



Know-how for Horticulture™

**Integrated
management of
greenhouse cucumber
and capsicum
diseases**

Len Tesoriero
NSW Agriculture

Project Number: VG00069

VG00069

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FINAL REPORT

Integrated management of greenhouse cucumber and capsicum diseases

HAL Project No. VG 00069

(2000 - 2004)

Len Tesoriero, & Fiona Bertus

**Elizabeth Macarthur Agricultural Institute, Menangle
& Gosford Horticultural Institute**

May 2004



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MEDIA SUMMARY

The project has focussed on major disease problems in greenhouse cucumbers and capsicums. Extensive disease surveillance over the three year course of the project has updated Australian pathogen records. New Australian records include the detection of a whitefly-transmitted virus, a fungal wilt disease and a fungal leaf spot on cucumbers.

Wilts associated with fungal root and stem rots were found to contribute to an estimated 30% of crop losses in cucumbers throughout Australia. The fungi, *Fusarium oxysporum* f. sp. *radicis-cucumerinum* and a range of *Pythium* species were found responsible for these extensive crop losses, often occurring in combination as a disease complex.

As a direct result of the crop surveys, trials into the control of this disease were initiated, in order to identify cultural, chemical and biological options. The integrated management of this disease through hygiene and sanitation, temperature and moisture control, chemical use and the introduction of microbial biological agents enabled greenhouse vegetable producers to reduce crop losses and maximise efficiency on-farm.

Hygiene and sanitation proved crucial to the management of *Fusarium* root and stem rot, as the fungus was shown to be spread aurally and with sciarid flies. Reducing extremes in temperature and root zone moisture limits the potential for infection. Any of eight different species of the water mould *Pythium* were identified. Some are known to be associated with high temperatures and others with low temperatures.

Of the chemical and microbial biocontrols evaluated, neither were found not to be curative. In some cases biocontrols halved losses in affected crops. Chemicals performed better (losses one-sixth of untreated plants) but there are problems with the lack of relevant crop registrations and untested compatibility with other biocontrols. These factors inhibit their adoption in disease management programs.

Trials were also aimed at the development of bioassays for rapid screening of microbial biocontrols but results were inconsistent. The addition of microbial biocontrols in some assays induced greater losses, and better results were achieved with small-scale greenhouse trials and on-farm trials.

Future research is required to develop improved chemical and microbial biocontrols, and appropriate use-patterns. Combinations of compatible microbial biocontrols that have different modes of action are required to obtain wider efficacy for disease complexes occurring on greenhouse cucumbers. Another approach is cross-protection, for example where non-pathogenic *Fusarium* isolates successfully control similar wilt diseases on tomatoes in overseas research.

TECHNICAL SUMMARY

A number of important diseases of greenhouse cucumbers and capsicums have been identified in this project through surveillance activities in major production areas of Australia. Of particular note was the first detection in Australia of a root and stem rot caused by the fungus *Fusarium oxysporum* f.sp. *radicis-cucumerinum*. This disease is now known to occur in all other major greenhouse cucumber production areas of the world and is considered to be one of the primary causes of crop losses. When affected plants were analysed further in this study, *Fusarium* was found to occur in combination with any of ten species of another root pathogen, *Pythium*. In many cases combinations of these pathogens were shown to accelerate disease development, hastening the onset of wilting symptoms and resulting in increased mortality.

Other highlights of the disease surveillance were the detection of the fungus, *Alternaria alternata* causing a leaf spot disease. Previously, another species of this fungus (*A. cucumerina*) was the only fungus causing a similar leaf disease on cucumbers in Australia. Plants sprayed for Downy Mildew with the strobilurin fungicide, Amistar®, were observed to have also been controlled of Alternaria Leaf Spots. An extension to the existing label or specific permits for these diseases is recommended.

Virus-like disease symptoms were observed in several NSW and SA crops. Plants were sometimes stunted, leaved displayed downward rolling and yellowing between veins. These symptoms could have easily been confused with certain nutritional disorders, but a number of observations of crops suggested they were caused by an infectious agent. Laboratory diagnosis revealed that a virus is responsible and is consistent with Beet pseudo-yellows virus. This virus is transmitted by greenhouse whiteflies and is known to occur in greenhouse cucumber crops overseas.

Several trials were conducted to evaluate the efficacy of chemical and biological controls for the root rot diseases. Economical control was achieved with chemical drenches of benomyl with either furalaxyl, or propamocarb. However, these chemicals have no current registrations or permits for use as drenches on cucumbers. Furthermore, benomyl was recently withdrawn from sale in Australia and has been shown to be incompatible with several biological controls (including certain predatory insects and mites). Alternative chemicals for control of *Fusarium* have been identified but need evaluation and permits for their use sought. Biological control of these diseases with a product containing the fungus, *Trichoderma harzianum*, was variable. In some bioassays and on-farm trials this product appeared to reduce disease severity of *Fusarium*. In some cases plant losses were halved with these treatments. However, *Trichoderma* afforded no suppression of a root rot disease caused by *Pythium aphanidermatum*. In contrast, a biocontrol product with the bacterium *Bacillus subtilis* as its active ingredient, did reduce symptoms of *P. aphanidermatum*, but had no effect on *Fusarium*. Combinations of these two biocontrols appeared to be incompatible and did not suppress disease in any of the on-farm trials where both *Fusarium* and *Pythium* were present. Similarly, a biostimulant (fulvic acid) failed to suppress these diseases in any on-farm trials. Another feature of these trials was that chemical and biological treatments were not effective when applied after disease symptoms had appeared (i.e. as curatives). In contrast, the best efficacy of these products was achieved when the first drench was applied to seedlings (i.e. as preventatives).

Various cultural controls for these diseases were identified in this project. Disease incidence and severity were reduced when environmental stresses were minimised, and vice versa. For example, extremes in temperatures were more conducive to the development of *Pythium* root rots. On one hand, high temperatures (>30°C) were associated with root rots caused by *P. aphanidermatum* and *P. deliense*, whereas low temperatures (<10°C) were conducive to disease expression by *P. irregulare* and an unidentified *Pythium* sp. In contrast, greenhouse structures with regulated environmental controls had much lower levels of these diseases. High moisture levels in the root zone were also strongly associated with increased incidence and severity of *Pythium* and *Fusarium* diseases, particularly in combination with temperature extremes.

Poor on-farm hygiene and sanitation enabled these diseases to spread rapidly. Even where new substrate media (sawdust and cocopeat) were used, these pathogens spread rapidly and resulted in approximately one-third of plants dying within sixteen weeks of transplanting. *Fusarium*, in particular, was shown to spread aerially from typical pink spore masses that formed on affected stems. This is consistent with overseas experience with this disease. Spread of *Fusarium* and *Pythium* was demonstrated with fungus gnats (sciarid flies). This exacerbated damage caused by their larvae feeding on cucumber roots and was often associated with the greatest losses in surveyed crops and some greenhouse trials. Handling infected plants was also identified to spread *Fusarium*, particularly via spores from the pink masses that cover affected stems. Wounds on stems facilitated *Fusarium* infections. Fresh cuts after pruning or picking fruit, and growth cracks on lower stems, were common infection points.

This project has identified a serious disease complex responsible for big losses in greenhouse cucumber production. Chemical, cultural and biological management strategies for this disease have been identified. We have commenced evaluation of chemical and biological options, but further work is needed to overcome their current limitations. Chemicals that are biorational (compatible with other IPM practices) and microbial biocontrols that work more consistently and with greater efficacy need to be developed urgently.

INTRODUCTION

Greenhouse production of cucumbers and capsicums has developed rapidly in the last decade. It provides larger urban areas of Australia with a ready supply of fresh products with minimal transport costs. Other advantages are the more efficient use of water resources, fertilisers and reduced environmental degradation. Protective structures provide a strategic capacity to feed large urban areas and market stability when adverse weather conditions affect outdoor production systems.

Plant diseases can cause significant losses to greenhouse cucumber crops and currently require a high chemical use for their control. Greenhouse-grown capsicums appear to suffer from fewer diseases to date but this situation is likely to change as the industry develops and plant pathogens spread. Soil-borne diseases have led some growers to rely upon fumigation treatments. Besides the high annual cost of these treatments, poor application practices and easy reintroduction of pathogens due to poor hygiene and sanitation have resulted in frequent disease outbreaks. Some fumigants are also known to have a negative impact on the environment by leaching into groundwater or depleting the ozone layer. Furthermore, they are highly toxic chemicals that pose a significant risk to farm workers and neighbours.

Many growers have adopted soilless production systems as a means of avoiding many diseases. However, diseases such as *Pythium* and *Fusarium* root rots are just as important in these production systems. This problem is exacerbated by the fact that there are no chemicals registered for most soil-borne pathogens in cucumbers. Similar problems exist for some foliar diseases (eg. *Botrytis* blights and rots).

There has been a rapid development and availability of biological control products worldwide, yet many of these products have not been objectively evaluated for efficacy. Similarly, many products have not been validated as part of integrated crop management systems for Australian conditions. Some research work in New Zealand demonstrated that the use of the microbial biocontrol, *Trichoderma*, had only little effect on controlling a *Pythium* root rot of cucumbers. Furthermore, the way the product was applied made a significant difference to its efficacy.

This project sought to identify and evaluate improved disease management strategies and facilitate their adoption through on-farm trials, educational resources and workshops. Surveillance in the major Australian production areas updated our records on the disease incidence and distribution on greenhouse cucumbers and capsicums.

Adoption of improved and safer disease management strategies based on IPM is necessary for the environmental and economic sustainability of the greenhouse cucumber and capsicum industries. We urgently need to developing safer chemical, biological and cultural options that: reduce direct losses; comply with food safety standards regarding chemical residues; lessen OH&S hazards to crop workers; and protect neighbours and the environment. This is especially pertinent in peri-urban areas of production.

I. National survey of greenhouse cucumber & capsicum diseases

Introduction

Over 240 samples were formally collected and diagnosed during the project from 2001-2004

These included:

- targeted surveys for rot rots and viral diseases
- samples collected from site visits and in response to grower concerns
- samples submitted to the Plant Health Diagnostic Service at EMAI

See Appendix 1 for list of samples and determinations.

Methods

Disease Surveys

Twenty-six properties were surveyed and/or samples were received from NSW (Sydney Basin, Gosford, Picton, Coffs Harbour, Milton and Sunrasia), seven from South Australia (North Adelaide Plains, Virginia), two from Queensland (Townsville & Bundaberg) and one from Western Australia (South Perth). Further state records were obtained from relevant government authorities.

Results and Discussion

Results of disease surveys and state records are listed in Tables 1 and 2. The first targeted survey in NSW coincided with the commencement of the winter 2001 crop. Many growers had commenced using cocopeat as a root substrate. This provided an opportunity to access and compare root diseases (rots, damping-off and wilts) in four media: cocopeat; sawdust; compost mix; and NFT. Several *Pythium* species and *Fusarium oxysporum* were the major root pathogens detected.

Excess root zone moisture was found to be a common factor associated with higher incidence and severity of *Pythium* rots and wilts. Certain farms using higher irrigation rates in cocopeat and compost media suffered significant losses. An experiment at the National Centre for Greenhouse Horticulture, Gosford showed a strong correlation between NFT and *Pythium* root infection that resulted in almost a complete plant losses. In contrast, plants growing in cocopeat bags in this facility were largely unaffected.

The fungal disease, Gummy stem blight, was commonly found associated with longitudinal splitting of lower stems. This often resulted in wilting and death of mature plants. The longitudinal splits were likely to have been caused by a combination of rapid plant growth and large diurnal temperature ranges.

The fungi *Alternaria alternata* and *A. cucumerina* were detected on leaf spots in NSW and SA. These are the first Australian records of a leaf spot disease caused by *A. alternata*. This pathogen has previously been recorded in Greece (Vakalounakis, 1990). Since no curative fungicides are specifically registered for these diseases in

Australia, the NSW greenhouse growers' association and the NSW vegetable IDO were contacted to seek a permit or an extension to the label registration of azoxystrobin (Amistar ®).

It should also be noted that there are a number of gaps in chemical registrations for both cucumbers and capsicum diseases. There are no chemicals registered for control of Botrytis rots, Black root rot, Fusarium root and stem rot, and Rhizoctonia root rot on cucumbers. There are no registrations for the control of Pythium root rots except for seedling mix amendments of metalaxyl as a granular formulation. This treatment is inappropriate where seeds are germinated in rockwool block, and does not afford sufficient protection for post-transplant onset of disease, especially where a disease complex is formed with *Fusarium*. The problem with chemical use in greenhouse cucumbers is that it is difficult to comply with withholding periods as fruit is picked regularly (every 2-3 days) at certain times of the year. There is a need to obtain registrations for 'soft' chemicals and microbial biocontrols.

The incidence of Black root rot was restricted to the Sydney Basin in this study and was much lower than in previous crop surveys in the early 1990s (Tesoriero, unpublished). This largely reflects the move from soil to soilless (bagged media and rockwool) production. The only incidence where this disease was recorded in a soilless medium (composted greenwaste bags) was adjacent to tunnel houses where soil production has persisted.

A virus disease found commonly on cucumbers in NSW and SA was identified as Beet pseudo-yellow virus (also called Cucumber yellow virus). This is the first report of this virus on cucumbers in Australia. It has been previously reported in Tasmania on weed species (Duffus & Johnstone, 1981). This virus is transmitted by greenhouse whiteflies.

Colour Page I:

Surveillance for greenhouse cucumber diseases

Captions

- Map of Australia with survey locations: Townsville, Bundaberg, Coffs Harbour, Gosford, Sydney, Bargo, Adelaide, Perth
- Fiona Bertus examines plants for disease symptoms
- Wilting cucumber plant associated with *Pythium* root rot
- Severely affected crop with *Pythium* root rot

TABLE 1. List for greenhouse cucumber diseases in Australian greenhouse cucumbers

NOTE: This list is not to be used for quarantine purposes. State records for several diseases are incomplete for greenhouse production.

DISEASE	PATHOGEN	NSW	SA	QLD	WA	COMMENTS
Fungal						
Alternaria leaf spots	<i>Alternaria cucumerina</i> <i>Alternaria alternata</i>	✓ ✓	✓ ✓	✓	✓	<i>A. alternata</i> not previously recorded
Anthrachnose	<i>Colletotrichum orbiculare</i>	✓	✓	✓	✓	
Botrytis Rots (Grey Mould)	<i>Botrytis cinerea</i>	✓	✓	✓	✓	Mainly found as flower infection and associated with abortion of young fruit. Also stem rots.
Black Root Rot	<i>Phomopsis sclerotioides</i>	✓				Records from compost media & soil
Black Root Rot	<i>Thielaviopsis</i> sp. (= <i>Chalara</i> sp.)	✓	✓			
Damping-off	<i>Rhizoctonia solani</i>	✓	✓	✓	✓	Wilting seedlings
Damping-off / Root rot	<i>Pythium</i> spp.	✓	✓	✓	✓	Species vary with seasons (temperature)
Fusarium foot rot & wilt	<i>Fusarium solani</i> <i>Fusarium oxysporum</i>	✓	✓	✓	✓	Disease complex with <i>Pythium</i> species
Root rot	<i>Phytophthora</i> sp.	✓				
Downy mildew	<i>Pseudoperonospora cubensis</i>	✓	✓	✓	✓	
Powdery mildew	<i>Sphaerotheca fuliginea</i> (anamorph = <i>Oidium</i> sp.)	✓	✓	✓	✓	
Gummy stem blight	<i>Didymella bryoniae</i> (anamorph = <i>Phoma cucurbitacearum</i>)	✓		✓		Associated with splitting of lower stems
Sclerotinia Rot	<i>Sclerotinia sclerotiorum</i>	✓	✓	✓	✓	
Bacterial						
Angular leaf spot	<i>Pseudomonas syringae</i> pv. <i>lachrymans</i>	✓	✓	✓	✓	
Bacterial leaf spot	<i>Xanthomonas campestris</i> pv. <i>cucurbitae</i> <i>Pseudomonas syringae</i> pv. <i>syringae</i>	✓ ✓		✓		Seedling leaf lesions
Bacterial seedling blight	<i>Acidovorax avenae</i> subsp. <i>Citrulli</i> <i>Acidovorax konjaci</i>	✓		✓		Seed-borne disease Cocktail cucumber seedlings

DISEASE	PATHOGEN	NSW	SA	QLD	WA	COMMENTS
Viruses						
Mosaic Diseases	Cucumber Mosaic Virus	✓	✓			
	Tomato Spotted Wilt Virus	✓	✓			Mild leaf mosaic masked by thrips injury
	Zucchini Yellow Mosaic Virus	✓	✓	✓		
Cucumber Yellows	Beet Pseudo-Yellows Virus	✓	✓			First record on cucumbers in Australia
Nematodes						
Root Knot	<i>Meloidogyne</i> sp.	✓	✓	✓	✓	

TABLE 2. LIST FOR GREENHOUSE CAPSICUM & CHILLI DISEASES

NOTE: This list is not to be used for quarantine purposes. State records for several diseases are incomplete for greenhouse production.

DISEASE	CAUSE	NSW	SA	QLD	WA	COMMENTS
Alternaria rots	<i>Alternaria</i> sp.	✓	✓	✓		
Anthracnose	<i>Colletotrichum</i> spp.	✓	✓	✓		
Downy Mildew	<i>Peronospora tabacina</i>	✓	✓			Old records, not from greenhouse crops
Collar & Root Rot	<i>Rhizoctonia solani</i>	✓	✓	✓	✓	
Grey Mould	<i>Botrytis cinerea</i>	✓	✓	✓	✓	
Powdery Mildew	<i>Leveillula taurica</i> (anamorph= <i>Oidiopsis</i> sp.)	✓	✓	✓	✓	
Root Rot	<i>Pythium</i> spp.	✓	✓	✓	✓	
Root Rot	<i>Fusarium</i> spp.	✓	✓	✓	✓	
Root Rot	<i>Phytophthora</i> spp.	✓	✓			
Sclerotinia Rot	<i>Sclerotinia sclerotiorum</i>	✓	✓	✓	✓	
Verticillium Wilt	<i>Verticillium dahliae</i>	✓		✓		
Bacteria						
Bacterial Wilt	<i>Ralstonia solanacearum</i>			✓		
Bacterial canker	<i>Clavibacter michiganensis</i>			✓		
Bacterial Leaf spot	<i>Xanthomonas campestris</i>	✓		✓	✓	
Bacterial Leaf spot	<i>Pseudomonas syringae</i>	✓				

DISEASE	CAUSE	NSW	SA	QLD	WA	COMMENTS
Viruses						
Mosaic	Pepper Mild Mottle Virus	✓	✓	✓		
	Cucumber Mosaic Virus	✓	✓	✓	✓	
	Potato virus Y	✓	✓	✓		
	Tomato Aspermy Virus		✓			
	Tomato Spotted Wilt Virus	✓	✓	✓	✓	
Nematodes						
Root Knot Nematodes	<i>Meloidogyne</i> spp.	✓	✓	✓	✓	

Alternaria

Anthracnose

Botrytis

black root rot

Pythium root rot

Fusarium

Downy & Powdery

Gummy stem blight

Angular leaf spot

CMV

TSWV

Potviruses & Closterovirus

Nematodes

II. Information Transfer

Extension activities and outputs were done collaboratively with two separately-funded HAL projects, and via Vegetable IDOs. Farm visits provided one-on-one discussion of specific disease management issues.

Below is a summary of group presentations, training, extension outputs and scientific publications arising from this project to date:

Industry workshops & seminars

- Rossmore, Sydney, June 2002 – Disease recognition workshop
- Virginia, SA, March 2002 - Disease recognition & project progress seminar
- Virginia, SA, April 2004 – Project summary seminar
- Bundaberg, Qld., 2004 – Project review/summary and IPM workshop
- Perth 2004 – Project summary, and disease recognition and management workshop

Contributions to technical resources

- *Know your diseases* (Section #6); *Plant health management* (in Section#1); and *Registered chemicals for common diseases in greenhouse vegetables* (Handy Guide #5) in *Integrated Pest Management in Greenhouse Vegetables – Information Guide* (Goodwin *et. al.*, 2002).
- *Fungal diseases, Bacterial diseases, Viral diseases & Nematodes* in *Pests, Diseases, Disorders and Beneficials in Greenhouse Vegetables: Field Identification Guide* (Goodwin *et. al.*, 2002).
- *Common diseases of greenhouse cucumbers*. Wall poster for growers (Tesoriero & James, 2004).

Conference papers, posters and abstracts

- Paper presented at the AHGA Conference, Central coast, NSW, 2001 (Tesoriero, 2001)
- Abstract & Poster presented at IX International Fusarium Workshop, Sydney, 2003 (Tesoriero *et. al.*, 2003)
- Paper presented at AHGA Conference, Melbourne, 2003 (Tesoriero, 2003)
- Paper & poster presented at the 3rd Australasian Soil-Borne Diseases Symposium, Barossa Valley, South Australia, 2004 (Tesoriero *et. al.*, 2004)

Colour Page II: Information transfer: IPM training

Captions

- Participants at IPM workshop, Bundaberg
- Training exercise in crop monitoring for diseases and pests, Shepparton

III. Tests to confirm identity & pathogenicity of *Fusarium* & *Pythium* isolates

Introduction

Farm surveys of Australian greenhouse cucumber crops identified a fungal wilt and stem rot as the most significant disease. A *Fusarium oxysporum* and various species of *Pythium* were consistently isolated from wilting plants. Observations of affected plants also noted that stems infected with *Fusarium* often developed pink-coloured fungal spore masses (sporodochia). Air-borne spread of the disease is not typical of diseases previously attributed to *Fusarium* (*F. oxysporum* f.sp. *cucumerinum*) in Australia. Disease symptoms are consistent with Greek (Vakalounakis, 1996) and Canadian (Punja & Parker, 2000) reports that have described a new strain of *Fusarium* (*F. oxysporum* f.sp. *radicis-cucumerinum*). Host range and taxonomic studies were required to characterise the Australian isolates. Further trials were designed to assess the relative aggressiveness of the different species of *Pythium*, and to determine the combined effects of combined infections of *Fusarium* and *Pythium* in cucumbers.

Methods

A series of experiments were designed to confirm pathogenicity of fungal isolates and to develop rapid and reliable bioassay methods. Diagnostic investigations of diseased plants in farm surveys revealed that most plants were infected with both *Fusarium oxysporum* and any one of eight different *Pythium* species. The first series of trials were conducted in various media, (Growool®, cocopeat or a commercial mix based upon composted green-waste). The second set of assays investigated a method where cucumber seedlings were suspended in plastic cups. Trial methods are detailed separately below.

A disease severity index (Table 3) was developed during the trials to score relative growth, wilting and hypocotyl rot symptoms. This was used for trials of pathogenicity and later for evaluation of microbial biocontrol efficacy.

Table 3. Disease Severity Index for *Fusarium* & *Pythium* Rots

SCORE	SYMPTOMS	AND/OR	AND / OR
0	No visible symptoms		
1	Pale-coloured hypocotyl or stem base	Stunting	No wilting; or leaves angled down
2	Yellow / brown hypocotyl or stem base	Stunting	Slight wilt and /or yellowing
3	Fungal mycelium visible or brown hypocotyl lesion or stem base	Stunting	Moderate wilt (leaves angled down, dehydration in 20-40% of leaves) and leaf yellowing
4	Brown, necrotic lesion or stem base	Stunting	Severe wilt (leaves angled down, dehydration in 40-100% of leaves) and leaf yellowing
5	Dead (Permanently wilted)		

Colour page III:

Rating cucumber seedlings for root and stem rot

Disease score 0: Healthy green hypocotyl; no visible disease

Disease score 1: Pale-coloured hypocotyl or stem base; stunting or leaves angled down

Disease score 2: Yellow/brown discoloured hypocotyl or stem base; stunting; slight wilt (leaves angled down, dehydration in <20% of leaves) and/or leaf yellowing

Colour page IV:

Rating cucumber seedlings for root and stem rot

Disease score 3: Fungal mycelium visible or brown hypocotyl or stem base; stunting; moderate wilt (leaves angled down, dehydration in 20-40% of leaves) and leaf yellowing

Disease score 4: Brown, necrotic hypocotyl or stem base; stunting; moderate wilt (leaves angled down, dehydration in 40-100% of leaves) and leaf yellowing

Disease score 5: Permanent wilt; plants dead

A. Trial 1

Cucumber susceptibility bioassay for *Fusarium* and *Pythium* isolates: Bioassay for seedling wilt in Growool blocks.

Methods

Three cultivars of Lebanese cucumber seedlings (cvs *Tadros*, *Mascot* and *Deena*) were established in Growool® blocks (75x75x65mm). Treatment plants were drenched through the surface of blocks with inocula, prepared by blending agar cultures of fungal isolates. *Fusarium* treatments received approximately 10^5 colony forming units (cfu) and *Pythium* received 10^3 cfu (determined by haemocytometer counts and/or most probable number estimates). Plants were set out in randomised complete blocks on greenhouse benches (average min/max temperatures: 8-25°C). Control treatments consisted of plant roots drenched with agar suspensions minus fungal inoculum. Plants were monitored regularly for 43 days. They were watered and fertilised as required and disease symptoms recorded. Tissue from permanently wilted plants was plated to agar media to confirm fungal infection.

Results:

The 3 combined *Fusarium* and *Pythium* treatments had mean mortality (days to 100% permanent wilting) of 21 days. In contrast, uninfected controls and plants receiving two of the three *Fusarium* isolates had not wilted at the conclusion of the trial. A *Pythium irregulare* isolate averaged 27 days to mortality; a *P. spinosum* (28 days); *Pythium*. sp. [globose swellings] (30 days); and *Fusarium oxysporum* [isolate 506] (31 days).

B. Trial 3

Cucumber susceptibility bioassay for *Fusarium* and *Pythium* isolates: Seedling bioassay in Growool® blocks

Method

Cucumber (cv. *Tarduna*) seedlings were established in Growool® blocks. Treatment plants were drenched through the surface of blocks. Inocula were prepared by blending agar cultures of fungal isolates. Treatments received 10^6 - 10^7 cfu (determined by haemocytometer counts) of *Fusarium* and 10^2 - 10^4 cfu of *Pythium* (determined by most probable number technique). Control treatments consisted of plant roots drenched with agar suspensions minus fungal inoculum. Plants were set out in randomised complete blocks (6 replications x 17 treatments) on greenhouse benches (Temperature range: 15-37°C). Roots of half of the plants that received fungal isolates, #1 & 2, were damaged by cutting with a scalpel. Plants were monitored regularly for 40 days after inoculation, and were watered and fertilised as required. Disease symptoms scored using disease severity index. Sub-samples of each treatment were plated to agar media to confirm fungal infection.

Results:

Results for damaged and undamaged plants were combined as were found not to be statistically different. Data is presented in Table 4.

Table 4. Mean Disease Score, Cumulative & % Mortality of cucumber seedlings at 40 days

Treatments	Mean Disease Score (0-5)	% Mortality
Nil inoculum control	0	0
<i>Fusarium oxysporum</i> #1	2.6	8
<i>Pythium</i> #1 (<i>P. irregulare</i>)	0.6	0
<i>Fusarium</i> #1 + <i>Pythium</i> #1	3.8	25
<i>Fusarium oxysporum</i> #2	2.6	0
<i>Pythium</i> #2 (<i>P. irregulare</i>)	1.7	8
<i>Fusarium</i> #2 + <i>Pythium</i> #2	3.7	33
<i>Fusarium oxysporum</i> #3	2.2	0
<i>Pythium</i> #3 (<i>P. irregulare</i>)	2.3	0
<i>Fusarium</i> #3 + <i>Pythium</i> #3	3.7	17

C. Trial 4

Cucumber susceptibility bioassay for *Fusarium* and *Pythium* isolates: Seedling bioassay in Growool® blocks

Method

As per Trial 3 except that different fungal isolates were used. Plants were observed for 42 days. There were 5 replications x 22 treatments.

Results:

There were no mortalities in this trial.

Table 5. Mean disease score of cucumber seedlings at 42days at Day 38

Treatments	Mean Disease Score
Nil inoculum control	0
<i>Fusarium oxysporum</i> #4	0.2
<i>Pythium</i> #4 (<i>P. irregulare</i>)	1.5
<i>Fusarium</i> #4 + <i>Pythium</i> #4	1.6
<i>Fusarium oxysporum</i> #5	0
<i>Pythium</i> #5 (<i>P. irregulare</i>)	1.5
<i>Fusarium</i> #5 + <i>Pythium</i> #5	1.9
<i>Fusarium oxysporum</i> #6	0.3
<i>Pythium</i> #6(<i>Pythium spinosum</i>)	2.8
<i>Fusarium</i> #6 + <i>Pythium</i> #6	3.1

D. Trial 5

Cucumber susceptibility bioassay for *Fusarium* and *Pythium* isolates: Seedling bioassay in Growool® blocks

Method

As per Trial 3 except that different fungal isolates were used. There were 5 replications x 26 treatments. Plants were monitored regularly for 38 days.

Results

Table 6. Mean Disease Score of cucumber seedlings at 38 days

Treatments	Mean Disease Severity Rating (0-5 scale) at 38 days	
	Undamaged + Damaged roots	% Mortality
Nil inoculum control	0.2	0
<i>Fusarium oxysporum</i> #1	2.6	10
<i>Pythium</i> #1 (<i>P. irregulare</i>)	0.3	30
<i>Fusarium</i> #1 + <i>Pythium</i> #1	3.2	0
<i>Fusarium oxysporum</i> #2	2.6	0
<i>Pythium</i> #2 (<i>P. irregulare</i>)	2.9	10
<i>Fusarium</i> #2 + <i>Pythium</i> #2	4.8	90
<i>Fusarium oxysporum</i> #7	3.4	10
<i>Pythium</i> #7 (<i>P. aphanidermatum</i>)	1.9	0
<i>Fusarium</i> #7 + <i>Pythium</i> #7	4.6	80
<i>Fusarium oxysporum</i> #8	3.4	20
<i>Pythium</i> #8	0.4	0
<i>Fusarium</i> #8 + <i>Pythium</i> #8	4.1	40

E Trial 6

Capsicum susceptibility bioassay for *Fusarium* and *Pythium* isolates: Seedling bioassay in Growool® blocks

Method

Capsicums were established in Growool® blocks and grown to flowering in a nursery (28 days). Inocula were prepared as in Trial 3. There were 6 replications of trays holding three plants. Average min/max temperatures were: 25-35°C. Plants were monitored regularly for 15 days. They were watered and fertilised as required and disease symptoms recorded. Tissue from permanently wilted plants was plated to agar media to confirm fungal infection. *Fusarium oxysporum* (isolates Fus 666 & 680), *Pythium aphanidermatum* (isolate Pyth 01/235)

Results

Plants of the combined inocula treatment: *Pythium* and *Fusarium oxysporum* (666) wilted in 7 days. The combination of *Pythium* with *Fusarium oxysporum* (680) caused plants to slightly wilt at 11 days. No disease symptoms were evident on either of the two treatments receiving *Fusarium oxysporum* alone. The *Pythium* treated plants showed an initial wilting at 11 days, then recovered.

F. Trial 10

Cucumber susceptibility bioassay for *Fusarium* and *Pythium* isolates in 2 cucumber varieties.

Methods

Two cultivars of Lebanese cucumber seedlings (*Tarduna* & *Montana*) were established in Growool® blocks. Inocula were prepared as in Trial 3. There were 5 replications x 2 plants x 6 treatments. Plant roots were not damaged. Temperature range: 16-37°C. Plants were monitored regularly for 36 days. All plants were plated to agar media to confirm fungal infection. Stem lengths of surviving plants were measured at Day 36. Stems and leaves were dried in oven for 4 days and weighed.

Results

Table 7. Mean disease score & % mortality of cucumber seedlings at 36 days

Treatments	Mean Disease Severity Rating (0-5 scale) at 36 days	
	<i>Tarduna</i> (% Mortality)	<i>Montana</i> (% Mortality)
Nil inoculum control	1.0 (10)	1.3 (20)
<i>Fusarium oxysporum</i> #2	3.8 (10)	3.6 (30)
<i>Pythium</i> #2 (<i>P. irregulare</i>)	3.3 (10)	2.9 (0)
<i>Fusarium</i> #2 + <i>Pythium</i> #2	5.0 (100)	5.0 (100)
<i>Fusarium oxysporum</i> #7	4.4 (70)	5.0 (100)
<i>Pythium</i> #7(<i>P.aphanidermatum</i>)	4.2 (60)	4.1 (50)
<i>Fusarium</i> #7 + <i>Pythium</i> #7	5.0 (100)	5.0 (100)

Table 8. Mean Dry Weights and Stem Lengths

Treatments	Dry Weights (g)		Stem Lengths (cm)	
	<i>Tarduna</i>	<i>Montana</i>	<i>Tarduna</i>	<i>Montana</i>
Nil inoculum control	3.63	3.00	47.44	34.07
<i>Fusarium oxysporum</i> #2	3.31	1.57	34.38	18.64
<i>Pythium</i> #2	2.22	1.17	26.78	11.20
<i>Fusarium</i> #2 + <i>Pythium</i> #2	0.83	0.47		
<i>Fusarium oxysporum</i> #7	2.24	0.98	38.33	
<i>Pythium aphanidermatum</i> #7	0.47	0.39	11.25	8.17
<i>Fusarium</i> #7 + <i>Pythium</i> #7	0.21	0.26		

G. Trial 35

Cucumber susceptibility bioassay for *Fusarium* and *Pythium* isolates: Seedling bioassay in Growool® blocks

Method

Cultivar *Mascot* planted into Growool® cubes. Inocula prepared as in Trial 3. *Pythium* inoculum had 1.2×10^5 colony forming units of *Pythium* (most probable number estimate). There were 15 replications x 3 plants x 4 treatments. Temperature range: 14-48°C (average 22.8 °C).

Plants were monitored regularly for 33 days. All dead plants were plated to agar media to confirm fungal infection. Stem heights of surviving plants were measured at Day 33. Stems and leaves were dried in oven for 4 days and weighed.

Results

Table 9. Mean Disease Severity Rating and % Mortality

TREATMENT	DISEASE SCORE DAY 33	% MORTALITY DAY 33
Control	0.09 a	0 a
<i>Fusarium</i> #7	4.76 c	87 b
<i>Pythium</i> #6	2.42 b	0 a
<i>Fusarium</i> #7+ <i>Pythium</i> #6	4.47 c	64 b

Table 10. Mean Dry Weights and Stem Lengths

TREATMENT	DRY WEIGHT (g)	STEM LENGTHS (cm)
Control	3.30 a	43 a
<i>Fusarium</i> #7	1.33 c	13 d
<i>Pythium</i> #6	2.16 b	32 b
<i>Fusarium</i> #7+ <i>Pythium</i> #6	1.35 c	25 c

Conclusions

We confirmed that *Fusarium* and *Pythium* isolates from diseased cucumbers were the causal agents. *Fusarium* isolates reproduced a root and stem rot disease in inoculated cucumber seedlings. Typical hypocotyl lesions formed as well as pink spore masses (sporodochia) on affected stems. Vascular colonisation by *Fusarium* led to plants collapsing as observed in field surveys. Some control plants became infected late in the trials, confirming aerial transmission of this pathogen. Transmission was also demonstrated with sciarid flies and their larvae. *Pythium* isolates (*P. irregulare*, *P. aphanidermatum* and *P. spinosum*) were also shown to cause root rot diseases and resulted in many plants permanently wilting. In three trials (#1, 5, & 10) there appeared to be synergistic interactions between certain *Fusarium* and *Pythium* isolates. The combined inoculation of *Fusarium* and *Pythium* greatly increased the severity of disease expression, and hastened permanent wilting. Further work is required to determine, more accurately, the nature of this interaction.

Colour page V:

Bioassays to test pathogenicity of *Pythium* and *Fusarium* isolates

Captions

- Arrangement of treatments in cucumber seedling trial

Control, *Fusarium*, *Pythium*, *Fusarium* + *Pythium*

- Comparison of plants from pathogenicity trial
- Cucumber seedling inoculated with *Fusarium* + *Pythium*

IV. Farm scores for incidence and distribution of wilting cucumber plants

Trial 7

Aims

Collect disease incidence and distribution data from commercial greenhouse crop. Make photographic records of disease symptoms and isolate pathogens.

Method

Wilting and healthy mature plants were counted in a multispan greenhouse. Cucumber plants (cvs *Montana* and *Cobra*) were growing in 'cucumber mix' (a commercially composted green-waste product) in 5-litre bags. Each bag had been sown with two seedlings at the first true leaf stage. One row of cv. *Montana* and eight rows of cv. *Cobra* filled one span. Rows consisted of 80 bags running in an East-West direction. Diurnal temperatures ranged from 5-30°C. Further counts were taken of seedlings planted into used 'cucumber mix' and an adjacent span containing cocopeat bags that had previously grown tomatoes. Treatments were: cvs *Mascot* and *Ornella* in 'cucumber mix' previously used for cucumbers; and cv. *Ornella* in cocopeat, previously used for tomatoes. Plants (except to cv. *Ornella* in used cocopeat) were scored at 66 days after transplanting for wilting and brown stem lesions.

Results

Typical symptoms of the disease were wilting plants with orange-pink stem cankers. Other plants wilted in their lower leaves only. They were mostly accompanied by a bronzing or brown lesion 0-20 cm from base of plants. Results are summarised in Table 11.

Table 11. Incidence of diseased plants in farm surveys

Cucumber cv.	Root substrate	Total plants	# Wilting	% Affected
<i>Montana</i>	Cuc mix (new)	160	33	21
<i>Cobra</i>	Cuc mix (new)	1280	807	63
<i>Mascot</i>	Cuc mix (used)	104	38	54
<i>Ornella</i>	Cuc. Mix (used)	66	38	58
<i>Ornella*</i>	Cocopeat (used)	1400	172	12

*counts at 4-leaf growth stage (14 days after transplanting). Rows 1-3 had a large disease 'hot-spot' towards the Western end of the house.

Conclusions

High plant losses were recorded (21-63% of plants wilting by 66 days). Twelve percent of plants (cv. *Ornella*) had wilting symptoms 14 days after transplanting. Previous use of media did not appear to influence the level of disease. The cultivar, *Cobra*, appeared to be more susceptible to this disease than cv. *Montana*. However, more objective work is required on cultivar susceptibility. There is no known resistance to these diseases in greenhouse cucumbers, but more tolerant cultivars may be useful in an integrated approach to reduce losses.

V. Host range tests to identify *Fusarium oxysporum* isolates

A. Trial 8. Cocopeat medium + residual roots infected with *Fusarium* and *Pythium*

This trial was a preliminary test of pathogen host range in cucurbits and tomatoes

Method: Bags (5 litre) of cocopeat media were collected from a farm where high infection rates prevailed (Trial 7). Seedlings (cotyledon stage) (see Table 12 for type and cultivar) were transplanted to bags and placed in a greenhouse (temperature range 10-32°C)

Results

Table 12. Relative survival of cucurbits and tomato to *Fusarium* and *Pythium*

Seedling	Cultivar	% Survival at 33 days
Rockmelon	Hales Best	25
	Navajo	75
	Amira	100
	Pablo	100
Cucumber	Crystal salad	92
	Marketmore	96
Watermelon	Warpaint	50
	Sensation	92
Melon	Aitana	100
	Gredos	100
Pumpkin	Qld. blue	100
Tomato	La belle	100

B. Trial 9. Cucurbit host range bioassay II: Growool® blocks +/- *Fusarium oxysporum*

Aim

Investigation of the host range for Australian isolates of *F. oxysporum* from diseased cucumbers

Method

Cucurbit seeds (see Table 13) were raised in Growool® cubes. Plants were drenched with 20 ml of *Fusarium oxysporum* (isolate 01/1092) inoculum. Fungal inoculum consisted of an aqueous suspension of freshly macerated PDA cultures. This was the equivalent to 10⁷ colony-forming units of fungal inoculum per plant. Control plants received an equivalent volume of blended PDA. Trays were arranged in a randomised block design on benches and maintained at 26°C in a greenhouse. Plants were watered so as to maintain a film (1-5mm) of moisture on the surface of trays. Complete fertilisers (Thrive^R1/10-strength) were used to maintain plant nutrition.

Results & Conclusions

Rockmelon (*Cucumis melo*) cultivars were the most susceptible hosts (Table 13). Cucumber (*Cucumis sativus*) and then Watermelon (*Citrullus vulgaris*) were next most susceptible. Other cucurbits and capsicums were tolerant to disease. These results are consistent with published host susceptibility determinations for *Fusarium oxysporum* f.sp. *radicis-cucumerinum* (Vakalounakis, 1996).

Table 13. Host Range of *Fusarium oxysporum* (isolate 01/1092 ex. Greenhouse cucumber)

Host Plant	Cultivar	% of plants permanently wilted		
		Day 12	Day 21	Day 35
Cucumber	Crystal salad	0	58	92
Rockmelon	Hales Best	58	100	100
	Pablo	Trace	75	92
Watermelon	Sensation	0	Trace	8
Pumpkin	Butternut	0	0	0
Squash	Green buttons	0	0	0
Zucchini	Black Beauty	0	0	0
Capsicum	Californian	0	0	0
	Wonder			

Colour page VI:

Seedling bioassays for host range of *Fusarium* isolates

Captions

- **Rockmelons were the most susceptible cucurbits to *Fusarium* isolates**

VI. Evaluation of microbial biocontrols, chemical & cultural controls for diseases

Introduction

Trials were carried out on NSW Agriculture sites and on greenhouse growers' properties to investigate biological control agents and to compare their effectiveness with conventional fungicides for control cucumber diseases. Trials are outlined below.

A. Efficacy of biocontrol products with *Trichoderma* and *Bacillus* to *Fusarium oxysporum* in cucumber seedlings.

Methods:

The biocontrols used were Trich-A-Soil® (*Trichoderma harzianum*) & Companion® (*Bacillus subtilis*). Two rates of each biological control were used:

Trich-A-Soil®, Rate 1 = 20g/L (1×10^9 spores/ plant = 2×10^7 spores/ml)

Trich-A-Soil®, Rate 2 = 40g/L (2×10^9 spores/ plant = 4×10^7 spores/ml)

Companion®, Rate1 = 20ml/L (1.5×10^7 CFU/plant = 3×10^5 CFU/ml)

Companion®, Rate2 = 40ml/L (3×10^7 CFU/plant = 6×10^5 CFU/ml)

The trial consisted of 8 treatments:

1. Control inoculum : Nil
2. Control inoculum : Trich-A-Soil®, Rate 2
3. Control inoculum : Companion®, Rate 2
4. Fusarium inoculum : Nil
5. Fusarium inoculum : Trich-A-Soil®, Rate 1
6. Fusarium inoculum : Trich-A-Soil®, Rate 2
7. Fusarium inoculum : Companion®, Rate 1
8. Fusarium inoculum : Companion®, Rate 2

Cucumber seeds (cv *Tarduna*) were planted in Growool® cubes and treated with biological controls the day after and then weekly for 5 weeks. Biological controls were poured onto surface of Growool® blocks. Water was used for the nil treatment.

Sixteen-day-old seedlings were inoculated with *Fusarium* culture 02/263 (9.4×10^6 conidia per plant = 4.7×10^5 conidia /ml). *Fusarium* culture had been isolated from a cucumber plant with root rot and wilt. Inocula was drenched through surface of Growool® blocks. Control treatments consisted of plant roots drenched with agar suspension minus fungal inoculum. Plants were arranged in randomised blocks (8 replications x 3 plants) in greenhouse on plastic trays, (min/max temperatures: 15-35°C). Plants were regularly monitored for 28 days after *Fusarium* inoculation. They were watered and fertilised as required and disease symptoms recorded using disease severity index. At the end of trial stem heights of surviving plants were measured and stems and leaves were dried in oven for 4 days and weighed. Root and stem tissue from permanently wilted plants and 1 plant from each replicate and treatment were plated on agar to confirm fungal infection.

Analysis of variance of the disease severity scores at day 28 was used to decide if there was any effect of treatment on the health of the plants. The scores were assumed to be normally distributed and diagnostic plots used to visually assess this assumption. The effect of the treatments on the dry weight of the plants was also assessed using analysis of variance. Treatment means were separated using the least significant difference procedure at the 5 % significance level.

At day 28 the plants were classified as being “permanently wilted” if they had a disease severity score of 3 or greater.

A Generalised Linear Model (GLM) with binomial errors and logit link was fitted to this data and the RPAIR procedure in GenStat was used to test treatment differences on the logit scale. Back-transformed means are presented in the results section.

Since all plants in treatments 7 and 8 were classified as being permanently wilted, treatment contrasts were used to compare them with other treatments.

Data were subjected to Analysis of Variance and pair-wise comparisons between treatments were tested using the standard procedure of Least Significant Difference (LSD).

Dry matter weights were analysed by an analysis of variance technique on the following model.

$$Y = \text{control} + \text{Factors}(\text{Pathogen} * \text{Fungi} * \text{Damaged} * \text{Variety}) + \text{error}$$

where Y = response variable, control = contrast against healthy plants, and the errors were assumed to have a normal distribution. Preliminary analyses showed the block effects were negligible in most cases. Differences between levels of a factor were compared using a least significant difference (LSD) test at 5% significant level.

Results

The effect of treatment on disease severity score, plant dry weight and proportion of permanently wilted plants was highly significant ($p < .001$) (Table 14).

Treatment effects on disease score, proportion of permanently wilted plants, dry weight and stem lengths are summarised in Table 14.

Disease score was significantly lower in *Fusarium* + *Trichoderma* Rate 2 treated plants as compared to *Fusarium* treated. *Fusarium* + *Bacillus* appeared to cause an increase in disease symptoms compared to *Fusarium* treated.

Plant dry weight was significantly higher in the *Bacillus* treated plants than the plants in any other treatments or control.

Table 14. Mean Disease Score, Proportion of Permanent Wilting and Dry Weight at Day 28.

TREATMENT	Number	DISEASE SCORE	proportion permanent wilting	DRY WEIGHT (g)
Control + Nil	1	0.13 a*	0.04 a	2.89 d
Control + <i>Trichoderma</i> Rate2	2	0.25 a	0.08 a	3.93 e
Control + <i>Bacillus</i> Rate 2	3	1.42 b	0.17 a	3.64 e
<i>Fusarium</i> + Nil	4	3.5 d	0.88 bc	1.36 a
<i>Fusarium</i> + <i>Trichoderma</i> Rate1	5	2.87 cd	0.70 b	1.94 bc
<i>Fusarium</i> + <i>Trichoderma</i> Rate2	6	2.21 c	0.21 a	2.43 cd
<i>Fusarium</i> + <i>Bacillus</i> Rate 1	7	4.63 e	1 c	1.77 ab
<i>Fusarium</i> + <i>Bacillus</i> Rate 2	8	4.96 e	1 c	1.39 ab

* Means in the same column with different letters beside them are significantly different at the 5% probability level.

B. Efficacy of *Trichoderma* and *Bacillus* biocontrol products against a *Pythium aphanidermatum* in cucumber plants grown in cocopeat bags.

Methods

The biological controls used were Trich-A-Soil® (*Trichoderma harzianum*) and Companion® (*Bacillus subtilis*).

Biological Control 1: Trich-A-Soil® = 10g/L (1 x 10⁹ spores/ plant = 1 x 10⁷ spores/ml)

Biological Control 2: Companion® = 10ml/L (1.5 x 10⁷ CFU/plant = 1.5 x 10⁵ CFU/ml)

The trial consisted of 8 treatments:

1. Control inoculum : Nil
2. *Pythium* inoculum : Nil
3. Control inoculum : Biological Control 1
4. Control inoculum : Biological Control 2
5. *Pythium* inoculum : Biological Control 1
6. *Pythium* inoculum : Biological Control 2

3 week old Cucumber seedlings (cv Montana) were planted into Cocopeat Easyfil™ Planterbags. 2 plants per bag with drip irrigation. Treatments were arranged in randomised blocks in plastic tunnel house (12 replicates x 2 plants). Min / max temperatures 8.8 – 38.5°C.

Companion® and Trich-A-Soil® were applied around plants weekly for 6 weeks. Water was applied for nil treatment.

Plants were inoculated with a blended *Pythium aphanidermatum* culture 02/ 749 5 days after second application of biological control. (2 x 10⁴ CFU / plant, using MPN estimates). Control treatments consisted of an agar suspension minus fungal inoculum. Plants were maintained as required and monitored for 36 days after

pathogen inoculation for disease symptoms. Fruit were harvested and counted. Root tissue from permanently wilted plants and 1 plant from each bag were plated to agar media to confirm fungal infection.

Data was analysed as in Trial A.

Results

Disease Score, mortality and fruit number results are shown in Table 15.

Table 15. Mean Disease Score, % Mortality and Number of Fruit Harvested

TREATMENT	DISEASE SCORE	% MORTALITY	NO. FRUIT PER PLANT
Control + Nil	0.13a	0	11.92b
<i>Pythium</i> + Nil	3.17c	17	9.92a
Control + <i>Trichoderma</i>	0.21a	0	13.04bc
Control + <i>Bacillus</i>	0.13a	0	14.04c
<i>Pythium</i> + <i>Trichoderma</i>	3.42c	25	9.17a
<i>Pythium</i> + <i>Bacillus</i>	2.75b	8	13.21bc

Plants treated with *Pythium* + *Bacillus* had significantly lower disease score than those treated with only *Pythium*. The disease score and mortality increased in *Pythium* + *Trichoderma* treated plants.

Bacillus treated plants had a significantly higher number of fruit per plant than other treatments.

C. Efficacy of *Trichoderma* and *Bacillus* biocontrol products to *Fusarium oxysporum* in cucumber plants grown in cocopeat bags

Methods

The biological controls used were Trich-A-Soil® (*Trichoderma harzianum*) and HayRite™ (*Bacillus amyloliquefaciens*)

Biological Control 1. Trich-A-Soil® = 10g /L (1 x 10⁹ spores/ plant = 1 x 10⁷ spores/ml)

Biological Control 2 HayRite™. = 2.5g/L (1.4 x 10⁹ CFU /plant = 1.4 x 10⁷ CFU/ml)

Three week old Cucumber seedlings (cv *Mascot*) were planted into Cocopeat Easyfil Planterbags™. 2 plants per bag with drip irrigation. Treatments were arranged in randomised blocks in plastic tunnel house (12 replicates x 2 plants). Min / max temperatures 4 –52°C.

Bacillus and *Trichoderma* were applied around plants weekly for 6 weeks.

Plants were inoculated with a blended *Fusarium* culture 02/263, 6 days after second application of biological controls. (2 x 10⁷ conidia/ plant, using haemocytometer). Control treatments consisted of an agar suspension minus fungal inoculum. Plants were maintained as required and monitored for 12 weeks after pathogen inoculation for disease symptoms. Fruit were harvested and counted. Root tissue from permanently wilted plants and 1 plant from each bag were plated at end of trial to confirm fungal infection. Final observations & plating were carried out 12 weeks (86 days) after *Fusarium* inoculation.

Disease score data were analysed using a Generalized linear model with an assumption of multinomial distribution underlying the frequencies of the scores. A logit link function was used to relate the multinomial data to the parameters of the interest (Treatment coefficients). Odd ratio statistic was then calculated to compare the treatment effects on plant health.

Fruit numbers were analysed by an analysis of variance technique on the following model: $Y = \text{control} + \text{Factors}(\text{Pathogen} * \text{Fungi} * \text{Damaged} * \text{Variety}) + \text{error}$, where Y = response variable, control = contrast against healthy plants, and the errors were assumed to have a normal distribution. Preliminary analyses showed the block effects were negligible in most cases. Differences between levels of a factor were compared using a least significant difference (LSD) test at 5% significant level.

Results

Disease score, mortality and fruit number results are shown in Table 16.

Table 16. Mean Disease Score, % Mortality and Number of Fruit Harvested

TREATMENT	DISEASE SCORE	% MORTALITY	FRUIT PER PLANT
Control + water	0	0	18.0
<i>Fusarium</i> + water	3.50	20.8	14.4
Control + <i>Trichoderma</i>	0.54	0	17.9
Control + <i>Bacillus</i>	0.63	12.5	16.7
<i>Fusarium</i> + <i>Trichoderma</i>	4.17	50	13.5
<i>Fusarium</i> + <i>Bacillus</i>	3.67	29.2	14.3

Disease Scores

As shown in Table 16, plants with treated with *Trichoderma* had a significantly higher disease score than control plants. There was no difference between *Bacillus* treated plants and controls.

Table 17. Odd ratios for disease scores of Biocontrols on inoculated plants.

Biocontrol	Odd ratio
Nil	1.00b
<i>Trichoderma</i>	4.31a
<i>Bacillus</i>	1.50ab

Fruit Numbers

Table 18. Summary of ANOVA on fruit number at Day 84 observed for Igloo trial 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogen	1	430.56	430.56	28.38	<.001
Biocontrol	2	11.17	5.58	0.37	0.693
Pathogen.+ Biocontrol	2	24.5	12.25	0.81	0.448
Residual	138	2093.71	15.17		
Total	143	2559.94			

Table 19. Pathogen and biocontrol means of total fruit number by Day 84 from Igloo trial 2

	Biocontrol			
Pathogen	Nil	<i>Trichoderma</i>	<i>Bacillus</i>	Means
Control	18.00	17.92	16.71	17.54a
<i>Fusarium</i>	14.38	13.54	14.33	14.08b
Means	16.19	15.73	15.52	

SED for Pathogen = 0.649; SED for bio-control = 0.795; SED for Pathogen by biocontrol = 1.124

Plants treated with biocontrols were not different to untreated control plants.

Fusarium treated plants had a significantly lower number of fruit per plant than those without any *Fusarium* inoculation.

D. Pilot study for control of Botrytis Rot of cucumbers

This study was undertaken to investigate method of establishing a *Botrytis* infection and evaluate biological controls.

Methods

6 week old cucumber (Cu462) seedlings were planted into used Cocopeat Easyfil Planterbags™ bags. Bags were organised randomly in blocks in greenhouse (5 replicates). 2 plants per bag were used with the following treatments.

1. Botrytis Control
2. Bioacumen - 1.16g / 200mls water + 0.25mls wetter.
3. *Gliocladium* – GR1 dry granules (April 02) 2g /200mls water
4. *Trichoderma* – (Trich-A-Soil®) 2g / 200mls water

Three leaves from each plant were cut off at the lower stem. Treatments 2-4 were sprayed onto cut surface. (Water was used for control). A small square of *Botrytis* culture 03/457D was placed on surface. Plants were maintained and scored for disease symptoms using following index: 0 = no symptoms; 1 = stem lesion; 2 = lesion + visible spores; 3 = lesion + visible spores + wilting; 4 = badly wilted + stem damage; 5 = dead

Results

Results for Disease score and mortality are shown in Table 20. There were no significant differences between treatments.

Table 20. Average Disease Score and %Mortality

Treatment	No. of Plants	Disease Score Day 16	Disease Score Day 32	% Mortality Day 32
<i>Botrytis</i> Control	12	1.58	2.83	25
<i>Botrytis</i> + <i>Gliocladium</i>	12	1.58	2.58	17
<i>Botrytis</i> + <i>Trichoderma</i>	11	1.82	2.55	18
<i>Botrytis</i> + Bioacumen	12	1.17	2.50	25

Colour page VII:

Evaluation of microbial biocontrols for *Fusarium* & *Pythium*

Igloo trials

Captions

- Microbial biocontrol products used in trials
- Wilting cucumbers infected with *Pythium*
- Wilting cucumbers infected with *Fusarium*

Colour page VIII:

Evaluation of microbial biocontrols for *Fusarium* & *Pythium*

Igloo trials with *Fusarium*

Caption

- **Lower stems and washed roots of igloo trial treatments evaluating *Trichoderma* (top) and *Bacillus* (bottom)**

Colour page IX:

On-Farm trials comparing efficacy of conventional fungicides and microbial biocontrols

Application of microbial biocontrol drenches

Trials (E-J) comparing efficacy of conventional fungicides and microbial biocontrols under varying cultural conditions

Methods

The following six trials were conducted in greenhouses at cucumber grower's properties in Bringelly and Rossmore.

For Trials 17 – 22 treatments were as follows:

1. Control - water
2. Stimulizer (Fulvic acid)
3. Trich –A-Soil® (Trichoderma) – 14g/L (1.4×10^9 spores /plant)
4. Companion® (Bacillus subtilis) - 10mls/L (1.4×10^7 CFU/plant)
5. Trich –A-Soil ® + Companion® (combination of treatment 3 & 4)
6. benomyl (7g/L) + propamocarb (3mls/L)

For Trial 22 treatments were:

1. Control
2. benomyl (7g/L)+ propamocarb (3mls/L)

Treatments were applied as 100mls drenches around base of each plant. There were 3 fortnightly applications of treatment, except Trial 18, which received 2 treatments.

Plants were scored fortnightly using cucumber crown and root disease severity index. Root & crown tissue of some dead plants and samples of each treatment were plated to agar media to detect the presence of *Pythium* and *Fusarium*.

Disease score data were analysed using a Generalized linear model with an assumption of multinomial distribution underlying the frequencies of the scores. A logit link function was used to relate the multinomial data to the parameters of the interest (Treatment coefficients). Odd ratio statistic was then calculated to compare the treatment effects on plant health.

Details of each trial follows:

E. Trial 17, On-farm 1

2 month old cucumber plants (Mascot cultivar) grown in bags of unused cucumber mix were organised in a complete randomised block design. (2 rows consisting of 15 replicates with 6 treatments). Each bag contained 1-2 plants and there were 2 bags per treatment replicate. Temperature 14-30°C.

Final observations recorded at 19 weeks after first treatment.

F. Trial 18, On-farm 2

Three-week-old cucumber plants (cv. *Rita*) grown in bags of cucumber mix in an unheated greenhouse were organised in a complete randomised block design. (2 rows, consisting of 18 replicates with 6 treatments). Each bag contained 1-2 plants and there were 2 bags per treatment replicate.

Final observations were recorded 19 weeks after first treatment.

G. Trial 19, On-farm 3

Two-week-old cucumber plants (cv. *Deena*) grown in containers of cocopeat in a were organised in a complete randomised block design. (2 rows, consisting of 10

replicates with 6 treatments). Each bag contained 3-4 plants and there were 2 bags per treatment replicate.

Final observations were recorded 20 weeks after first treatment.

H. Trial 20, On-farm 4

Two-week-old cucumber plants (cv. *Deena*) grown in bags of volcanic rock were organised in a complete randomised block design. (2 rows, consisting of 15 replicates with 6 treatments). Each bag contained 2 -3 plants and there were 2 bags per treatment replicate.

Final observations were recorded 20 weeks after first treatment.

I. Trial 21, On-farm 5

One-week-old cucumber plants (cv. *Deena*) grown in trays were treated with biocontrols. 2 weeks later they were in grown bags with sawdust and organised in a complete randomised block design (3 rows consisting of 7 replicates with 6 treatments). Each bag contained 2-4 plants and there were 2 bags per treatment replicate. They received 2 more applications of biocontrols a fortnight apart.

Final observations were recorded 21 weeks after first treatment.

J. Trial 22, On-farm 6

Cucumber plants (cv. *Deena*) grown in bags of new cocopeat were organised in a complete randomised block design (2 rows, consisting of 8 replicates with 2 treatments). Each bag contained 3-4 plants and there were 2 bags per treatment replicate.

Final observations were recorded 15 weeks after first treatment.

Results

Odd ratio values in Table 21 show how likely the treatments can cause severe damage to the plants in comparison to Control group.

Table 21. Odd ratio values of treatment over the control for disease score

	Odd ratio values					
Treatments	Trial17	Trial 18	Trial19	Trial 20	Trial 21	Trial 22
Control	1.000ab	1.000a	1.000a	1.000ab	1.000a	1.000a
Fulvic acid	1.137ab	0.676ab	0.602a	1.403a	0.376ab	*
<i>Trichoderma</i>	0.754b	0.430b	1.068a	0.948ab	0.170bc	*
<i>Bacillus</i>	1.615a	0.936a	1.000a	1.532a	0.268b	*
<i>Trichoderma</i> + <i>Bacillus</i>	0.821ab	1.235a	1.144a	1.026ab	0.498ab	*
chemical	0.674b	0.163c	0.240b	0.610b	0.108c	0.176b

Fulvic acid - no different to control

Trichoderma –Trials 2 & 5 significantly reduced disease.

Bacillus – Trial 5 significantly reduced disease

T + B – no different to control

Chemical – Trial 2, 3, 5 & 6 reduced disease.

Discussion and conclusions

Economical control was achieved with chemical drenches of benomyl with either furalaxyl, or propamocarb. However, these chemicals have no current registrations or permits for use as drenches on cucumbers. Furthermore, benomyl was recently withdrawn from sale in Australia and has been shown to be incompatible with several biological controls (including certain predatory insects and mites). Alternative chemicals, such as fludioxanil, for control of *Fusarium* need evaluation and permits for their use sought.

Biological control of these diseases with a product containing the fungus, *Trichoderma harzianum*, was variable. This product appeared to reduce disease severity in two of the five on-farm trials. In some cases plant losses were halved with these treatments. The bacterium *Bacillus subtilis* reduced disease in only one of the five trials where it was assessed. Combinations of the two biocontrols appeared to be incompatible and did not suppress disease in any of the on-farm trials where both *Fusarium* and *Pythium* were present. Similarly, a biostimulant (fulvic acid) failed to suppress these diseases in any on-farm trials. Another feature of these trials was that chemical and biological treatments were not effective when applied after disease symptoms had appeared (i.e. as curatives). In contrast, the best efficacy of these products was achieved when the first drench was applied to seedlings (i.e. as preventatives).

Poor on-farm hygiene and sanitation enabled these diseases to spread rapidly. Even where new substrate media (sawdust and cocopeat) were used, these pathogens spread rapidly and resulted in approximately one-third of plants dying within sixteen weeks of transplanting. *Fusarium*, in particular, was shown to spread aurally from typical pink spore masses that formed on affected stems. This is consistent with overseas experience with this disease.

Colour page X:

Evaluation of microbial biocontrols for *Fusarium* & *Pythium*

On-farm trials

Captions

- Untreated plants with typical brown rot of lower stems (3 pictures)

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Appendix 1

Cucurbit and Capsicum samples diagnosed for disease 2001-2004

	Sample No.	Sample	Location	Symptoms	Result
2001	01/235	cucumber	Kemps Creek	dying in groups	<i>Pythium aphanidermatum</i>
	01/279	cucumber	Rossmore	fruit browning	<i>Phoma cucurbitacearum</i>
	01/291	cucumber	Syd Markets	corky stem, few fruit, 20-30%dying	Root knot nematodes
	01/345	cucumber	Berkshire Park		Potyvirus & nutritional imbalance
	01/402	cucumber	Woolgoolga	bleaching near veins	oedemas
	01/410	cucumber	Rossmore	viral symp on plants near doorway	<i>P. aphanidermatum</i> & potyvirus
	01/415	cucumber	Rossmore	root rot leafspot	<i>Fusarium</i> & <i>Pythium irregulare</i>
	01/417	cucumber	Rossmore	wilting gummy stem blight	<i>Phoma cucurbitacearum</i>
	01/430	rockmelon	Mildura		<i>Fusarium oxysporum</i>
	01/441	cucumber	Rossmore	wilt, root rot	<i>Phomopsis sclerotioides</i>
	01/442	cucumber	Rossmore	wilt crown / foot rot	<i>Fusarium oxysporum</i>
	01/443	cucumber	Rossmore	leafspot, fruit & stem Sclerotinia	Potyvirus <i>Alternaria Sclerotinia</i>
	01/444	cucumber	Rossmore	viral, fruit rot, leafspot	Virus neg <i>Alternaria Sclerotinia Botrytis</i> fruit rot
	01/445	cucumber	Austral	leaf mosaic	Potyvirus <i>Sclerotinia</i> on fruit
	01/446	cucumber	Rossmore	seedling wilt	<i>Pythium spinosum</i>
	01/460	cucumber	Berkshire Park		Cucumber mosaic virus
	01/479	cucumber	SA	Virus?	QD negative Nutritional?
	01/480	cucumber	SA	root rot	<i>Fusarium oxysporum</i> & <i>Pythium</i>
	01/481	cucumber	SA	wilting crown / foot rot	<i>Fusarium Pythium Rhizoctonia</i>
	01/482	capsicum	SA		TSWV by ELISA
	01/483	cucumber	SA	Thrips damage	Root knot nematodes
	01/484	cucumber	SA	viral	TSWV by ELISA
	01/485	cucumber	SA	wilt	<i>Pythium Fusarium</i>
	01/486	cucumber	SA	viral	QD negative /greenhouse whitefly larvae/ thrips
	01/504 A	cucumber	Rossmore	mottled light green	CMV
	01/504 B	cucumber	Rossmore	puckered light green	QD neg
	01/504 C	cucumber	Rossmore	blotchy burnt look	Potyvirus Negative ZYMV
	01/505	cucumber	Rossmore	leaf spot wilt	<i>Fusarium oxysporum Pythium mamillatum Alternaria alternata</i>
	01/506	cucumber	Rossmore	wilt	<i>Fusarium oxysporum Pythium</i>
	01/507	cucumber	Rossmore	thrips & viral	Thrips damage

Sample No.	Sample	Location	Symptoms	Result
01/508	coco peat	Rossmore	clean' from potting up area	<i>Pythium</i> (not pathogenic) <i>Fusarium</i>
01/509	cucumber	Rossmore	Bacterial	Angular leaf spot (<i>Pseudomonas</i> sp.) BIOLOG <i>P.syringae</i> pv <i>syringae</i>
01/514	cucumber	Londonderry	leaf spot	Angular leaf spot
01/518-1	cucumber	Austral	downy	Downy mildew
01/518-2	cucumber	Austral	viral	Potyvirus Negative ZYMV
01/519	cucumber	Austral	wilt	<i>Phytophthora drechsleri</i> & <i>Pythium ultimum</i>
01/520	cucumber	Rossmore	wilt / leafspot	<i>Fusarium</i> & <i>Pythium</i> <i>irregulare</i> <i>Phomopsis</i> <i>sclerotoides</i>
01/521	cucumber	Rossmore	<i>Alternaria</i> on old lesions	Downy mildew & <i>Alternaria</i>
01/522	cucumber	Rossmore	viral, wilt	Potyvirus ZYMV by ELISA
01/523	cucumber	Rossmore	leafspots	Angular leaf spot BIOLOG <i>P.syringae</i> pv <i>tabaci</i> B
01/524	cucumber	Rossmore	leaf markings	Downy mildew
01/526	cucumber	Rossmore	wilt	<i>Pythium</i> & <i>Fusarium</i>
01/532	coco peat	Rossmore	unused	negative for pathogens
01/533	Dina	Rossmore	white fungal growth	<i>Fusarium</i> sp
01/534	Dina	Rossmore	wilt	<i>Pythium spinosum</i>
01/535	cucumber	Rossmore	wilt	<i>Fusarium solani</i> & <i>Pythium</i>
01/536	cucumber	Rossmore	viral	1 particle poty
01/538	cucumber	Rossmore	wilt / vascular tiss no symptoms	<i>Fusarium</i> & <i>Pythium</i>
01/539	cucumber	Rossmore	wilt	<i>Fusarium</i> & <i>Pythium</i>
01/540	cucumber	Rossmore	unplanted seedlings	no pathogens detected
01/541	cucumber	Rossmore	young seedlings dying	<i>Pythium</i>
01/542	cucumber	Rossmore	wilt / rotting on stem roots Cu on base	<i>Pythium</i>
01/555	cucumber	Tahmoor	leaf spotting	<i>Alternaria</i> leaf spot
01/556	cucumber	Tahmoor	crown & root rot	<i>Fusarium</i> & <i>Pythium</i> pH=5.76 EC=1.23mS
01/566	cucumber	Milton	root disease, leaf spotting, wilt, abortion	gummy stem blight, <i>Alternaria</i> leafspot, WFT injury, <i>Pythium</i> root rot
01/568	cucumber	Badgery's Creek	wilt over scattered plants	gummy stem blight (<i>Phoma cucurbitarium</i>)
01/666	capsicum	Buronga		
01/690	cucumber	Kemps Creek		Undetermined (Phytotoxicity/ environmental?)
01/728	cucumber	Bringelly	yellowing, wilt	<i>Fusarium</i>
01/737	cucumber	West Hoxton	wilt	<i>Fusarium</i> & <i>Pythium</i>
01/738	cucumber	West	wilt	<i>Fusarium</i> & <i>Pythium</i>

Sample No.	Sample	Location	Symptoms	Result
		Hoxton		
01/739	cucumber	West Hoxton	wilt & leaf spots	<i>Fusarium</i> & <i>Pythium</i> . <i>Alternaria alternata</i> , <i>A. cucumerina</i> powdery mildew
01/740 - 1	cucumber cv Deena	West Hoxton	wilt	<i>Pythium irregulare</i> ?
01/740-2	cucumber cv Deena	West Hoxton	wilt	<i>Pythium spinosum</i>
01/741	cucumber cv Mascot	West Hoxton	wilt	<i>Fusarium</i> & <i>Pythium</i>
01/742	cucumber	West Hoxton	wilt	<i>Fusarium</i> sp. <i>Pythium ultimum</i> & <i>P spinosum</i>
01/768	cucumber	Narara		
01/781	cucumber cv Mascot	Leppington	stunted	<i>Pythium</i> nutritional problem, salt injury, powdery mildew, mites
01/782	cucumber	Leppington		small amt <i>Fusarium</i> & <i>Pythium</i>
01/783	cucumber	Austral		<i>Botrytis cinerea</i>
01/784	cucumber	Sydney	lacking thrift, stunted, narrow stem /crown	<i>Fusarium</i> sp.
01/829	cucumber	Sydney	wilt	<i>Pythium</i> , <i>Fusarium</i> root rot, sciarid fly damage
01/830	cucumber	Narara	vascular browning, wilt	low level <i>Fusarium</i> , undetermined
01/831	cucumber	Narara	wilt	Undetermined
01/839	cucumber	Narara		<i>Fusarium</i> , <i>Pythium</i>
01/840	cucumber	Narara		<i>Fusarium</i> , <i>Pythium</i>
01/847	chilli	Mildura		TSWV by test strip
01/883	cucumber	Rossmore	wilt	<i>Pythium</i> sp. <i>Fusarium</i> and <i>Thielaviopsis</i> sp.
01/884	cucumber	Rossmore	wilt	<i>Pythium</i> sp.
01/885	cucumber	Rossmore	leafspots	<i>Alternaria leaf spot</i>
01/1071	cucumber	Mildura	wilting / virus	<i>Fusarium</i> sp. <i>Pythium</i> sp CMV WFT
01/1082	cuc & scoria	Virginia SA	wilting	<i>Fusarium</i> , <i>Pythium</i>
01/1083	mix	Virginia SA		<i>Fusarium</i> (not pathogenic)
01/1084	cucumber	Virginia SA	virus like symptoms	negative for virus. Powdery mildew
01/1085	soil (mature)	Virginia SA		<i>Rhizoctonia solani</i> pathogenic to cuc seedlings
01/1086	soil (old)	Virginia SA		<i>Rhizoctonia solani</i> pathogenic to cuc seedlings
01/1087	cucumber	Virginia SA	mildew	Powdery mildew
01/1088	cuc (road shed)	Virginia SA	wilting	<i>Fusarium</i> , <i>Pythium</i> . Poor or nil germination of bioassay cucs.
01/1089	cuc (mature)	Virginia SA	wilting	<i>Fusarium</i> , <i>Pythium</i> . Root knot nematodes

	Sample No.	Sample	Location	Symptoms	Result
	01/1090	cuc (young)	Virginia SA	wilting	<i>Fusarium, Pythium</i>
	01/1091	cucumber	Virginia SA	wilting	<i>Pythium, Fusarium & Thielaviopsis root rot</i>
	01/1092	cucumber	Virginia SA	wilting	<i>Fusarium & Pythium</i>
	01/1093	cucumber	Virginia SA	Yellowing & puckering	Negative for virus
	01/1094	cucumber	Virginia SA	leafspots	<i>Alternaria (alternata?)</i> leaf spot
	01/1095	soil	Virginia SA		no pathogens detected from cuc baits
	01/1149	cucumber	Narara	leaves brittle	<i>Pythium irregulare</i>
	01/1190 #1&2	cucumber	Rossmore	wilting & stunting	<i>Fusarium, Pythium and Rhizoctonia.</i> Lot of nematodes on cuc baits
	01/1205	cucumber	Gol Gol	yellow edges on leaves	Salt toxicity or excess boron ?
	01/1206	cucumber	Gol Gol	yellowing wedge shaped sectors on leaves	Powdery mildew & <i>Alternaria</i>
	01/1207	cucumber	Gol Gol	wilt	Wilt & root rot, <i>Rhizoctonia, Fusarium</i> and <i>Pythium</i>
	01/1208	cucumber	Gol Gol	wilt	Wilt & root rot, <i>Fusarium</i> and <i>Pythium</i>
	01/1209	cucumber	Gol Gol	viral	Cucumber mosaic virus
	01/1210	cucumber	Gol Gol	nematodes?	Root knot nematodes
	01/1211	capsicum	Gol Gol	brown streaks on fruit	Cucumber Mosaic virus
2002	02/2	cucumber	Yanco	viral	Cucumber mosaic virus
	02/90	cucumber	Londonderry	fruit rot	<i>Pseudomonas syringae pv lachrymans</i>
	02/263	cucumber	Rossmore	wilt	<i>Fusarium & Pythium</i>
	02/299	cuc seedlings	Rossmore	7 samples	<i>Fusarium Pythium Rhizoctonia</i>
	02/300	cuc seedlings	Rossmore	6 samples	<i>Fusarium Pythium Rhizoctonia</i>
	02/301	cuc seedlings	Rossmore	4 samples	<i>Fusarium Pythium Rhizoctonia</i>
	02/302	cuc seedlings	Rossmore	1 sample	no result.
	02/303	cucumber	Rossmore	56 samples	<i>Fusarium & Pythium</i>
	02/304	cuc fruit	Rossmore	fruit	fungal / bacterial soft rot
	02/436	cucumber	Rossmore		<i>Fusarium</i>
	02/441	cucumber	Baldivis WA	wilt	<i>Fusarium & Pythium</i>
	02/444	cucumber	Hurley St Rossmore	foot / crown rot	<i>Fusarium</i>
	02/445	cucumber	Rossmore	foot / crown rot	<i>Fusarium & Pythium</i>
	02/446	cucumber	Rossmore	foot / crown rot	<i>Fusarium & Pythium</i>
	02/484-A	cucumber A	Baldivis WA		<i>Fusarium oxysporum</i>

	Sample No.	Sample	Location	Symptoms	Result
	02/484-B	cucumber B	Baldivis WA		<i>Fusarium oxysporum</i> & <i>Pythium</i>
	02/541	cucumber	Rossmore		sciarid larvae damage to taproot
	02/550	cucumber	Daylesford VIC	splitting of lower stems	Physiological / change in temp. Secondary fungal infection.
	02/551	cucumber	Daylesford Vic	yellow leaf spots	Mite injury & environmental / nutritional disorder
	02/555	cucumber	Rossmore		<i>Fusarium</i> & <i>Pythium</i>
	02/556	cucumber	Rossmore		<i>Fusarium</i> & <i>Pythium</i>
	02/558	cucumber	Narara		Negative CMV / nutritional
	02/572	cucumber	91 Third Rd Berkshire Park		<i>Fusarium</i> & <i>Pythium</i>
	02/581	cucumber	Rossmore		<i>Fusarium</i> & <i>Pythium</i>
	02/582	cucumber	Rossmore	wilt	<i>Fusarium</i> & <i>Pythium</i> , gummy stem blight
	02/583	cucumber	Rossmore	leaf spots	<i>Pseudomonas syringae</i>
	02/586	pumpkin		stem markings	Physical injury, non pathogenic
	02/593	cucumber	Rossmore	crown rot, leaves with spots & bleached tissue between veins	<i>Pythium</i> & <i>Fusarium</i> , <i>Alternaria</i> & nutritional symptoms
	02/602	cuc leaves	Warnervale		Non pathogenic Heat scorch symptoms
	02/609	cucumber	Rossmore	wilt	<i>Fusarium</i> & <i>Pythium</i>
	02/616	cucumber	Rossmore	wilt	<i>Fusarium</i> & <i>Pythium</i>
	02/639	cucumber	Austral	aborting fruit	<i>Botrytis cinerea</i>
	02/703	cucumber	Badgery's Creek		Chemical injury?
	02/704	cucumber	Badgery's Creek	wilt	<i>Fusarium</i> & <i>Pythium</i>
	02/723	cucumber	Kemps Creek		salt injury & <i>Pythium</i>
	02/749	cucumber	Rossmore		<i>Fusarium</i> & <i>Pythium</i>
	02/750	cucumber	Rossmore		<i>Fusarium</i> & <i>Pythium</i>
	02/751	cucumber	Rossmore		<i>Fusarium</i>
	02/775	cucumber	Austral	virus symptoms	Undetermined, chemical injury?
	02/782	cucumber	Berkshire Park		Physiological stem splitting
	02/783	cucumber	Glenorie	yellow & distorted fruit	Undetermined, physiological / environmental
	02/786	cucumber	Two Wells, SA		Undetermined, <i>Pythium</i> & <i>Fusarium</i> associated with root symptoms
	02/792	cucumber	Rossmore		potyvirus
	02/863	cucumber	Mildura		<i>Pythium</i> root rot
	02/908	cucumber	Oakhurst		<i>Fusarium oxysporum</i>
	02/964	rockmelon	Sydney		<i>Fusarium</i> sp

	Sample No.	Sample	Location	Symptoms	Result
2003	03/35	cucumber	Seven Hills		<i>Fusarium & Pythium</i>
	03/43	cucumber			Heat / moisture stress damage?
	03/66	luffa			
	03/159	cucumber	Rossmore		<i>Fusarium & Pythium</i> , Root knot nematodes
	03/237	watermelon			
	03/273	cucumber	Narara		Gummy stem Blight
	03/340	cucumber	Thuringowa QLD		<i>Pythium aphanidermatum</i>
	03/370 A	cucumber cv Rosanna	Bringelly	wilt, root rot	<i>Phomopsis sclerotioides</i>
	03/370 B	cucumber cv Deena	Bringelly	wilt, root rot	<i>Fusarium & Pythium</i>
	03/370 C	cucumber cv Deena	Bringelly	wilt, root rot	<i>Fusarium & Pythium</i>
	03/370 D	cucumber cv Deena seedlings	Bringelly		<i>Pythium</i>
	03/372	cucumber	Bringelly	root rot	<i>Rhizoctonia & Fusarium</i>
	03/400	cucumber	Thuringowa QLD		<i>Pythium aphanidermatum</i>
	03/407	cucumber	Bringelly		<i>Fusarium & Pythium</i>
	03/409	cucumber	Tahmoor		
	03/410	cucumber	Tahmoor		
	03/438	cucumber seedling	Rossmore		<i>Fusarium</i>
	03/439	cucumber	Rossmore		<i>Pseudomonas syringae</i> p.v. <i>syringae</i>
	03/449	cucumber	Pheasants Nest	poor tap root development	<i>Oedemas & Rhizoctonia</i>
	03/451	cucumber			<i>Pythium</i>
	03/457 A	cucumber	Kemps Creek	stem & fruit rot	<i>Sclerotinia</i>
	03/457 B	cucumber	Kemps Creek		<i>Downey Mildew</i>
	03/457 C	cucumber	Kemps Creek	stem & fruit rot	<i>Sclerotinia</i>
	03/457 D	cucumber	Kemps Creek	stem & fruit rot	<i>Botrytis</i>
	03/457 E	cucumber	Kemps Creek	stem & fruit rot	<i>Sclerotinia</i>
	03/502	cucumber	Lilyfield / Duranbah	root rot	<i>Fusarium / Pythium</i>
	03/512	cucumber	Yoogoli		Thrips injury
	03/546	cucumber	Seven Hills	Black root rot	<i>Fusarium / Pythium / Phomopsis sclerotioides</i>
	03/585	cucumber	Coffs Harbour		<i>Thrips injury / Powdery Mildew</i>
	03/590	cucumber	Rossmore	seedling rot	<i>Fusarium</i>

	Sample No.	Sample	Location	Symptoms	Result
	03/591	cuc leaves	Rossmore	angular leaf spot	<i>Pseudomonas syringae p.v. syringae</i>
	03/594	cucumber	Pheasants Nest	foliar damage	Heating /unburnt gas, <i>Pythium</i> / <i>Fusarium</i> , <i>Botrytis</i> on fruit
	03/639	cucumber seedling	Seven Hills	root rot / wilt	<i>Pythium</i> (<i>P. irregulare</i> , <i>P. spinsum</i> , <i>P. aphanidermatum</i>) & <i>Fusarium</i>
	03/666	cucumber cv Mascot	Woolgoolga	stem rot & fruit tips pinching	<i>Sclerotinia</i> and <i>Botrytis</i> , nutritional & physiological
	03/679	cucumber	Rossmore		<i>Pythium</i> , negative for plant parasitic nematodes
	03/687	cucumber	Seven Hills		<i>Pythium</i>
	03/694	cucumber		root rot / wilt	<i>Pythium</i>
	03/695	cuc leaves	Rossmore	virus -like	Closterovirus, possible Beet Pseudo Yellows Virus
	03/697	cuc soil	Rossmore		No parasitism observed
	03/713	mini cuc leaves	Mangrove Mountain	bacterial	bacterial
	03/777	cucumber	Taree	burnt symptoms	possible environmental / chemical injury
	03/803	cucumber	Bringelly	brown roots	<i>Fusarium</i> / <i>Pythium</i>
	03/829	mini cuc fruit	Kemps Creek	fruit	<i>Phoma</i> Gummy stem blight
	03/852	capsicum	Marsden Park	virus	Tomato Spotted Wilt Virus
	03/868	cucumber leaves	Rossmore	virus symptoms	Undetermined
	03/869	capsicum	Rossmore	vein clearing & mosaic	Potyvirus
	03/890	cucumber	Narara	distorted leaves	environmental
	03/902	cucumber	Rossmore	discoloured leaves	Undetermined / possible chemical damage
	03/918	cucumber	Leppington	root rot	<i>Fusarium</i> / <i>Pythium</i>
	03/1007	cucumber	Orange		Thrips juveniles & injury
2004	04/49	cucumber	Seven Hills	root rot / wilt	<i>Fusarium</i> / <i>Pythium</i>
	04/57	cucumber	Seven Hills	stem rot	<i>Fusarium oxysporum</i>
	04/71	cucumber	Bonville	stunted fruit / marked leaves	Undetermined / Nutritional
	04/94	capsicum	Narara		
	04/101-1	watermelon			<i>Fusarium</i> & <i>Pythium</i>
	04/101-2	watermelon			<i>Fusarium</i>
	04/123	cucumber	Seven Hills	root & stem rot	<i>Pythium aphanidermatum</i> & <i>F oxysporum</i>
	04/126	cucumber	Rossmore	root rot	<i>F oxysporum</i> / <i>Pythium irregulare</i>
	04/136	chilli	Windsor		