roland, Olivade, Tradiro, Violin, Grosse Lisse, La Belle, and Ivanhoe. Once all seedlings had reached the developmental stage of first true leaf emergence, plants and pots were placed in sealed polyurethane bags overnight to induce guttation on the leaves. Plants were inoculated with Cmm isolate DAM 1996, by spraying 5mL of a bacterial suspension (approx. 5 x 10^{8}cfu/mL). After inoculation plants were returned to the sealed bags for 24 hours.

Plants were grown in a heated glasshouse with a temperature range of 20-28^{o}C. Plants were grown and disease assessed as previously described in pathogenicity experiment 1. There were four replicates of each tomato variety.

Pathogenicity experiment 3. Assessment of the effect of Cmm isolates on two tomato cultivars
Tomato seeds (cvs. Clarence and La Belle) were germinated in seedling trays containing UC Mix. Once all seedlings had reached the developmental stage of first true leaf emergence, plants were placed in sealed polyurethane bags overnight to induce guttation on the leaves. Seedlings were placed in the bags at a rate of four seedlings per bag.

Plants were inoculated with Cmm isolates DAM 1986, 1996, 2000, 2020, and 2028 by spraying 5mL of a bacterial suspension (approx. 1-5 x 10^{8}cfu/mL). Different spray bottles were used for each isolate of Cmm. Spray bottles were previously calibrated in the laboratory to ensure that equal amounts of bacteria were added to each bag. After inoculation plants were returned to the sealed bags overnight, before being planted out into larger pots containing UC mix. There were six replicates with the La Belle cultivar and three replicates with the Clarence cultivar. Plants were grown in a heated glasshouse and disease assessed as previously described.

Pathogenicity experiment 4. Assessment of the effect of Cmm isolates on different solanaceous host crops

Seeds of capsicum (cvs. Californian wonder, Giant Bell and Patio red), tomato (cvs. Grosse Lisse and La Belle) and eggplant were germinated in seedling trays containing UC Mix. Once all seedlings had reached the developmental stage of first true leaf emergence, plants were placed in sealed polyurethane bags overnight to induce guttation on the leaves. Seedlings were placed in the bags at a rate of four seedlings per bag.

Plants were inoculated with Cmm isolates DAM 2000, 2089, 2097, and 4009 by spraying 5mL of a bacterial suspension (approx. 1-5 x 10^6 cfu/mL). Different spray bottles were used for each isolate of Cmm. Spray bottles were previously calibrated in the laboratory to ensure that equal amounts of bacteria were added to each bag. After inoculation plants were returned to the sealed bags overnight, before being planted out into larger pots containing UC mix. Plants were grown in a heated glasshouse and disease assessed as previously described. There were five replicates of each cultivar-treatment combination.

Results and Discussion

Isolate collection

Approximately 130 different isolates of Cmm were collected from around Australia throughout this project. In addition 26 different international isolates from 13 different countries were sourced from the Belgium culture collection. The majority of isolates collected were isolated from tomato although there were two isolates from Cmm obtained from diseased capsicum.

DNA genotyping

Preliminary results indicate that there is variation amongst Australian isolates, with there being at least 6 different genotypes. Further research will also be undertaken to determine if there are any patterns in where the different genotypes come from.

Pathogenicity experiment 1. Preliminary screening

Bacterial canker was observed on all of the treated tomato seedlings after one month. There did not appear to be any difference in disease levels associated with the different isolates of Cmm or the different inoculation methods.

Pathogenicity experiment 2. Assessment of the effect of tomato cultivars on disease symptoms

Bacterial canker was observed on all of the treated tomato varieties. There was no statistical difference in disease levels between any tomato variety. The most severe symptoms were seen earliest in the La belle cultivar.

Pathogenicity experiment 3. Assessment of the effect of Cmm isolates on two tomato cultivars

Statistical differences were observed in the levels of disease caused by different isolates of Cmm on the two tomato cultivars (Figure 6.1). There were no differences between the reaction of the two different tomato cultivars so for ease of display both the results for both have been combined. It appears that Cmm isolate 2028 is hypovirulent causing minimal disease.

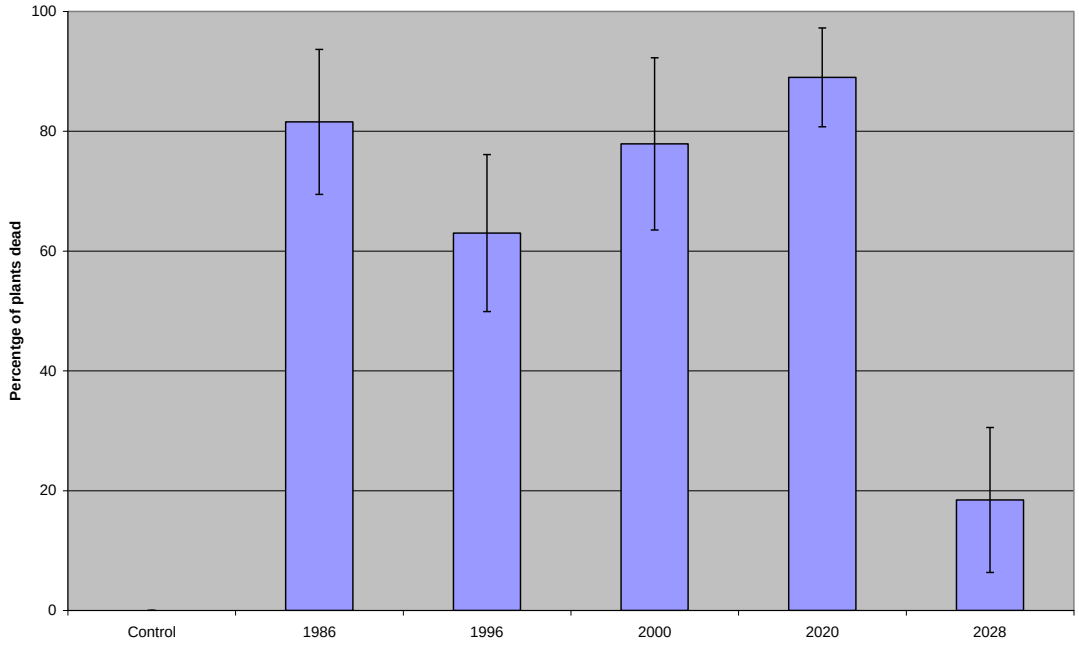

Figure 6.1 The effect of different isolates of Cmm (DAM 1986, 1996, 2000, 2020 and 2028) on tomato plant death.

Pathogenicity experiment 4. Assessment of the effect of Cmm isolates on different solanaceous host crops

Minimal disease symptoms were observed on the alternative solanacous host plants. On all of the capsicum cultivars were the bacterium was directly applied small necrotic lesions ridged by a raised edge were observed (Figure 6.2). Cmm bacteria were reisolated from these lesions, although the disease did not spread further through the plants. Possible reasons for the discrepancy between our experimental work with Cmm on capsicum and the observed field symptoms may be due to watering only being from underneath the pot via saucers (i.e. no splash on the leaves allowing the bacteria to spread from different parts of the plant), and also the age and growth stage of the plants used in this experiment. The experiment only lasted 10 weeks, so the capsicum had no fruit load or change in phytohormone status which may have encouraged systemic disease development. Interestingly no symptoms were observed on the eggplant, through the plant health diagnostic service there have frequently been bacterial ooze from the vasculature of diseased eggplant test positive for Cmm by immunostrip (Agdia, USA), however the bacterium Cmm has not been isolated. These diagnostic results may now be viewed as potential cross reactions of the immunostrip as inoculated eggplant tested negative to Cmm.

Figure 6.2 Symptoms of a local infection of Cmm on the leaves and cotyledons of a capsicum plant

Conclusions

The molecular results confirm the presence of multiple genotypes of Cmm found across Australia, indicating multiple incursions of Cmm into Australia, probably on contaminated seed batches, or plants. Pathogenicity testing also confirmed that the genetic variation affects the disease development. Further testing is required to compare the effect of the different genotypes against the different solanaceous host plants and to also compare disease development on solanaceous host plants grown under sprinkler irrigation, or in low grade glasshouse structures with frequent condensation drips, to determine why disease severities observed in field grown capsicums was not observed in the glasshouse experiment.

Chapter 7. Outputs and outcomes of the project

Within this project there have been countless farm visits within the Sydney basin between 2005-2009, there have been 17 field days held across Australia (Figure 7.1), three interstate scientist visits to the NSW Sydney greenhouse production area to learn disease recognition (Figure 7.2), seven published conference abstracts and two grower primefacts produced. These project extension activities and outputs have frequently been undertaken in collaboration with other projects within NSW and interstate (Figure 7.3). The major projects collaborated with have been:

- VG03098 Regional extension strategy for managing western flower thrips and tomato spotted wilt virus in the Sydney region.
- VG05094 Sustainable integrated control of foliar diseases in greenhouse vegetables.
- VG07118 Greenhouse growers reducing crop losses with preventative disease management practices.
- VG07119 Identification and monitoring of resistance in Australian vegetable crops.
- VG07125 Best-practice IPM strategies for control of major soil borne diseases of vegetable crops throughout Australia.
- VG07128 Integrated viral disease management in vegetable crops.
- ACIAR 2007/066 Strategies for the management of wilting diseases of solanaceous crops in Mindanao and Australia.

Figure 7.1 Picture of a grower field day held at Kemps Creek, Sydney in May 2008, more than 50 growers and consultants attended.

Grower group meeting, field days and interstate collaborator visits:

- Tahmoor, NSW growers meeting field day to introduce project, November 2005.
- Rossmore, Sydney NSW growers meeting field day to introduce project, November 2005.
- Sydney, NSW disease recognition training day, April, 2006.
- Tahmoor, NSW growers meeting field day, June 2006.
- Rossmore, Sydney NSW growers meeting on IPM in cucumbers, June 2006.
- Brisbane, QLD field days and farm visits, May 2006.
- Rossmore, NSW disease recognition and training, September, 2006.
- Rossmore, NSW field day, November, 2006.
- Perth, WA field day and farm visits, February, 2007.
- Launceston, Tas farm visits, July, 2007.
- Virginia, SA field day and farm visits, October, 2007.
- Rossmore, NSW, interstate scientists visit, November 2007.
- Kemps Creek, Sydney, field day, May, 2008.
- Darwin, NT field day and farm visits, June, 2008.
- Coffs Harbour, NSW field day and farm visits, October 2008.
- Hawkesbury-Hills area, NSW field day and farm visits, September 2008.
- Rossmore, NSW, interstate scientists visit, November 2008.
- Rossmore, NSW field day, December 2008.
- Rossmore, NSW interstate scientists visit, April, 2009.
- Virginia, SA field and farm visits, June 2009.
- Coffs Harbour, NSW field days and farm visits, November 2009 (planned)

Figure 7.2 Interstate scientists visit the Sydney basin greenhouse growing area to improve disease recognition abilities and collaborations.

Conference and pathology meeting presentations:

- Tesoriero LA, Forsyth LM and Burgess LW (2009) *Fusarium oxysporum* and *Pythium* species associated with vascular wilt and root rots of greenhouse cucumbers. 16th Biennial Australasian Plant Pathology Conference, Newcastle, Australia.
- Forsyth LM, Crowe T, Deutscher A and Tesoriero L. (2009) Bacterial canker of tomato: Australian diversity of *Clavibacter michiganensis* subsp. *michiganensis.* 16th Biennial Australasian Plant Pathology Conference, Newcastle, Australia.
- Forsyth LM, and Tesoriero L (2009) Bacterial canker of tomato in Australia. Australian Hydroponic Greenhouse Association Conference, Sydney, Australia.
- Forsyth LM, and Tesoriero L (2009) New and emerging disease threats to the Australian greenhouse and ornamental industry. Australian Hydroponic Greenhouse Association Conference, Sydney, Australia.
- Tesoriero L (2008) Wilting diseases of greenhouse tomatoes. Hydroponic farmers federation April 2008, Lilydale, Australia.
- Forsyth LM and Tesoriero L (2007) Exotic disease threats to the Australian greenhouse and ornamental industry. Australian Hydroponic Greenhouse Association Conference, Launceston, Australia.
- Forsyth LM and Tesoriero L (2007) Integrated disease management of Fusarium wilt of greenhouse cucumbers. Vegetable pathology meeting about integrated management of soilborne diseases, Sydney, November 2007.
- Forsyth LM, Lidbetter F, Carrus R and Tesoriero L (2007) Identification of chemical and biological control strategies for managing Fusarium wilt of cucumber. 15th Biennial Australasian Plant Pathology Conference, Adelaide, Australia.

Grower primefacts produced:

- Forsyth LM, Tesoriero L and Jelinek S (2009) Healthy farms: good on-farm hygiene and sanitation. Industry and Investment NSW Primefact.
- Forsyth LM and Tesoriero L (2009) Managing tomato canker in Australia. Industry and Investment NSW Primefact.

Figure 7.3 Project member Leanne Forsyth and a number of interstate collaborators participated in the HAL pathology showcase at the Werribee field days, May 2009.

Chapter 8. Future research recommendations

The following are suggestions for future research:

- Implementation and motivation of grower groups to ensure uptake of the recommendations within the hygiene manual produced in VG07118 Greenhouse growers reducing crop losses with preventative disease management practices. Improving grower knowledge of good sanitation and hygiene practices should assist with preventing some disease outbreaks, and more importantly managing disease outbreaks so that they are less devastating.
- Further testing is needed of more water disinfestation systems, this will be undertaken in collaboration with the Smart Farms project within NSW and in collaboration with Graham Smith and VG09073 National greenhouse waste-water recycling project.
- Further evaluations are needed of grafting techniques and further training is required of seedling suppliers on the appropriate grafting techniques to ensure healthy grafted cucumber plants.
- Further evaluation is needed on Bion®, potassium silicate, Octave® and Amistar® in a commercial glasshouse to get further data to support applications for permits through AgAware Consulting Pty. Ltd..
- Further characterisation is needed of novel new biological control options, and plant defence inducing compounds, including the use of essential oils to reduce the inoculum load of *Fusarium* and *Pythium* in greenhouse crops, and to reduce the effect on the growth and yield of the cucumber plants.
- Further testing is required to compare the effect of the different genotypes of *Clavibacter michiganensis* subsp. *michiganensis* against the different solanaceous host plants and to also compare disease development on solanaceous host plants grown under sprinkler irrigation, or in low grade glasshouse structures with frequent condensation drips, to determine why disease severities observed in field grown capsicums was not observed in the glasshouse experiment.

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Appendix 1. Farm survey forms

Form 1. Initial survey of knowledge and practice of hygiene. Each practice was given a score, all practices were tallied and growers assessed.

Glasshouse hygiene survey

Recorded by: _____ Date: / /

Farm location:

Name:
Address:
Postcode:
Phone:
GPS location:

Farm details:

Area of production:
Greenhouse type:
Growing media:
Water storage tanks:
Heating/cooling system/temperature range:
Humidity range:
Light levels:
Nutrient mixture: commercial mix, own mixture

Survey:

	Points	
A. Preseason		
1. Maintain a 1 month crop free, weed free period	10	
2. Disinfect structure (all surfaces), removing all plant, algae and organic debris	10	
3. Discard or steam sterilize growing medium used for crop production	10	
4. Disinfect irrigation system	10	
B. Transplant production		
1. Purchase pest/disease free seedlings and or maintain spray regime upon seedling arrival	10	
2. Use new or steam sterilized growing medium for transplant production	10	
3. Use disease free growing media (tested)	5	
4. Store growing medium in a soil-free/disease-free area	10	
5. Where market allows choose most disease resistant variety(ies)	5	
6. Grow transplants in separate greenhouse from other crops	10	

C. Crop production		
1. Keep daily records of maintenance procedures: fertilizer frequency, date of growth stages, max and min temperatures, and humidity	5	
2. Test water for presence of carbonates and other minerals that can interfere with maintaining appropriate pH and solubility of fertilizer salts	3	
3. Determine pH of water before adding fertilizer to determine whether fertilizer components will remain in solution, and test pH of final drip solution regularly	3	
4. Conduct monthly foliage analysis to determine proper nutrition program	10	
5. Base nutrient program on foliage analysis reports	5	
6. Use EC meter to formulate and test nutrient solutions	3	
7. Test water for presence of pathogens	10	
8. Are water lines flushed to reduce build up of nutrient scum	5	
9. Are any additional fertilizer supplements used (e.g. silicon/calcium)	10	
10. Is the water recycled/treated before recycling	+10	
D. General pest management		
1. Grow no other crop in production greenhouse	10	
2. Maintain weed free greenhouse	10	
3. Scout weekly for insects and diseases	10	
4. Maintain scouting and spray records	10	
5. Maintain plant free zone around greenhouse	5	
E. Disease management		
1. Maintain adequate plant spacing for air circulation	10	
2. Ventilate when to minimize condensation and exchange with outside air daily (one full air exchange/day)	10	
3. After fruit set, remove senescing leaves below fruit* and remove from greenhouse area (*snap off at natural abscission zone)	10	
4. Remove any diseased foliage or fruit and remove from greenhouse area	10	
5. Remove flowers from bottom of fruit to minimize disease spread	5	
6. No smoking by anyone touching foliage or greenhouse structure	3	
7. Anyone touching plants should was hands and disinfect tools before entering and between houses	10	
8. Apply fungicides only if disease is present	10	
F. Insect management		
1. Screen openings whenever possible	5	
2. Monitor insects with yellow sticky cards. Check weekly. Change when needed.	10	
3. Begin releases of appropriate natural enemies at recommended rates and intervals at the first sign of insect pests.	10	
4. Use insecticides only against those pests for which effective natural enemies are not available.	10	

G. Contamination minimization		
1. Footbaths used to prevent walking of pathogens in from outside	10	
2. Dedicated PPE for workers in each greenhouse	5	
3. Concrete paths separating greenhouses	5	
4. Rouging of disease plants	10	
5. Ability to isolate contaminated lines/rows of plants	10	
H. Training		
1. Workers are trained on what pests and diseases to look for	10	
2. Workers are trained on plant hygiene	10	
3. Staff communicating with growers regularly when there is a plant abnormality	10	
4. Staff turnover is low	1	

Appendix 2. Weekly/fortnightly greenhouse disease audit

Date: _____
Farm: _____
Shed: _____
Crop: _____
Age of Crop: _____

Overall Observations:

_____
_____
_____
_____
_____
_____
____

Disease levels

Diseases/causes	Percentage plants affected	Varieties affected	Growing conditions
Fusarium/Pythium root rot			
Powdery mildew			
Downy mildew			
Angular leaf spot			
Gummy stem blight			
Viral?			
Bacterial canker			
Other			

Insect levels

Insect	Percentage plants affected	Varieties affected	Growing conditions
Whitefly			
Thrips			
Aphids			
Mites			
Moths/Caterpillers/Eggs			
Fungus gnats			
Other			