Project 2.1 Integrated management of soilborne pathogens (Sclerotinia beans, lettuce, carrots, celery and other)

Victorian Department of Primary Industries (VICDPI)

Project Number: VG07126

VG07126

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Integrated Management of Soilborne Pathogens 2.1 (Sclerotinia)

Villalta *et al*

Final Report
Horticulture Australia Project VG07126
(31 August 2010)

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Project details
Integrated Management of Soilborne Pathogens (Sclerotinia)
Horticulture Australia Final Report Project VG07126
August 2010

The purpose of this project was to develop new management options for the integrated control of Sclerotinia diseases of vegetables. Sclerotinia is a persistent and costly disease problem for most vegetable growers in Australia. The project evaluated new disease control materials and cultural practices for their potential to reduce inoculum carry-over in soil, reduce disease and increase yield, and developed decision support tools for disease forecasting and economic analysis. This research will provide growers with more IPM options and the most appropriate and effective use of chemical treatments for managing Sclerotinia on their farms.

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- Many other people contributed to this project by supporting researchers and by providing products and materials tested in laboratory, glasshouse and field trials and they are acknowledged in the many technical reports included in this final report.

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Contents

MEDIA SUMMARY

This national project, a collaborative research between DPI Victoria, Peracto, UTAS/TIAR and DEEDI Qld, has developed four new strategies for the integrated control of Sclerotinia diseases in vegetables.

Sclerotinia is the most damaging soilborne disease of vegetables crops causing the industry over $10m of crop losses annually, particularly in lettuce and green bean production. The best strategy to manage Sclerotinia is to use a number of control methods including cultural practices, disease forecasting and fungicides. However, high losses still occur in Australia because there are no suitable and cost-effective treatments to manage Sclerotinia, especially to eliminate disease carry-over in soil and protect plants against infection.

This project, therefore, focused on developing new control strategies, using lettuce and green beans as model crops, which can be integrated into Integrated Pest Management (IPM) and Best Management Prcatices (BMP) programs for Sclerotinia and other soilborne pathogens.

1. Three new fungicide treatments were identified as potential replacements for Filan™, the only fungicide presently available for effective Sclerotinia control.

- In field trials on beans under high disease pressure, the best fungicides for white mould control were Switch™ and Filan™, which reduced disease by 81% and 83%, respectively, followed by Shirlan at 58%. Shirlan™ combined with Filan™ reduced disease by 91%.
- For lettuce drop control, the best fungicides were Filan™, Switch™ and Shirlan™, which reduced disease by 60-80%. A new fungicide (AE C656948) was also effective against *S. minor* lettuce drop.

2. Two new experimental soil treatments containing plant-derived anti-microbial compounds were very effective in destroying sclerotia of *S. minor* and *S. sclerotiorum.*

3. Four new brassica green manure crops (Caliente 199, Gladiator, Nemfix and Mustclean) were shown to have excellent biofumigant activity against four major soilborne pathogens (*S. minor*, *P. dissotocum*, *R. solani* and *F. oxysporum)* of vegetables. Biofumigation with Mustclean reduced lettuce drop by 62% and bean root rots by 35% while Caliente 199 increased spring onion yield by 16%.

4. Three new methods were developed for detection and assessment of white mould risk on beans.

- Molecular-based detection of airborne ascospores of *S. sclerotiorum*.
- Efficient samplings protocols for assessing white mould before harvest.
- Site-specific risk factors that drive white mould development identified.

The three methods are being developed as decision support tools to improve spray application and Sclerotinia management.

The new strategies are being delivered to growers through demonstration sites, workshops and extension materials to fast track adoption. Efficacy data for new controls has been provided to AgAware for development of minor use permits.

TECHNICAL SUMMARY

Sclerotinia is a major soilborne disease challenge for vegetable growers in Australia, which causes over $10m loss annually. There are currently very limited management options available for controlling this disease. Consequently, this national project, a collaborative work between DPI Victoria, Peracto, UTAS/TIAR and Agri-Science Qld, was funded to develop new management options for the integrated control of Sclerotinia in Australia.

A series of laboratory, glasshouse and field trials were conducted in Victoria, Tasmania and Queensland to evaluate new disease control materials and integrated management strategies including rotation, cultural practices and disease forecasting for Sclerotinia control in lettuce and beans.

In field trials on beans under high disease pressure, the best products for control of white mould (*Sclerotinia sclerotiorum*) were Switch™ and Filan™ applied during flowering, which reduced disease by 81% and 83%, respectively, followed by Shirlan at 58%. Under low disease pressure, Folicur™ was also effective. When applied to the soil surface before canopy closure, Shirlan™ reduced disease by 55% and Filan™ by 72%. A soil application of Shirlan™ combined with foliar applications of Filan™ reduced disease by 91%. For lettuce drop control, the best products were Filan™, Switch™ and Shirlan™ applied as plant drenches, which reduced disease by 60-80%. The new fungicide AE C656948 was also effective against *Sclerotinia minor* on lettuce.

Six plant-derived products (Voom, Vigor®, fennel oil, Promax®, Bioweed®, Fumafert®) were tested in laboratory, glasshouse and field trials as potential soil treatments against several soilborne pathogens of vegetables including *S. minor*, *S. sclerotiorum* and *S. cepivorum*. Although Voom, Vigor® and fennel oil all effectively killed mycelium of these three pathogens, only Voom at 3-5% was able to kill sclerotia of the same three pathogens. When tested under field conditions, a pre-plant soil application of 50 L/ha of Voom provided a significant reduction of bean root rot severity which was equivalent to that provided by Basamid™.

This project demonstrated for the first time in Australia the potential of using rotations with new biofumigation crops for disease management in vegetables crops. Laboratory and glasshouse studies showed that the volatiles compounds, particularly 2-propenyl-GSL, released from freeze dried tissue of Caliente 199, Mustclean, Gladiator and Nemfix were inhibitory and/or biocidal to four major pathogens of vegetables (*S. minor*, *F. oxysporum*, *P. dissotocum* and *R. solani*). Field trials conducted in Victoria showed that by using Mustclean and Caliente 199 as green manure crops bean root diseases were significantly reduced. In Tasmania, Mustclean reduced lettuce drop by 62%. Other effects from using biofumigant crops were excellent weed suppression and increased yields. The agronomic and biofumigant potential of another nine new biofumigant varieties was evaluated in field trials in Tasmania and Queensland.

In Tasmanian field trials, six commercial green bean cultivars were assessed for their susceptibility to *S. sclerotiorum* infection. Valentino, a tall variety with large canopy and long flowering period was the most susceptible. In commercial bean crops, more than 5-10% white mould incidence may result in whole crop rejection, therefore sampling strategies were developed and site-specific risk factors that drive disease development identified. This will enable growers to identify their high risk fields/crops early enough to make decisions regarding management and harvest. In addition, a molecular-based tool was also developed to detect airborne inoculum. The new predictive methods require commercial validation before

they can be used by industry to improve spray application and overall Sclerotinia management.

Economic analysis was conducted on the key strategies evaluated and an Excel-based model developed to study the long-term economic consequences of changing management practices for Sclerotinia.

Although this project has developed four new IPM strategies for managing Sclerotinia in lettuce and bean, further work is needed to complete development of these strategies and validate their performance on farms in different regions of Australia to ensure high adoption by industry. This work will include assessing the long-term impact of rotation with biofumigant and other green manure crops and other non-chemical strategies (e.g. biocontrols) on pathogen suppression, soil biology, yields and farm profitability.

CHAPTER 1 - EVALUATING PLANT PRODUCTS AS POTENTIAL SOIL TREATMENTS FOR THE CONTROL OF SCLEROTINIA AND OTHER SOILBORNE DISEASES

Oscar Villalta, Denise Wite, Alice Ames, Cassie Scoble, Caroline Donald and Ian J. Porter

SUMMARY

This study evaluated six plant-derived products for the control of soilborne pathogens of vegetables. Laboratory experiments showed that volatile products released from solutions containing 3% and 5% of Voom® were the most effective treatments killing mycelium and sclerotia of *S. cepivorum*. Voom® at 3% was also very effective in killing sclerotia of *S. rolfsii* but not resting (sclerotial) structures of three other pathogens (*S. minor, B. cinerea, R. solani*). Lower concentrations of Voom® were only inhibitory of mycelial growth and less effective in reducing the viability of *S. cepivorum* sclerotia. Volatile products released from solutions of Voom® at 1%, 3% and 5% and Vigor® at 4%, 6% and 8% were biocidal to mycelium of *S. minor* and *S. sclerotiorum* while bitter fennel oil at 2%, 4% and 6% was only inhibitory of mycelial growth. Voom® at 3% and 5% caused significant reductions of sclerotia viability of both *S. minor* and *S. sclerotiorum* in soil. Vigor® at 5%, 8% and 10% had no effect on sclerotia viability and bitter fennel oil was effective only at 80%. In field trials most plant products evaluated were ineffective in controlling diseases caused by *S. minor* on lettuce and a complex of soilborne pathogens on green beans, with the exception of Voom®. Pre-plant soil applications of Voom® at 50 L/ha provided a reduction of root infection severity equivalent to Basamid® on green beans. The potential use of these treatments for the management of soilborne disease of vegetables is discussed.

Introduction

Sclerotinia sclerotiorum and *S. minor* are two of the most damaging soilborne pathogens of vegetable crops including lettuce, cabbage, bean and carrot in Australia. These pathogens survive in soils for many years as melanised sclerotia, often together with other pathogens that cause root infections including *Fusarium* spp., *Rhizoctonia* spp., and *Pythium* spp. Growers use fungicide treatments to control Sclerotinia diseases but this practice does not eliminate inoculum carry-over in soil. Despite the use of fungicides growers still experience significant losses to Sclerotinia, especially when climatic conditions are most conducive to disease development.

Management of soilborne pathogens is best achieved by reducing inoculum carry-over and by preventing inoculum build up over time using beneficial farming practices such as crop rotation and biofumigation. Growers are concerned that there are currently no registered cost-effective and Integrated Pest Management (IPM) compatible soil treatments to reduce inoculum in soils used for vegetable growing in

Australia. Therefore, one of the long-term goals of our research is to develop and evaluate new soil treatments for soilborne pathogens which are environmentally safe such as biological control agents, natural plant products and cultural methods. Many plant products (extracts and essential oils) have been reported to have good antimicrobial activity against soilborne pathogens such as *Fusarium oxysporum* (Bowers and Locke 2000) and *Phytophthora* spp. (Bowers and Locke 2004). Soylu *et al.* (2007) laboratory work also showed that oregano and fennel essential oils have antifungal activity against mycelium and sclerotia of *S. sclerotiorum.*

In this study several commercially formulated plant extracts and essential oils were evaluated for their effectiveness in reducing the survival of inoculum of Sclerotinia and other soilborne pathogens of vegetables in Petri dish and soil bioassays. The study also aimed to evaluate several plant products for their ability to control key soilborne pathogens including *Sclerotinia* spp. of vegetables in the field and for possible use in integrated management programs.

Materials and Methods

Evaluating new soil treatments *in vitro* and soil assays

Treatments

A series of laboratory experiments were conducted to test the effects of volatile products released from six treatments on mycelial cultures and sclerotia of three soilborne fungi (*S. minor*, *S. sclerotiorum* and *S. cepivorum).* The treatments were two commercial plant products (Voom™ and Vigor™), one synthetic oil (diallyl disulphide, DADs™), one essential oil (bitter fennel oil, Essential oils of Tasmania) and two active components (fenchone and anethole) of fennel oil (Table 1). Some *in-vitro* experiments included allyl isothiocyanate (98% allyl-ITCs w/v, Fluka) as the volatile positive control**.** Mycelial cultures and sclerotia used in the tests were produced and maintained on potato dextrose agar (PDA). The fungal isolates of *S. minor*, *S. sclerotiorum* and *S. cepivorum* were isolated from soil (sclerotia) collected from vegetable farms in Victoria. Other isolates were obtained from culture collections at Knoxfield.

Table 1. Products and rates tested in laboratory and glasshouse experiments.

Product	Active	Source
Untreated control		
Bitter fennel oil	Composition analysis available	Essential Oils Tas.
Voom®	mustard and other essential oils	Akhil
DADs®	90% diallyl disulphide	Aceto, USA
Vigor®	mustard oil and capsaicinoids	Champon, USA
Fenchone	Active of *Foeniculum vulgare*	Sigma
Anethole	Active of *Foeniculum vulgare*	Sigma

Mycelial growth

A series of *in-vitro* experiments evaluated a variety of treatments (Table 1) for their ability to inhibit and destroy pathogen mycelium. Two methods (vapour and contact) were used to expose inoculum of the pathogens tested to volatile products released from treatments in culture. In the vapour method, aliquots of sterile distilled water solutions (1 mL/plate) containing the different treatments were placed onto 3 cm diameter filter paper discs in the centre of upturned Petri dish lids. PDA in the bottom of Petri dishes were inoculated with a 5mm plug of a 4-6 day-old- culture of each pathogen, then placed upside down over the upturned lids containing the treatments. The plates were sealed with plastic film to prevent escape of volatile products and incubated in the dark at 20ºC (±3ºC) for the required period in each experiment. In the contact method, aliquots of treatment solutions (1 mL/plate) were added directly to the PDA media (temperature 40-45ºC) before pouring into Petri dishes and then a plug with the pathogen was added. The growth of each fungus was measured periodically by either taking two radial transects of the colonies at various incubation times after adding the treatments or by measuring the whole colony diameter. The effect on growth inhibition (fungistatic) was compared to growth of untreated controls and biocidal (fungicidal) effect concluded if the plug with pathogen failed to show any growth after being transferred to fresh PDA plates.

Sclerotia viability

The treatments in Table 1 were also tested on sclerotia of *S. minor*, *S. sclerotiorum* and *S. cepivorum* in culture and in soil assays. The aim was to learn more about the potential of the treatments for reducing the viability of sclerotia. In culture, sclerotia of *S. minor* and *S. sclerotiorum* produced in PDA plates were exposed to volatiles of treatments using the vapour and contact methods previously described. Sclerotia of *S. cepivorum,* also produced in PDA plates, were exposed to volatiles of treatments using the same methods, except that sclerotia were treated inside small plastic cups (30 ml-cc, Huhtamaki). In this method, Sclerotia were placed inside mesh bags (20 sclerotia/bag) which were attached to the inside of plastic cup lids (vapour phase) or bottom of the cup (contact). Treatment solutions were added to filter paper (0.5 mL/cup) placed on the bottom of the cup. The cup was sealed and incubated at room temperature for 24-hrs. In the second method (drench), sclerotia in mesh bags were placed directly on the filter paper before adding the treatments. In all experiments with sclerotia, after application of treatments sclerotia were surface sterilised using the modified method of Hunger *et al.* (2002) and then sclerotia placed singly on PDA droplets to determine their viability. Plates were incubated and assessed periodically for 14 days at 20°C or room temperature.

In soil assays, sclerotia of *S. minor* and *S. sclerotiorum* were placed inside a nylon mesh bag which was placed in the middle of a one kg plastic container filled with sterile river sand. Treatments were delivered below the mesh bag and then containers sealed to prevent the escape of volatiles. After treatment all sclerotia were surface sterilised and their viability tested on PDA as previously described.

Data analysis

The laboratory experiments were set up as replicated complete randomised blocks with three to four replicates per treatment. The pot experiments were set up as complete randomised blocks with four replicates. Data were analysed by analysis of variance using Genstat for Windows (Lawes Agricultural Trust, Rothamsted Experimental Station).

Evaluating new soil treatments in the field

Treatments

Two field trials were conducted to compare the efficacy of five plant products (Table 2) on control of diseases caused by soilborne pathogens. The treatments included five commercially available plant products (Promax®, Vigor®, Pine oil®, Voom® and Fumafert Pine oil®). In a third trial, Voom® was compared to Fumafert® and Basamid® for the control of allium white rot of spring onions. Basamid® and Filan® were the standard treatments. These soil treatments were applied pre-plant to reduce soilborne inoculum and then the disease caused by surviving inoculum was measured. The aim was to learn more about the potential of commercial rates of these soil treatments for reducing the viability of inoculum and to examine the commercial practicality of application of products for disease control.

Table 2. Products and treatments tested in field trials.

Product	Active	Rate of product[1]	Source
Untreated control			
Bioweed®	Pine oil	90 L/ha	Certified Org.
Promax®	Thyme oil	19 L/ha	Bio HumaNetics
Voom®	mustard and other essential oils	50 L/ha	Prem Akhil
Vigor®	mustard oil and capsaicinoids	50 L/ha	R&R Fumigation
Fumafert®	mustard seed meal, neem kernel	2 t/ha, broadcasted	Organic C. P.
Perlka®	calcium cyanamide	1 t/ha, broadcasted	James Cathcart
Basamid®	dazomet	500 kg/ha, broadcasted	R&R Fumigation
Filan®	boscalid	1 kg/ha, 1000 L/ha	Nufarm

[1] Liquid solutions were drenched onto soil followed by mixing into soil with a rotary hoe and rolling at Lindenow and just rotary hoeing at Heatherton. Filan® applied banded across plant rows with a knapsack fitted with boom sprayer.

Field sites

The trials were conducted in three commercial farms with different vegetable cropping systems. In the first field site (Lindenow, Vic), green beans are cropped in a 2-year rotation with cabbage, sweet corn and other crops in an alluvial soil. The major diseases targeted at this site were bean white mould, caused by *S. sclerotiorum* and bean root infections caused by *Rhizoctonia* spp., *Fusarium* spp. and *Pythium* spp. which were identified previously at this site. The soil treatments were applied 4 weeks prior to sowing green beans. In the second site (Heatherton, Vic), lettuce had been cropped in a short rotation with brassica speciality vegetables in a loam sandy soil. The major diseases targeted at this site were *S. minor* lettuce drop and clubroot of brassica crops which were identified previously as the most damaging diseases at this

site. The treatments were applied 6 weeks prior to planting lettuce which was followed by a crop of Pak Choy. In the third site (Clyde South, Vic), spring onions are cropped in short rotation with radish and continental parsley in a sandy soil. *Allium* white rot, caused by *S. cepivorum*, was the disease investigated. The soil treatments were applied 8 weeks prior to sowing spring onions. Crops were managed by growers using their own practices, except that researchers supervised the application of fungicides.

Application

At Lindenow and Heatherton, liquid products (Pine oil®, Vigor®, Promax® and Voom®) were applied with watering cans using 27 L water per plot (3 m wide by 10 m long) at Lindenow and 9 L per plot (1.5 m by 9 m) at Heatherton. Solid products (Perlka®, Basamid® and Fumafert®) were broadcasted onto soil. The soil was rotary hoed and rolled immediately after treatment application to prevent the escape of volatiles from soil, except for Heatherton where the soil was only rotary hoed. Plots are Lindenow were 3 m wide by 10 m long and at Heatherton 1 m wide by 9 m long. At Clyde, Voom™ was shank-injected into soil using 500 L of water per ha. Plots (1 m wide by 5 m long) were covered with plastic sheeting to prevent the escape of treatment volatiles. At Heatherton and Clyde treatments were applied to raised beds of sandy soil.

Measurements and analysis

The effect of soil treatments on inoculum levels was investigated in soil from plots at Lindenow and Heatherton. The samples were collected with a hand trowel to a depth of 10-15 cm by combining ten sub-samples taken along each plot into a one bulked sample. Samples were collected one and four weeks after treatment application at Heatherton and Lindenow, respectively. Populations of sclerotia of *S. minor* and *S. sclerotiorum* were measured in 200 g of the soil of each plot using the wet sieving method and sclerotia viability tested on PDA media. Soil samples were also sent to SARDI to determine levels of *Pythium clade* f and *R. solani* in soil using a qDNA soil test.

Data analysis

The field trials were set up using complete randomised blocks with five to six replicates per treatment. Data were analysed by analysis of variance using Genstat for Windows (Lawes Agricultural Trust, Rothamsted Experimental Station).

Results and Discussion

Evaluating new soil treatments in culture and soil assays

Effect of Voom® treatments on S. cepivorum (vapour phase)

Volatile products released from solutions of Voom® at concentrations of 0.75%, 1.0% and 1.25% for 24 hrs exposure significantly reduced mycelial growth of *S. cepivorum* compared to untreated controls (Tables 3 and 4). Cultures that did not grow in the presence of Voom® at concentrations equal or lower than 1.25% resumed growth once agar blocks were transferred to unamended PDA plates (Tables 3 and 4). Voom® at concentrations of 3% and 5% were biocidal to mycelium of *S. cepivorum* (Table 4).

There were no significant differences in the levels of inhibition of mycelial growth among cultures treated with two concentrations of Voom® (0.5% and 1%) for four exposure times (3, 6, 12 and 24hrs) (Figure 1). Voom® at 0.25% for 12 and 24 hrs exposures was significantly more effective in inhibiting mycelial growth than for 3 and 6 hrs exposures.

Volatile products released from solutions containing 1.25%, 3% and 5% of Voom® (24 hrs exposure) were the most effective treatments in reducing viability of *S. cepivorum* sclerotia (Figures 2 and 3). Voom® at 0.75% and 1.0% also gave significant reductions of sclerotia viability compared to the untreated control (Figures 2 and 3). There were no significant differences in the levels of sclerotia killed between the two methods of applying the treatments (vapour and contact phases), except for Voom® at 0.5% (Figures 2 and 3).

There was a significant interaction between concentration of Voom® (0.25% - 1.0%) and exposure time (vapour phase) on the levels of sclerotia mortality (Figure 4). For example, sclerotia treated with Voom at 1% for 24 hrs had significantly more sclerotia killed than sclerotia treated with Voom at 1% for 3 hrs.

Table 3. Effect of different concentrations of Voom®, applied using the vapour phase method for 24-hrs, on radial mycelial growth of *S. cepivorum* on PDA agar plates.

Treatment (% v/v)	Mycelial growth (mm)	
	7 days	14 days
Untreated control	35.0 a	35.0 a
Voom 0.50%	35.0 a	35.0 a
Voom 0.75%	21.3 b	35.0 a
Voom 1.00%	3.8 c	35.0 a
Voom 1.25%	0.0 c	35.0 a

Means with the same letter are not significantly different at P = 0.05.

Table 4. Effect of different concentrations of Voom®, applied using the vapour phase method, on radial mycelial growth of *S. cepivorum* on PDA agar plates.

Treatment (% v/v)	Mycelial growth (mm)	
	7 days	14 days
Untreated control	34.9 a	35.0 a
Voom 0.5%	16.8 b	35.0 a
Voom 1.0%	0.0 c	35.0 a
Voom 3.0%	0.0 c	0.0 b
Voom 5.0%	0.0 c	0.0 b

Means with the same letter are not significantly different at P = 0.05.

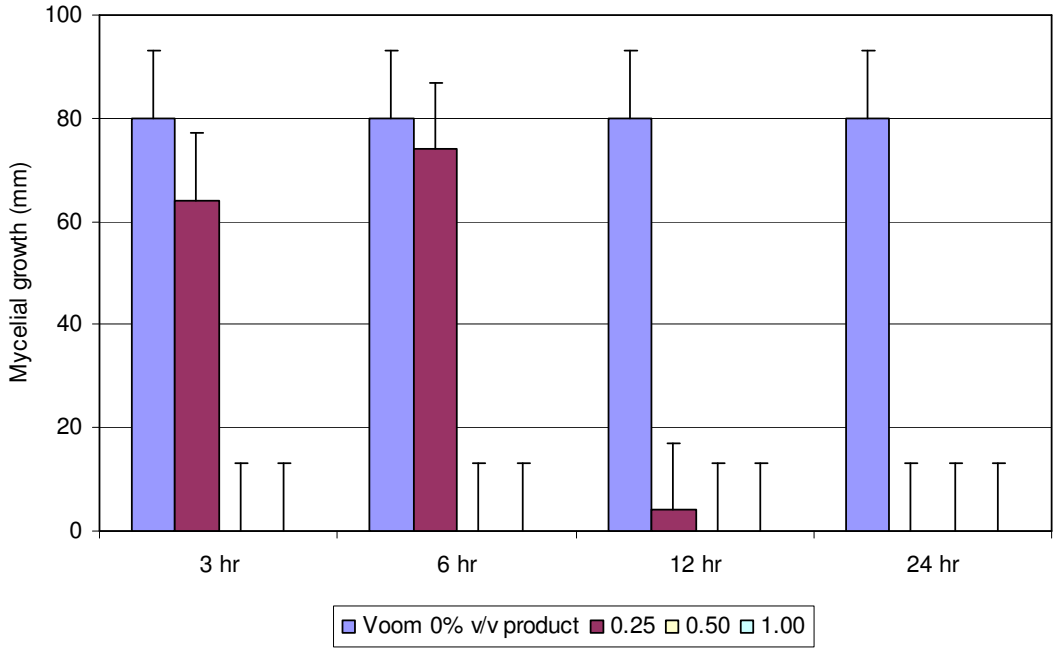

Figure 1. Effect of three rates of Voom (0.25%, 0.5% and 1% v/v of product), applied using the vapour phase method for four exposure times, on mycelial growth of *S. cepivorum* (Error bars are lsds at P=0.05).

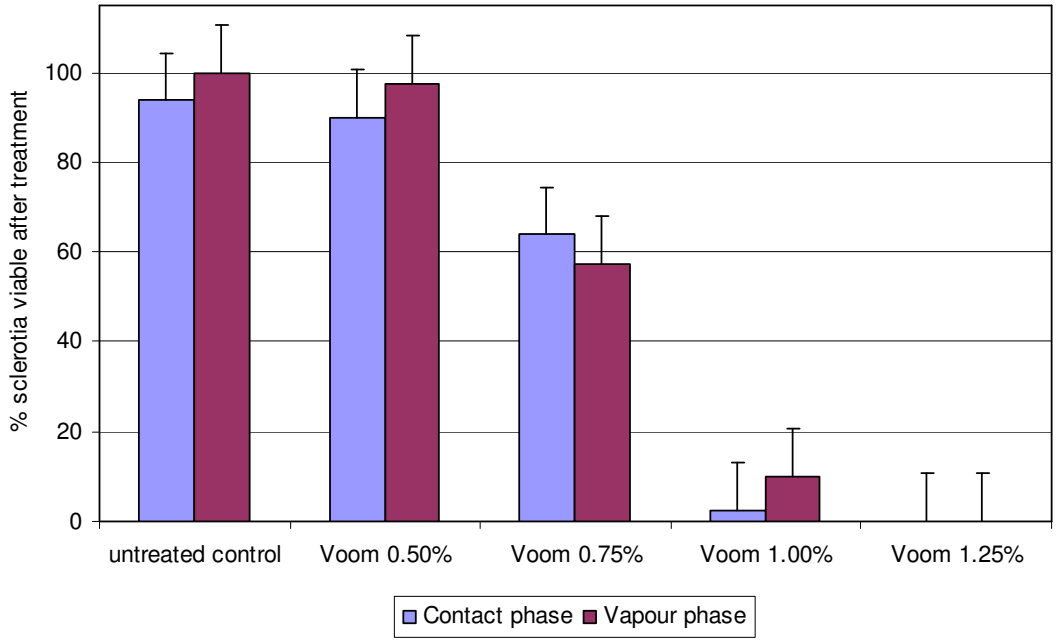

Figure 2. Effect of Voom treatments (0.5% – 1.25% v/v of product) on the percentage of *S. cepivorum* sclerotia (10/rep) that survived the treatments applied for 24-hrs using contact and vapour phase methods. (Error bars are lsds at P=0.05)

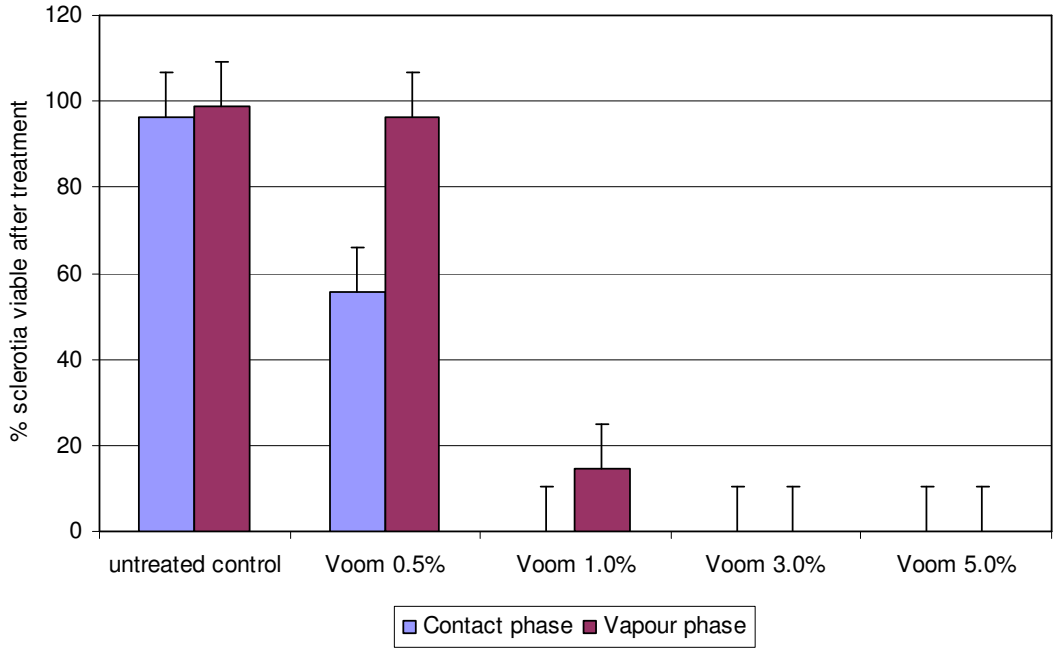

Figure 3. Effect of Voom treatments (0.5% – 5.0% v/v of product) on the percentage of *S. cepivorum* sclerotia that survived the treatments applied for 24-hrs using contact and vapour phase methods. (Error bars are lsds at P=0.05)

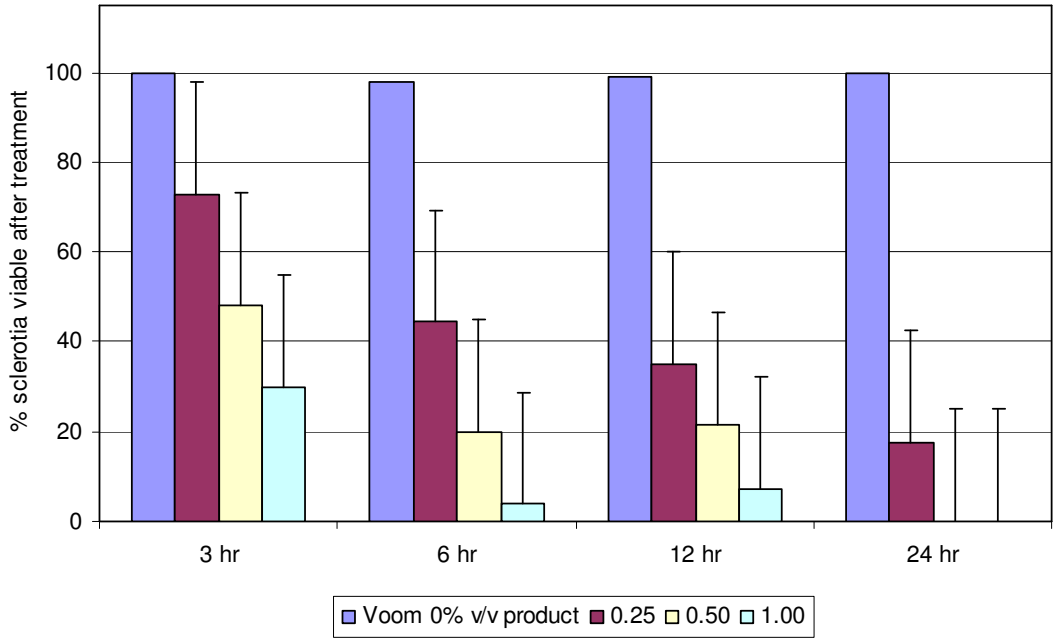

Figure 4. Effects of three concentrations of Voom (0.25%, 0.5% and 1% v/v of product), applied using the vapour phase method and four exposure times, on the survival of sclerotia of *S. cepivorum*. (Error bars are lsds at P=0.05)

Effect of Voom® treatments on five soilborne pathogens

Volatile products released from solutions containing 3% Voom significantly reduced mycelial growth of *S. cepivorum, Fusarium oxysporum and Rhizoctonia solani,* compared to their respective untreated controls, for three of the four exposure times (6, 12 and 24 hrs) tested during the first 4 days of incubation (Table 5). The reductions provided by other treatments were inconsistent for the other pathogens tested (*Sclerotinia minor, Botrytis cinerea and Sclerotium rolfsii)* during the same period. After this period, Voom at 3% did not affect the mycelial growth of all pathogens, except for *F. oxysporum* which grew slower than other pathogens in culture.

Experiments with resting (sclerotial) structures showed that volatile products released (24-hrs exposure) from a solution containing 3% of Voom only significantly reduced the viability of sclerotia of *S. rolfsii* by 85%, *S. cepivorum* by 25% ad *S. minor* by 15% compared to their respective untreated controls (Figure 5).

Table 5. Effect of Voom at 3% v/v of product, applied using the vapour phase method for four exposure times, on mycelial growth of six soilborne pathogens recorded after 4 and 7 days incubation on PDA agar plates.

Pathogen	Treatment (3% v/v of product)	Mycelial growth colony diameter (mm) 4 days 7 days Exposure to volatiles of Voom (hrs)							
		1	6	12	24	1	6	12	24
S. rolfsii	Untreated	23.0	20.0	22.3	15.0	80.0	80.0	80.0	80.0
	Voom	18.3	16.0	24.3	18.3	80.0	80.0	80.0	80.0
S. cepivorum	Untreated	45.0	35.7	35.3	27.0	80.0	80.0	80.0	80.0
	Voom	30.3	32.0	23.3	16.7	80.0	80.0	80.0	80.0
S. minor	Untreated	80.0	80.0	57.3	59.7	80.0	80.0	80.0	80.0
	Voom	62.3	61.3	66.7	55.3	80.0	80.0	80.0	80.0
B. cinerea	Untreated	35.7	61.7	36.7	32.7	80.0	80.0	80.0	80.0
	Voom	33.3	37.3	40.7	28.0	80.0	80.0	80.0	80.0
R. solani	Untreated	19.7	23.0	25.3	18.3	80.0	80.0	80.0	80.0
	Voom	15.0	20.0	13.7	15.0	80.0	80.0	80.0	80.0
F. oxysporum	Untreated	4.3	13.0	13.0	7.7	49.7	53.0	54.7	50.3
	Voom	3.3	5.6	7.3	3.7	42.3	43.3	46.3	40.7

Lsd values for pathogen effect = 1.8 (<0.001); pathogen x treatment effect = 2.2 (<0.001); pathogen x treatment x exposure effects = 3.6 (<0.001); pathogen x treatment x exposure x assessment time effects = 5.0 (<0.001).

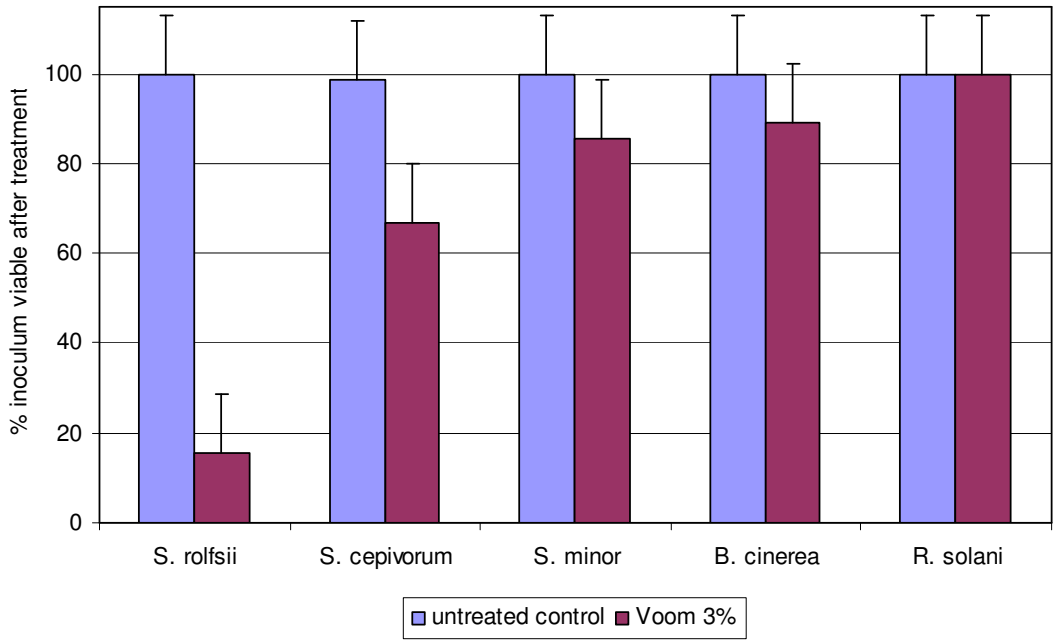

Figure 5. Effect of Voom™ at 3.0% v/v of product applied using the vapour phase method, on the percentage survival of sclerotial inoculum of five soilborne pathogens . (Error bars are lsds at P=0.05)

Effect of diallyl disulphide (DADS) on five soilborne pathogens

Experiments with resting (sclerotial) structures of the same five pathogens showed that volatile products released for 24 hrs from solutions containing 1% of DADS only significantly reduced the viability of sclerotia of *S. minor* by 50% (Figure 6). This rate of DADS was tested because it is the highest field rate (e.g. 10 L/ha in 1000 litres of water) recommended for use as biostimulant of *S. cepivorum* sclerotial germination in the management of onion white rot.

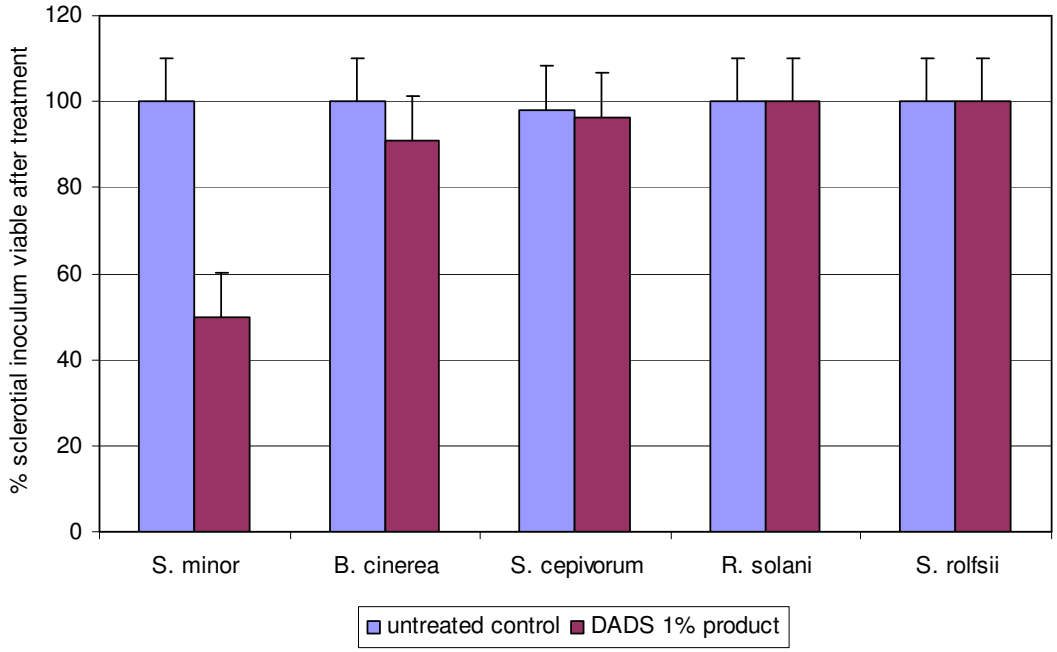

Figure 6. Effect of diallyl disulphide (DADs) at 1.0% v/v of product on the percentage of sclerotial inoculum of five soilborne pathogens that survived the treatment applied using the vapour phase method. (Error bars are lsds at P=0.05).

Effect of plant products on mycelial growth of S. minor and S. sclerotiorum

Volatile products released from three solutions containing 4%, 6% and 8% of Vigor® and 1%, 3% and 5% of Voom® were biocidal to mycelium of *S. minor* (Table 6.) The standard volatile treatment allyl-ITC was also biocidal at 2.5%. Bitter fennel oil at 2%, 4% and 6% significantly inhibited the growth of *S. minor* compared to the untreated control during the first 4 days of incubation and at 6% for 7 days incubation in sealed plates (Table 6). None of these treatments were biocidal to mycelium. The results were similar when solutions of treatments were added to the PDA media (contact phase) before placing a block of agar with the *S. minor* pathogen, except for Vigor® at 4% which was inhibitory of mycelial growth (Table 7.)

The results for *S. sclerotiorum* were similar to those observed with *S. minor* for both vapour and contact phases (Tables 8 and 9).

The effects of the same treatments at concentrations of 1%, 5% and 10% were tested on mycelial growth of *S. minor* using the vapour phase. Two chemical components of bitter fennel oil (fenchone and anethole) were included. The standard treatment allyl-ITC and all Voom® treatments killed mycelium of *S. minor* at all exposure times (2, 3 and 7 days) (Table 10). Fenchone and Vigor® were also biocidal at 5% and 10%. All other treatments significantly reduced mycelial growth compared to untreated controls, except fenchone and Vigor® at 1% for 2 and 3 days of exposure to volatiles.

Table 6. Effect of three concentrations of two plant-derived commercial products (Voom® and Vigor®) and one essential oil (fennel oil), applied using the vapour phase method for 7 days, on mycelial growth of *S. minor* in PDA plates.

Product[1]	**Treatment (% of product)**	**Mycelial growth (mm) first 7 days sealed plates**			
		2 days	**4 days**	**7 days**	**Fresh PDA**[2]
Untreated control	water	16.3	84.0	84.0	84.0
Control Tween	0.5%	49.0	70.3	84.4	84.4
Control Ethanol	20%	0.0	0.0	0.0	84.0
Standard	2.5%	0.0	0.0	0.0	0.0
Vigor	4%	0.0	0.0	0.0	0.0
Vigor	6%	0.0	0.0	0.0	0.0
Vigor	8%	0.0	0.0	0.0	0.0
Fennel oil	2%	0.0	0.0	84.0	84.0
Fennel oil	4%	0.0	0.0	18.3	84.0
Fennel oil	6%	0.0	0.0	0.0	56.0
Voom	1%	0.0	0.0	0.0	0.0
Voom	3%	0.0	0.0	0.0	0.0
Voom	5%	0.0	0.0	0.0	0.0
F-test		<0.001	<0.001	<0.001	
lsd (P=0.001)		1.1	3.5	0.97	

[1] Tween 20 used as emulsifier for oils.. Ethanol used to dissolve standard treatment (allyl-isothiocyanates) before mixing in water.

[2] PDA agar blocks without growth after 7 days incubation in the presence of treatments were transferred to fresh PDA plates to assess viability for a further 7 days.

Table 7. Effects of three rates of two plant-derived commercial products (Voom and Vigor) and one essential oil (fennel oil), applied using the contact phase for 1 week, on mycelial growth of *S. minor* in PDA plates.

Product[1]	Treatment (% of product)	Mycelial growth (mm) first 7 days sealed plates			
		2 days	4 days	7 days	Fresh PDA[2]
Untreated control	water	15.0	84.0	84.0	84.0
Control Tween	0.5%	53.0	84.0	84.0	84.4
Control Ethanol	20%	29.3	51.0	84.0	84.0
Standard	2.5%	0.0	0.0	0.0	0.0
Vigor	4%	0.0	0.0	30.0	84.0
Vigor	6%	0.0	0.0	0.0	0.0
Vigor	8%	0.0	0.0	0.0	0.0
Fennel oil	2%	0.0	0.0	26.0	84.0
Fennel oil	4%	0.0	0.0	84.0	84.0
Fennel oil	6%	0.0	0.0	0.0	84.0
Voom	1%	0.0	0.0	0.0	0.0
Voom	3%	0.0	0.0	0.0	0.0
Voom	5%	0.0	0.0	0.0	0.0
F-test		<0.001	<0.001	<0.001	
lsd (P=0.001)		2.8	8.7	14.5	

[1] Tween 20 used as emulsifier for oils.. Ethanol used to dissolve standard treatment (allyl-isothiocyanates) before mixing in water.

[2] PDA agar blocks without growth after 7 days incubation in the presence of treatments were transferred to fresh PDA plates to assess viability for a further 7 days.

Table 8. Effects of three rates of two plant-derived commercial products (Voom and Vigor) and one essential oil (fennel oil), applied using the vapour phase for 1 week, on mycelial growth of *S. sclerotiorum* in PDA plates.

Product[1]	Treatment (% of product)	Mycelial growth (mm) first 7 days sealed plates			
		2 days	4 days	7 days	Fresh PDA[2]
Untreated control	water	15.7	84.0	84.0	84.0
Control Tween	0.5%	48.0	71.7	84.4	84.4
Control Ethanol	20%	8.0	8.0	31.3	84.0
Standard	2.5%	0.0	0.0	0.0	0.0
Vigor	4%	0.0	0.0	0.0	0.0
Vigor	6%	0.0	0.0	0.0	0.0
Vigor	8%	0.0	0.0	0.0	0.0
Fennel oil	2%	0.0	6.7	37.7	84.0
Fennel oil	4%	0.0	0.0	29.3	84.0
Fennel oil	6%	0.0	0.0	16.0	28.0
Voom	1%	0.0	0.0	0.0	0.0
Voom	3%	0.0	0.0	0.0	0.0
Voom	5%	0.0	0.0	0.0	0.0
F-test		<0.001	<0.001	<0.001	
lsd (P=0.001)		6.4	9.8	31.1	

[1] Tween 20 used as emulsifier for oils.. Ethanol used to dissolve standard treatment (allyl-isothiocyanates) before mixing in water.

[2] PDA agar blocks without growth after 7 days incubation in the presence of treatments were transferred to fresh PDA plates to assess viability for a further 7 days.

Table 9. Effects of three rates of two plant-derived commercial products (Voom and Vigor) and one essential oil (fennel oil), applied using the contact phase for 1 week, on mycelial growth of *S. sclerotiorum* in PDA plates.

Product[1]	Treatment (% of product)	Mycelial growth (mm) first 7 days sealed plates			
		2 days	4 days	7 days	Fresh PDA[2]
Untreated control	water	15.0	84.0	84.0	84.0
Control Tween	0.5%	54.0	84.0	84.0	84.4
Control Ethanol	20%	35.7	84.0	84.0	84.0
Standard	2.5%	0.0	0.0	0.0	0.0
Vigor	4%	0.0	0.0	0.0	84.0
Vigor	6%	0.0	0.0	0.0	0.0
Vigor	8%	0.0	0.0	0.0	0.0
Fennel oil	2%	0.0	0.0	20.0	84.0
Fennel oil	4%	0.0	0.0	72.3	84.0
Fennel oil	6%	0.0	0.0	0.0	84.0
Voom	1%	0.0	0.0	0.0	0.0
Voom	3%	0.0	0.0	0.0	0.0
Voom	5%	0.0	0.0	0.0	0.0
F-test		<0.001		<0.001	
lsd (P=0.001)		3.2		1.8	

[1] Tween 20 used as emulsifier for oils. Ethanol used to dissolve standard treatment (allyl-isothiocyanates) before mixing in water.

[2] PDA agar blocks without growth after 7 days incubation in the presence of treatments were transferred to fresh PDA plates to assess viability for a further 7 days.

Table 10. Effect of three rates of two plant-derived commercial products (Voom and Vigor), one essential oil (fennel oil) and two components in fennel oil (fenchone and anethole), applied using the vapour phase for threw exposure times (2, 3 and 7 days), on mycelial growth of *S. minor* in PDA plates.

		Mycelial growth after 7 days sealed plates			
Product[1]	**Treatment (% product)**	**Exposed to volatiles (days)**			
		2	**3**	**7**	**Fresh PDA (2, 3,7 days)**[2]
Untreated control	water	84.0	84.0	84.0	84.0, 84.0, 84.0
Control Tween	0.5%	84.0	84.0	84.0	84.0, 84.0, 84.0
Control Ethanol	20%	5.0	0.0	0.0	84.0, 84.0, 84.0
Standard	1%	0.0	0.0	0.0	0.0, 0.0, 0.0
Standard	5%	0.0	0.0	0.0	0.0, 0.0, 0.0
Standard	10%	0.0	0.0	0.0	0.0, 0.0, 0.0
Fenchone	1%	84.0	84.0	84.0	84.0, 84.0, 84.0
Fenchone	5%	0.0	0.0	0.0	0.0, 0.0, 0.0
Fenchone	10%	0.0	0.0	0.0	0.0, 0.0, 0.0
Anethole	1%	27.0	18.3	28.0	84.0, 84.0, 84.0
Anethole	5%	7.7	0.0	0.0	84.0, 84.0, 84.0
Anethole	10%	19.3	0.0	0.0	84.0, 84.0, 56.0
Vigor	1%	84.0	84.0	0.0	84.0, 84.0, 84.0
Vigor	5%	0.0	0.0	0.0	0.0, 0.0, 0.0
Vigor	10%	0.0	0.0	0.0	0.0, 0.0, 0.0
Fennel oil	1%	79.7	38.3	69.3	84.0, 84.0, 84.0
Fennel oil	5%	24.3	8.3	6.7	84.0, 84.0, 84.0
Fennel oil	10%	0.0	0.0	0.0	84.0, 84.0, 56.0
Voom	1%	0.0	0.0	0.0	0.0, 0.0, 0.0
Voom	5%	0.0	0.0	0.0	0.0, 0.0, 0.0
Voom	10%	0.0	0.0	0.0	0.0, 0.0, 0.0
F-test		<0.001	<0.001	<0.001	
lsd (P=0.001)		1.1	3.5	0.97	

[1] Tween 20 used as emulsifier for oils. Ethanol used to dissolve standard treatment (allyl-isothiocyanates) before mixing in water.

[2] PDA agar blocks without growth after 7 days incubation in presence of treatments for 2, 3 and 7 days were transferred to fresh PDA plates to assess viability for a further 7 days.

Effect of plant product treatments on viability of sclerotia of S. minor and S. sclerotiorum

In soil assays, volatile products released from solutions containing 3% and 5% Voom® were the most effective treatments (24-hrs exposure) in reducing the viability of sclerotia of both Sclerotinia pathogens (Figure 7). Voom® at 3% and 5% significantly reduced the viability of sclerotia of *S. minor* by 93% and 100%,

respectively, compared to the untreated control (Figure 7). Voom® at 3% and 5% also significantly reduced the viability of sclerotia of *S. sclerotiorum* by 78% compared to the untreated control.

All concentrations of Vigor® (5%, 8% and 10%) did not reduce the viability of sclerotia of both pathogens when compared to untreated controls (Figure 8).

Volatile products released from the solutions of 80% fennel oil was the most effective treatment in reducing the viability of sclerotia of both *Sclerotinia* pathogens (Figure 9). This treatment significantly reduced the viability of sclerotia of *S. minor* and *S. sclerotiorum* by 80% and 73%, respectively, compared to untreated controls. Fennel oil at 50% significantly reduced the viability of *S. sclerotiorum* by only 42%.

Sclerotia were also exposed to volatile products of plant treatments on PDA plates using the vapour and contact exposure methods. The standard treatment allyl-ITC at 2.5% effectively killed sclerotia of both *Sclerotinia* pathogens for both exposure methods (Table 11). Vigor® at 6% and 8% was more effective in reducing the viability of *S. minor* sclerotia than sclerotia of *S. sclerotiorum* for both exposure methods. Voom® at 3% and 5% was more effective in reducing the viability of sclerotia of both pathogens for the vapour exposure method.

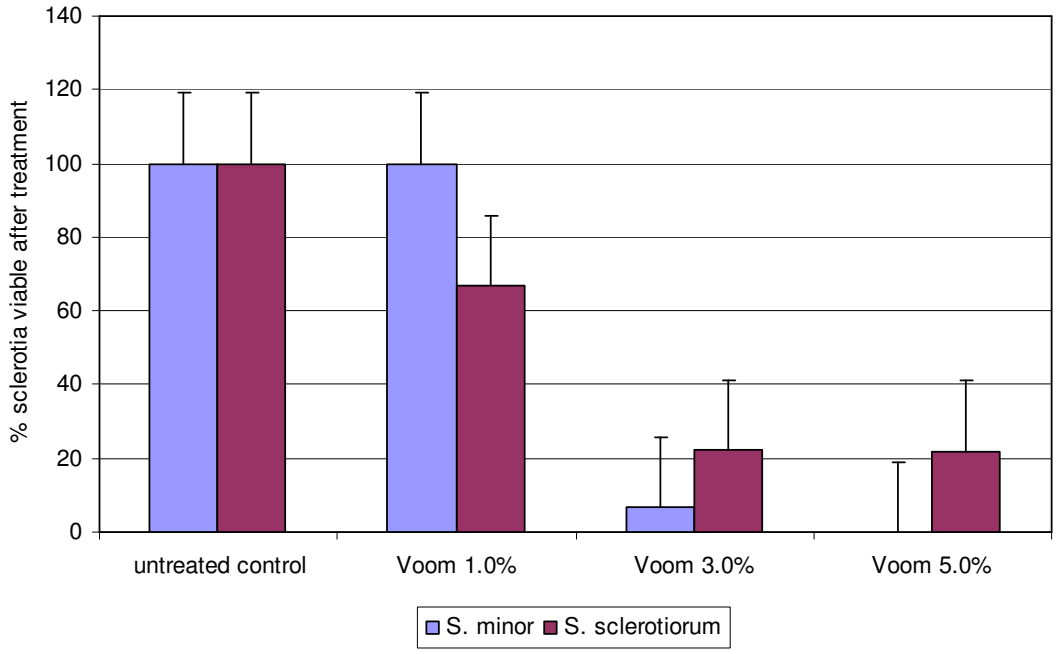

Figure 7. Effect of three rates of Voom (1%, 3% and 5%), applied using the vapour phase method, on the viability of sclerotia of *S. minor* and *S. sclerotiorum* in soil assays (Error bars are lsds at P=0.05)

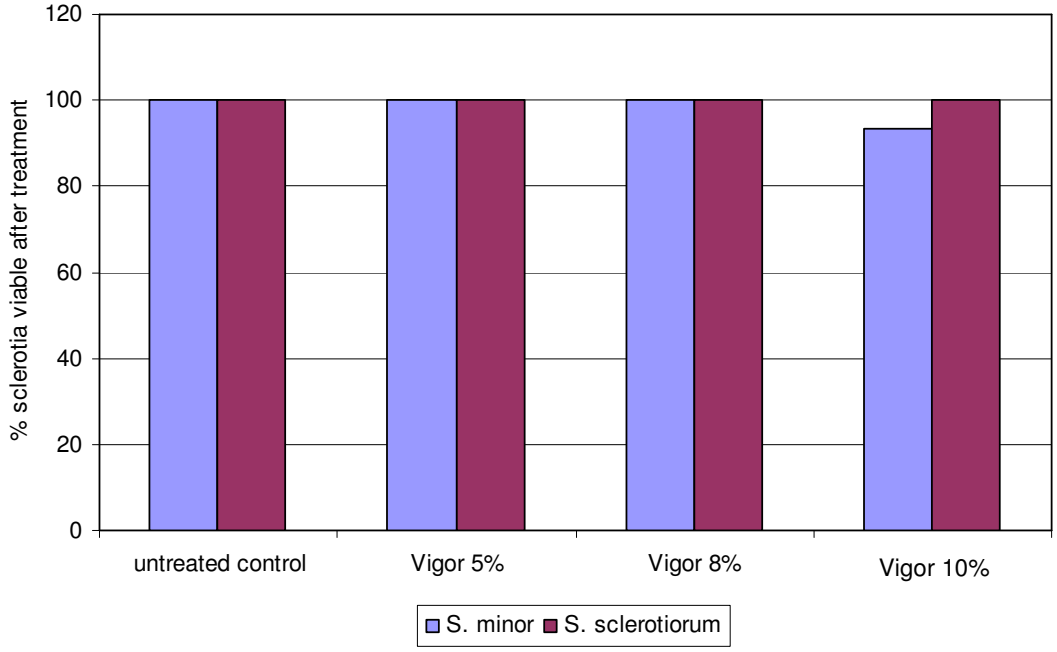

Figure 8. Effect of three rates of Vigor (5%, 8% and 10%), applied using the vapour phase method, on the viability of sclerotia of *S. minor* and *S. sclerotiorum* in soil assays.

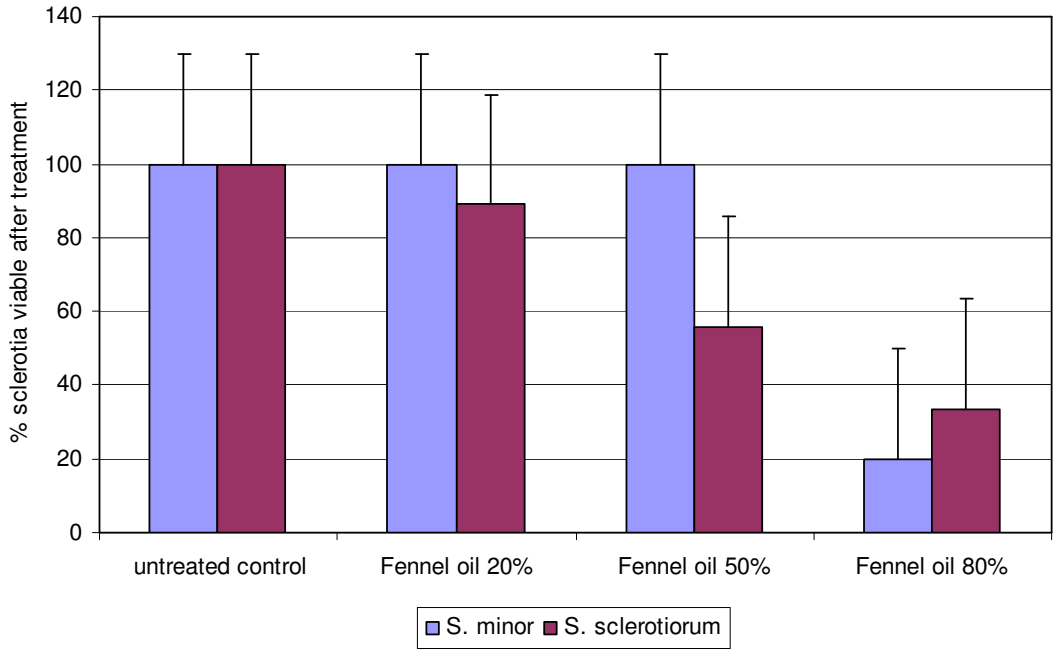

Figure 9. Effects of three rates of fennel oil (20%, 50% and 80%), applied using the vapour phase method, on the viability of sclerotia of *S. minor* and *S. sclerotiorum* in soil assays (Error bars are lsds at P=0.05).

Table 11. Effects of three rates of three plant-derived commercial products (Voom, Vigor, Promax), one essential oil (fennel oil) and two components of fennel oil (fenchone, anethole), applied using contact and vapour phases for 1 week, on the viability of sclerotia of *S. minor* and *S. sclerotiorum* in PDA plates.

Product[1]	**Treatment (% of product)**	**Mean number sclerotia viable from total (3/rep) tested**[2]			
		S. minor		***S. sclerotiorum***	
		contact	**vapour**	**contact**	**vapour**
Untreated control	water	3.0	3.0	3.0	3.0
Control Tween	0.5%	3.0	3.0	3.0	3.0
Control Ethanol	20.0%	3.0	3.0	3.0	3.0
Standard	2.5%	0.6	0.0	0.0	0.0
Vigor	4.0%	2.0	0.6	3.0	0.6
Vigor	6.0%	0.0	0.0	2.0	0.6
Vigor	8.0%	0.0	0.0	3.0	1.0
Fennel oil	2.0%	3.0	3.0	3.0	3.0
Fennel oil	4.0%	3.0	3.0	3.0	3.0
Fennel oil	6.0%	3.0	3.0	3.0	3.0
Fennel oil	7.5%	-	3.0	-	-
Fennel oil	10.0%	-	3.0	-	-
Fennel oil	15.0%	-	3.0	-	-
Voom	1.0%	2.0	3.0	3.0	0.0
Voom	3.0%	2.3	0.0	2.3	0.0
Voom	5.0%	0.0	0.0	1.3	0.0
Promax	2.5%	-	3.0	-	-
Promax	5.0%	-	3.0	-	-
Promax	7.5%	-	3.0	-	-
Anethole	2.5%	-	3.0	-	-
Anethole	5.0%	-	3.0	-	-
Anethole	7.5%	-	3.0	-	-
Fenchone	2.5%	-	3.0	-	-
Fenchone	5.0%	-	3.0	-	-
Fenchone	7.5%	-	3.0	-	-

[1] Tween 20 was used to emulsify oils.Ethanol used to dissolve standard treatment (allyl-isothiocyanates) before mixing in water.

[2] Sclerotia placed on PDA plates then treated with treatments using contact and vapour phases for 7 days. Sclerotia that did not germinate on original plate (3/rep/plate) were transferred to fresh PDA plates to assess viability for a further 7 days.

Evaluating plant products as potential soil treatments in the field

Effect of a pre-plant soil application of Voom® (5% v/v) on the incidence of allium white rot of spring onions (Clyde site)

The standard soil treatment Basamid® was the only treatment that gave a significant reduction of white rot incidence in a field trial with sandy soil (Table 13). There were no significant differences in disease levels between plots covered with plastic sheeting and those without cover during the period of the soil treatment, indicating that overhead irrigation probably provided a good seal which prevented volatile products from escaping from soil.

Table 13. Effect of a pre-plant soil application of Voom on the incidence of onion white rot, caused by *S. cepivorum,* in a spring onion crop, Cranbourne Victoria.

Soil treatment[1]	% spring onions with white rot	
	No plastic[1]	Plastic
Untreated	13.0 a	13.8 a
Voom (5% of product v/v)	10.4 a	10.3 a
Fumafert (400 g m^2)	11.7 a	13.1 a
Basamid (50 g m^2)	5.2 b	5.4 b

[1] Voom was shank-injected into soil using 500 L of water per ha followed by covering with plastic sheet (for 2 weeks) and overhead irrigation.

The effects of pre-plant soil treatments on control of root rot infections of green beans (Lindenow site)

Basamid® and Voom® were the only two treatments that significantly reduced the severity of root infections of green bean plants, compared to the untreated control (Figure 10). Other soil treatments did not provide significant reductions of root infections compared to the untreated control. Soil DNA analysis confirmed the presence of *R. solani* and *Pythium clade f* in soil samples from this site. The levels of *R. solani* were significantly lower in soil treated with Basamid® than in soil treated with other treatments (data not shown). There were no significant differences in the levels of *Pythium clade f* in soil among all treatments.

The incidence of Sclerotinia white mould was too low at this site to allow comparison of treatments

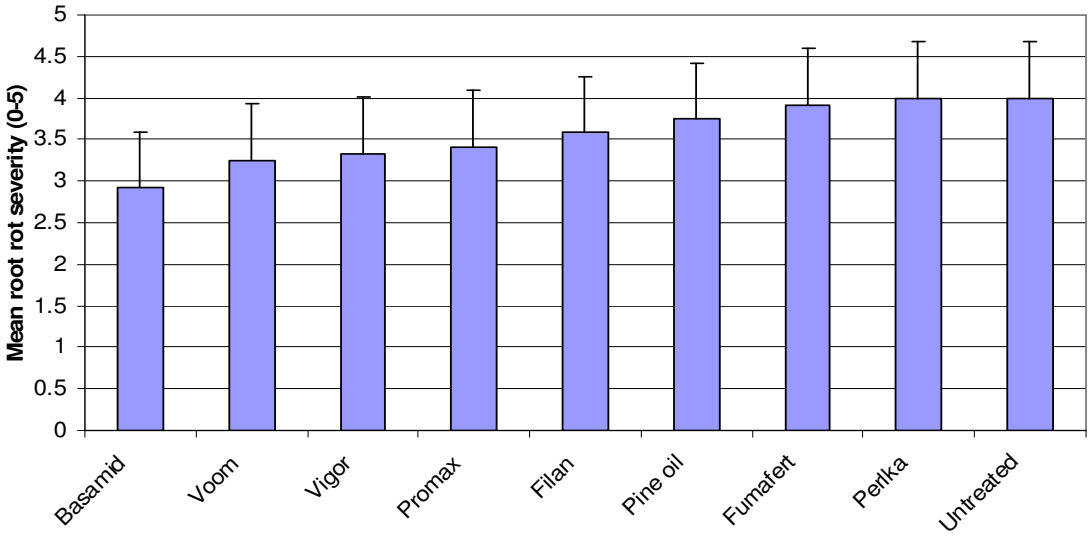

Figure 10. Effects of pre-plant soil treatments on root rot severity of green bean plants grown at Lindenow, Victoria. (Error bars are lsds at P=0.05). Treatments were four plant oil derived products (Vigor, Voom, Promax, Pine oil), one fungicide (Filan), two soil amendments (Perlka and Fumafert) and the standard soil fumigant (Basamid). See table 2 for chemical concentration.

The effect of pre-plant soil treatments on control of sclerotinia lettuce drop and clubroot of Pak Choy (Heatherton site)

On a Pak Choy crop, Basamid® was the only soil treatment that gave a significant reduction of clubroot infection compared to all other treatments (Figure 11). There were no significant differences in clubroot infections among other treatments.

On a lettuce crop, Basamid® and the fungicide Filan® were the only two treatments that provided significant reductions of lettuce drop incidence, caused by *S. minor* (Figure 12).

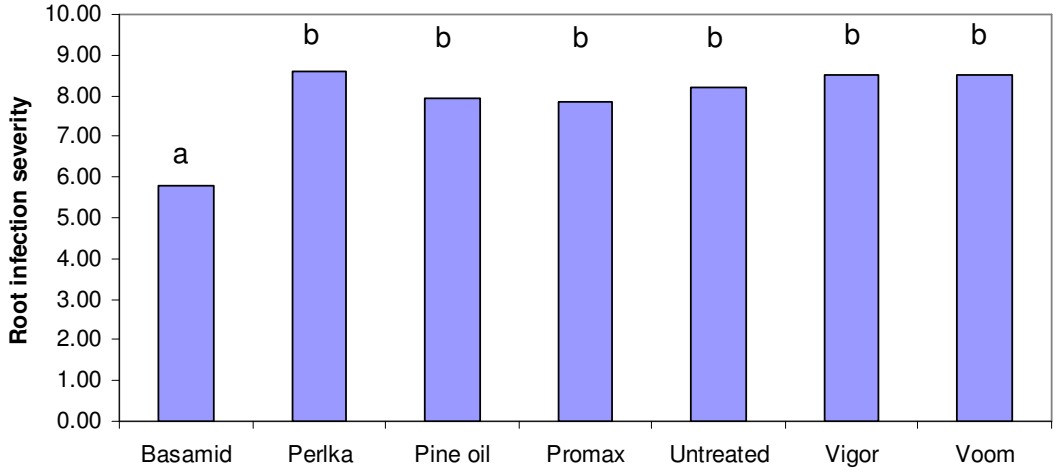

Figure 11. Effects of pre-plant soil treatments on the severity of clubroot of a Pak Choy crop at Heatherton, Victoria. The treatments were four plant oil derived products (Vigor, Voom, Promax, Pine oil), one soil amendment treatments (Perlka) and the standard soil fumigant (Basamid).

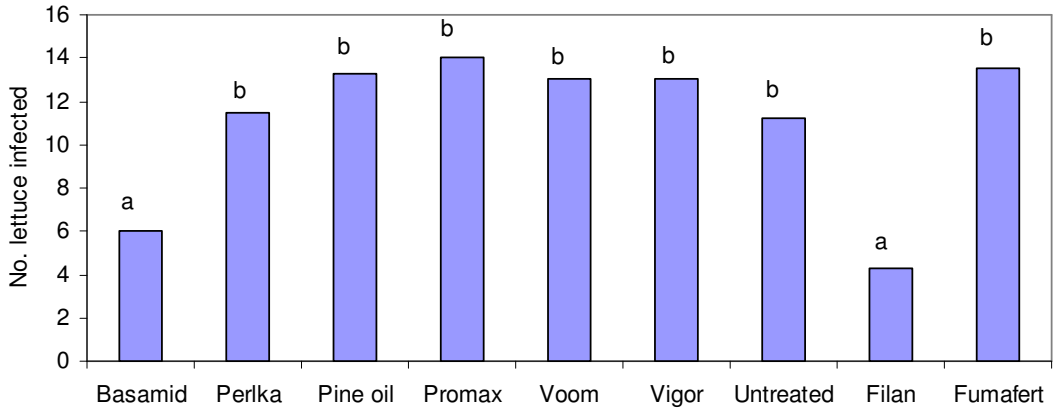

Figure 12. Effects of pre-plant soil treatments on the incidence of Sclerotinia lettuce drop at Heatherton, Victoria. The treatments were four plant oil derived products (Vigor, Voom, Promax, Pine oil), two soil amendment treatments (Perlka and Fumafert) and the standard soil fumigant (Basamid).

Conclusions and Recommendations

In this study six plant products were evaluated for their ability to reduce the viability of mycelial and sclerotial inoculum of important soilborne pathogens of vegetables and for possible use in integrated management programs.

In laboratory and glasshouse experiments, inhibitory and biocidal effects of volatile and contact phases of three plant products including one essential oil were determined on mycelium and sclerotia of plant pathogens. Volatile products released from aqueous solutions containing 3% and 5% of Voom® were the most effective treatments killing mycelium and sclerotia of *S. cepivorum*. Voom® at 3% was also very effective in killing sclerotia of *S. rolfsii* but not resting (sclerotial) structures of three other pathogens (*S. minor, B. cinerea, R. solani*). Lower concentrations of Voom (e.g. 0.75% and 1%) were only inhibitory of mycelial growth and less effective in reducing viability of sclerotia of *S. cepivorum*.

Plant product treatments tested as volatile and contact phases had a significant effect on the viability of mycelium of *S. minor* and *S. sclerotiorum*. The three concentrations of Voom® (1%, 3% and 5%) and Vigor® (4%, 6% and 8%) tested were biocidal to mycelium of *S. minor* and *S. sclerotiorum*. Three concentrations of bitter fennel oil tested (2%, 4% and 6%) were only inhibitory of mycelial growth.

Plant product treatments added to soil had a significant effect on the viability of sclerotia of *S. minor* and *S. sclerotiorum*. For instance, Voom at 3% and 5% caused significant reductions of sclerotia viability of both Sclerotinia pathogens in a soil assay. The three concentrations of Vigor® (5%, 8% and 10%) had no effect on viability of sclerotia in soil. Bitter fennel oil was only effective at reducing viability of sclerotia of *S. minor* and *S. sclerotiorum* at 80% concentration, which is unlikely to be cost-effective for growers.

Fenchone, a component of fennel oil, was biocidal to mycelium of *S. minor* at concentrations of 5% and 10%. Anethole, another component of fennel oil, had no effect on mycelium of *S. minor*. This suggests that fenchone (batch of oil tested had about 10% fenchone) may be the active component giving fennel oil the anti-fungal activity observed (Soylu *et al*. 2007).

Results from laboratory experiments demonstrated the potential of Voom® and Vigor® for use as soil treatments to reduce inoculum of *Sclerotinia* spp. and *S. cepivorum* in soil and this may influence the development of disease in the field.

Three field trials investigated the effects of six plant products including Voom® and Vigor® on inoculum reduction and disease development. In the first field trial, Voom®, applied as a pre-plant treatment to sandy soil at 5% concentration, did not reduce allium white rot incidence compared to the standard soil treatment Basamid®. In the second trial, Voom®, applied at 50 L/ha resulted in a significant reduction of severity of root infections of green beans, caused by *P. clade* f, *R. solani* and probably other soilborne pathogens, and this reduction was statistically similar to the reduction caused by Basamid®. In the third trial, only Basamid® provided a significant reduction of clubroot infection severity and *S. minor* lettuce drop incidence.

The observed reductions in pathogen viability in laboratory experiments and root infections in the field indicate that some of the plant products could have important roles in IPM based management strategies for control of soilborne pathogens of vegetables. Further work is therefore required to continue evaluating these and newer

plant products to identify field rates that provide effective control of soilborne pathogens and other beneficial effects (e.g. nematode control). The benefit:cost and the antifungal mechanisms on hyphae and sclerotia also need to be investigated.

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CHAPTER 2 - EVALUATING FUNGICIDE AND ALTERNATIVE TREATMENTS FOR SCLEROTINIA CONTROL IN LETTUCE AND GREEN BEANS

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SUMMARY

In field trials in Victoria, two applications of Filan® provided consistent good control of *S. minor* lettuce drop under both high and low disease pressure. Switch® and Shirlan® also gave good control of *S. minor* infection in low disease incidence sites and therefore are potential alternatives to Filan® for lettuce drop control. Two applications of Folicur® (tebuconazole) were ineffective in reducing the incidence of *S. minor* lettuce drop at two of three sites with low disease. In a bean trial, however, two applications of Folicur® gave good control of white mould, caused by *S. sclerotiorum,* under low disease pressure. None of the alternative products evaluated for Sclerotinia control were effective in controlling *S. minor* infection in field and pot trials.

Introduction

There are only a limited number of fungicide treatments available with minor permits (Filan®, Folicur®, Amistar®) for the management of Sclerotinia diseases in lettuce and green beans in Australia. Because there is limited efficacy data for Folicur® and Amistar® growers mostly prefer to use Filan® (a.i. boscalid), which is very expensive, to control lettuce drop, caused by *S. minor*, and bean white mould, caused by *S. sclerotiorum*. Reports of unsatisfactory disease control with Filan® in high disease conditions and development of resistance in other pathogens (*Alternaria alternata*) overseas are two additional concerns for growers (Donald and Villalta 2007; Avenot and Michailides 2007). Hence a priority for researchers and growers in Australia is to develop improved control strategies for Sclerotinia, including identification of alternative fungicide treatments for Filan® and development of non-chemical treatments.

Several fungicide treatments including Switch® (cyprodinil and fludioxonil), Filan® (boscalid), Pristine® (boscalid and pyraclostrobin) and Rovral® (iprodione) have been reported to reduce the incidence of Sclerotinia lettuce drop, caused by *S. minor* (Matheron and Porchas 2005; Miller *et al*. 2005; Wright 2007). Wright (2007) found that Perlka® (calcium cyanamide) caused significant reduction of *S. minor* infection on field lettuce in New Zealand. Multiple applications of Contans®, formulated with the biocontrol agent *Coniothyrium minitans*, also provided useful control of lettuce drop caused by *S. minor* and *S. sclerotiorum* in field lettuce in Arizona and California,

USA (Matheron and Porchas 2005; Pryor 2007). Folicur® (tebuconazole), Filan® and Amistar® (azoxystrobin) gave good control of Sclerotinia stem rot on winter oil seed rape (Gladders and Oxley 2007). These fungicides and alternative treatments have not been widely evaluated in Australia for control of diseases caused by *S. minor* and *S. sclerotiorum* in vegetables. On green beans, white mould is caused mainly by air-borne ascospores of *S. sclerotiorum*, and control requires application of fungicides during flowering to protect senescing petals and damaged tissue against infection. On the other hand, *S. minor* attacks host plants through mycelial contact. In Victoria, lettuce drop is caused mainly by *S. minor* but in other parts of Australia eg. the Lockyer Valley in Qld, it can be caused by both *S. minor* and *S. sclerotiorum*. This can confound current disease management strategies as the two fungi require different control tactics.

Field trials were conducted in vegetable farms to evaluate Filan® and alternative fungicide treatments for the control of lettuce drop, caused by *S. minor*, and bean white mould, caused by *S. sclerotiorum*. Two fertilisers (Perlka™ and Stand SKH™) and one biocontrol (Contans®) treatment were also evaluated for their ability to control *S. minor* infection on field lettuce. A glasshouse study investigated the efficacy of several nutrient amendment and self-defence inducer treatments on control of *S. minor* infection on lettuce seedlings in pots.

Material and Methods

Field study

Field trials

In total, seven fungicide trials were conducted to evaluate fungicide and alternative treatments for Sclerotinia control. Filan™ was used as the standard control treatment in all trials. Five of the seven trials compared the efficacies of nine fungicide treatments on control of *S. minor* lettuce drop (Table 1). One of these five trials was carried out by Classy Solutions. Two of the five trials also evaluated the effect of a pre-plant soil application with calcium cyanamide (Perlka™) on control of *S. minor* lettuce drop. The sixth trial investigated the ability of the biocontrol agent *C. minitans* (Contans®) and the foliar fertiliser Stand SHK® (AgriChem) to reduce *S. minor* lettuce drop. The seventh trial evaluated the efficacy of Folicur® alone and integrated with Filan® on control of bean white mould, caused by *S. sclerotiorum*. The trials were carried out in vegetable farms in Bacchus Marsh (1), Heatherton (2), Werribee (3) and Lindenow (1), Victoria. The trials were established within commercial crops grown in soils naturally infested with sclerotia of *S. minor*, with the exception of Lindenow where soil was infested by sclerotia of *S. sclerotiorum.*

Treatments

Table 1 shows the fungicide treatments evaluated including rates tested. A maximum of two sprays were applied for each treatment per crop. In lettuce trials, fungicide

treatments were applied after transplanting lettuce and repeated 2 weeks later, with the exception of Bacchus Marsh where the second spray was applied 4 weeks after the first application. In the third trial at Werribee, two late applications of Filan® (Nufarm), applied 2 and 4 weeks after planting, were compared to the standard Filan® treatment. All fungicide treatments were applied using a knapsack and a boom sprayer fitted with three cone (drench) nozzles calibrated to deliver the appropriate amount of fungicide solution to each plant using 1000 L water/ha. Sprays were applied banded to plant rows followed by overhead (sprinkler) irrigation to distribute the chemicals into the root zone and base of plants. In the bean trial, fungicide treatments were applied with the grower's precision sprayer, calibrated to deliver the treatments using 300 litres water/ha. Agral (Syngenta) was used as the wetter at 60 mL/100L water. The first spray was applied when bean plants were at 20-30% flowering and the second 7 days later.

Table 1. Fungicide treatments evaluated for Sclerotinia control in field trials in Victoria.

Treatment	Active ingredient	Field rate (product)
1. untreated control		
2. Filan® - Nufarm	500 g a.i./kg boscalid	1.0 kg/ha
3. Sumisclex® - Sumitomo	500 g a.i./L procymidone	1.0 L/ha
4. Shirlan® - Nufarm	500 g a.i./L fluazinam	350 ml/ha
5. Switch® - Syngenta	375 cyprodinil + 250 fludioxonil g/kg	1.0 kg/ha
6. Folicur® - Bayer	430 g a.i. /L ha tebuconazole	350 ml/ha
7. Rovral® - Bayer	500 g a.i./L iprodione	1.0 L/ha
8. Bavistin® - Dupond	500 g a.i./L carbendazim	1.0 L/ha
9. Scala® - Bayer	400 g a.i./L pyrimethanil	1.25 L/ha

Perlka™ (calcium cyanamide) was tested as a pre-plant soil treatment at 500 kg product per ha. It was incorporated into the top 10 cm of soil (root zone) with a rake 7 days before lettuce transplanting followed by irrigation with overhead sprinklers. This was done to induce early release of large amounts of nitrogen to destroy sclerotia and avoid plant phytotoxicity. The fertiliser Stand SHK™ (20% silica, 15% potassium and 1% humic acids; Agrichem) was tested as a foliar treatment at 3 and 5 L product/ha using 800 L water/ha. It was applied 2 and 4 weeks after planting with the knapsack method previously described, with the exception that spray nozzles were used.

An isolate of *Coniothyrium minitans* (Contans®), with proven antagonistic activity against *S. sclerotiorum*, (Luth, 2001), was evaluated in this study Contans® was supplied by Prophyta (www.prophyta.de), Germany, and tested at 2, 4 and 8 kg/ha. This was a solid substrate formulation of spores which was mixed with water prior to application with a watering can using 4000 L of water/ha. The population density of *C. minitans* (colony forming units) in the product used was determined using a standard dilution plating technique and selective medium (Jones and Stewart, 2000). Contans® had 5.6-6.4 x 10^8 cfu/g product. The first application of Contans® was applied as a drench onto lettuce plant residue prior to incorporation into soil on 18/04/2008. Cauliflower was then planted on 30/06/2008. The second application was applied also as a drench but banded to rows of lettuce seedlings immediately after planting on 12/12/2008.

Lettuce and green bean crops were grown using standard grower practices. Fertilisers, pesticides and non-sclerotinia fungicides were applied as per local commercial practices. Trials were laid out in randomised blocks with each treatment replicated four to five times. Each plot consisted of a raised (lettuce) or flat (bean) bed 1.0-1.3 m wide by 9-10 m long (lettuce) and 6 m by 38 m (bean), respectively. Each lettuce plot contained 90-144 lettuce plants spaced along three (Heatherton) or four rows (Werribee, Bacchus Marsh) per bed. Bean plants were grown in rows spaced 50 cm.

Assessments

The population of sclerotia of *S. minor* and *S. sclerotiorum* (Lindenow) were determined in soils from all field trials prior to treatment application and/or bed formation. Ten soil samples were collected arbitrarily from each field to recover sclerotia using the wet sieving method and their viability tested on potato dextrose agar medium. In lettuce trials, the cumulative number of plants infected by *S. minor* was recorded at weekly intervals until harvest. The fresh weight of six lettuce plants harvested from inside one square meter in each plot was recorded at harvest in three fungicide trials. In the bean trial, the number of plants infected with white mould were counted along a two metre linear transect inside each plot (n = 6). Inoculum, disease and fresh weight data were analysed using analysis of variance (Genstat®). Data were transformed as required before analysis and when the variance analysis indicated a significant treatment effect, Fisher's LSD tests (5% level) were used to compare the means of treatments.

Glasshouse study

Two pot trials investigated the ability of five nutrient amendments and three self-defence inducers treatments, tested at commercial rates, to control *S. minor* infection on lettuce seedlings. One biocontrol agent of *S. minor* was also tested. The aim of this work was to identify new treatments that could be applied to lettuce transplants in the nursery and/or at transplanting for the control of *S. minor* infection in the field.

Treatments

The treatments, rates and application methods tested are described in Tables 8 (pot trial 1) and 9 (pot trial 2). The six nutrient amendment treatments were: AgroMate® (80% OM, 70% humic acids; Agrichem), SupaHumus® 26 humic acids liquid (Agrichem), Stand SHK® (potassium silicate; Agrichem), KCS Enhance® (7% Ca, 11% silica, 5% potassium; Agrichem), Perlka® (calcium cyanamide) and Rustica® (Campbells, Australia). The self-defence inducer treatments were Bion® (acibenzolar-S-methyl, Syngenta), Chitin (1% liquid formulation; Ellis and Associates) and Aminogrow® (Chitosan, OCP). *Trichoderma hamatum* was the biocontrol agent of *S. minor* (LettuceMate®; Agrimm Technologies). Treatments included untreated, pathogen and substrate (grain only) controls. Filan™ was used as the standard control treatment.

Lettuce transplants (Iceberg) were purchased from Boomaroo's nursery two weeks before setting up the trials. The transplants were kept in a glasshouse until used and

had no fungicide treatments applied for Sclerotinia. Inoculum of *S. minor* was prepared on autoclaved barley grain with an isolate of *S. minor,* collected from an infected Cos lettuce and incubated for 3 weeks at 20ºC. The commercial potting mix (Biogrow) used was steam sterilised then mixed with infested grain at 1% inoculum (w/w). The fungus in the colonised grains consisted mainly of mycelium. Treatments were applied using a variety of methods including soil amendment, transplant and at planting treatments and a combination of the latter two (Tables 7 and 8). One lettuce seedling was planted in each pot (14 x 12 cm). Soil amendment treatments were applied to inoculated potting mix prior to planting transplants. Transplant treatments were applied to lettuce transplants in nursery trays seven days before planting into pots with potting mix inoculated. Drench treatments were applied immediately after planting transplants using 100 ml of solution added as a drench to each lettuce transplant. Bion® was sprayed to run-off as a foliar treatment. After application of treatments, pots were arranged on glasshouse benches in randomised blocks with each treatment replicated four times.

Assessments

The number of seedlings that were infected and killed by *S. minor* was recorded four weeks after planting into pots. In the first pot trial, a second set of lettuce transplants was planted into soil without inoculum to determine the effect of nine of the treatments on plant growth. Plant fresh weight was measured on four lettuce plants per treatment two weeks after planting. In the second pot trial, soil pH was measured on two samples of soil (n = 2) for selected treatments. The experiments were analysed by ANOVA using Genstat for Windows 12th edition (Lawes Agricultural Trust, Rothamsted Experimental Station).

Results

Field study

Sclerotia

S. minor sclerotia extracted from soil prior to the trials were 12-38/kg soil at the Bacchus Marsh site and 3-8/kg soil at the other field sites (data not shown). At the bean trial, sclerotia of *S. sclerotiorum* were 3/kg soil.

Bacchus Marsh trial

Incidence of *S. minor* lettuce drop in untreated control plots was 18.1% at harvest (Table 2). Significant control of lettuce drop was obtained with all products tested at this site, except Scala®. Filan®, Sumisclex® and Bavistin® significantly reduced disease incidence from 18.1% to 1.6%, 3.5% and 6.1%, respectively (Table 2). Filan® and Sumisclex® caused the greatest reduction of disease (80-91%). Teldor® and Bavistin® provided equivalent disease control. Rovral® and Scala® were the least effective. There were no significant differences in the fresh weights of lettuce plants (Green Butterhead, cv 'Jerka') at harvest at this site (Table 2).

Table 2. Trial 1 mean incidence of Green Butterhead lettuce plants infected by *S. minor* at harvest in a trial conducted at Bacchus Marsh in autumn 2002, Victoria.

Treatment	Mean % infected lettuce plants	Fresh weight kg
Untreated control	18.1 d	6.38
Scala® 1.25 L/ha	13.2 cd	6.56
Rovral® 1L/ha	12.1 c	7.28
Teldor® 1 L/ha	10.0 bc	5.20
Bavistin® 1L/ha	6.1 ab	6.45
Sumisclex® 1L/ha	3.5 a	6.27
Filan® 1 kg/ha	1.6 a	7.84

Means followed by the same letter are not significantly different at $P = 0.05$ according to LSD test.

Heatherton and Werribee trials

There were low incidences of *S. minor* lettuce drop (5.7-7.6%) in the other four fungicide trials carried out during 2008-2010 (Tables 3 and 4). All treatments significantly reduced disease incidence in the spring trial, but not in the autumn trial at Heatherton. In the first trial, Shirlan®, Switch® and Filan® gave modest but significant disease control (40-66%) (Table 3). These treatments significantly reduced disease from 5.7% to 1.7%, 3.2% and 3.4%, respectively, in a Cos lettuce crop (cv. 'Ezmina') grown in a sandy soil during autumn 2008 (Table 3). Shirlan® caused the greatest disease reduction, which was statistically similar to that provided by Filan® and Switch®. In the second Heatherton trial, Shirlan®, Filan® and Switch® also gave a modest but significant disease control (61-70%) (Table 3). These treatments significantly reduced disease from 6.0% to 1.8%, 1.9% and 2.3%, respectively, in Cos lettuce grown during spring 2008. Folicur® gave significant control (63%) only in the spring trial.

Table 3. Trials 2 and 3 mean incidence of Cos lettuce plants infected by *S. minor* at harvest in two field trials carried out in Heatherton in autumn and spring 2008, Victoria.

Treatment	Mean % infected lettuce plants	
	Autumn[1]	Spring
Untreated control	5.7 a	6.0 a
Folicur®, 0.35 L/ha	7.1 a	2.2 b
Perlka®, 500 kg/ha	5.9 a	-
Filan® 0.8 kg/ha	3.4 ab	1.9 b
Switch®, 1.0 kg/ha	3.2 ab	2.3 b
Shirlan®, 0.35 L/ha	1.9 b	1.8 b

Means followed by the same letter are not significantly different at $P = 0.05$ according to LSD test.
[1] There was no significant difference in fresh weight of plants (six plants/plot) among treatments in the autumn trial.

In the autumn trial at Werribee, Shirlan®, Sumisclex®, Switch® and Filan® gave significant control (73-82%) of *S. minor* lettuce drop (Table 4). These treatments significantly reduced disease from 7.6% to 1.3%, 1.4%, 2.0% and 1.3%, respectively, in an iceberg lettuce crop (cv 'Casino') grown in a clay loam soil during autumn 2008

(Table 4). In the spring trial at Werribee, Shirlan®, Filan® and Switch® gave equivalent disease control (52-78%) in iceberg lettuce grown during late spring and early summer 2008-2009 (Table 4). Rovral®, Bavistin® and Folicur® were not effective in trials at Werribee. There were no significant differences in fresh weight of lettuce plants among treatments in the autumn trials at Heatherton and Werribee (data not shown).

Table 4. Trials 4 and 5 mean incidence of Iceberg lettuce plants infected by *S. minor* at harvest in two field trials carried out at Werribee in autumn 2008 and spring-summer 2008-09, Victoria.

Treatment	Mean % infected lettuce plants	
	Autumn[1]	Spring-summer
Untreated control	7.6 a	6.0 a
Rovral®, 1L/ha	-	4.2 a
Bavistin®, 0.5L/ha	-	3.1 a
Folicur®, 0.35 L/ha	6.0 a	3.5 a
Perlka®, 500 kg/ha	7.4 a	-
Filan® 1 kg/ha	1.3 b	1.9 b
Sumisclex®, 1 L/ha	1.4 b	-
Switch®, 1 kg/ha	2.0 b	1.3 b
Shirlan®, 0.35 L/ha	1.3 b	2.9 ab

Means followed by the same letter are not significantly different at $P = 0.05$ according to LSD test.
[1] There was no significant difference in plant fresh weight (six plants/plot) between treatments in the autumn trial.

Alternative treatments

Perlka™ (calcium cyanamide) did not reduce *S. minor* infection compared to the untreated controls in both the Heatherton (sandy soil) and Werribee (clay loam soil) trials carried out during autumn 2008 (Tables 3 and 4).

The incidence of *S. minor* lettuce drop was also relatively low (6.2%) in the Werribee trial that evaluated the biological control agent *C. minitans* (Contans™) and Stand SHK (Table 5). In the first lettuce crop assessed at this site, Filan® treatments gave good control of disease (56-69%) compared to the untreated control in an iceberg crop (cv 'Casino') grown during late spring and early summer 2008-2009 (Table 5). The biocontrol and nutrient (Stand SHK™) treatments were not effective. In the second crop assessed, disease levels were too low to allow comparison of treatments (Table 5).

Table 5. Trial 6 mean incidence of Iceberg lettuce plants infected by *S. minor* at harvest in a trial carried out during 2008-2010 at Werribee, Victoria.

Treatment	Mean percentage infected lettuce plants	
	Spring-summer 2008-09	Summer-autumn 2009-10
Untreated control	6.2 a	3.3
Contans® 2 kg/ha	4.7 a	2.3
Contans® 4 kg/ha	6.0 a	2.5
Contans® 8 kg/ha	6.4 a	2.8
Stand SHK® 3 L/ha	5.2 a	-
Stand SHK® 5 L/ha	5.6 a	-
Filan® 1 kg/ha (standard)	1.9 b	1.2
Filan® 1 kg/ha (late application)	2.7 b	1.3

Means followed by the same letter are not significantly different at $P = 0.05$ according to LSD test.

Bean trial

In the bean trial at Lindenow, good control of white mould, caused by *S. sclerotiorum*, was obtained with the three fungicide treatments tested under low disease pressure conditions (Table 6, Figure 1). Two applications of Filan® over the flowering period gave the best disease control (95%). Similarly, either two applications of Folicur® or one of Folicur® plus one of Filan® gave significant control reducing disease by 82% and 74%, respectively, compared to the untreated control.

Table 6. Trial 7 mean incidence of green bean plants infected by *S. sclerotiorum* at harvest in a trial at Lindenow during spring-early summer 2009, Victoria.

Treatment	Mean percentage infected green bean plants[1]
Untreated control	9.5 a
Filan® 1 kg/ha	0.4 c
Folicur®, 0.35 L/ha	1.7 bc
Folicur® + Filan®	2.5 b

Means followed by the same letter are not significantly different at $P = 0.05$ according to LSD test.

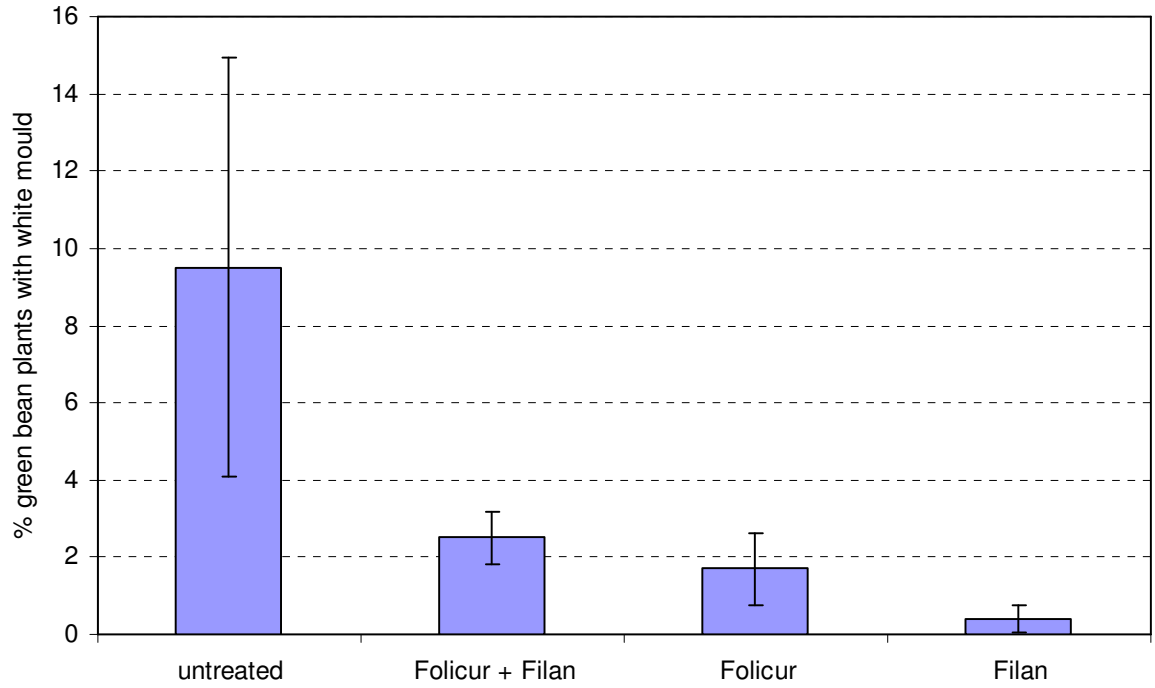

Figure 1. Trial 7 - Mean percentage of green bean plants infected by *S. sclerotiorum* at Lindenow, Victoria (error bars are SEM).

Pot trials

In the first pot trial, only Filan® applied to lettuce transplants as a drench at planting protected two out of four seedlings against *S. minor* infection (Table 7). All SKH Ca® treatments gave some level of protection during the first ten days after planting but by day 14 all seedlings were infected. All other treatments were ineffective at protecting seedlings against infection. Bion® significantly reduced the fresh weight of seedlings compared to other treatments (Table 7).

In the second pot trial, only Filan® at 2 kg/ha and Perlka®, applied as a soil amendment treatment at 3% and 5% (w/w), protected three out of four seedlings against *S. minor* infection (Table 8). All other treatments were ineffective at protecting seedlings against infection. The three rates of Perlka® tested (1%, 3% and 5% w/w) increased soil pH from 6.1 (control) to 8.1, 9.8 and 10.4, respectively, and this was extremely phytotoxic to lettuce seedlings. Soil pH in other treatments ranged from 5.8 to 6.3 including the untreated control.

Table 7. Pot trial 1 -Effects of nutrient amendment and self-defence inducer treatments on control of *S. minor* infection on lettuce seedlings in a pot trial.

Product	Treatment[1]	Average no plants killed by *S. minor* after planting[2]			Plant weight (grams)[3]
		Day 3	Day 10	Day 14	
Untreated control	Potting mix only	0.0	0.0	0.0	-
Pathogen control	P. mix + inoculated grain	1.0	3.0	4.0	-
Substrate control	P. mix + grain only	0.0	0.0	0.0	22.12
Filan™	Drench, 1 kg in 1000L/ha	0.0	0.0	2.0	21.52
T. hamatum (LettuceMate™)	Transplant (4 g/L)	0.0	3.0	4.0	20.97
T. hamatum (LettuceMate™)	Transplant+potting (4 g/L)	0.0	3.0	4.0	-
H. acids liquid (Agrichem)	Transplant, 1L in 100L/ha	0.0	2.0	4.0	-
H. acids liquid (Agrichem)	Transplant, 2L in 100L/ha	1.0	3.0	4.0	24.60
H. acids liquid (Agrichem)	Transplant+drench, 1 L	1.0	3.0	4.0	-
H. acids liquid (Agrichem)	Transplant+drench, 2 L	1.0	4.0	4.0	-
H. acids dry (Agrichem)	Soil amendment, 1% w/w	0.0	2.0	4.0	-
H. acids dry (Agrichem)	Soil amendment, 2% w/w	0.0	3.0	4.0	-
Stand SKH® (Agrichem)	Transplant, 3 L in 1000L/ha	1.0	3.0	4.0	-
Stand SKH® (Agrichem)	Transplant, 5 L in 1000L/ha	0.0	3.0	4.0	23.75
Stand SKH® (Agrichem)	Drench, 3 L in 1000L/ha	1.0	4.0	4.0	-
Stand SKH® (Agrichem)	Drench, 5 L in 1000L/ha	1.0	3.0	4.0	-
Stand SKH® (Agrichem)	Transplant+drench, 3 L	1.0	3.0	4.0	-
Stand SKH® (Agrichem)	Transplant+drench, 5 L	1.0	3.0	4.0	-
SKH Ca® (Agrichem)	Transplant, 3 L in 1000L/ha	0.0	2.0	4.0	-
SKH Ca® (Agrichem)	Transplant, 5 L in 1000L/ha	0.0	1.0	4.0	24.95
SKH Ca® (Agrichem)	Transplant+drench, 3 L	0.0	1.0	4.0	-
SKH Ca® (Agrichem)	Transplant+drench, 5 L	0.0	1.0	4.0	-
Chitin liquid (1%)	Transplant, 5 ml/L	1.0	3.0	4.0	-
Chitin liquid (1%)	Transplant, 10 ml/L	1.0	2.0	4.0	20.60
Chitin liquid (1%)	Transplant+drench, 5 ml/L	1.0	3.0	4.0	-
Chitin liquid (1%)	Transplant+drench, 10 ml/L	0.0	3.0	4.0	-
Aminogrow® (OCP)	Transplant, 5 ml/L	0.0	2.0	4.0	-
Aminogrow® (OCP)	Transplant, 10 ml/L	1.0	2.0	4.0	24.37
Aminogrow® (OCP)	Transplant+drench, 5 ml/L	0.0	2.0	3.0	-
Aminogrow® (OCP)	Transplant+drench, 10 ml/L	0.0	2.0	4.0	-
Bion® (Syngenta)	Foliar, 25 µgrams/500 water	1.0	3.0	4.0	10.25
Bion® (Syngenta)	Foliar, 25 µgrams/500 water	0.0	3.0	4.0	-
F-test					<0.001
lsd (P = 0.05)					4.33

[1] Transplant treatments applied 7 days before transplanting. Lettuce transplants planted into pots immediately after mixing amendments into soil with mycelial inoculum. Drench treatments applied immediately after transplanting using 100 mL of solution added as a drench onto each transplant, except Bion® (foliar application).

[2] One lettuce transplant planted into each 14 x 12 cm pot (total 4 pots per treatment) to simulate application of treatments in the field.

[3] Plant weight is the average fresh weight of four lettuce plants taken 4 weeks after transplanting.

Table 8. Pot trial 2 - Effects of nutrient amendment and self-defence inducer treatments on control of *S. minor* infection on lettuce seedlings in a pot trial.

Product	Treatment[1]	Average no. plants killed by *S. minor*[2]	Soil pH[3]	SEM[3]
Untreated control	Potting mix only	0.0	6.08	0.035
Pathogen control	P. mix + inoculated grain	4.0	-	-
Substrate control	P. mix + grain only	0.0	-	-
Filan™	1 drench, 0.5 kg in 1000L/ha	4.0	-	-
Filan™	2 drench, 0.5 kg in 1000L/ha	4.0	5.80	0.045
Filan™	1 drench, 1.0 kg in 1000L/ha	3.0	-	-
Filan™	2 drench, 1.0 kg in 1000L/ha	3.0	5.96	0.137
Filan™	1 drench, 2.0 kg in 1000L/ha	1.0	-	-
Filan™	2 drench, 2.0 kg in 1000L/ha	2.0	5.93	0.038
Rustica™ fertiliser	Soil amendment, 1% w/w	4.0	6.01	0.037
Rustica™ fertiliser	Soil amendment, 3% w/w	4.0	6.05	0.196
Rustica™ fertiliser	Soil amendment, 5% w/w	4.0	6.08	0.248
Perlka™, fertiliser	Soil amendment, 1% w/w	3.0	8.08	0.663
Perlka™, fertiliser	Soil amendment, 3% w/w	1.0	9.74	0.068
Perlka™, fertiliser	Soil amendment, 5% w/w	1.0	10.37	0.133
SKH Ca® (Agrichem)	1 drench, 1% v/v	4.0	-	-
SKH Ca® (Agrichem)	2 drench, 1% v/v	4.0	6.01	0.052
SKH Ca® (Agrichem)	1 drench, 3% v/v	4.0	-	-
SKH Ca® (Agrichem)	2 drench, 3% v/v	4.0	6.02	0.098
SKH Ca® (Agrichem)	1 drench, 5% v/v	4.0	-	-
SKH Ca® (Agrichem)	2 drench, 5% v/v	4.0	6.24	0.008
Stand SKH® (Agrichem)	1 drench, 1% v/v	4.0	-	-
Stand SKH® (Agrichem)	2 drench, 1% v/v	4.0	6.07	0.046
Stand SKH® (Agrichem)	1 drench, 3% v/v	4.0	-	-
Stand SKH® (Agrichem)	2 drench, 3% v/v	4.0	6.11	0.062
Stand SKH® (Agrichem)	1 drench, 5% v/v	4.0	-	-
Stand SKH® (Agrichem)	2 drench, 5% v/v	4.0	6.31	0.051
H. acids dry (Agrichem)	Soil amendment, 1% w/w	4.0	5.96	0.010
H. acids dry (Agrichem)	Soil amendment, 3% w/w	4.0	5.93	0.038
H. acids dry (Agrichem)	Soil amendment, 5% w/w	4.0	5.75	0.119
H. acids liquid (Agrichem)	1 drench, 1% v/v	4.0	-	-
H. acids liquid (Agrichem)	2 drench, 1% v/v	4.0	5.97	0.009
H. acids liquid (Agrichem)	1 drench, 3% v/v	4.0	-	-
H. acids liquid (Agrichem)	2 drench, 3% v/v	4.0	6.01	0.042
H. acids liquid (Agrichem)	1 drench, 5% v/v	4.0	-	-
H. acids liquid (Agrichem)	2 drench, 5% v/v	4.0	6.05	0.008
Chitin liquid (Ellis & Assoc)	1 drench, 1% v/v	4.0	-	-
Chitin liquid (Ellis & Assoc)	2 drench, 1% v/v	4.0	5.87	0.129
Chitin liquid (Ellis & Assoc)	1 drench, 3% v/v	4.0	-	-
Chitin liquid (Ellis & Assoc)	2 drench, 3% v/v	4.0	5.83	0.075
Chitin liquid (Ellis & Assoc)	1 drench, 5% v/v	4.0	-	-
Chitin liquid (Ellis & Assoc)	2 drench, 5% v/v	4.0	6.00	0.027
Aminogrow® (OCP)	1 drench, 1% v/v	4.0	-	-
Aminogrow® (OCP)	2 drench, 1% v/v	4.0	5.93	0.057
Aminogrow® (OCP)	1 drench, 3% v/v	4.0	-	-
Aminogrow® (OCP)	2 drench, 3% v/v	4.0	5.99	0.075
Aminogrow® (OCP)	1 drench, 5% v/v	4.0	-	-
Aminogrow® (OCP)	2 drench, 5% v/v	4.0	5.96	0.039

[1] Lettuce transplants planted into pots immediately after mixing amendments into soil with mycelial inoculum. Drench treatments applied immediately after transplanting using 100 mL of solution added as a drench onto each transplant.

[2] One lettuce transplant planted into each 14 x 12 cm pot (total 4 pots per treatment) to simulate application of treatments in the field.

[3] Soil pH and SEM are the average of two soil measurements (2 reps) taken 12 hrs after transplanting.

Discussion and conclusions

Field trials

Fungicide efficacy

In field trials, two applications of Filan® provided consistent good control of *S. minor* lettuce drop under both high and low disease pressures. Bavistin® was effective at the high disease site but not under low disease. Sumisclex® also gave good control of disease at the high and one of the low disease sites. The future use of Bavistin® and Sumisclex® for Sclerotinia management in vegetables is doubtful due to residue and other issues. Thus, it is very unlikely that these two fungicides will be available to growers for Sclerotinia control.

Switch® and Shirlan® also gave good control of *S. minor* infection in low disease sites and therefore are potential alternatives to Filan® for lettuce drop control under low disease pressure. These two treatments did not affect the fresh weight of lettuce plants at harvest indicating that the rates and number of applications tested were not phytotoxic to plants. These two fungicide treatments currently do not have minor use permits for Sclerotinia management in vegetables. Further evaluation is therefore warranted, especially in high disease conditions, to collect additional efficacy data for these products.

Two applications of Folicur® (tebuconazole), which had a minor use permit for Sclerotinia control, were ineffective in reducing incidence of *S. minor* lettuce drop at two of the three sites with low disease. In the bean trial, however, two applications of Folicur® gave good control of white mould caused by *S. sclerotiorum*. Under low disease pressure conditions, control provided by Folicur® was statistically similar to that provided by two applications of Filan®. A similar schedule using Folicur® plus Filan® also provided similar good control of white mould. This result suggests that Folicur® could be a good replacement for Filan®, which is more expensive than Folicur®, to control white mould in green beans when the risk of disease is known to be low. Trials in Tasmania (see chapter 3), however, have shown that Folicur® will not provide good control of white mould if disease pressure is high (see Tasmanian trials).

The efficacy of fungicide treatments can be affected by disease risk, spray timing and fungicide efficacy. The risk of disease was influenced by populations of sclerotia in soil and time of the year when crops were grown. Spray timing is critical for sclerotinia control because fungicide treatments, included those tested in this study, have little or no curative activity. This was evident in the lettuce trials where some disease was recorded even in plots treated with the best treatments. In the bean crop, applications were made at the right times during flowering yet some disease also developed in the best treatments. Actives in Filan® (boscalid), Switch® (fludioxonil) and Shirlan® (fluazinam) are known to have good residual activity in soil and this may have contributed to the good control against *S. minor* infection in soil by preventing mycelial growth and contact with plant tissue.

Alternative treatments

None of the alternative treatments evaluated were effective in these trials. Two nutrient treatments were evaluated for their ability to reduce *S. minor* lettuce drop in the field. Perlka™ (calcium cyanamide) was ineffective in reducing disease in two sites with different soil types. Recent field research in New Zealand showed that Perlka™ was very effective in reducing the incidence of *S. minor* lettuce drop (Wright 2007). It is possible that concentrations of nitrogen released into our soils prior to planting lettuce did not affect survival of populations of *S. minor* sclerotia available in the root zone for infections. Stand SHK® (20% silica, 15% potassium), applied as a foliar treatment at 3 and 5 L/ha, also did not reduce lettuce drop incidence. Previous work conducted in Tasmania indicated that potassium silicate at concentrations of 3000-5000 µgrams/ml were suppressive to mycelial growth of *S. sclerotiorum* in culture (F. Hay pers. comm.). Liang et al. (2005) work demonstrated that foliar-applied Si effectively controlled infection by *Podosphaera xanthii* (powdery mildew) on cucumber plants, only via the physical barrier of Si deposited on leaf surfaces and/or osmotic effect of the silicate applied. This work also showed that continuously root-applied Si can enhance defence resistance in response to infection by *P. xanthii in* cucumber. Based on these and other findings potassium silicate has been suggested to have potential for Sclerotinia control, for example, to prevent infections caused by ascospores of *S. sclerotiorum,* landing on tissue (bean flowers) treated with potassium silicate. Another potential use would be to prevent infections on lettuce caused by ascospores of *S. sclerotiorum* and mycelium of *S. minor*. In our work, Stand SHK™ was applied to the plant foliage using low volumes of water. It is therefore possible that very little solution reached the base of plants and soil surface under leaves where protection against *S. minor* infection was required.

Three rates of *C. minitans* (Contans™) were also evaluated for their ability to reduce the incidence of *S. minor* lettuce drop in two lettuce crops grown in a clay soil. In the first lettuce crop assessed, Contans™ treatments did not reduce disease incidence compared to the untreated control. In the second crop assessed, disease levels were too low to allow treatment comparisons. *C. minitans* (Contans™) is a commercially available fungal parasite with proven efficacy against *S. sclerotiorum*, but not *S. minor* (P. Luth 2001). Recently, however, this biocontrol agent was reported to reduce the incidence of lettuce drop, caused by *S. minor* and *S. sclerotiorum,* under field conditions in California and Arizona (Matheron and Porchas 2005; P. Luth pers. comm.). We will continue monitoring our trial site to determine if there are long-term effects of Contans™ treatments on populations of *S. minor* sclerotia and disease.

Screening fortransplant treatments

The majority of nutrient and self-defence inducer treatments evaluated at commercial rates did not protect lettuce seedlings against *S. minor* infection. Seedlings were transplanted in soil with abundant mycelium of *S. minor* and probably roots and other plant parts were in direct contacted with mycelium. Pot treatments were therefore evaluated under conditions of extremely high inoculum pressure, which may not occur in soils cropped with lettuce in rotation with other non-host crops. In the field, *S. minor* survives in soil not as mycelium but mostly as sclerotia which germinate in response to exudates emitted by the roots of host plants. Under high inoculum

pressure, only the fungicide Filan® and Perlka® were capable of protecting some of the plants against *S. minor* infection probably by preventing mycelial growth and contact with plant tissue or destroying mycelium, respectively. Previous research in Tasmania demonstrated that soil applied calcium hydroxide increased soil pH above 8 which inhibited *S. minor* sclerotia germination and reduced lettuce drop incidence in field lettuce (Wilson *et al.* 2005). However, this treatment has not been adopted by growers because it is not practical for lettuce production. Laboratory work with potassium silicate also demonstrated the capacity of this nutrient to change culture conditions which were suppressive to mycelial growth of *S. sclerotiorum* (F. Hay pers. comm.). In our pot trials there was no evidence of substantial changes in soil pH, except Perlka®, after amending or drenching soil with the nutrient treatments, including potassium silicate (Stand SHK™) at 1%, 3% and 5%. Further research is required to optimise conditions so that the effects of these alternative treatments reported in glasshouse trials can be translated into effectiveness in the field.

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CHAPTER 3 - DEVELOPMENT OF STRATEGIES FOR MANAGING SCLEROTINIA DISEASES IN GREEN BEANS AND LETTUCES IN TASMANIA

Hoong Pung and Susan Cross

SUMMARY

In Tasmania, green beans and lettuces are highly susceptible to *Sclerotinia* diseases. A series of applied research studies were conducted by researchers at Peracto Pty Ltd, in order to investigate and develop strategies that are practical and could be readily adopted for use by the green bean and lettuce industries in Tasmania. Effective disease control by fungicides is vital. In many paddocks, growers will sustain total crop losses if no fungicide sprays are applied. Currently, in Tasmania, green bean and lettuce producers solely rely on boscalid fungicide (Filan™) to manage the *Sclerotinia* diseases. There is an urgent need to provide the industry with more choices in new and safer fungicides that can be used in alternation for good fungicide resistance and residual management. In field trials conducted in this project, we identified cyprodinil + fludioxonil (Switch™) applied at 625 g ai/ha as bioequivalent to boscalid at 500 g ai/ha. Fluazinam (Shirlan™) applied at 125 g ai/ha was moderately effective under high disease pressure. AE C656948, a new fungicide, was found to be highly effective for lettuce drop control.

The potential of an early application of tebuconazole (Folicur™) and fluazinam (Shirlan™), just before row closure on green beans was investigated. The early applications were aimed at the soil surface in order to inhibit the pathogen at soil level. Folicur™ had little or no effect on white mould, when applied before row closure. Shirlan™, applied at 125 and 250 g ai/ha before row closure reduced white mould incidence by 55% compared to the untreated control. Filan™ applied at 500 g ai/ha reduced white mould by 72%. The combined treatment of Shirlan™, applied at 125 g ai/ha before row closure, followed by two sprays of Filan™ at flowering, gave the highest level of disease control, reducing *Sclerotinia* incidence by 91%. This demonstrates the potential of using an early application onto the soil surface with Shirlan™ to reduce disease the pressure, followed by Filan™ at flowering to prevent flower infections.

Disease control by fungicides can vary due to differences in the spraying efficacy in green bean crops. Du-Wett™, a new blend of organosilicone and organic adjuvants, is believed to improve spray coverage and adsorption of fungicides. Two trials were conducted on green bean crops in Tasmania to compare the effects of Du-Wett to two commonly used non-ionic adjuvants (Activator™ and Agral™) to see if it can improve the efficacy of Filan™ for the control of white mould. When applied under dry conditions, Du-Wett was found to improve the performance of Filan™ by slightly increasing white mould control and reducing the levels of variability in the disease control in different replicate plots. But, under constant wet conditions, there was no obvious benefit in applying Filan™ with Du-Wett™.

Biocontrol agents generally did not provide the level of disease control that is acceptable to commercial crops under high disease pressure. However, they may have a place in an integrated control strategy, if they are used in combination with fungicide applications. Contans™ applied after sowing followed by three sprays of Filan™ was shown to slightly reduce white mould incidence and severity compared to Filan applications only.

The growth habits of five commercial green bean varieties (Celtic, Stanley, Flavor Sweet, Montano and Valentino) and their effects on white mould incidence and severity were investigated. Valentino has the largest size bean seed, and has a slightly longer flowering period, taller plants and larger leaves, which made it more susceptible to high white mould incidence. Among the other green bean varieties, there were no obvious differences in their susceptibility to white mould. Flowers of all the varieties were located close to the main stems and beneath the leaves canopies. This demonstrates the difficulty in preventing flower infections with conventional fungicide spray applications and that complete disease control is impossible in a vigorous growing crop with a dense canopy.

Brassica biofumigant crops sown as green manures in between crops have the potential to replenish organic matter, improve soil health and help suppress soilborne pathogens including *Sclerotinia*. The selection of biofumigant crops that are suitable for the cold Tasmanian conditions is very limited. Therefore, nine new biofumigant varieties were evaluated at three sites in Tasmania for their suitability in winter and spring to observe their growth habits and to determine their potential for *Sclerotinia* control. The new varieties evaluated were Indian mustard (*B. juncea* - Mustclean), white mustard (*Sinapis alba* - Architect™, Abraham™, Attack™), forage rape (*B. napus* - Greenland™), oilseed radish (*Raphanus sativus* - Adios™, Arena™ and Doublet™) and Ethiopian mustard (*B. carinata*). Growth of all the biofumigant varieties sown in winter were very slow, where crops sown in May took five months to reach the flowering stage, whereas those sown in October reach it in only three months. The susceptibility of the new varieties to frost damage in winter and their growth habits were determined. The biomass of all the biofumigant varieties sown in spring was 3 to 6 times higher than that of ryegrass. The brassica green manure crops produced vastly different root systems compared to the ryegrass root systems. In a replicated field trial in a paddock that had a very high disease level, lettuce planted after Mustclean had less than 62% lettuce drop incidence compared to those planted after ryegrass. It should be noted, however, that as with all biological systems, changes due to the biofumigant crops are complex and may be subject to changes depending on their varieties, sowing time, soil types and weather conditions. Changes to the agronomic management of the subsequent crop also need to be considered.

INTRODUCTION

Green beans are produced in north-west Tasmania mainly for processing into frozen vegetables. Tasmania supplies 75% of the Australian frozen green bean market. The most destructive disease of green beans is white mould caused by *Sclerotinia sclerotiorum*. In addition to direct losses in the field, detection of more than 10 per cent diseased plants may result in rejection of the whole crop. Even a low incidence of white mould could lower the bean quality or increase the cost of processing. Crop

yields are typically highest in high plant density and vigorous growing bean crops. These crops, however, are especially prone to serious loss because of white mould disease. *S. sclerotiorum* produces sclerotia that can survive for three or more years in soil. In Tasmania, bean crops are typically sown in rotation with many other horticultural crops which are also susceptible to *Sclerotinia* infections. In recent years, there has been an increase in the incidence of the disease in other major crops in north-west Tasmania as well, such as potatoes, brassicas, carrots and pyrethrum, because their dense crop canopies in summer conditions also created the ideal environment for the pathogen.

Lettuce is a major crop in south-east Tasmania, and lettuce drop due to *Sclerotinia minor* is threatening the sustainability of the crop production. *S. minor* produces vast numbers of sclerotia that can survive in soil. After intensive crop production over many years, the pathogen level in soil is relatively high in most paddocks. Lettuce crops that have more than 20% plants infected are often not harvested. Short break crops and fungicide application are the only methods available to manage the disease. Long breaks between the susceptible lettuce crops are not economically feasible due to limited land available for crop rotations. In some paddocks, the pathogen level in soil is so high that no satisfactory disease control can be obtained with both methods.

Currently, both the green bean and lettuce producers rely on fungicide applications to manage the *Sclerotinia* diseases. In 1990s to 2004, many producers in crops relied solely on the fungicide procymidone for white mould control. But in 2004, procymidone was withdrawn from uses in green beans in Australia because of safety concerns and was replaced by the new fungicide boscalid. Boscalid is safe to beneficial insects like bees and predatory mites and is low in mammalian toxicity, and hence its registered use has been expediated in the USA and many other countries. Boscalid had been shown to be effective in preventing *Sclerotinia* infections (Pung et al 2005). However, *Sclerotinia* disease management is still a major challenge because of difficulty in obtaining complete spray coverage under dense crop canopy and/or in a combination of wet, humid and warm weather conditions. A better understanding on the impact of crop conditions on the current *Sclerotinia* control strategies is required so that producers can adopt appropriate measures to better manage the disease.

There are very few fungicides that are effective in controlling white mould under high disease pressure in Australia. Apart from procymidone and boscalid, there are no other fungicides currently available for controlling the pathogen. Iprodione, the only other fungicide available is not as effective for white mould control under high disease pressure on green beans, and it was found to have no effect for lettuce drop control in south-east Tasmania. There is an urgent need to provide the industry with alternative fungicides that can be used in alternation for good fungicide resistance and residual management.

Fungicides can only provide short-term *Sclerotinia* control during a susceptible crop period, but have no effects on the sclerotia of *Sclerotinia* in soil. Therefore the uses of integrated control strategies that can integrate short-term and long-term control measures are more desirable for sustainable disease control. However, apart from crop rotation and fungicide applications, there is a lack of other strategies, particularly

for producers of green beans and lettuces, which are proven to be effective, consistent, timely, practical and economical. Therefore the aims of studies conducted by researchers at Peracto Pty Ltd were to evaluate and identify other strategies that may be readily integrated or incorporated into commercial practice within a short period. These include the monitoring of crop environment over different growing seasons to gain a better understanding on their impact on the levels of disease control by fungicides, examining the influence of commercial green bean plant varieties and susceptibility to the disease, investigating the potential of new biofumigant crop varieties, evaluating the efficacies of alternative fungicides, biological products and other treatment application methods.

THE EFFECTS OF SPRAY ADJUVANTS ON BOSCALID EFFICACY FOR WHITE MOULD CONTROL ON GREEN BEANS

Summary

Two trials were conducted at Moriarty and Merseylea in Tasmania, in 2007 and 2008 to examine the effects of spray adjuvants: Activator and Agral (non-ionic adjuvants) and Du-Wett (an organosilicone blend adjuvant) on the efficacy of boscalid for the control of white mould (*Sclerotinia sclerotiorum*) on green beans. Boscalid, was applied with or without adjuvants at 125, 250 and 500 g ai/ha. All fungicide treatments were applied in three sprays during the flowering period. Leaf wetness, air and soil temperatures and soil moisture were monitored for crop conditions. White mould incidence and the levels of disease control by fungicides were influenced by the crop and weather conditions. Under dry conditions, disease incidence was relatively low and disease control by fungicides met expectations. Under constant wet conditions during the critical flowering and pod maturity period, boscalid did not prevent white mould, but was shown to substantially reduce disease severity to the levels where the crop may still be harvestable. Boscalid applied at 500 g ai/ha was more effective than the lower rates. The benefits of spray adjuvants are dependent on the crop and weather conditions. When applied under dry conditions, Du-Wett improved the performance of Filan by reducing the levels of variability in its control, while Activator slightly increased disease incidence. Under constant wet conditions, there was no obvious benefit in applying boscalid with Du-Wett or Agral.

Introduction

White mould caused by *Sclerotinia sclerotiorum* can cause serious crop losses on green beans. The disease is typically initiated by flower infections, which then lodge onto other parts of the plant, and the fungus spreads via mycelial growth onto healthy tissue on leaves, stems or pods. Bean crops that have a dense canopy and are sown in fields that have a history of the disease can have serious crop losses of 30% to 100% due to widespread and severe white mould. Although fungicides such as boscalid had been shown to be highly effective in preventing *Sclerotinia* infections, the disease control may not meet expectations because of variability in the flowering period, poor spray coverage, penetration and retention in green bean crops. High volume sprays, generally believed to help improve spray coverage, have been shown previously to increase spray run-off and overlap in spray droplets on outer leaves of bean crops, with no significant improvement in spray penetration through the plant canopy (Pung & O'Brien, 2000). Spray adjuvants are known to increase spray coverage and retention. Organosilicone adjuvants are known for their superior spreading and stomatal penetrating properties and have been widely used in herbicide applications. However, pure organosilicone adjuvants are mainly developed for use with herbicides. They are not desirable for use with fungicides because their superior stomatal penetration and leaf wax disruption properties are damaging to plant tissues. Recently, a new organosilicone and organic adjuvant blend, Du-Wett, was developed specifically for use with fungicides. Its unique blend was developed to retain the

superior organosilicone spreading properties, while excluding their other foliage damaging effects.

Two field trials were conducted in this study to examine and compare two commonly used non-ionic surfactants on beans, Activator and Agral with the new organosilicone adjuvant blend, Du-Wett, for use with Filan™ spray applications for *Sclerotinia* control on beans.

Materials & Methods

The two trials were conducted within commercial processing bean crops, with cv. Montano at Moriarty and cv. Flavor Sweet at Merseylea. The trial design was randomised complete block with four replicates and plot size of 5 m x 3 plant rows. All fungicide treatments were applied in three sprays during the flowering period as described in Tables 1, 2 and 3. Spray treatments were applied with a knapsack sprayer fitted with a 1.5 m boom and cone jet nozzles. At close to harvest, green bean plants in 3 m x 2 plant rows were assessed for white mould incidence and severity due to *S. sclerotiorum* infections.

Table 1. Product details

Product Name	Active Ingredient	Concentration of Active Ingredient	Formulation	Product Type
Folicur - Bayer	tebuconazole	430 g/L	Suspension Concentrate	Fungicide
Filan - Nufarm	boscalid	500 g/kg	Wettable Granules	Fungicide
Shirlan - Nufarm	fluazinam	500 g/L	Suspension Concentrate	Fungicide
Switch - Syngenta	cyprodinil + fludioxonil	375 g + 250 g	Water Dispersible Granule	Fungicide
Du-Wett - Nufarm	trisiloxane ethoxylate (blend of organosilicone & organic surfactants)	500 g/L	Liquid	Spray adjuvant
Agral - Syngenta	nonyl phenol ethylene oxide condensate non-ionic organic	600g/L	Soluble Concentrate	Spray adjuvant
Activator - Nufarm	Surfactant	non-ionic surfactant	900 mL/L	-

Table 2. Treatment details in the trial at Moriarty, Tasmania in 2007

No.	Treatment	Product Rate		Application Schedule
		Product + Adjuvant Rate (per ha)	Active Ingredient (g ai/ha)	
1	Untreated control	Nil	Nil	Nil
2	Filan 125 g ai	250 g	125	Three spray applications with 280 L water/ha with TX12 hollow cone nozzles at flowering stage. First application at 70-80% plants with first flowers, and then 7 days interval.
3	Filan 250 g ai	500 g	250	
4	Filan 500 g ai	1000 g	500	
5	Filan 125 g ai + Du-Wett	250 g + 200 mL	125	
6	Filan 250 g ai + Du-Wett	500 g + 200 mL	250	
7	Filan 500 g ai + Du-Wett	1000 g + 200 mL	500	
8	Filan 125 g ai + Activator	250 g + 50 mL/100 L	125	
9	Filan 250 g ai + Activator	500 g + 50 mL/100 L	250	
10	Filan 500 g ai + Activator	1000 g + 50 mL/100 L	500	

Table 3. Treatment details in the trial at Merseylea, Tasmania in 2008

No.	Treatment	Product Rate		Application Schedule
		Product + Adjuvant Rate (per ha)	Active Ingredient (g ai/ha)	
1	Untreated control	Nil	Nil	Nil
2	Filan 250 g ai	500 g	250	3 sprays at 7-10 day interval with 440 L water with TX 18 hollow cone nozzles at 50 cm spacing 1st spray at 10-20 % plants with first flowers
3	Filan 500 g ai	1000 g	500	
4	Filan 250 g ai + Agral	500 g + 200 mL/100 L	250	
5	Filan 500 g ai + Agral	1000 g + 200 mL/100 L	500	
6	Filan 250 g ai + Du-Wett	500 g + 200 mL	250	
7	Filan 500 g ai + Du-Wett	1000 g + 200 mL	500	

The numbers of plants infected by *Sclerotinia* in each plot were counted in two plant rows x 3 m along the rows in the middle of each plot. There was an average of 105 plants assessed per plot. The disease incidence was then tabulated as the percentage of plants infected by dividing the number of plants infected with the total number of plants assessed in each plot and then multiplying by 100.

The disease severity of infected plants was assessed according to the following severity rating:
1 = mild - infection of single stem, leaf or bean pod
2 = moderate - infection of multiple stem branches
3 = severe - infection affecting whole plant

Disease severity index was then tabulated according to the formula:
Disease index = ((1 x no. plants in rating 1) + (2 x no. plants in rating 2) + (3 x no. plants in rating 3))/(total plants assessed).

Analysis of variance was conducted on the data set using ARM and Statgraphic Plus 2.0, and pairwise comparisons were made of the mean values using Least Significant Difference Test (LSD).

Figure 1. Leaf wetness beneath plant canopy at Moriarty

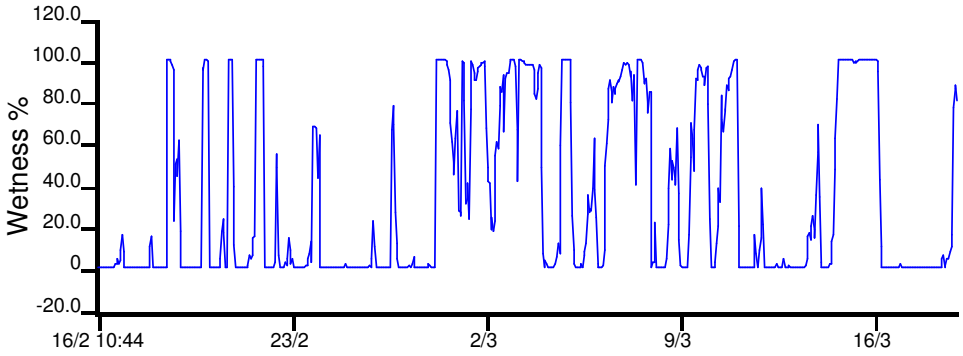


Figure 2. Soil moisture recorded at 15 and 30 cm deep at Moriarty

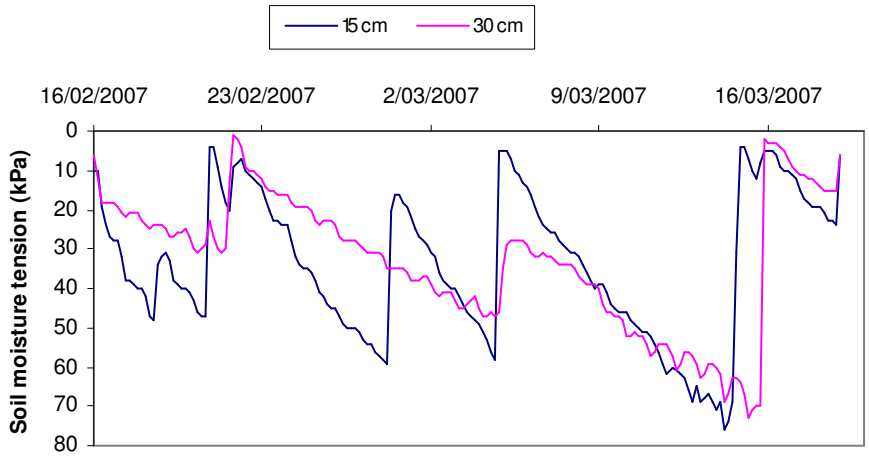


Figure 3. Leaf wetness beneath plant canopy at Merseylea

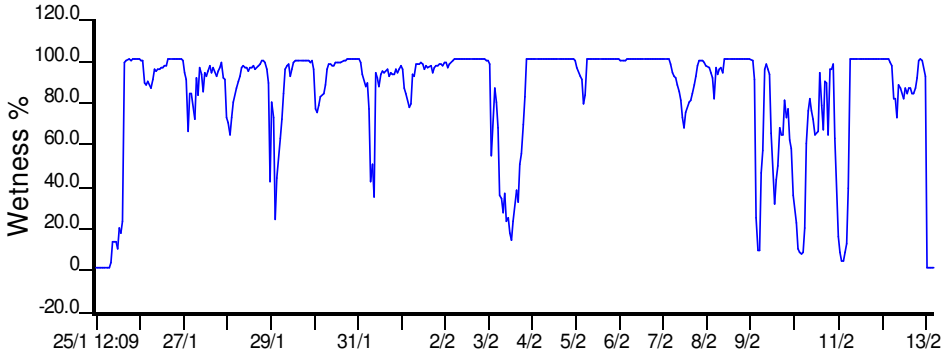
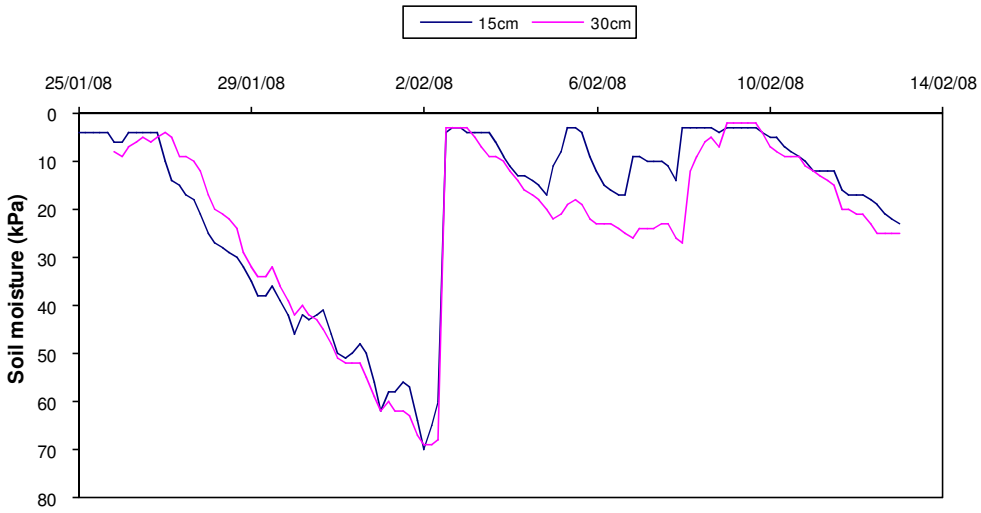


Figure 4. Soil moisture recorded at 15 and 30 cm deep at Merseylea



Results

Table 4. Treatment effects on Sclerotinia incidence and severity at Moriarty

No.	Treatment	69DAS, 13DAA3					
		Sclerotinia incidence[1 2]		*Sclerotinia* severity rating (% infected plants in each rating)			Disease severity index rating
		% infected plants	SE[3]	1 Mild	2 Moderate	3 Severe	
1	Untreated control	6.09 c	0.61	1.6	3.5	1.0	0.12
2	Filan 125 g ai	3.89 bc	1.06	1.0	1.0	1.9	0.09
3	Filan 250 g ai	1.38 ab	0.60	0.0	0.5	0.9	0.04
4	Filan 500 g ai	1.40 a	1.12	0.2	1.0	1.2	0.04
5	Filan 125 g ai + Du-Wett	1.46 ab	0.33	0.7	0.2	0.5	0.03
6	Filan 250 g ai + Du-Wett	2.18 ab	0.28	1.2	0.5	0.5	0.04
7	Filan 500 g ai + Du-Wett	0.98 a	0.56	0.5	0.5	0.5	0.01
8	Filan 125 g ai + Activator	2.90 abc	0.66	1.2	0.5	1.2	0.06
9	Filan 250 g ai + Activator	3.70 bc	0.97	1.2	1.4	1.1	0.07
10	Filan 500 g ai + Activator	2.91 ab	1.35	1.7	0.5	0.8	0.05
p-value		0.015	-	-	-	-	0.722

1 Data analysis was conducted on transformed data using square root of (x+0.5). Untransformed mean values are presented in the table.
2 Means within columns followed by the same letter are not significantly different at the 5% level according to LSD test.
3 SE: standard error of 4 replicate plots
DAS: days after sowing; DAA3: days after the third fungicide application

Table 5. Spray adjuvant effects on *Sclerotinia* incidence and severity at Moriarty

Treatment	*Sclerotinia* incidence[1 2]		*Sclerotinia* severity rating (% infected plants in each rating)			Disease severity index rating
	% infected plants	SE[3]	1 Mild	2 Moderate	3 Severe	
Untreated control	6.1 c	0.55	1.6	3.5	1.0	0.12
Filan (no adjuvant)	2.2 ab	0.61	0.4	0.5	1.3	0.05
Filan + Activator	3.2 b	0.26	1.3	0.8	1.0	0.03
Filan + Du-Wett	1.5 a	0.61	0.8	0.4	0.3	0.06
p-value	0.003	-	-	-	-	-

1 Data analysis was conducted on transformed data using square root of (x+0.5). Untransformed mean values are presented in the table.
2 Means within columns followed by the same letter are not significantly different at the 5% level according to LSD test.
3 SE: standard error of replicate plots

Figure 5. The levels of variability in *Sclerotinia* control due to treatments applications of Filan +/- spray adjuvants at Moriarty

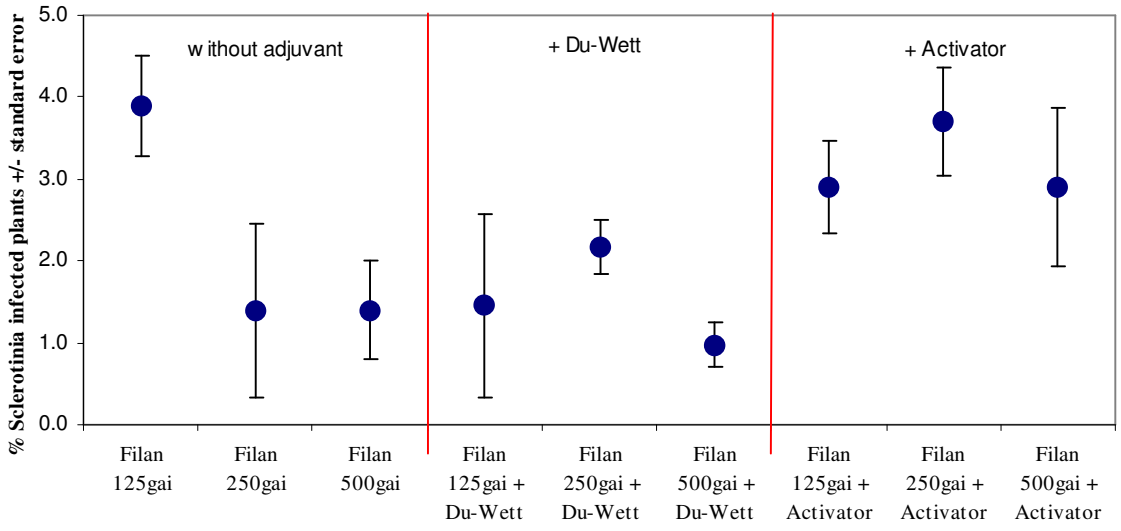
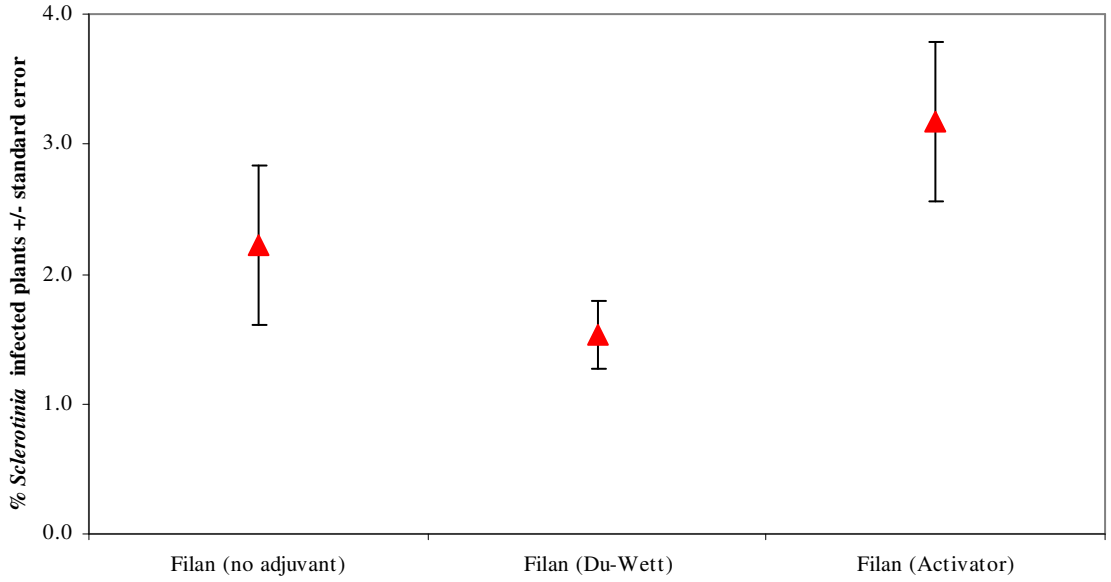


Figure 6. The combined effects of Filan and spray adjuvants in the variability of *Sclerotinia* control at Moriarty



At Moriarty, the trial was conducted within a late commercial green bean crop cv. Montano, grown for processing into slicing beans in frozen vegetables. The crop was ready for harvest at 69 days after sowing (69DAS). Crop conditions were relatively dry during the flowering and pod maturity stage in February to March of 2007 with relatively high air and soil temperatures in February. Even though the crop was irrigated once a week, the leaf wetness recorded indicated that the crop was dry most of the time (Figure 1). Soil moistures recorded at 15 and 30 cm were relatively low even after irrigation (Figure 2), because of little or no rainfall throughout 2006 and 2007.

There were no *Sclerotinia* infections in the crop during the spray applications. Relatively low incidences of white mould (*S. sclerotiorum)* appeared only after petal drop and at close to harvest in the trial, with 6% plants infected in the untreated control plots at 69DAS, when the crop was ready for commercial harvest.

In the analysis on treatment effects, there were significant differences in white mould incidence between the treatments ($p = 0.0151$) (Table 4). Filan, applied in three spray applications at the bean flowering period, generally reduced white mould due to *Sclerotinia* infections. The fungicide treatments reduced white mould by 36% to 84% compared to the untreated control. Filan, applied on its own at the lowest rate of 125 g ai/ha, gave relatively poor disease control compared to the higher rates at 250 and 500 g ai/ha (Figure 5). But, when applied with the Du-Wett adjuvant, the low rate at 125 g ai/ha gave a similar level of disease control as the two higher rates.

In the analysis on adjuvant effects, there were significant differences in white mould incidence between adjuvants ($p = 0.0033$) (Table 5). With Filan, Du-Wett gave better *Sclerotinia* control than Activator. Activator adjuvant, applied with Filan appeared to reduce the level of disease control by the fungicide (Figures 5-6). This finding is very significant, as Activator was the most popular spray adjuvant used by growers at the time. It was applied with Filan and other fungicides for disease control in Tasmania. The product formulation of Filan also contains some surfactant. This indicates that it may be better to use Filan on its own rather than applying it with Activator. In contrast, Du-Wett appeared to enhance white mould control by Filan.

In a further assessment on the performance of Filan + adjuvant combinations (Figures 5-6), the standard errors (SE) of the different treatments and adjuvants were calculated based on the variabilities between different replicate plots. It is interesting to note that Filan + Du-Wett had relatively low SE values compared to Filan alone or Filan + Activator. This indicates that Du-Wett improved the consistency of the fungicide performance by reducing the levels of variability in the disease control. The enhanced disease control may be related to improved distribution and penetration to lower parts of the dense bean plants at the second and third applications. Studies by Gaskin et al (2000 and 2002) showed that Du-Wett can enhance spray coverage and retention, as well as improving penetration through dense plant canopies in onions, potato and grape foliage and fruit bunches at relatively low volume applications.

Table 6. *Sclerotinia* disease incidence, severe infected plants and plant weight at Merseylea

No.	Treatment	67DAS, 8DAA3				
		Sclerotinia incidence [1] (% infected plants)	% Severe infected plants [1 2]		Plant fresh weight (kg/4 m row) [2]	
1	Untreated control	100	48	a	3.04	d
2	Filan 0.5kg/ha	98	16	bc	3.97	abc
3	Filan 1.0kg/ha	98	8	c	4.56	a
4	Filan 0.5kg + Agral	96	11	c	4.27	ab
5	Filan 1.0kg + Agral	98	9	c	4.43	a
6	Filan 0.5kg + Du-Wett	99	13	c	4.31	abcd
7	Filan 1.0kg + Du-Wett	99	12	c	4.48	a
p value		0.282	0.004		0.010	

1 Data analysis was conducted on transformed data using log (x+1). Untransformed mean values are presented in the table.
2 Means within columns followed by the same letter are not significantly different at the 5% level according to LSD test.
DAS: days after sowing; DAA3: days after the third fungicide application

Table 7. *Sclerotinia* disease severity at Merseylea at 67DAS

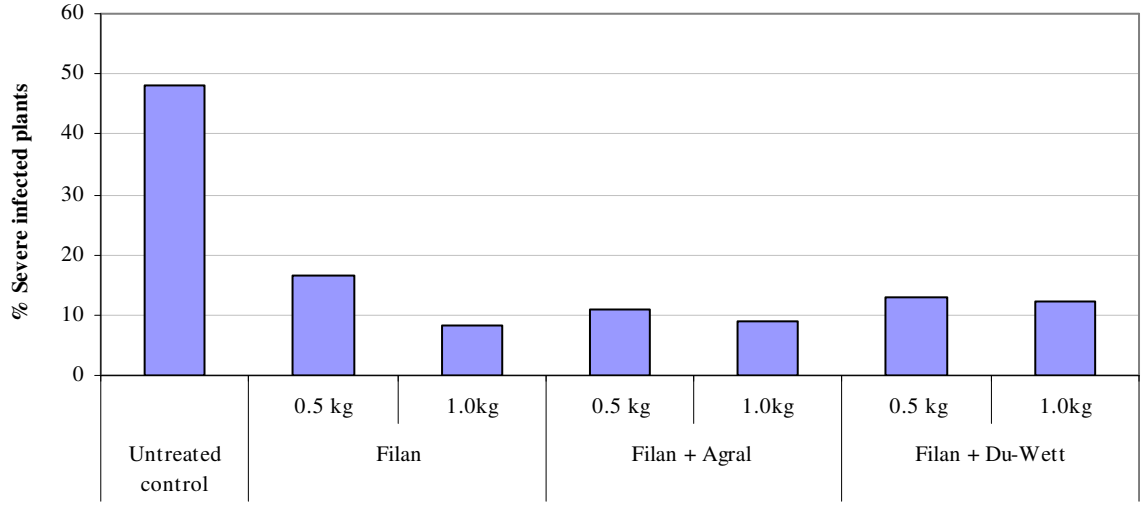
No.	Treatment	*Sclerotinia* severity rating (% infected plants at each rating)			Disease severity index rating [1]	
		1 Mild	2 Moderate	3 Severe		
1	Untreated control	2	50	48	2.5	a
2	Filan 0.5kg/ha	5	77	16	2.1	bc
3	Filan 1.0kg/ha	17	73	8	1.9	c
4	Filan 0.5kg + Agral	21	64	11	1.8	c
5	Filan 1.0kg + Agral	19	69	9	1.8	c
6	Filan 0.5kg + Du-Wett	12	74	13	2.0	c
7	Filan 1.0kg + Du-Wett	18	69	12	1.9	c
p value		-	-	-	0.003	

1 Means within columns followed by the same letter are not significantly different at the 5% level according to LSD test.
DAS: days after sowing

The second trial at Merseylea was also conducted within an early commercial green bean crop (cv. Flavor Sweet) that was grown for processing into frozen vegetables. The trial was located in an area that is both ideal for bean crops and very prone to severe white mould. The crop was ready for harvest at 68DAS. The crop conditions were often wet during the flowering and pod maturity stage in January to February of 2008, along with relatively high air and soil temperatures. Frequent showers, 100% cloud cover, lack of wind and high temperatures created highly favourable conditions for *Sclerotinia* infections in the crop. The leaf wetness recorded indicated that the crop was wet most of the time beneath the plant canopy (Figure 3). Soil moistures recorded at 15 and 30 cm were also relatively high at petal drop and pod maturity in February (Figure 4). As a consequence, white mould disease incidence was very high in the trial. At one day before commercial harvest and 67DAS, almost all plants were infected by *Sclerotinia*. No fungicide treatments reduced the disease incidence (p = 0.282) (Table 3).

There were differences, however, in the disease severity between the fungicide treatments and untreated control (Tables 6 and 7). Filan, substantially reduced the percentage of severe infected plants and hence, the disease severity index. Filan applied at the recommended product rate of 1 kg/ha (500 g ai/ha) appeared to give more consistent disease control with less variability than the lower product rate of 0.5 kg/ha (250 g ai/ha).

Figure 7. Treatment effects on the percentage of severe *Sclerotinia* infected plants at Merseylea



Under the combination of high disease pressure and highly disease favourable conditions, the Agral and Du-Wett adjuvants did not improve disease control by Filan. Although not significant, plants treated with the Du-Wett adjuvant appeared to have slightly more disease. Du-Wett is recommended for use only when applied in dry conditions. It is not recommended for use in wet conditions; as the fungicide may be washed off the plant surfaces by showers or rainfall. This indicates the importance of understanding the properties of an adjuvant and its suitability under various crop or weather conditions.

Even with Filan fungicide spray applications at the appropriate timing, almost all plants were infected in the trial. This shows that when the weather and crop conditions are highly favourable to widespread and severe *Sclerotinia* infections, it is extremely difficult to prevent disease. The fungicide applications, however, did substantially reduce the disease severity (Figure 7). At harvest, the percentage of severe infected plants is the key factor in determining whether an infected crop is worthwhile harvesting or not. In processing green bean crops in Tasmania, any crop that has 10% or more severe infected plants will not be harvested. Therefore, in this study, Filan applied at 1 kg/ha was able to reduce the severity of the disease to levels that will enable the crop to be harvested. Outside the trial area, the commercial crop was sprayed with Filan at 1 kg/ha and the entire crop was harvested commercially as the disease severity was within the limit. If untreated, there would be a total crop loss.

Conclusions

White mould incidence and the levels of disease control by fungicides were influenced by the crop and weather conditions. Under dry conditions, disease incidence was relatively low and disease control by Filan met expectations. Under constant wet conditions during the critical flowering and pod maturity period, almost all plants were affected by white mould, and Filan was shown to substantially reduce disease severity to the levels where the crop may still be harvestable. Filan applied at 500 g ai/ha, gave more consistent disease control than lower rates of 125 and 250 g ai/ha. The benefits of spray adjuvants are dependent on the crop and weather conditions. When applied under dry conditions, Du-Wett improved the performance of Filan by reducing the levels of variability in its control, while Activator slightly increased disease incidence. Under constant wet conditions, there was no obvious benefit in applying Du-Wett or Agral with Filan.

THE EFFICACY OF ALTERNATIVE PRODUCTS FOR *SCLEROTINIA* CONTROL IN GREEN BEANS AND LETTUCES

Summary

Two trials were conducted at Merseylea and Richmond in Tasmania, in 2008 and 2010 to compare alternative products against boscalid for *Sclerotinia* disease control in green beans and lettuces. The products evaluated were fungicides: cyprodinil + fludioxonil (Switch), fluazinam (Shirlan), tebuconazole (Folicur) and AE C656948, a soil fumigant: Agfix, and a mycoparasite: *Coniothyrium minitans* (Contans). Agfix and Contans were applied as pre-plant soil treatments. All fungicide treatments were applied in three sprays. Switch applied at 625 g ai/ha was as effective as Filan at 500 g ai/ha for *S. sclerotiorum* control on green beans. Shirlan gave moderate level of the disease control on green beans, while Folicur had no effect. AE C656948, a new fungicide, was shown to be highly effective and persistent in controlling *S. minor* on lettuces. The soil fumigant, Agfix, and Contans, applied as pre-plant soil applications had little or no effect in controlling *S. minor* on lettuces.

Introduction

Green beans and lettuces are highly susceptible to *Sclerotinia* diseases. Growers mainly depend on fungicide spray applications for *Sclerotinia* control. However, with increases in the intensity of crop productions, the pathogen also increases to levels that continually challenge the efficacies of fungicides. In the 1990s to 2004, many growers relied solely on procymidone for *Sclerotinia* control, as it is highly effective even under high disease pressure. In 2004, following the withdrawal of procymidone from use in green beans and lettuces, many growers turned to boscalid, a relatively new fungicide at the time. Apart from boscalid, there is no other suitable alternative fungicide that has similar or better levels of efficacy. This over reliance on boscalid is risky to growers, as the number of boscalid applications must be limited per year in the same paddock as part of a good long term chemical residual management strategies. The availability of several effective fungicides or other products that can be used in alternation is essential for *Sclerotinia* disease management. Two trials were conducted in this study to evaluate and identify products that can be used as alternative to boscalid for *Sclerotinia* control.

Materials & Methods

The two trials were conducted in Tasmania at Merseylea in 2007/08 and Richmond in 2010. The trial at Merseylea was set up within a commercial processing bean crop cv. Flavor Sweet in grey sandy loam with plot size of 5 m x 3 plant rows. All fungicides Filan, Folicur, Shirlan and Switch were applied as described in Table 2, using a knapsack sprayer fitted with a 1.5 m boom and cone jet nozzles. At close to harvest, green bean plants in 3 m x 2 plant rows were assessed for white mould incidence and severity due to *S. sclerotiorum* infections.

Table 1. Product details

Product Name	Active Ingredient	Concentration of Active Ingredient	Formulation
Folicur	tebuconazole	430 g/L	Suspension Concentrate
Filan	boscalid	500 g/kg	Wettable Granules
Shirlan	fluazinam	500 g/L	Suspension Concentrate
Switch	cyprodinil + fludioxonil	375 g + 250 g	Water Dispersible Granule
AE C656948	fluopyram	500 g/L	Suspension Concentrate
Agfix	2-propenyl isothiocyannate	10%	Suspension Concentrate
Contans	*Coniothyrium minitans*	1 x 10^6 cfu/g	Water soluble granules

Table 2. Treatment details on green beans at Merseylea, Tasmania in 2008

Treatment	Product Rate		Application Schedule
	Product (per ha)	Active Ingredient (g ai/ha)	
Untreated control	Nil	Nil	Nil
Filan	1000 g	500	3 sprays at 7-10 day interval with 440 L water using TX 18 hollow cone nozzles 1st spray at 10-20 % plants with first flowers
Shirlan	250 ml	125	
Switch	1000 g	625	
Folicur	350 ml	150	

Table 3. Treatment details on lettuces at Richmond, Tasmania in 2009

Pre-plant/After planting Treatments	Product Rate		Application Schedule
	Product + Adjuvant Rate (per ha)	Active Ingredient (g ai/ha)	
Untreated control	Nil	Nil	Nil
Agfix / Nil	30 L/ha	-	A
Contans / Contans fb 2x Filan	4 kg Contans and 1.0 kg Filan	-	ABCD
Nil / 2x Filan	1.0 kg	500	CD
Nil / 2x AE C656948 @ 300 mL	300 ml	150	BCD
Nil / 2x AE C656948 @ 500 mL	500 ml	250	

The trial at Richmond was set up within a commercial lettuce crop cv. Explore in black clay loam with plot size of 5 m x 1.2 m. All products were applied as spray applications using a knapsack sprayer fitted with a 1.5 m boom and DG8004 fan jet nozzles with 530 L/ha water at 400 kPa before and/or after lettuce planting at the application timings as shown in Table 3. Agfix is a biopesticide based on concentrated plant biofumigant extract and Contans is based on Coniothyrium minitans, a mycoparasite of *Sclerotinia* pathogens. Filan is a fungicide that is currently used for *Sclerotinia* control on lettuces and AE C656948 is a new experimental fungicide. In application timing A, a pre-plant soil application of Agfix and Contans was applied 19 days prior to lettuce planting by spraying onto bare soil

surface and then mixed into soil to 20 cm deep with a rotary hoe. In application timings B, C and D, products were applied at 5, 21 and 36 days after lettuce planting, respectively. After each application timings B, C and D, the trial area was irrigated using overhead sprinklers with 10 mm water in order to drench the product to plant base and top soil in order to optimise disease control.

The trial design of both trials was randomised complete block with 4 replicates. Treatment details were as described in Tables 2-3. At close to harvest, plants were assessed for *Sclerotinia* incidence and severity.

At Merseylea, bean plants were assessed for white mould incidence and severity in two plant rows x 2 m in each plot as described in the previous green bean trials (p49-59). At Richmond, all plants within each plot were assessed for *Sclerotinia* wilt or lettuce drop. All infected plants were severe and not marketable. The disease incidence was then tabulated as the percentage of plants infected by dividing the number of plants infected with the total number of plants assessed in each plot and then multiplying by 100.

Analysis of variance was conducted on the data set using ARM and Statgraphic Plus 2.0, and pairwise comparisons were made of the mean values using Least Significant Difference Test (LSD).

Results & Discussion

Table 4. White mould incidence, severe infected plants and weights of green bean plants at Merseylea

Treatment	67DAS, 8DAA3		
	***Sclerotinia* incidence (% Plants infected)**	**% Severe infected plants [1 2]**	**Plant fresh weight (kg/4 m row) [2]**
Untreated control	100	48 a	3.04 c
Folicur 0.35 L/ha	98	41 ab	3.27 c
Shirlan 0.25 L/ha	100	20 bc	3.38 bc
Switch 1.0 kg/ha	94	9 c	3.86 bc
Filan 1.0 kg/ha	98	8 c	4.56 ab
p value	0.282	0.0038	0.0097

[1] Data analysis was conducted on transformed data using log (x+1). Untransformed mean values are presented in the table.
[2] Means within columns followed by the same letter are not significantly different at the 5% level according to LSD test.
DAS: days after sowing; DAA3: days after the third fungicide application

Table 5. Sclerotinia disease severity of green bean plants at Merseylea

Treatment	67DAS, 8DAA3				
	Sclerotinia severity rating (% plants infected at each rating)			Disease severity index rating	
	1 Mild	2 Moderate	3 Severe		
Untreated control	2	50	48	2.5	a
Folicur 0.35 L/ha	5	53	41	2.3	ab
Shirlan 0.25 L/ha	8	71	20	2.1	bc
Filan 1.0 kg/ha	17	73	8	1.9	c
Switch 1.0 kg/ha	14	72	9	1.8	c
p value	-	-	-	0.0027	

Means within columns followed by the same letter are not significantly different at the 5% level according to LSD test.
DAS: days after sowing; DAA3: days after the third fungicide application

The first trial was conducted within an early commercial green bean crop cv. Flavor Sweet, grown for processing into frozen vegetables, at Merseylea, Tasmania. In January to February of 2008, the constant wet weather conditions were highly favourable to white mould due to *S. sclerotiorum*. White mould disease incidence was very high in the trial, with almost all plants infected at 67 days after sowing, one day before commercial harvest. All fungicide treatments did not reduced the disease incidence ($p = 0.282$) (Table 4). There were differences, however, in the disease severity between the treatments (Tables 4-5). Filan and Switch, both applied at 1 kg/ha, were very effective in reducing *Sclerotinia* disease severity. Shirlan applied at 0.25 L/ha, also did reduce *Sclerotinia* disease severity, but it was not as effective as Filan or Switch. The lower efficacy by Shirlan may be due to its low application rate. Folicur, applied at 0.35 L/ha, as recommended in a temporary permit for lettuces, had little or no effects for *Sclerotinia* control.

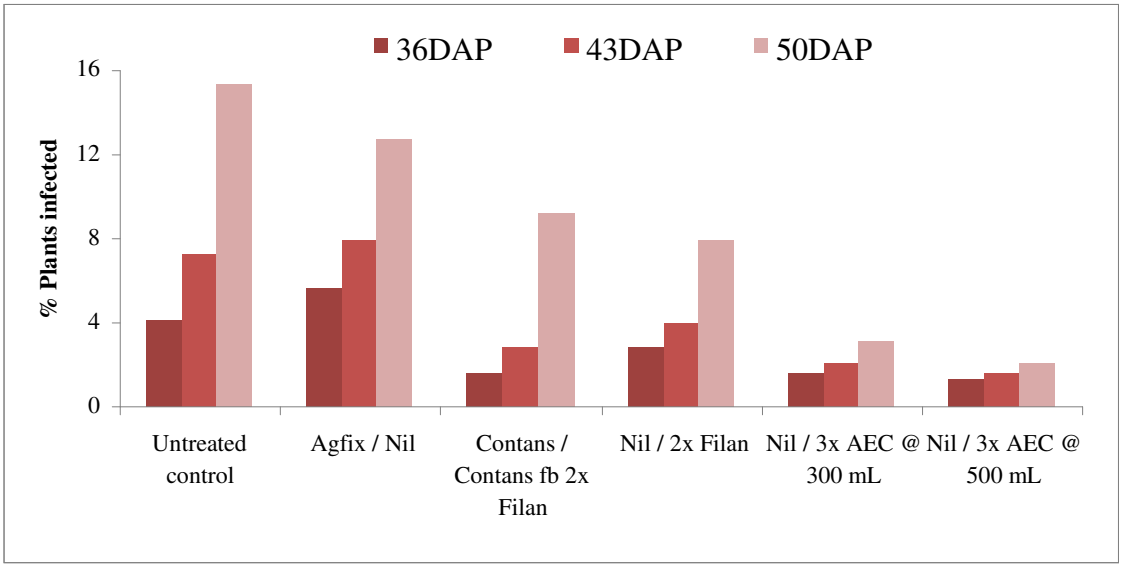
This trial showed that when the weather conditions are highly favourable to widespread and severe *Sclerotinia* infections, it is extremely difficult to control the disease. At harvest, the percentage of severe infected plants is the key factor in determining whether an infected crop is worthwhile harvesting or not. In processing green bean crops in Tasmania, any crop that has 10% or more severe infected plants will not be harvested. In this trial, Filan or Switch applied at 1 kg/ha were able to reduce the severity of the disease to levels that will enable the crop to be harvested. If untreated, there would have been a total crop loss.

Table 6. Treatment effects on lettuce drop at Richmond

Treatment	Total no. plants assessed/plot	Lettuce drop incidence (% Plants infected)		
		18/03/10 36DAP	25/03/10 43DAP	1/04/10 50DAP
Untreated control	95	4.1 ab	7.2 a	15.3 a
Agfix / Nil	87	5.6 a	7.9 a	12.7 ab
Contans / Contans fb 2x Filan	95	1.6 c	2.9 b	9.2 b
Nil / 2x Filan	95	2.9 bc	4.0 ab	7.9 b
Nil / 3x AE C656948 @ 300 mL	95	1.6 c	2.1 b	3.1 c
Nil / 3x AE C656948 @ 500 mL	96	1.3 c	1.6 b	2.1 c
p value	-	0.0050	0.0047	0.0001

Means within columns followed by the same letter are not significantly different at the 5% level according to LSD test.
DAP: days after planting; DAA3: days after the third post-plant application
Arsine square root transformation applied to the data set for analysis of variance, untransformed data presented
fb = followed by

Figure 1. Treatment effects on lettuce drop incidence over time at Richmond.



The second trial at Richmond was conducted in an area, where the previous lettuce crop could not be harvested in the wet autumn conditions of 2009 because of very high incidence of lettuce drop of 70% due to *S. minor* infections. This trial was conducted under the drier summer conditions in February to March of 2010. Lettuce drop incidence was much lower than before, but still considered to be high by commercial standard with 15% plants infected in the untreated control (Table 6). Filan was applied in only two applications because of concerns on the persistence of boscalid in the soil that has very high clay content. Pre-plant soil applications of the soil biofumigant Agfix did not have any impact in reducing lettuce drop. Contans, applied as pre-plant soil treatment, followed by Filan applications after lettuce planting, did not improve lettuce drop control (Figure 1). The new fungicide AE C656948 was shown to be highly effective as well as persistent in reducing lettuce drop.

Conclusions

Switch applied at 1 kg/ha was as effective as Filan at 1 kg/ha for *S. sclerotiorum* control on green beans. Shirlan (0.25 L/ha) gave moderate level of the disease control on green beans, while Folicur had no effect. AE C656948, a new fungicide, was shown to be highly effective and persistent in controlling *S. minor* on lettuces. The soil fumigant, Agfix, and Contans, applied as pre-plant soil applications had little or no effect in controlling *S. minor* on lettuces.

THE POTENTIAL OF EARLY TREATMENT APPLICATIONS FOR WHITE MOULD CONTROL IN GREEN BEANS

Summary

The efficacies of the early treatment applications with biocontrol agents (Contans and Micro Plus) and fungicides (Folicur, Shirlan and Des-O-Germ) were evaluated, alone or in combination with Filan applications at flowering for white mould control on green beans. The effects of the spray adjuvants, Du-Wett and Du-Wett Rain Master, were also evaluated. Contans and Des-O-Germ applied after sowing followed by two applications of Filan appeared to slightly improve disease control compared to only two applications of Filan. Micro Plus had little or no effects on the disease. Folicur had little or no effect on white mould, when applied before closure, and it did not improve disease control when applied with Filan. Shirlan, applied at 250 and 500 ml/ha before row closure reduced white mould incidence by approximately 55%. Filan applied at 1 kg/ha reduced white mould by 72%. The combined treatment of Shirlan, applied at 250 ml/ha before row closure, followed by two sprays of Filan at flowering, gave the highest level of disease control, reducing *Sclerotinia* incidence by 91%. Du-Wett was more effective than Du-Wett Rain Master in improving the efficacy of Filan.

Introduction

Contans is based on *Coniothyrium minitans*, and it is registered for use overseas for white mould control in the USA and Europe. As *C. minitans* is a mycoparasite of sclerotia of *Sclerotinia*, it must be applied early into soil, before or at sowing, so that it could parasitise and reduce the sclerotia level, hence reducing disease pressure. Many studies conducted in the past had shown that while it may reduce the disease pressure, it did not provide the level of disease control that is acceptable to commercial crops in regions that are prone to high incidence of *Sclerotinia* infections. Micro Plus is another biocontrol agent, based on *Bacillus lydicus* that can survive well in soil and has activity in inhibiting many soilborne pathogens. Des-O-Germ is a chemical disinfectant based on quartenary ammonium that is used for disinfecting vegetable produce in processing and has broad spectrum activity against many soilborne fungal pathogens. These are all 'soft products' that are non-toxic and have no long term chemical residual effects. However, they are typically not as effective as fungicides and when used on their own, provide inadequate disease control. It is possible, however, that if they are used in combination with fungicide applications, the combined effects of reducing or inhibiting the pathogen in soil followed by flower protections by Filan (boscalid) may enhance disease control. Pre-plant applications several weeks or months prior to green bean sowing are not feasible in farm practice in Tasmania. Hence, early application of these products immediately after green bean sowing was explored in this study.

Early fungicide applications with products that are more suitable for soil applications such as Folicur (tebuconazole) and Shirlan (fluazinam) may potentially also help inhibit the pathogen in soil and hence reduce disease pressure. These early fungicide

applications, however, are also expected to be more effective if their applications are followed by flower protection by Filan.

This study aimed to determine the efficacies of the early treatment applications, alone and in combination with Filan applications at flowering for white mould control on green beans.

Materials & Method

This study was conducted within a commercial processing green bean crop cv. Flavor Sweet in grey sandy loam soil at Merseylea, Tasmania in 2008/09. The trial design was randomised complete block with 4 replicates. Each replicate plot was 5 metre long with 2 plant rows and a buffer row. Products were applied as described in the table below. Plants in the middle of the plot (3 m x 2 plant rows) were assessed at close to harvest at 78 and 86 days after sowing. Analysis of variance was conducted on the data set using ARM and Statgraphic Plus 2.0, and pairwise comparisons were made of the mean values using Least Significant Difference Test (LSD). The percentage of plants with severe infection was transformed using arsine square root transformation before analysis in order to normalised the data set. Untransformed means were presented in the results.

Table 1: Products, active ingredients and application rates

Product	**Active ingredients**	**Rate of active ingredient/ha**	**Type**
Contans	*Coniothyrium minitans*	4 x 10^6 cfu/g	mycoparasite
Des-O-Germ	Quartenary ammonium compounds	100 g/L	disinfectant
Filan	boscalid	500 g/kg	fungicide
Folicur	tebuconazole	430 g/L	fungicide
Micro Plus	*Bacillus lydicus*	2 x 10^6 cfu/g	bacteria biocontrol
Shirlan	fluazinam	500 g/L	fungicide

Table 2. Treatment Details

Treatment	Product Rate	Application after sowing [A]	Application before row closure [B]	Two applications at flowering [C]
Untreated control	N/a			
Contans at sowing followed by (fb) Filan	4 kg/ha fb 1 kg/ha	+		+
Des-O-Germ fb Filan	100 ml/100 L fb 1 kg/ha	+		+
Micro Plus fb Filan	2.0 kg/ha fb 1 kg/ha	+		+
Shirlan	250 ml/ha		+	
Shirlan	500 ml/ha		+	
Folicur	1.0 L/ha		+	
Shirlan fb Filan	250 ml/ha + 1.0 kg/ha		+	+
Folicur fb Filan	1.0 kg/ha + 1.0 kg/ha		+	+
Filan	1.0 L/ha			+
Filan + Du-Wett	1.0 kg/ha + 200 ml/ha			+
Filan + Du-Wett Rain Master	1.0 kg/ha + 400 ml/ha			+

[A] Drench application was applied at initial seedling emergence (9 days after sowing) as a band application along the plant rows with a watering can at 6 L per plot.

[B] One application at very high water volume of 5 L per plot using a watering can to cover the whole plot, before row closure (49 days after sowing).

[C] Two foliar spray applications were applied with 310 L water/ha at 400 kPa using a knapsack precision sprayer fitted with a 2 m boom and hollow cone nozzles TX12. The first spray was applied at 20-30% plants with first flowers (64 days after sowing) and then 7 days later.

Results & Discussion

Table 3. Treatment effects on white mould incidence and severity

Treatment	White mould incidence (% infected plants)		Disease severity (% severe infected plants)	
	78DAS	86DAS	78DAS	86DAS
Untreated control	19.5 a	49.9 a	3.0 ab	12.5 ab
Folicur 1.0 L/ha	23.5 a	43.3 a	4.7 a	13.3 a
Shirlan 500 ml/ha	5.2 cd	22.5 b	1.0 bc	5.0 cd
Shirlan 250 ml/ha	10.2 b	22.1 bc	1.4 abc	6.6 bc
Filan 1 kg/ha	2.2 de	14.2 cd	0.3 c	5.6 cde
Folicur fb Filan	5.0 cd	14.1 cd	0.5 bc	2.8 cde
Micro Plus fb Filan	5.8 bc	13.4 d	0.3 c	3.1 cd
Filan + Du-Wett Rain Master	4.3 cde	10.6 de	0.0 c	1.3 def
Des-O-Germ fb Filan	2.8 de	9.9 de	0.0 c	1.2 ef
Contans fb Filan	3.8 cde	9.7 de	0.3 c	1.3 def
Filan + Du-Wett	1.8 e	6.3 de	0.0 c	0.0 f
Shirlan 250 ml fb Filan	1.8 e	4.7 e	0.0 c	0.0 f
p-value	0.0001	0.0001	0.0001	0.0001

Means within columns followed by the same letter are not significantly different at the 5% level according to LSD test.
Treatment was sorted in a descending order according to white mould incidence at 86DAS
DAS: Days after sowing of green beans

White mould incidence in the trial was considered to be very high, but many of the infected plants have mild to moderate infections, when the crop was ready for harvest (Table 3). Many of the treatments that substantially reduced the percentages of infected plants, also reduced the percentage of plants with severe infections. At harvest, the percentage of severe infected plants is the key factor in determining whether an infected crop is worthwhile harvesting or not. In processing green bean crops in Tasmania, any crop that has 10% or more severe infected plants will not be harvested. Therefore, in this study, plants in the untreated control and Folicur treatment would not be harvested.

Contans and Des-O-Germ applied after sowing followed by two applications of Filan appeared to slightly improve disease control compared to only two applications of Filan. Micro Plus appeared to have little or no effects. Folicur had little or no effect, when applied before closure. At 86DAS, Shirlan, applied at 250 and 500 ml/ha before row closure significantly reduced *Sclerotinia* infection by approximately 55%. Shirlan, applied at 250 ml/ha before row closure, followed by two sprays of Filan at flowering, reduced *Sclerotinia* incidence by 91%. Filan applied at 1 kg/ha reduced white mould by 72%. This indicates that the combined treatment of Shirlan followed by Filan did improve white mould control.

Filan + Du-Wett appeared to improve disease control. Du-Wett Rain Master, which has a sticker and was developed for use under wet condition, did not improve the fungicide control. There was no long period of crop wetness and the crop conditions

followed a typical wet and dry daily cycle according to daily water condensations in cold nights and rapid drying during the day as temperature increases. Previous study on Du-Wett also showed that it was beneficial under relatively dry crop conditions.

THE EFFECTS OF COMBINATION TREATMENT APPLICATIONS FOR LETTUCE DROP CONTROL

Summary

A trial was conducted to investigate the potential of integrating alternative biocontrol agents (Contans and Micro Plus) and fungicides (Shirlan and Switch), in combination with Filan for *S. minor* control on lettuces. Lettuce drop incidence was very high with 40 to 65% plants infected when plants were ready for harvest at 61 days after sowing. None of the products had much impact on the disease. Although Filan applied at 1 kg/ha in three applications appeared to have the lowest lettuce drop incidence with 42% plants infected, the disease incidence was still too high. The high disease incidence was due to a combination of wet and cold climatic conditions in autumn and high pathogen level in soil. This highlights the importance of planting time and the limits to the level of *Sclerotinia* control with fungicide applications alone.

Introduction

Lettuce drop is mainly caused by *S. minor*, and sometimes by *S. sclerotiorum* or both pathogens. Both pathogens produce sclerotia that stay dormant and survive in soil until a susceptible crop is grown. Contans, based on *Coniothyrium minitans*, is a mycoparasite of sclerotia of *Sclerotinia*, and it must be applied early into soil, before or at sowing, so that it could parasitise and reduce the sclerotia level, hence reducing disease pressure. Previous studies had shown that while it may reduce the disease pressure, it did not provide the level of lettuce drop control that is acceptable to commercial crops in areas where *S. minor* disease pressure is very high. It is possible that Contans may be more effective if used in an integrated management with fungicide applications than when it is used alone under high disease pressure. Similarly, another biocontrol product, Micro Plus, based on *Bacillus lydicus* may be more effective when applied in combination with a fungicide. Therefore, this study aimed to evaluate the potential of these products, when used in combination with fungicide application.

At the time this study was conducted in March to May 2009, only Filan (boscalid), Rovral (iprodione) and Folicur can be used for lettuce drop control. In this farm, Rovral had been shown several times over the years to have no effect for *S. minor* control. Folicur was shown to have little or no control for *S. sclerotiorum* on green beans, but its efficacy for lettuce drop control was not tested. Filan is the only effective fungicide for use in lettuces, but its use had been restricted to two applications per year at a site because of its persistence in soil. Therefore, this study was conducted to determine if Folicur and another fungicide, Shirlan, have efficacy for *S. minor* control.

Materials & Methods

Treatments were applied soon after planting at 0, 13 and 39 days after planting. The crop was ready for harvest at 61 days after planting. Spray treatments were applied with a hand boom fitted with fan jet drift guard nozzles operated at pressures of 290 kPa and spray volumes of 250 L/ha. The trial area was irrigated (5 mm) immediately after each spray application, in order to drench the product to the base of the plants and the soil surface. The trial design was randomised complete block with 4 replicates. At 27, 39, 49 and 61 days after planting, all plants within each plot were assessed for lettuce drop. Plant density was approximately 80 plants per plot. Analysis of variance was conducted on the data set using ARM and Statgraphic Plus 2.0, and pairwise comparisons were made of the mean values using Least Significant Difference Test (LSD).

Table 1: Products, active ingredients and application rates

Product	Active ingredients	Product Rate/ha	Rate of active ingredient/ha	Type
Contans	*Coniothyrium minitans*	4.0 kg	4 x 10^6 cfu/g	mycoparasite
Filan	boscalid	1.0 kg	500 g	fungicide
Micro Plus	*Bacillus lydicus*	2.0 kg	2 x 10^7 cfu/g	bacteria biocontrol
Shirlan	fluazinam	0.25 L	125 g	fungicide
Switch	cyprodinil + fludioxonil	1.0 kg	375 g + 250 g	fungicide

Table 2: Treatment applications timing

No.	Treatment	After transplanting 0DAP	2nd application 13DAP	3rd application 39DAP
1	Untreated control			
2	Contans, nil, Contans	Contans	Nil	Contans
3	Contans, Filan, Contans	Contans	Filan	Contans
4	Micro Plus, Nil, Micro Plus	Micro Plus	Nil	Micro Plus
5	Micro Plus, Filan, Micro Plus	Micro Plus	Filan	Micro Plus
6	Shirlan, Nil, Shirlan	Shirlan	Nil	Shirlan
7	Shirlan, Filan, Shirlan	Shirlan	Filan	Shirlan
8	Switch, Nil, Switch	Switch	Nil	Switch
9	Switch, Filan, Switch	Switch	Filan	Switch
10	Filan, Filan, Filan	Filan	Filan	Filan

Figure 1: Soil temperature (10 cm deep) at the trial site

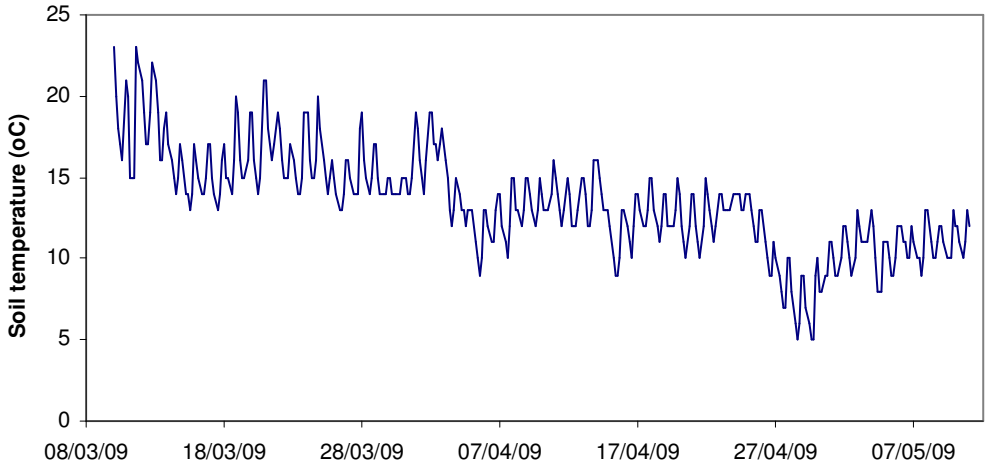
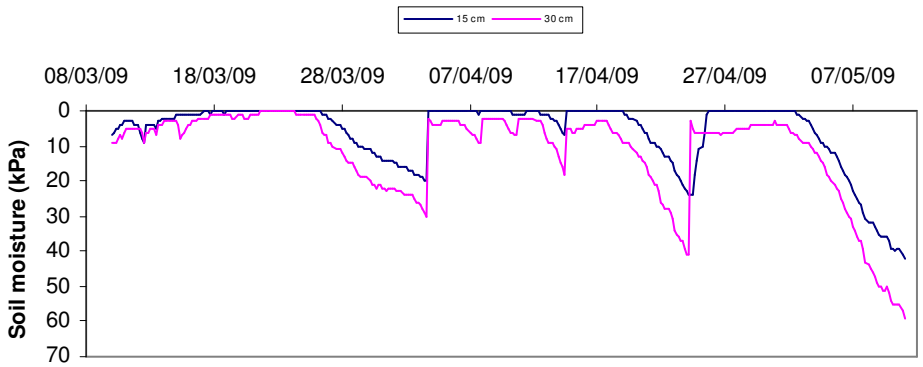


Figure 2: Soil moisture recorded at 15 and 30 cm deep at the trial site



Results & Discussion

Table 3: Treatment effects on lettuce drop incidence in the trial at Richmond in 2009

No.	After three applications	Lettuce drop incidence (% Plants infected)			
		27DAS	39DAS	49DAS	61DAS
1	Untreated control	6	23	44	64
5	Micro Plus, Filan, Micro Plus	7	23	45	62
4	Micro Plus, Nil, Micro Plus	5	21	39	60
6	Shirlan, Nil, Shirlan 250 ml	4	20	39	58
2	Contans, nil, Contans	4	25	40	57
8	Switch, Nil, Switch	5	17	36	50
7	Shirlan, Filan, Shirlan 250 ml	4	15	31	48
9	Switch, Filan, Switch	3	19	36	47
3	Contans, Filan, Contans	5	16	30	45
10	Filan, Filan, Filan	2	15	29	42
p-value		0.302	0.376	0.124	0.152

Lettuce drop incidence was very high in the trial area at close to harvest 61 days after sowing. The soil conditions were constantly wet because of frequent rainfall in March and April of 2009 (Figures 1-2). The trial area was known to have high disease pressure in the previous lettuce crop planted in 2008. The disease incidence was relatively low in March, but increased rapidly as crop growth slowed down with the onset of cold conditions in April. Lettuce crops are grown in autumn and winter for a much longer period of 9 to 12 weeks compared to 6 to 7 weeks in spring and summer time. Lettuce crops in Tasmania are typically known to be pre-disposed to higher *Sclerotinia* infections because of the combinations of wet and cold conditions, which prolonged crop growth. Therefore, areas known to have high *Sclerotinia* disease levels are typically sown with ryegrass or other less susceptible crops by the growers.

Filan applied at 1 kg/ha in three applications, which was the commercial standard for lettuce drop control at the time, did not provide satisfactory disease control. None of the other products, including Contans, Micro Plus, Shirlan or Switch had much impact on the disease either. This study demonstrates the importance of climatic conditions, disease inoculum levels in soil, and planting time for *Sclerotinia* management. There are limits to the level of *Sclerotinia* control with fungicide applications alone.

GROWTH HABITS OF GREEN BEAN VARIETIES AND THEIR SUSCEPTIBILITY TO WHITE MOULD DISEASE

Summary

The growth habits of five green bean varieties (Celtic, Stanley, Flavor Sweet, Montano and Valentino) and their effects on white mould incidence and severity were investigated. Valentino, which has the largest seed, produce larger and taller plants and had a slightly longer flowering period, was the most susceptible to *Sclerotinia* infections. Among the other bean varieties, there were no obvious differences between their susceptibility to white mould. There was little or no difference in the flowering habits between the varieties. Flowers of all the green bean varieties were located close to the main stems and beneath the leaves canopies. This demonstrates the difficulty in preventing flower infections with conventional fungicide spray applications. In a study within a commercial green bean crop (Flavor Sweet), seedling density was reduced by 30%, to determine whether a lower plant density may reduce its susceptibility to white mould. The reduction in plant density had no effects on its susceptibility to white mould, because the plant bushes became larger and wider covering any space between plants at the lower density.

Introduction

There are currently no green bean plant varieties that are resistant to white mould. However, there has been reports that there may be differences in white mould incidence and severity among the varieties that are sown for processing green beans and for the fresh market beans in Tasmania. These differences are believed to be linked to their plant growth habits such as plant sizes and/or flowering characteristics. This study was therefore conducted to compare the growth habits of seven seed lots of five green bean varieties and determine their effects on white mould incidence and severity. All the varieties used in the study were commonly sown varieties and were supplied by the major beans producers in Tasmania. A separate study was also set up within a commercial crop to determine whether a lower plant density may reduce its susceptibility to white mould.

Materials & Method

The trial was set up in grey sandy loam within a commercial processing bean crop at Merseylea, Tasmania in 2008/09. Seven plant varieties were supplied by the major green bean producers in north-west Tasmania. The trial design was randomised complete block with 4 replicates. Seeds from each variety were sown in 2 plant rows x 5 m in each replicate plot at 50 mm seed spacing and 580 mm row spacing. The trial area was not treated with any fungicide. The plants were managed in the same manner as in the commercial crop.

Table 1. Green bean varieties and seed treatments

Variety	Commercial seed treatment	Bean Type
Celtic (1)	Fludioxinil/Streptomycin	Processing bean
Celtic (2)	Captan/Thiram	Processing bean
Stanley (1)	Maxim/Apron/Dynasty	Processing bean
Stanley (2)	Maxim/Apron XL	Processing bean
Flavor Sweet	Thiram	Processing bean
Montano	Maxim/Apron XL	Processing bean
Valentino	Captan	Fresh market bean

At 19 days after sowing, the plant density was recorded. Growth habits of each variety were noted. White mould incidence and severity were assessed at 77 and 86 days after sowing. Green beans were ready for harvest at 86 days after sowing. The disease incidence and disease severity index were tabulated as described in previous green bean trials (p49-59). Analysis of variance was conducted on the data set using ARM and Statgraphic Plus 2.0, and pairwise comparisons were made of the mean values using Least Significant Difference Test (LSD).

In the study on the effects of reduced plant density, four small areas within the commercially sown green bean variety cv. Flavor Sweet were marked and alternate seedlings in plant rows were removed in order to reduce plant density. A record of the plant numbers indicate that the plant density in the thinned plant rows had been reduced by 30%. At harvest, plants in 2 x 2 m rows were assessed for white mould incidence and severity.

Results & Discussion

Table 2. Plant densities and white mould incidence and severity on the green bean varieties

Bean Variety	Seed weight (g/100 seeds)	% Plant density 19DAS [1]	Sclerotinia incidence % plants infected [1]		Disease severity index [2]		Plant height (cm)
			77DAS	86DAS	77DAS	86DAS	86DAS
Celtic (1)	13.63	71 a	5.6 b	20.1	0.1 b	0.4 b	28
Celtic (2)	13.73	71 a	5.8 b	16.1	0.1 b	0.3 b	33
Stanley (1)	19.27	76 a	9.5 b	21.3	0.2 b	0.4 b	41
Stanley (2)	19.21	76 a	3.9 b	16.4	0.1 b	0.3 b	37
Flavor Sweet	16.90	79 a	6.2 b	19.0	0.1 b	0.3 b	33
Montano	22.90	60 b	4.1 b	23.4	0.1 b	0.4 b	38
Valentino	28.19	76 a	20.9 a	34.0	0.4 a	0.8 a	43
p value		0.0152	0.021	0.156	0.054	0.065	-

[1] Means within columns followed by the same letter are not significantly different at the 5% level according to LSD test.
[2] Means within columns followed by the same letter are not significantly different at the 10% level according to LSD test.
DAS: Days after sowing

At 77 days after sowing (DAS), the white mould on Valentino was significantly higher than all the other varieties (p = 0.021) (Table 2). At 86DAS, although not

significantly different (p = 0.156), the highest disease incidence was recorded on Valentino plants. The disease severity on this variety was also higher than the other varieties. Valentino is grown by the largest fresh market bean producer in Tasmania. It has the largest size bean seed, and its growth habit is quite different to all the other varieties that are grown for processing. Its plant bushes have slightly longer flowering period, were taller and its leaves were larger compared to the other varieties. All these factors may have created an ideal environment for the pathogen beneath the plant canopy, making it more susceptible to *Sclerotinia* infections. This variety, however, is mainly grown at Forthside in Tasmania, where paddocks are exposed to windy conditions. Therefore, crops tend to stay relatively dry and are less prone to high incidence or severity of white mould.

Among the processing green bean varieties, there were no significant differences in the white mould incidence or severity (Table 2). Although the processing beans are not as susceptible as Valentino to white mould, many crops are grown in relatively sheltered areas that are ideal for growing beans. These locations however, are also favourable to the disease. White mould incidence increased very rapidly from 77DAS to when the plants were ready to harvest, when the disease incidence was almost three times higher. No fungicide was applied. Celtic plants tended to be smaller than the other varieties. In commercial crops, Celtic is known to be less prone to severe white mould. Montano and Stanley plants tended to be taller. Flavor Sweet produced wider and heavier bushes with more bean pods.

Sclerotinia infections started when ascospores landed on open flowers and infected the petals. The ascospores cannot infect healthy leaves or stems. The white mould symptoms only became obvious as the plant bushes become denser and spent infected flowers started to lodge, and the pathogen spreads onto stems, leaves or bean pods. Flowers of all the green bean varieties were located close to the main stems and beneath the leaves canopies. Vigorous growing plants are usually taller and have longer flowering period. An extended flowering period is likely to predispose plants to higher and more severe *Sclerotinia* infections. Fungicide spray applications are often timed at the beginning of the flowering period, when with small bushes, the crop canopies are still relatively sparse, allowing greater fungicide penetration and spray coverage to prevent flower infections. The flowering habits of all the varieties demonstrate the difficulty in spraying fungicide onto flowers to prevent the primary spore infections. There was little or no difference in the flowering habits between the commercial green bean varieties.

Table 3. Effects of green bean plant density cv. Flavor Sweet on white mould incidence and severity

Plant density	**Number of plants/m row**	***Sclerotinia* incidence % plants infected**	**Disease severity index**
Normal plant density	19	20.4	0.5
Low plant density	13	16.8	0.7

Means of four plots

When the Flavor Sweet plant density was reduced by approximately 30% after seedling emergence, there was no obvious difference in the plant canopies (Table 3). In the plant rows with lower plant density, plant bushes were larger and still fill up the

spaces between plants at flowering. Flowers remained mostly hidden beneath the plant canopy at the low plant density. Therefore, reducing plant density did not substantially reduce the disease incidence. Crop yield is closely related to bean plant density. Higher plant density tends to produce higher yield. In commercial crops, the sowing rate and row spacing are selected in order to maximise crop yield.

INVESTIGATIONS IN SELECTING AND EVALUATING THE POTENTIAL OF BIOFUMIGANT GREEN MANURE CROPS FOR *SCLEROTINIA* DISEASE CONTROL

Summary

The potential of new biofumigant crop varieties as green manure break crops for *Sclerotinia* control in two subsequent green bean crops and a lettuce crop were examined. The varieties evaluated were Indian mustard (*B. juncea*), white mustard (*Sinapis alba*), forage rape (*B. napus*), oilseed radish (*Raphanus sativus*) and Ethiopian mustard (*B. carinata*). Growth of all the biofumigant varieties sown in winter were very slow, where crops sown in May took five months to reach the flowering stage, whereas those sown in October reach it in only three months. The white mustards, Architect and Abraham were highly susceptible to frost damage, whereas Attack was tolerant to it. Mustclean, an Indian mustard, was moderately tolerant. All the oilseed radish varieties, Adios, Arena and Doublet, and BQ Mulch were highly tolerant. The plant biomass of all the biofumigant varieties sown in spring was 3 to 6 times higher than that of ryegrass. Plant stems and root growth were affected by plant density, soil type and drainage. Ryegrass produces fibrous root systems, while the brassica crops produced a main tap root, with or without lateral root branching as well as fine fibrous roots. Increases in the biomass of green bean plants were recorded at one of the two sites because of changes in soil conditions due to the biofumigant crops. With the changes in soil conditions due to increase organic matter, changes such as irrigation and fertiliser applications must also be made. Otherwise, if the crop was irrigated and managed the same way as the area previously sown with ryegrass, changes such as greater water retention may also encourage the spread of white mould on green beans. Mustclean was shown to reduce *S. minor* infected plants in the subsequent lettuce crop compared to ryegrass and other biofumigant varieties.

Introduction

As land values and production costs increase, growers must be able to maximise returns from their land by adopting moderately intensive to intensive cropping systems with shorter breaks between crops. This pressure on land use often leads to yield decline due to increased soilborne disease and pest incidence, soil degradation, and organic matter (carbon) depletion. In the past, new land, long crop rotations or soil fumigation with methyl bromide or other chemical fumigants were used to prevent yield decline and control soilborne diseases and pests. These measures are no longer feasible and alternative options are needed. With the phasing out of methyl bromide for use in soil fumigation, there has been a lot of active research in the use of brassica biofumigant crops for soilborne disease and pest management. Previous research (Matthiessen & Kirkegaard 2006, Pung et al 2008, Aird & Long 2008) showed that brassica biofumigant crops sown as green manures have the potential to improve soils and reduce *Sclerotinia* and other diseases. When the previous studies were conducted on biofumigant varieties in Tasmania (Pung *et al.,* 2004), plant

breeding and selection of brassica varieties for biofumigant activities were relatively new and the number of varieties developed for commercial use was very limited. Since then, great advances have been made in selective plant breeding to develop varieties for commercial use as biofumigant crops. There are also increasing records of the positive effects of brassica green manure crops that are unrelated to biofumigation such as increasing organic matter, reducing soil compaction and improving soil properties. In Australia, previous studies have been mainly conducted on two biofumigant varieties, Fumus (*Brassica juncea*) and BQ Mulch (*B. napus*/*B. campestris*). Currently, BQ Mulch is the only variety that is sold commercially, while Fumus is not available to growers. In recent years, many biofumigant crop varieties had been developed for growers' use in Europe. There are now a range of different brassica types developed to suit various climatic, seasonal and soil conditions in various European countries. There have been no studies conducted to evaluate these new varieties in Australia. Therefore, this study, aimed to evaluate brassica green manure varieties that may be suitable for use as a break crop under the cold climatic conditions in Tasmania. The potential of these varieties as part of an integrated pre-plant management strategy for *Sclerotinia* control in the subsequent green beans and lettuces were examined. The varieties evaluated were Indian mustard (*B. juncea*), white mustard (*Sinapis alba*), forage rape (*B. napus*), oilseed radish (*Raphanus sativus*) and Ethiopian mustard (*B. carinata*). If shown to be suitable in Tasmania, these new varieties will provide growers with a greater choice in the types of brassica green manure crops, which can be used in crop rotations to replenish organic matter as well as for soilborne disease control.

Materials & Methods

New biofumigant varieties were evaluated in Tasmania in three sites, Merseylea, Sassafras and Richmond (Table 1). The paddocks at Merseylea and Richmond were known to have very high *Sclerotinia* disease pressure. At Meseylea and Sassafras, the varieties were sown in May 2009, to determine their suitability as winter break crop in paddocks that were later sown with green beans. The varieties were sown in large non-replicated blocks, each measuring 7 m x 100 m, with a commercial seed drill. The rest of the paddock was sown with ryegrass at Merseylea and oats at Sassafras. At Merseylea, two by one metre quadrants in the middle of each of the biofumigant crops were assess for plant density, biomass and height. The crops at Sassafras were not assessed for plant biomass and height as they were slashed by the grower before they could be assessed, because of great concern of cross pollinations by bees with a brassica seed crop in the vicinity. The green manure crops were not irrigated and relied on rainfall, while the green bean crop was irrigated. The sequence of events are described in Table 2.

At Richmond, a replicated trial study was conducted to determine the suitability of the new biofumigant varieties sown in spring (October 2009) and their impact on lettuce drop in a paddock that was later planted with lettuces. The varieties were sown in small plots of 1.2 m x 8 m with a precision seed drill. The control plots were sown with ryegrass on the same day. The trial design was a randomised complete block with four replicates. Plant density and biomass were assessed in 0.5 m bed area in the middle of each plot. The green manure crops were mulched and incorporated at flowering and then planted with lettuces as described in the chronology of events

(Table 2). The green manure and lettuces were irrigated with overhead sprinklers. The trial site had approximately 70% lettuce drop incidence and hence Filan was applied at 1 kg/ha in two spray applications at 14 and 28 days after lettuce planting. Lettuces were assessed for *Sclerotinia* infection in 3 m bed area in the middle of each plot. An analysis of variance was conducted on the results from the Richmond trial using ARM and Statgraphic Plus 2.0, and pairwise comparisons were made of the mean values using Least Significant Difference Test (LSD).

Table 1. Biofumigant plant varieties

Variety Name	Scientific Name	Common Name
Architect	*Sinapus alba*	white mustard
Abraham	*Sinapus alba*	white mustard
Attack	*Sinapus alba*	white mustard
Mustclean	*Brassica juncea*	Indian mustard
B. carinata	*Brassica carinata*	Ethiopian mustard
BQ Mulch	*Brassica napus + B.campestris*	rape + turnip
Greenland	*Brassica napus*	rape
Adios	*Raphanus sativus*	oilseed radish
Arena	*Raphanus sativus*	oilseed radish
Doublet	*Raphanus sativus*	oilseed radish

Table 2. Chronology of events

Date	Chronology of Events
Merseylea, Tasmania	
22/05/09	Biofumigant varieties sown in large single non-replicated plots.
05/08/09	Plant density recorded.
16/10/09	Assessed plant biomass.
01/11/09	All biofumigant varieties slashed and incorporated into soil.
14/12/09	Green beans cv. Flavor Sweet sown.
01/02/10	1st application of Filan, followed by another two applications at 7 day intervals.
18/02/10	White mould disease assessment.
Sassafras, Tasmania	
18/05/09	Biofumigant varieties sown in large single non-replicated plots.
11/10/09	All biofumigant varieties slashed early to prevent cross contamination of a brassica seed crop nearby.
08/12/09	Green beans cv. Orlando sown.
02/02/10	Obvious differences noted in the growth of green beans.
17/02/10	Assessed for plant biomass and yield of green beans prior to commercial harvest.
Richmond, Tasmania	
28/10/09	Biofumigant varieties and grass sown in replicated plots.
17/11/09	Plant density recorded.
15/12/09	All white mustard varieties were in full flower, while Indian mustard was at the beginning of flowering. All mustard plant varieties were assessed for plant biomass and plant density at flowering. Oilseed radish varieties were not flowering yet.
16/12/09	All white mustard and Indian mustard plant varieties slashed and incorporated into soil.
30/12/09	All oilseed radish varieties were in full flower. The rest of the green manure crops, including ryegrass were assessed for plant biomass and plant density.
05/01/10	All biofumigant varieties and ryegrass were slashed and incorporated into soil.
08/02/10	Soil rotary hoed to prepare for lettuce planting.
10/02/10	Multileaf lettuce planted cv. Explore.
24/02/10	1st application of Filan, followed by another application at 14 day interval.
25/03/10	Lettuce drop disease assessment.
01/04/2010	Lettuce drop disease assessments.

Results & Discussions

Table 3. Observations on the growth of the biofumigant varieties sown in winter and spring in 2009

Variety name	Common name	Frost tolerance (May – August)	Crop maturity		Growth habit
			Sown in May, flowering in October	Sown in October, flowering in December	
Architect	white mustard	Very low	Mid	Early	Tall single stem
Abraham	white mustard	Low	Mid	Early	
Attack	white mustard	High	Early	Early	
Mustclean	Indian mustard	Medium	Late	Mid	
B. carinata	Ethiopian mustard	-	-	Late	Short rosette
BQ Mulch	rape + turnip	High	Late	Mid	Medium multi stem
Greenland	rape	-	-	Late	
Adios	oilseed radish	High	Early	Mid to late	Short rosette
Arena	oilseed radish	High	Early	Mid to late	
Doublet	oilseed radish	High	Early	Mid to late	

The biofumigant varieties showed difference in their tolerance to frost conditions (Table 3). The white mustard, Architect and Abraham were highly susceptible to frost damage, whereas Attack was tolerant to it. Mustclean, an Indian mustard, was moderately tolerant. All the oilseed radish varieties, Adios, Arena and Doublet, and BQ Mulch were highly tolerant.

All the brassica green manure crop growth was relatively slow during winter, but plant biomass increased rapidly at the onset of warmer spring conditions in September. Oilseed radish varieties, rape and rape/turnip appeared to be better suited for sowing in the autumn and winter, when frost conditions may occur in Tasmania. Early sowing in March and April, prior to the onset of the very cold conditions may be more conducive to growing oilseed radish, rape and turnip varieties for greater plant biomass. The mustard varieties were more suitable for sowing in spring, where they established and grew very rapidly in October to December, reaching the flowering stage in approximately 3 months at Richmond compared to approximately 5 months at Merseylea and Sassafras (Tables 4 and 5). With green manure crops sown in winter, the white mustards (Architect, Abraham and Attack) and oilseed radishes (Adios, Arena and Doublet) matured about the same time. The Indian mustard (Mustclean) and rape/turnip (BQ-Mulch) matured about two weeks later.

Table 4. Crop growth over winter and spring in 2009 and white mould incidence in the subsequent green bean plants at Merseylea

Variety Name	Sowing Rate kg/ha	Plant density/m2 (5/08/09)	Average crop height (m) (16/10/09)	Biomass plant density/m2 (16/10/09)	Biomass fresh weight (kg/m2)	Biomass dry weight (kg/m2)
Architect [1]	10	37	1.00	27	0.55	0.14
Abraham	10	44	1.50	30	2.18	0.46
Attack	10	116	1.80	56	3.18	0.73
Mustclean	10	123	1.40	59	3.04	0.50
BQ Mulch	10	142	1.50	123	6.18	0.86
Adios	15	56	1.60	45	8.32	1.52
Arena	15	96	1.50	55	n/a	n/a
Doublet	15	97	1.40	75	6.48	0.99

[1] Sparse and stunted plants due to frost

With spring sown crops in October at Richmond, the white mustard varieties (Architect and Attack) matured early reaching their optimum plant biomass and full flowering about two weeks earlier than all the other varieties. Mustclean an Indian mustard had intermediate maturity, about one week after the white mustard varieties. The oilseed radish, rape and turnip matured about two weeks after the white mustards. *B. carinata* and Greenland rapes had not produce any bud or flowers when they were mulched and incorporated along with the oilseed radish on 30/12/09.

Table 5. Growth of biofumigant varieties that was sown in spring in October 2009 at Richmond

Variety Name	Sowing Rate kg/ha	Mean no. of plants/m bed (17/11/09)		Average plant height (m) *	Plant biomass *			
					Biomass plant density /m bed	Fresh weight (kg/m2)		Dry weight (kg/m2)	
Architect	11	117	e	0.88	125	4.95	c	0.69	bcd
Attack	10	180	d	1.04	179	5.19	c	0.73	a-d
Mustclean	10	254	bc	1.03	280	7.69	bc	0.77	ab
B. carinata	11	240	c	0.58	220	8.58	ab	1.37	a
BQ Mulch	9	281	b	0.93	272	6.52	c	0.78	cd
Greenland	9	440	a	0.54	449	10.02	ab	1.30	ab
ryegrass	12	279	b	0.55	164	1.89	d	0.43	d
Adios	11	92	e	0.92	85	10.24	ab	1.13	abc
Doublet	11	174	d	0.98	157	10.76	a	1.29	ab

Means within columns followed by the same letter are not significantly different at the 5% level according to LSD test.
* White mustard and Indian mustards were assessed on 15/12/09. Ethiopian mustard, oilseed radish, rape, rape/turnip and ryegrass were assessed on 30/12/09

At Richmond, the plant biomass of all the biofumigant varieties was significantly higher with approximately 3 to 6 times that of the biomass produced by ryegrass. This showed that the brassica green manure crops have great potential to substantially increase organic matter in carbon depleted soil and hence improve soil fertility and properties. The oilseed radish, Adios and Doublet, produced the highest plant

biomass, because of their thick tap root systems. The plant biomass of Greenland was very high because of its very high plant density, but this also resulted in thin stems and smaller root systems as a result of overcrowding. At Richmond, the sowing rates for most of the brassica crops appeared to be too high. Architect and Adios, which have lower plant densities tended to have larger plants with thicker stems and bigger roots than the other varieties.

Currently, on many farms, ryegrass is the most commonly used break crop in autumn and winter, between vegetable crops in spring and summer, in Tasmania. The brassica green manure crops produced vastly different root systems compared to the ryegrass root systems. Ryegrass produces fibrous root systems, while the brassica crops produced a main tap root, with or without lateral root branching as well as fine fibrous roots. Oilseed radish and *B. carinata* have enlarged tap roots and are recommended for use to reduce soil compaction. The soil benefits from each type of root systems are expected to be different. Therefore, the use of different types of break crops is preferable to using only one type of green manure crop. In Europe, the use of combinations of two or three brassica green manure varieties are recommended to growers in order to obtain the multiple benefits of their different root structures.

At Merseylea and Sassafras, plant density and growth were also affected by the soil type and waterlogging under wet winter conditions. At Sassafras, the biofumigant crops had high plant densities and produced relatively large plants in the well-drained deep red ferrosol soil in the northern part of the site compared to the sparse and stunted plants in the poorly drained duplex soil in the southern part of the site. At Merseylea, the plant density of Attack was reduced by approximately 50% when it was assessed for plant biomass at flowering (Table 4), because of foot rot due to saturated soil conditions after frequent rainfall in September and October.

Plant biomass was not available for the brassica green manure crops at Sassafras, because the crops were slashed by the grower on a Sunday, one day before the crops were scheduled to be assessed for plant density and biomass, because of concerns of cross pollination and contamination by bees of a cauliflower seed crop that was nearby. This highlights the precaution that growers need to consider, when growing biofumigant crops to ensure that there is no brassica seed crop nearby.

At Merseylea and Sassafras, the green manure crops were incorporated in October 2009, and green beans were sown in December 2009. At 3 months after the green manure crops had been slashed and rotary hoed into the soil, there was still a lot of undecomposed crop residue at Merseylea in late January 2010. But there was little or no crop residue at Sassafras. This was not surprising as the crop residue fragments were much larger at Merseylea compared to those at Sassafras. At Merseylea, many of the tap roots of oilseed radish remained intact and contained sclerotia of *S. sclerotiorum*. No sclerotes were found in the mustard or rape crop residues. Before incorporation, only very low levels of infected plants of less than 0.1% were noted in the biofumigant crops at Merseylea. No *Sclerotinia* was noted at Sassafras in the biofumigant crops, before or after incorporation. Chopping the plants into fragments is not sufficient to extract the full benefits of the biofumigation process. Brassica plant tissue must be broken down at cellular level with a mulching implement that is equipped with hammers to pulverize the above plant material to generate the biofumigant compounds (Matthiessen & Kirkegaard 2006). Large pieces of undecomposed brassica crop residue may re-grow or become colonized by the *Sclerotina* pathogens.

Table 6. Effects of green manure crops sown in winter in 2009 on white mould incidence in the subsequent green bean crop in 2010 at Merseylea

Variety Name	White mould % Plants infected (18/02/10)
Architect	63
Abraham	85
Attack	74
Mustclean	83
BQ Mulch	88
Adios	82
Arena	88
Doublet	75

The bean crop at Merseylea was irrigated with a centre pivot irrigator, and there was no visual difference in the green bean plant growth between inside and outside the biofumigant crop site. When the green beans were ready for harvest, the white mould incidence at this site was very high, ranging from 63% to 88% plants infected (Table 6). The disease incidence outside the trial site was similar. However, we noted that the white mould severity in the whole biofumigant site was much higher with many wilting plants compared to fewer wilting plants outside the trial area. Green beans in the paddock, including the trial site were treated with three commercial applications of Filan at 1 kg/ha. The biofumigant crops did not reduce the disease incidence, and instead, appeared to cause the disease to spread more rapidly on the infected plants. This is believed to be due to the higher crop residue and greater water retention in the

green manure crop area, hence creating a more ideal environment for the rapid spread of the disease in infected plants at close to harvest.

At Sassafras, the green bean crop was irrigated with travelling gun irrigation. The growth of green bean plants in the biofumigant area was more vigorous with larger plants compared to the areas previously sown with oats in the same paddock. The differences in green bean plant growth were recorded in the plant biomass assessment (Table 5). In field observations, the soil beneath the bean plant canopy was moist in the biofumigant area. In the oat area, soil surface was dry and compacted. The surface soil temperature between the rows was measured with an infra red heat sensor was considerably different: 20-21^{o}C in between the large plant rows in the biofumigant crop area versus 35-40^{o}C in between the small plants in the oat area. Changes in soil and crop conditions due to the biofumigant crops appeared to promote growth of the bean crop. Unfortunately, *Sclerotinia* disease is also favoured by the moist soil conditions. In the biofumigant area, there was a small hot spot area with many *Sclerotinia* infected plants. Elsewhere, there was no disease. This indicates that changes in crop management strategies, particularly with irrigation intervals and fertiliser application, may have to be considered following biofumigant green manure crops.

Table 7. Effects of biofumigant crops sown in winter on the subsequent green bean crop at Sassafras

Variety Name	Sowing Rate kg/ha	Plant biomass (09/02/10)	
		Fresh weight (kg/20 plants)	Dry weight (kg/20 plants)
Oat - control		1.83	0.48
Architect	10	2.14	0.57
Abraham	10	2.36	0.66
Attack	10	2.02	0.50
Mustclean	10	2.48	0.57
Adios	15	2.53	0.77
Arena	15	2.02	0.52
Doublet	15	2.03	0.52

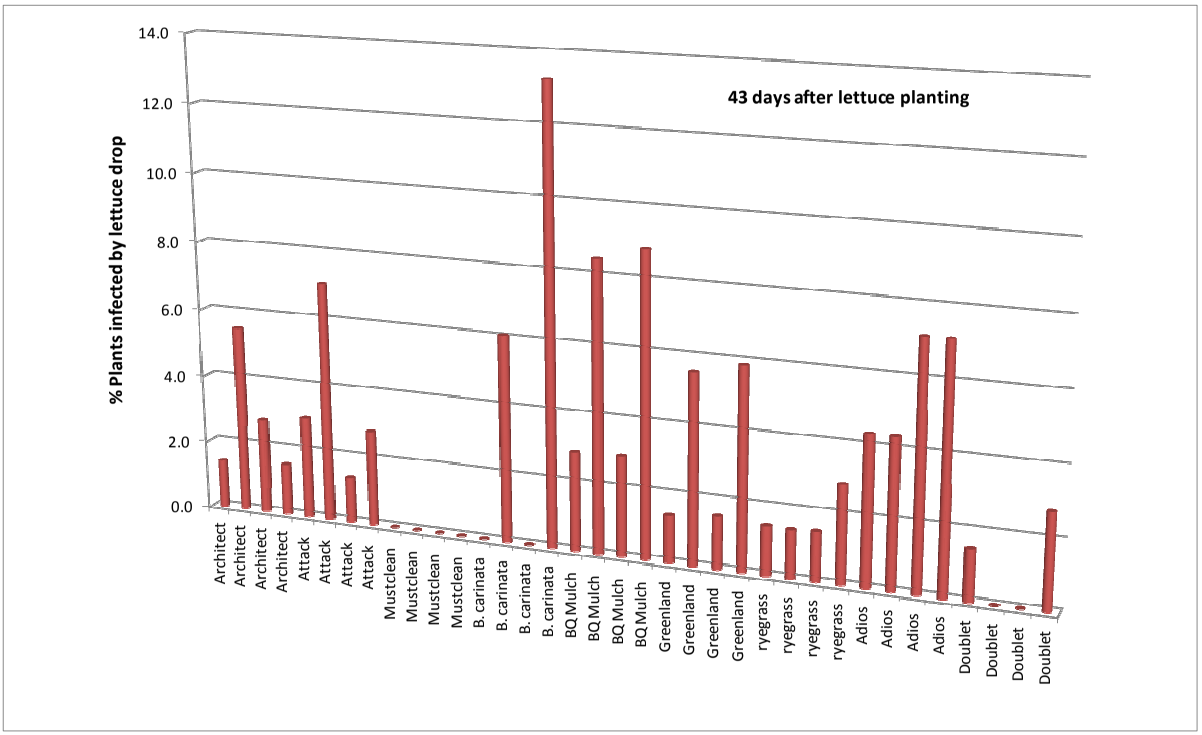
Table 8. Effects of green manure crops sown in spring in 2009 on lettuce drop incidence in the subsequent lettuce crop in 2010 at Richmond

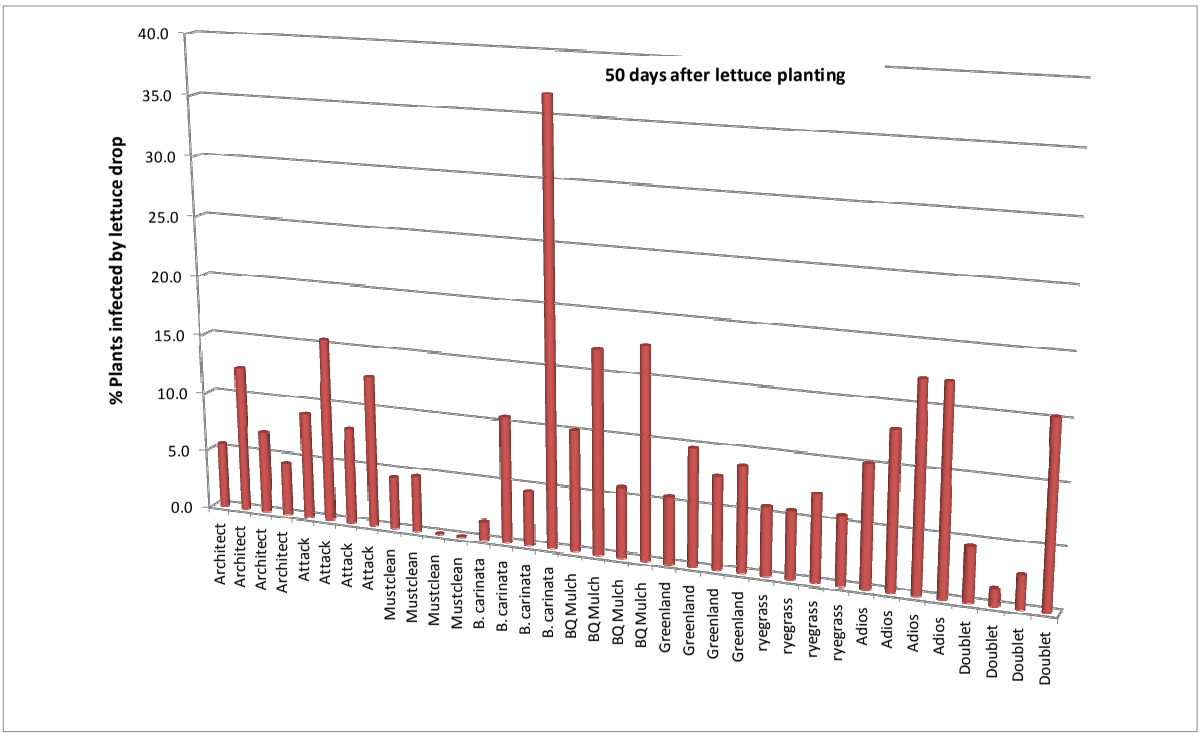
Green manure	Lettuce drop incidence (% plants infected ± standard error)					
	43DAP (25/03/10)			50DAP (01/04/10)		
ryegrass	1.8	bc	± 0.38	6.1	abc	± 0.38
Architect	2.8	abc	± 0.95	7.3	ab	± 1.73
Attack	3.6	ab	± 1.20	11.3	ab	± 1.71
Mustclean	0.0	d	± 0	2.3	c	± 1.3
B. carinata	4.8	abc	± 2.03	13.4	ab	± 2.67
BQ Mulch	5.8	ab	± 1.66	12.6	ab	± 2.81
Greenland	3.6	ab	± 1.24	8.0	ab	± 0.88
Adios	5.7	a	± 0.81	14.3	a	± 1.71
Doublet	1.1	cd	± 0.68	6.0	bc	± 3.17
p value	0.0020			0.0243		

Arsine square root transformation - data not homogeneous unable to normalise
DAP: Days after planting

At Richmond, the lettuce drop incidence was relatively high at 50 days after planting, when plants were ready for harvest (Table 8). The whole trial area was sprayed at 14 and 28 days after sowing, with two Filan applications at 1 kg/ha. There were significant differences in the lettuce drop incidence between the previous green manure crop varieties. Lettuce drop incidence was lowest after Mustclean, followed by Doublet and ryegrass. The consistency of the disease control in each of the green manures is shown in Figure 1. Disease control by Mustclean was very consistent, indicating that its effect was not a random event. All the other varieties did not reduce lettuce drop incidence, when compared to ryegrass.

Figure 1. The consistency of the effects of green manure crops on lettuce drop control in four replicate plots at Richmond at 43 and 50 days after lettuce planting





GENERAL DISCUSSION

For crops that are highly susceptible to *Sclerotinia* diseases, such as green beans and lettuces, growers are dependent on fungicides to prevent and reduce infections. Green bean is a major crop in north-west Tasmania. In many paddocks, growers will sustain total crop losses if no fungicide sprays are applied. Currently, in Tasmania, green bean and lettuce producers solely relied on boscalid fungicide (Filan™) to manage the *Sclerotinia* diseases. Before 2004, they relied solely on the procymidone (Sumisclex™ or Fortress™) until it was withdrawn from uses in both crops in Australia because of safety concerns. Boscalid is safe to beneficial insects such as bees and predatory mites, and is low in mammalian toxicity. However, it binds to soil particles and can persist in certain soil types. Therefore, its use must be carefully managed to ensure its availability for its long term use. Filan's use is recommended to two or three applications per year in a paddock. Iprodione, also available for *Sclerotinia* control, is not as effective for white mould control under high disease pressure on green beans, and it was found to have no effect for lettuce drop control in south-east Tasmania. The lack of choice in fungicides that are effective under high disease pressure is a major risk to growers. There is an urgent need to provide the industry with more choices in new and safer fungicides that can be used in alternation for good fungicide resistance and residual management. In field trials conducted in this project, we identified cyprodinil + fludioxonil (Switch™) applied at 625 g ai/ha as bioequivalent to boscalid at 500 g ai/ha. Fluazinam (Shirlan™) applied at 125 g ai/ha was moderately effective under high disease pressure. Folicur had little or no effect for *Sclerotinia* control on vegetables. AE C656948, a new fungicide, was found to be highly effective for lettuce drop control.

Sclerotia of *S. sclerotiorum* will germinate and produce apothecia and ascospores after row closure. Fungicides have no effects on the sclerotia when they are dormant, but can inhibit fungal growth after it germinates and grows. We therefore, examined the potential of an early fungicide application aimed at the soil surface, with tebuconazole (Folicur™) and fluazinam (Shirlan™), just before row closure on green beans, in order to inhibit the pathogen at soil level. The two fungicides were choosen because they are suitable for use as soil applications. Folicur™ had little or no effect on white mould, when applied before closure, and it did not improve disease control when applied with Filan™. Shirlan™, applied at 125 and 250 g ai/ha before row closure reduced white mould incidence by approximately 55%. Filan applied at 500 g ai/ha reduced white mould by 72%. The combined treatment of Shirlan, applied at 125 g ai/ha before row closure, followed by two sprays of Filan at flowering, gave the highest level of disease control, reducing *Sclerotinia* incidence by 91%. This demonstrates the potential of using an early application onto the soil surface with Shirlan™ to reduce disease pressure, followed by Filan™ at flowering to prevent flower infections.

The effects of alternating two sprays of Shirlan™ at 125 g ai/ha and Switch™ at 625 g ai/ha, with one spray of Filan™ at 500 g ai/ha, for *S. minor* control on lettuces were also examined. But even though lettuce drop incidence was reduced by these alternating spray applications, the disease levels were still too high with 47 to 50%

plants infected at harvest compared to 64% plants infected in the untreated control. The high disease incidence was due to a combination of wet and cold climatic conditions in autumn and the high pathogen level in the soil. This demonstrates the influence of planting time and disease levels in soil in the success of fungicide control. Other factors such as location, wet weather conditions and crop vigour have also been observed to be key factors affecting disease control. Until recently, many such poor disease controls are often blamed on the loss of fungicide efficacy or fungicide resistance.

Although fungicides such as boscalid had been shown to be highly effective in preventing *Sclerotinia* infections, the disease control can be variable due to variability in the flowering period, spray coverage, penetration and retention in green bean crops. High volume sprays, generally believed to help improve spray coverage, have been shown previously to increase spray run-off and overlap in spray droplets on outer leaves of bean crops, with no significant improvement in spray penetration through the plant canopy (Pung & O'Brien, 2000). Spray adjuvants are known to increase spray coverage and retention. Organosilicone adjuvants are known especially for their superior spreading and stomatal penetrating properties. However, pure organosilicone adjuvants are mainly developed for use with herbicides, because their strong stomatal penetration and leaf wax disruption properties can be damaging to plant tissues. Hence, organosilicone adjuvants are not desirable for use with fungicides. Recently, a new organosilicone and organic adjuvant blend, Du-Wett, was developed specifically for use with fungicides. Its unique blend was developed to retain the superior organosilicone spreading properties, while excluding their other foliage damaging effects. Two trials were conducted on green bean crops in Tasmania to compare the effects of Du-Wett to two commonly used non-ionic adjuvants Activator and Agral, on the efficacy of boscalid for the control of white mould on green beans. When applied under dry conditions, Du-Wett was found to improve the performance of Filan by slightly increasing white mould control and reducing the levels of variability in the disease control in different replicate plots. Under the same conditions, Activator slightly increased disease incidence. But, under constant wet conditions, there was no obvious benefit in applying boscalid with Du-Wett or Agral.

Many studies conducted in the past have shown that biocontrol agents generally did not provide the level of disease control that is acceptable to commercial crops under high disease pressure. Contans™, a mycoparasite based on *Coniothyrium minitans*, is one of a few biocontrol agents that have proven activity against *Sclerotinia* pathogens. It is registered for use overseas for white mould control in the USA and Europe. The biocontrol product must be applied early into soil, before or at sowing, so that it can parasitise and reduce the sclerotia level, hence reducing disease pressure. Micro Plus is a bacteria biocontrol agent, based on *Bacillus lydicus* that can survive well in soil and has activity in inhibiting many soilborne pathogens. Des-O-Germ is a chemical disinfectant based on quaternary ammonium that is used for disinfecting vegetable produce in processing and has broad spectrum activity against many soilborne pathogens. These are all 'soft products' that are non-toxic and have no long term chemical residual effects. However, typically, when they are used on their own under high disease pressure, they had little or no effect for disease control. It is possible, however, that they may have a place in an integrated control strategy, if they are used

in combination with fungicide applications. The combined effects of reducing or inhibiting the pathogen at soil level followed by flower protections by Filan™ may enhance disease control. These products were selected because they are available for commercial use overseas. The applications of biocontrol agents several weeks or months before sowing and maintaining moisture to field capacity to maintain their populations until the sowing of green beans are not feasible in farm practice in Tasmania. Therefore, we applied these products immediately after green bean sowing or after lettuce planting. In a green bean crop, Contans and Des-O-Germ applied after sowing followed by three sprays of Filan™ appeared to slightly reduce white mould incidence and severity compared to Filan applications only. The advantage of the small improvement however, needs to be weighed against the cost of the additional application. A major factor on their use commercially will ultimately depend on how consistent these 'soft products' are in controlling the disease in different crops and conditions. Like fungicides, the efficacy of all these 'soft products' also depends on the disease levels and crop conditions. With biocontrol agents, there is an additional challenge of maintaining relatively high populations of the fungus or bacteria under different soil types and conditions in order to have any beneficial effect.

The growth habits of five commercial green bean varieties (Celtic, Stanley, Flavor Sweet, Montano and Valentino) and their effects on white mould incidence and severity were investigated. Valentino is grown for fresh market beans in Tasmania. All the other varieties were grown for processing green beans. Valentino has the largest size bean seed, and its growth habit was quite different to all the other varieties, with slightly longer flowering period, taller plants and larger leaves. All these factors contributed to a more favourable environment for the pathogen beneath the plant canopy. Therefore, it is not surprising that Valentino had the highest white mould incidence. Among the processing green bean varieties, there were no obvious differences in their susceptibility to white mould. There was little or no difference in the flowering habits between all the varieties. Flowers of all the varieties were located close to the main stems and beneath the crop canopies. This demonstrates the difficulty in preventing flower infections with conventional fungicide spray applications and that complete disease control is impossible in a vigorously growing crop with dense canopy. Reducing plant density by 30% did not substantially reduce white mould, as the crop compensates by producing larger bushes, covering any space between plants at the lower density.

In north-west Tasmania, green bean is a major crop, sown in rotations with other horticultural crops such as potato, brassica, carrot, pea, poppy and pyrethrum. All these crops are susceptible to white mould caused by *S. sclerotiorum*, because of their dense crop canopies and summer conditions creates the ideal environment for the pathogen. Lettuce is a major crop in south-east Tasmania, and lettuce drop due to *S. minor* is threatening the sustainability of long term production. After intensive crop production over many years, the pathogen level in soil is often high and increasingly the level of disease control by fungicide alone is unsatisfactory under long wet weather conditions. Many crops are now sown in 2 to 3 year crop rotations as a necessity in modern production. Very long breaks of 5 to 10 years between susceptible crops are no longer economically feasible. Over a long term, this intensive cropping system often leads to yield decline because of increased soilborne disease and pest incidence. Typically, a great diversity in soil microbial populations

can provide natural checks and balances to the levels of plant pathogens in the soil. A healthy soil that has high organic carbon and the desirable properties to support many saprophytic and beneficial organisms can help suppress soilborne pathogens. The depletion of organic matter and carbon is believed to be the source of changes in soil conditions to favour soilborne plant pathogens. The depletion of organic matter causes soil degradation, compaction, reduce fertility and water retention. Recent studies showed that brassica biofumigant crops sown as green manures prior to the planting of lettuces have the potential to suppress and reduce *S. minor* infections in lettuce crops. BQ Mulch™, a blend of rape and turnip (*B. napus* and *B. campestris*) was found to be the most effective variety for *Sclerotinia* control (Pung et al 2004). In addition to the toxic biofumigant chemical compounds produced in their root tissues, other factors such as increased organic matter and reduced sub-soil compaction due to the brassica green manure crops are believed to be also important (Matthiessen & Kirkegaard 2006, Pung et al 2008, Aird & Long 2008). BQ Mulch is now commonly sown in Tasmania as a biofumigant green manure crop. The choice for biofumigant crops suitable for the cold Tasmanian conditions that are available commercially is still very limited. Recently, many new brassica varieties have been developed elsewhere for commercial use. Therefore, we examined nine different varieties at three sites in Tasmania for their suitability in winter and spring sowing, to observe their growth habits and determine their potential for *Sclerotinia* control. The new varieties evaluated were Indian mustard (*B. juncea* - Mustclean), white mustard (*Sinapis alba* - Architect™, Abraham™, Attack™), forage rape (*B. napus* - Greenland™), oilseed radish (*Raphanus sativus* - Adios™, Arena™ and Doublet™) and Ethiopian mustard (*B. carinata*). Growth of all the biofumigant varieties sown in winter were very slow, where crops sown in May took five months to reach the flowering stage, whereas those sown in October reach it in only three months. The white mustards, Architect and Abraham were highly susceptible to frost damage, whereas Attack was tolerant to it. Mustclean, and Indian mustard, was moderately tolerant. All the oilseed radish varieties, Adios, Arena and Doublet, and BQ Mulch were highly tolerant. Therefore, the oilseed radish varieties and BQ Mulch are more suitable for autumn/winter sowing, when frost conditions occur. All the varieties are suitable for spring sowing under warmer conditions.

The plant biomass of all the biofumigant varieties sown in spring was 3 to 6 times higher than that of ryegrass. The brassica stems and root growth were affected by plant density, soil type, drainage and weather conditions. The brassica green manure crops produced vastly different root systems compared to the ryegrass root systems. Ryegrass produces fibrous root systems, while the brassica crops produced a main tap root, with or without lateral root branching as well as fine fibrous roots. Oilseed radish and *B. carinata* have enlarged tap roots, which are useful in reducing soil compaction. The soil benefits from each type of root systems are expected to be different. Therefore, the use of different types of break crops is preferable to using only one type of green manure crop. In Europe, the use of combinations of two or three brassica green manure varieties are recommended to growers in order to obtain the multiple benefits of their different root structures. Different blends of the brassica types could be use at different sowing season for cold autumn/winter versus warm spring/summer conditions.

Increases in the biomass of green bean plants were recorded at one of the two sites, because of changes in soil conditions due to the biofumigant crops. With the changes in soil conditions due to increased organic matter, changes such as irrigation and fertiliser applications also need to be considered. This demonstrates that changes in the agronomic management of the soil and the subsequent crop are also critical in order to benefit from the green manure crop. If the subsequent crop was irrigated and managed the same way following a traditional break crop such as ryegrass, the soil and crop conditions may encourage the spread of white mould on green beans.

In a replicated trial at Richmond, the effects of eight varieties of brassica biofumigant crops (Architect™, Attack™, Mustclean™, *B. carinata*, BQ Mulch™, Greenland™, Adios™ and Doublet™) sown in October were compared to a traditional ryegrass break crop for *S. minor* control in a severe infected paddock. Lettuce drop incidence was the lowest in Mustclean plots. The disease control by Mustclean was significantly higher than that of ryegrass. All the other varieties did not reduce lettuce drop incidence, when compared to ryegrass.

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CHAPTER 4 - INFLUENCE OF ROTATION AND BIOFUMIGATION ON SOILBORNE DISEASES, YIELD AND SOIL IN VEGETABLE PRODUCTION IN VICTORIA

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SUMMARY

This is the first major study of the influence of crop rotation and biofumigation on disease and yield in vegetable production. Laboratory experiments identified four new brassica green manure crops with biofumigant activity against four major soilborne pathogens of vegetables. Volatile compounds or ITCs (isothiocyanates) released from freeze dried shoot tissue of Caliente 199, Mustclean and Gladiator and root tissue of Nemfix were inhibitory and/or biocidal to four pathogens (*S. minor*, *S. sclerotiorum*, *P. dissotocum* and *R. solani*) isolated from vegetable crops. The biocidal activity appears to be related to the concentration of glucosinolates (eg 2-propenyl-GSL) in tissue. Caliente 199 and Nemfix were the most biocidal at the lowest rate of tissue tested. *P. dissotocum* was the most sensitive pathogen to allyl-ITCs liberated by the hydrolysis of 2-propenyl-GSL. Pot trials confirmed that Nemfix, Gladiator, Caliente 199 and Mustclean have potential for controlling *S. minor* infection and Nemfix and Mustclean for controlling *R. solani* in soil.

Four rotation trials were established in commercial farms in Victoria to determine the feasibility and benefits of incorporating green manure crops in between vegetable crops for the management of soilborne pathogens of vegetables. Preliminary results indicate that amending soil with crop residues from some brassica green manure crops can result in positive effects on disease control and yield. In one site, for instance, amending soil with residue of Mustclean and Caliente 199 provided significant reductions of root rot infections, caused by *P. clade* f*, R. solani* and others, on green beans. At another site, amending soil with residue of Caliente 199 significantly increased the fresh weight of spring onions. Another beneficial effect provided by rotating with brassica crops was excellent weed suppression. Growers are encouraged to begin trialling the most promising brassica green manure crops identified by our research.

Introduction

Soilborne diseases are persistent and costly problems for growers in intensive vegetable production. In most production systems in Victoria, vegetables are cropped year after year mostly without a break. The problem is that many of the crops rotated in the same field are often susceptible to the same soilborne pathogen such as *Sclerotinia minor* and a multitude of other soilborne pathogens that cause root infections. In the long term, success controlling soilborne diseases is more likely to be achieved if a more holistic (systems) approach to managing soilborne pathogens is adopted. This approach includes the use of beneficial farming practices including crop

rotation and biofumigation that reduce inoculum carry-over in soil and thus the risk of disease outbreaks, supported by effective and environmentally friendly IPM tools.

There has been little research on rotations and biofumigation in vegetable cropping in both Victoria and Australia (Villalta *et al.* 2004). Particularly, there is lack of knowledge of how crop rotation practices influence disease management, yield and soil biology in vegetable production in Victoria. Most research on the potential of *Brassica* crops to control pathogens in Australia has been focused on field crops and potatoes. There are many reports in the literature showing that Brassica tissue can inhibit bacterial, fungal and nematode pathogens in laboratory and the glasshouse (Mattner *et al.*, 2008, Mora and Kirkegaard 2002, Smolinska and Horbowicz 1999). Kirkegaard *et al.* (1998) demonstrated that volatile compounds released from freeze dried root and shoot samples of canola (*B. napus*) and Indian mustard (*B. juncea*) inhibited the growth of several important cereal pathogens. There is also evidence of the possible role of biofumigation in controlling take-all in cereal in Australia (Kirkegaard et al. 1998). Hao and Subbarao (2003) reported that rotations with broccoli can be a practical management strategy for *S. minor* lettuce drop in California because crop residue of broccoli reduced both sclerotia in soil and disease incidence.

The objectives of this study were to:
1) evaluate the influence of crop rotations with biofumigant and other green manure crops on soilborne pathogens, disease, yield and soil in different vegetable cropping systems in Victoria; and
2) evaluate the biofumigant potential of existing and new brassica manure crops for controlling soilborne pathogens and root diseases of vegetables in controlled experiments and in the field.

The research reported here is part of an on-going study and involves:

- laboratory, glasshouse and field trials to investigate the biofumigant and agronomic potential of existing and new brassica green manure crops for managing soilborne pathogens in vegetable cropping;
- long-term field trials in which different crop rotation schemes with biofumigant and other green manure crops are being evaluated to control *Sclerotinia* and other pathogens causing root rots including *Fusarium* spp., *Rhizoctonia* spp. and *Pythium* spp.; and
- host susceptibility studies to explore how biofumigant and other green manure crops are influenced by soilborne pathogens

Materials and Methods

Plant treatments

Table 1 describes the green manure treatments evaluated in laboratory and pot trials. Seed of brassica treatments was supplied by J. Kirkegaard (CSIRO, Canberra), with the exception of Gladiator, Caliente 199 and Mustclean which was obtained from seed suppliers in Victoria and New Zealand (Caliente 199, a mustard blend containing mainly ISCI 99). Table 2 describes the brassica and non-brassica green manure crop treatments evaluated in replicated field trials. Seed was obtained from seed suppliers in Victoria. Plant material in table 1 was grown in replicated large pots (50 L) in the glasshouse and in small plots in a sand bed outdoors at Knoxfield. Plant biomass (fresh and dry weight) and glucosinolate (GSL) content was determined on plant material grown in pots, sand bed and field trials. In the field trials, plant biomass and weed density were measured inside one square meter quadrat in each plot prior to incorporation into soil and after drying in an oven at 50ºC. The growth habit, flowering times and various agronomic aspects were also recorded.

Table 1. Brassica species/varieties grown in pots and sand bed evaluated in laboratory and pot trials.

Treatment	Species/variety	Origin
Standard control	Fumafert™ seed meal	Organic Crop Protectants Pty Ltd
Addagio - Fodder radish	*Raphanus sativus*	Nematode resistant, Germany/PHPetersen/Schlatholter
Nemat	*Eruca sativus*	Biofumigant - Italy/Triumph Italia Spa/ Patalano-Lazzeri
Maxima-Plus	*Brassica napus*	Fodder rape - Kirkegaard
BQ-Mulch	*B. napus/campestris*	Rape turnip, Biofumigant, NZ/Wrightson
Idagold White mustard	*Brassica juncea*	Oilseed (meal for biofumigant) USA/Uni Idaho/Morra
Arid	*Brassica juncea*	Low-GSL oilseed mustard Canada/Kirkegaard
Nemfix	*Brassica juncea*	Biofumigant, Australia, Seedmark Robertson
Mustclean	*Brassica juncea*	Graham's Seeds
Caliente 199	*Brassica juncea*	Biofumigant, USA, D. Gies, Andrew Culley F & S services
Gladiator	*Brassica juncea*	Biofumigant, Australia, Jacobs

Adios (fodder radish), Architekt (white mustard) and Doublet (fodder radish) is additional material supplied by SeedForce being tested *in vitro* experiments.

Table 2. Brassica and non-Brassica green manure treatments evaluated in field trials.

Treatment	Rate	Source
Fallow		
Mustclean	8-10 kg/ha	Graham's Seeds, Vic
B.Q. Mulch	8-10 kg/ha	Wrightson Seeds, Vic
Caliente 199	8-10 kg/ha	High Performance Seeds, USA
Faba beans	80-90 kg/ha	Graham's Seeds, Vic
Triticale (Monstress)	80 kg/ha	Graham's Seeds, Vic
Ryegrass (Tetila-USA)	25 kg/ha	Graham's Seeds, Vic
Rye-corn	75 kg/ha	Graham's Seeds, Vic
Vetch (Popany)	80 kg/ha	Graham's Seeds, Vic
Oats	80 kg/ha	Graham's Seeds, Vic
Sudan grass		(used at Heatherton only)

Glucosinolate (GSL) concentrations

The concentrations of GSL in brassica plant material used in laboratory experiments, pot trials and crops grown in field trials was estimated using HPLC. Plant samples were collected from pots and sand bed when plants were at full flowering. Samples from field plots also were collected (5-6 reps/treatment/site) when plants were at full flowering at three field trials (Lindenow, Clyde north and Clyde south). Root and shoot material were separated from a sample of whole plants and placed into cotton bags and stored in a freezer at -20ºC then freeze dried. Freeze-dried material was pulverised using an electric grinder and then stored in sealed plastic jars until used. GSL concentrations were determined using the method of West *et al.* (2002). We report only results for 2-propenyl-GSL (Sinigrin). Other GSL compounds are currently being determined and will be reported later.

Pathogen suppression *in vitro*

A series of laboratory experiments was conducted to test the effects of volatile hydrolysis products released from defatted mustard seed meal tissue (Fumafert™) and from freeze dried root and shoot tissue, collected from glasshouse and field (Clyde south) grown brassica plants (Table 1 and 2), on the growth of four fungal pathogens isolated from vegetable farms. The methods were based on those of Mattner *et al.* (2008) and Kirkegaard *et al.* (1998). In total ten brassica treatments were evaluated in petri dish assays for their ability to destroy or inhibit mycelial growth of *Sclerotinia minor*, *Fusarium oxysporum*, *Pythium dissotocum* complex and *Rhizoctonia solani* AG-1. Fumafert™ (66% mustard seed meal of *B. juncea* and 33.3% Neem kernel *Azadirachia indica*) was used as a standard control.

Freeze dried brassica tissue in amounts of 0.25 and 0.50 grams was placed into one side of split petri plates. Sterile distilled water was then added to the tissue in each plate at volumes required (1.0-2.0 mL/plate) to produce a paste or slurry, with 1.0 ml added to plates with no tissue. A 5 mm plug of a 4-6 day-old culture of each pathogen was then placed on the other half of the plate containing potato dextrose agar (PDA). The plates were sealed with parafilm to prevent escape of volatile products and incubated in the dark at 20ºC. The radial growth of the fungus was measured by

taking two radial transects of the colonies at various times after adding water to hydrolyse the brassica tissue. PDA plugs that did not show any mycelial growth after 7 days incubation were transferred to fresh PDA to check their viability. The mycelial growth of each fungus in the presence of treatments was compared to growth of controls without brassica tissue to determine treatment effects (inhibition or biocidal activity).
The experiments were designed as replicated complete randomised blocks and analysed by ANOVA using Genstat for Windows 12th edition (Lawes Agricultural Trust, Rothamsted Experimental Station).

Host susceptibility studies

Three pot trials were conducted to determine the susceptibility of brassica and non-brassica green manure treatments, being evaluated in laboratory and field trials (Table 1 and 2), to isolates of two *Sclerotinia* pathogens (*S. minor*, *S. sclerotiorum*) and one of *R. solani*. As a first step, a series of petri dish experiments were conducted to test the commercial seed used in pot and field trials for the presence of pathogenic fungi using three selective media (PDA, V8 and WA).

Inoculum of the three pathogens was prepared on autoclaved wheat and barley grain inoculated with culture pathogens and incubated for 3 weeks at 20ºC before use. Isolates of *S. minor, S. sclerotiorum* and *R. solani* used were isolated from soil and infected plants from vegetable farms in Victoria. Seed raising mix (Biogrow, Bayswater Victoria) Potting mix (Biogrow) was inoculated with inoculum bulked up in grain at 1% inoculum w/w and included un-inoculated and substrate controls. The commercial potting mix was steam sterilised before adding the inoculum. The fungus in the colonised grains consisted mainly of mycelium. The number of seed sown in each pot was determined by their relative sizes. For *Sclerotinia* spp. and *R. solani* trials, five seeds were sown in each pot. The pots were arranged on glasshouse benches using a complete randomised block design, with four replicate pots for each treatment/pathogen combination. The number of infected and healthy plants in each pot was assessed periodically for 30 days after inoculation. Plant density reduction due to infections was then estimated based on the number of healthy plants that grew in the respective untreated controls to compensate for un-germinated seeds.

The effect of brassica treatments on pathogen control in pots

Two pot trials were conducted to determine the effects of brassica treatments on the control of *S. minor* infection on green beans and *R. solani* (damping off) on broccoli seedlings.

Inoculum for both pathogens was prepared as previously described in host susceptibility studies. Isolates of *S. minor* and *R. solani* used were from soil and infected plants from vegetable farms in Victoria. Seedling mix was inoculated with inoculum bulked up in grain at 1% inoculum (w/w) and included untreated and substrate controls. The fungus in the colonised grains consisted mainly of mycelium. The commercial potting mix was steam sterilised before adding the inoculum and brassica treatments. The potting media with inoculum was then amended with macerated fresh tissue from eight brassica treatments (Table 1) at rates of 5% and

10% of soil (w/w). The plant material used was grown in small plots in the sand bed at Knoxfield and harvested at full flowering. Whole plants were placed in a blender to macerate the tissue so that when it was mixed with soil and rolled the sap would be released into soil. Potting mix was amended with macerated whole plant tissue at 5% and 10% w/w. Fumafert™ was used as the standard control. Five seed of green bean (cv 'Valentino') and five of Broccoli (cv 'Marathon') were sown in each pot. The experimental design and assessment methods were similar to those described for the host susceptibility pot trials.

Field trials - influence of crop rotation and biofumigation on inoculum, disease, yield and soil condition

Four rotation trials were established in commercial farms in Victoria to determine the feasibility and benefits of incorporating green manure crops in between vegetable crops for the long-term management of soilborne pathogens of vegetables. In particular the trials evaluated the impact of single crop species (legume, cereal, grass or brassica) on pathogen populations in soil, disease incidence, yield and soil condition over 2-3 year crop rotation cycles.

Field sites and treatments

The rotation trials began in early 2009. The first trial was established in eastern Victoria at Lindenow in a silty clay loam (alluvial) soil, which has a hard pan and a poor structure and drained slowly when wet. The other three trials were established in the coastal areas of Melbourne in sandy soils (Clyde south and Heatherton) with low fertility and in a clay loam soil which has poor structure, formed a hard surface crust when dry and drained slowly when wet (Clyde north). Preliminary results (1st year rotation) from three of the four sites that had commercial crops in experimental plots are reported here.

Treatments

At the four sites, four types of green manure crops (brassica, legume, cereal and grass – Table 2) are being rotated with crops susceptible to several soilborne pathogens including lettuce (*S. minor*), green beans (*S. sclerotiorum, Fusarium spp., Pythium spp., Rhizoctonia spp.*), brassica speciality vegetables (clubroot) and spring onions and parsley (*S. cepivorum*, *Pythium* spp.) in 1-2 year crop rotation cycles.

BQ Mulch, Caliente 199 and Mustclean were the brassica treatments investigated. These varieties were selected in plant breeding programs for high isothiocyanate (ITCs) production. The first is a fodder rape and the latter two are mustards. The non-brassica green manure treatments were two legumes (faba bean and vetch), one grass (rye-grass) and three cereal crops (rye-corn, oats and triticale). At all sites break and commercial crops were grown using grower's standard practices, with the exception that applications of chemical controls for the pathogens under investigation were supervised by researchers. Below is a brief description of activities at each site.

Lindenow

On the 9th of March 2009, seed of brassica and non-brassica treatments (Table 2) were drilled into replicated plots on a field previously in long-term rotation with green beans, sweet corn, capsicum and cabbage. The experiment with break crops was designed as complete randomised block of eight treatments including fallow and six replicates. Plots were 6 m wide and 38 m long, with a row spacing of 30 cm. Experimental plots were not fertilised. When the brassica crops reached 50% flowering on the 20th of May (Mustclean) and on the 16 of July (Caliente 199 and BQ Mulch), the crops were pulverised with a hammer pulveriser prior to incorporation into moist soil with a rotary hoe and mouldboard plough to a depth of 30-40 cm. On the 15th of September all plots were sown with green beans (cv 'Valentino'). Fungicide treatments (Filan™) were applied to half of each plot, the first on the 9th of December (15-20% flowering) and the second on the 15th of December 2009 (>50% flowering). The sprays were applied with the grower's boom sprayer using 300 L water/ha and a wetter (60 mL Agral/100 L water). Plots were irrigated with a pivot irrigation system. Green beans were harvested on the 26th of December.

Clyde south

Seed of brassica and non-brassica treatments (Table 2, oats used instead of rye-grass) were drilled into replicated plots on the 5th of April 2009. The field was in short-term rotation with spring onions, radish and parsley. The trial was design as complete randomised block of seven treatments including fallow and six replicates. Plots were raised beds 1 m wide and 40 m long, with a row spacing of 25 cm. Experimental plots were topped with poultry manure (10-15 tonnes/ha) immediately after sowing the break crops. When the brassica crops reached 20-30% flowering on the 3rd of June (Mustclean) and 22th of July (Caliente 199 and BQ Mulch), the crops were pulverised with a hammer pulveriser prior to incorporation into soil with a rotary hoe to a depth of 30 cm followed by overhead irrigation. On the 19th of August plots were sown with spring onions (cv 'Paragon'). Fungicide treatments (Filan™) were applied to a 10 m long section of each 40 m plot, the first applied immediately after sowing and the second 4 weeks later. The sprays were applied with the grower's boom sprayer using 700 L water/ha. Spring onions were harvested on the 19th of November 2009. Radish were sown a month later and harvested in late January 2010. Following radish, a second spring onion crop was grown and harvested in early April 2010.

Heatherton

Seed of the first three treatments evaluated (BQ Mulch, rye-corn, sudan grass) were drilled into replicated plots on the 20th of February 2009. The other treatments evaluated (Caliente 199 and Mustclean) were sown on the 19th of May 2009. The field used was in short-term rotation with lettuce (Cos and fancy lettuce), spring onions and speciality Asian vegetables (Chinese broccoli, baby Pak Choy, etc). All treatments including fallow were replicated four times. Plots were also raised beds 1 m wide and 9 m long, with a row spacing of 25 cm. Plots were fertilised with Rustica™ at 125 kg/ha. When BQ Mulch reached full flowering on the 16th of March the first three crops (BQ Mulch, rye-corn, Sudan grass) were incorporated with a rotary hoe followed by overhead irrigation. Two passes were needed to cut plants into

small sections but full pulverisation of tissue was not achieved. Fifty percent of BQ Mulch plants were heavily infected with clubroot but plant growth (above ground biomass) was not affected. The other two treatments (Caliente 199 and Mustclean) were incorporated into soil as previously described on the 3rd of June 2009. On the 20th of August plots were sown with Chinese broccoli, which was harvested on the 19th of September 2009. This was followed by a crop of lettuce (multi-leaf butter type cv 'Emerson') which was harvested on 7th of December 2009.

Measurements

Isothiocyanate (ITC) analysis

Soil samples were collected from three field trials (Lindenow, Clyde south and north) to determine types and concentrations of ITC released into soil. The soil samples were collected four hours after brassica treatments were incorporated and stored frozen at -20ºC until analysed by Gas Chromatography (Varian 3400). ITCs measured are presented in Table 9. A separate report is available for method development and validation (Rose *et al*. 2010).

Inoculum

Soil samples were collected after incorporation of break crops but prior to sowing commercial crops in experimental plots to determine the effect of treatments on soil inoculum levels. The samples were collected with a hand trowel to a depth of 10-15 cm by combining ten sub-samples taken along each plot into one bulked sample.
Populations of sclerotia of *S. minor* and *S. sclerotiorum* were measured in 200 g of the soil of each plot using the wet sieving method and sclerotia viability tested on PDA media. Soil samples were also sent to SARDI to determine levels of *Pythium clade* f and *R. solani* DNA in soil using a quantitative PCR based test to estimate pathogen DNA.

Biomass

In each trial, plant biomass (fresh weight) of green manure treatments was measured inside a square metre quadrat in each plot. Dry weights were also recorded after drying a sub-sample of plant material in an oven at 50ºC. Weed composition and weight were also measured inside each square meter.

Disease and yield

Disease incidence and severity of root rot infections were recorded at commercial harvest at Lindenow (green beans), Heatherton (lettuce, Chinese broccoli) and Clyde (spring onions). In Lindenow, green bean plants were harvested from one square metre (3 linear metres of rows) in each plot. All plants and pods were counted to determine infections caused by *Sclerotinia* white mould. Disease incidence was recorded as the percentage of plant or pods with disease symptoms. Roots were scored for the degree of root rot infection using a rating scale of 0-5 where plants with no root infections and severe infections were assigned ratings of 0 and 5, respectively. At Clyde south, all spring onion plants were harvested from one square metre (3 linear

metres of rows) in each plot and examined individually for root infection and then fresh weights and bunch numbers determined. At Heatherton, five Chinese broccoli plants were harvested from each plot and examined for clubroot infections and then fresh weights taken. For lettuce crops, all plants in plots were monitored regularly to determine the number of plants killed by *S. minor* over the crop's life. Other problems that affected marketability were also recorded at all sites.

Soil parameters

A sub-sample of the bulk soil sample collected from each plot was used to determine the effect of green manure treatments on soil chemical and biological properties. Soil microbial communities (total bacteria, fungi and actinomycetes) were determined using standard serial soil dilution plating and selective media. Soil chemical properties and nutrient levels were determined by standard procedures used by SWEP analytical laboratory. Soil compaction was assessed in all trials by measuring soil penetration resistance with a mechanical cone soil penetrometer (CP40II). Three readings were taken from each plot on soil at field capacity and then the average of soil penetration (kPa) on the top soil (0-250 mm) and sub-soil (250-450 mm) estimated for each plot. Only results for Lindenow are presented in this report.

Statistical analysis

The results were analysed by general analysis of variance (ANOVA) using Genstat for Windows 12th edition (Lawes Agricultural Trust, Rothamsted Experimental Station). Data was transformed, when required, before analysis to normalise and stabilise the variance. When significant treatment and interaction effects were determined the means of treatments and interactions were compared using LSD tests (5%).

Results and discussion

Concentrations of glucosinolate (GSL) in brassica tissue

Field samples

Only results for 2-propenyl-GSL are currently available and presented (Table 3). At Clyde south, Caliente 199 (*B. juncea*) had the highest concentration of GSL in leaf tissue, which was significantly higher than concentrations measured in leaf tissue of Mustclean (*B. juncea*) but not in BQ Mulch (*B. napus/B. camprestris*). GSL levels were similar in stem tissue for the three brassica crops at this site. At Clyde north, Caliente 199 also had the highest concentration of GSL in leaf and stem tissue, which were significantly higher than GSL levels measured in tissue from the other two brassica crops. BQ Mulch had significantly more GSL in the stem than Mustclean but not in leaf tissue. At the two Clyde sites, there were significant differences in GSL concentrations between the two tissue types. At Lindenow, there were only significant

differences in stem GSL levels between the three brassica treatments. Caliente 199 had, generally, lower levels of leaf and stem GSL than those measured in similar shoot tissue at the other two sites. Plants of Caliente 199 had uneven growth at Lindenow due to poor soil structure and a severe attack by cabbage aphids. As a result Caliente 199 did not produce the same amount of biomass observed at the other two sites. This was probably the reason for not producing higher levels of 2-propenyl GSL in shoot tissue at this site. Nevertheless, on average Caliente 199 had the highest levels of 2-propenyl GSL on both leaf and stem tissue across the three sites (Table 3).

Pot and sand bed samples

Preliminary results from analysis of first samples of tissue from pot plants showed that shoot tissue of Nemfix (*B. juncea*) and Caliente 199 had the highest levels (72-96 µmol/g dry weight) of Sinigrin or 2-propenyl-GSL (Figure 1). Mustclean, BQ Mulch, and Gladiator had lower levels of GSL, decreasing from 32 µmol/g to 8 µmol/g of dry weight, respectively. The rest of brassica material tested had very low or no GSL detected. A sample of the standard control (Fumafert™) had 149 µmol/g of dry weight.

Preliminary results from analysis of first samples of tissue from plants grown in a sand bed showed that Nemfix and Caliente 199 also had the highest levels of 2-propenyl-GSL in shoot (25-35 µmol/g) and root (6-8 µmol/g) tissue (Figure 2). Shoot tissue of Gladiator, Mustclean and BQ Mulch had levels ranging from 19 to 13 µmol/g), respectively while the other brassicas did not have detectable 2-propenyl-GSL. Concentrations of 2-propenyl-GSL were relatively lower (8.3-0.7 µmol/g) in the root tissue of five brassica treatments, with Nemat, Caliente 199 and Nemfix having the greatest levels.

HPLC tests have also revealed the presence of non-sinigrin GLS in root and shoot tissue which is currently being investigated.

Table 3. Mean concentration of glucosinolates (sinigrin or 2-propenyl GSL) measured in leaves and stems of three biofumigant *brassica* cultivars/varieties grown in replicated plots at three field sites in Victoria. Other GSL currently being determined in shoot and root tissue.

		2-propenyl GSL (µmole/g DW)[1] Tissue		
Trial site	Cultivar (species/variety)	leaf	stem	Root[2]
Clyde south				
	Mustclean (*B. juncea*)	20.0 a	8.7 a	-
	BQ-Mulch (*B. napus/campestris*)	33.2 a	6.7 a	-
	Caliente 199 (*B. juncea*)	57.2 b	16.8 a	-
Clyde north				
	Mustclean (*B. juncea*)	25.5 a	5.1 a	-
	BQ-Mulch (*B. napus/campestris*)	30.8 a	13.9 b	-
	Caliente 199 (*B. juncea*)	74.1 b	40.8 c	-
Lindenow				
	Mustclean (*B. juncea*)	30.6 a	8.7 a	-
	BQ-Mulch (*B. napus/campestris*)	25.8 a	17.9 a	-
	Caliente 199 (*B. juncea*)	28.2 a	11.3 a	-
Mean of three trials				
	Mustclean (*B. juncea*)	25.4	7.5	-
	BQ-Mulch (*B. napus/campestris*)	29.9	12.8	-
	Caliente 199 (*B. juncea*)	53.2	23.0	-

Mean values within a column and site with the same letter are not significantly different according to LSD test (5%).

[1] Six replicate samples used for GSL analysis, except for Lindenow where five replicates were used for BQ-Mulch and Mustclean. DW = dry weight

[2] Root tissue is currently being been analysed for GSL concentrations.

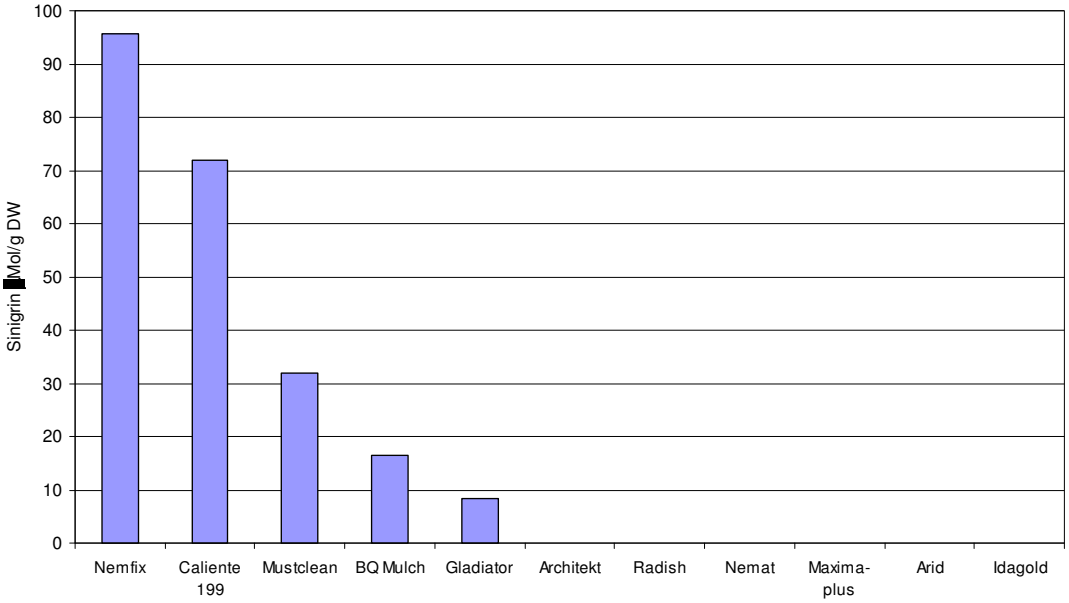

Figure 1. Concentrations of Sinigrin (2-propenyl glucosinolate) measured in freeze dried shoot tissue (leaves plus stems) from eleven brassica varieties/species grown in large pots in the glasshouse. (Only one sample has been analysed; Fumafert™ had 149 µmol/g DW).

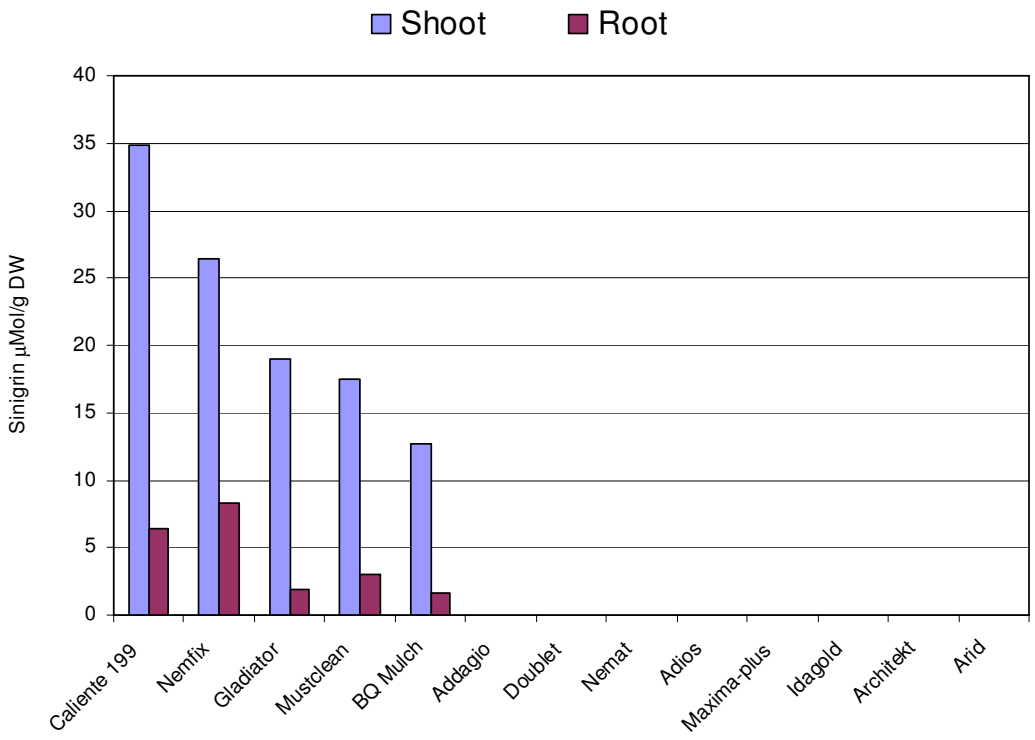

Figure 2. Concentrations of Sinigrin (2-propenyl glucosinolate) measured in freeze dried shoot and root tissue from twelve brassica varieties/species grown in a sand bed at Knoxfield. (Only the first sample has been analysed). Adios (fodder radish), Architekt (white mustard) and Doublet (fodder radish) has not been tested *in vitro*, pot or field trials yet.

The effect of brassica treatments on pathogen mycelial growth in culture

Tables 4, 5 and 6 show the results of laboratory experiments that investigated the effects of root and shoot tissue from ten brassica species/cultivars on mycelial growth of four pathogens (*S. minor*, *Fusarium oxysporum*, *Pythium dissotocum* complex and *Rhizoctonia solani* AG 2-1*)*. Fumafert™ and oats were the GSL and non-brassica controls, respectively. Freeze dried tissue from brassica plants grown in pots was used in the experiment and included freeze dried tissue from three brassica crops (Caliente 199, BQ Mulch and Mustclean) grown in replicated plots at Clyde south.

Pathogen suppression

There were significant differences in the efficacy of both root and shoot tissue treatments on mycelial growth and differences in sensitivity of pathogens to treatments.

In the first test with *S. minor*, Fumafert™ at 0.5g/plate was biocidal to mycelium of *S. minor* but at 0.25g/plate was only partially biocidal (not all cultures killed) and inhibitory. When testing root tissue, Nemfix at 0.5g was biocidal, but at 0.25g only partially biocidal and inhibitory to mycelial growth of *S. minor*. Other treatments (root tissue) were ineffective in inhibiting *S. minor* growth in culture (Table 4). There was a significant interaction between amount of root tissue and inhibitory effect for fumafert and nemfix, but no obvious difference in efficacy between pot and field grown root plant material for Caliente 199, BQ Mulch and Mustclean. When testing shoot tissue, Mustclean (pot only) and Gladiator at 0.5g were biocidal to *S. minor* and Caliente 199 (pot) biocidal at 0.25g and 0.5g. Caliente 199 (field), Nemfix and BQ Mulch (field) at 0.5g were partially biocidal or inhibitory and Caliente 199 (field), Mustclean (pot) and BQ Mulch (field) at 0.25g only inhibitory (Table 5).

In the second test with all four pathogens and using plants from Clyde south, Fumafert™ treatments were biocidal to all pathogens at 0.25g and 0.5g/plate (Table 6). Caliente 199 was biocidal to all four pathogens at 0.5g and at 0.25g biocidal to *R. solani* but only inhibitory to the other three pathogens. Both rates of BQ Mulch only inhibited growth, except *R. solani.* Mustclean inhibited growth of *P. dissotocum* at 0.5g but had no effect on other pathogens. *P. dissotocum* was the most sensitive pathogen to volatile products released from shoot and also root tissue. For instance, increasing the amount of tissue from 0.5g to 1.0g/plate improved the efficacy (biocidal effect) of BQ Mulch on *P. dissotocum* complex but not on *S. minor* (data not shown).

Efficacy of treatments in culture

Results from this experiment indicate that isothiocyanate liberating GSL with biofumigation potential were mostly present in shoot tissue, except for Nemfix. Caliente 199 showed the greatest potential to kill mycelium of *S. minor* at the lowest rate of tissue tested. However, shoot tissue of Caliente 199 plants grown in pots were more effective in killing mycelium than tissue from field plants. It is possible that pot grown plants, which were fertilised regularly and produced very lush and tender tissue, accumulated more GSL than field plants which had very fibrous stems and lignified plant material. Brassica treatments with the highest levels of GSL in shoot tissue (Figure 1) were the only treatments to show potential for biofumigation in culture (Table 5 & 6). Further testing is required to find the lowest amount of tissue required for these crops to kill mycelium and other inoculum structures such as melanised sclerotia of *S. minor*.

Plant pathogenic fungi tested appeared to have different sensitivity to the different types of ITCs released from freeze dried brassica tissue (Sarwar *et al.* 1998). Different parts of the brassica plant produce different glucosinolates, which produce different ITCs that have different properties (Sarwar *et al*. 1998). Leaf glucosinolates produce volatile ITCs such as 2-propenyl-ITC that are lost rapidly, whereas root GSL often produce non-volatile ITCs such as 2-phenylethyl-ITC that can also persist over long periods of time in soil. Generally, aromatic ITCs such as benzyl-ITC and 2-phenyethyl-ITC are less volatile, but more toxic, than aliphatic ITCs such propenyl-ITC and butenyl-ITC (Sarwar *et al*. 1998).

Table 4. Inhibition of *in vitro* mycelial growth of *S. minor* by volatile products released from freeze-dried root tissue of different *Brassica* cultivars/species grown in pots and field plots.

	Mycelial growth (mm)[1]			
	After 5 days of incubation		After 14 days incubation	
Cultivar (species/variety)[3]	**0.25 g/plate[2]**	**0.50 g**	**0.25 g**	**0.50 g**
Untreated	28.00 a	-	28.00 a	-
Fumafert	6.00 c	0.00 d	7.00 b	0.00 c
Nemfix (*B. juncea*)	19.12 b	0.00 d	28.00 a	0.00 c
Caliente 199 (*B. juncea*)	27.00 a	25.62 a	28.00 a	28.00 a
Caliente 199 (*B. juncea*) - field	26.25 a	25.75 a	28.00 a	28.00 a
Mustclean (*B. juncea*)	26.50 a	27.12 a	28.00 a	28.00 a
Mustclean (*B. juncea*) - field	26.50 a	26.62 a	28.00 a	28.00 a
BQ-Mulch (*B. napus/campestris*)	26.88 a	26.49 a	28.00 a	28.00 a
BQ-Mulch – field	26.00 a	23.62 a	28.00 a	28.00 a
Gladiator (*B. juncea*)	27.38 a	26.75 a	28.00 a	28.00 a
Arid (*B. juncea*)	27.12 a	26.75 a	28.00 a	28.00 a
Idagold (*Sinapsis alba*)	26.88 a	27.12 a	28.00 a	28.00 a
Maxima-Plus (*B. napus*)	26.75 a	26.62 a	28.00 a	28.00 a
Addagio (*Raphanus sativus*)	26.38 a	26.25 a	28.00 a	28.00 a
Nemat (*Eruca sativus*)	27.62 a	26.88 a	28.00 a	28.00 a
Oats (*Avena sativa*)[3]	26.38 a	26.50 a	28.00 a	28.00 a

Mean values, within each assessment time, with the same letter are not significantly different according to LSD tests (5%). There was a significant interaction between treatment and amount of tissuetested.

[1] PDA plug without mycelial growth after 5 days incubation were transferred to fresh PDA plates to determine if effect was fungistatic or biocidal. Red = biocidal; yellow = did not kill all cultures; green = only inhibitory.

[2] Amount of freeze dried tissue added to each petri dish (split plates).

[3] Brassica plants were grown in large pots in a glasshouse. Fumafert purchased from Organic Crop Protectants (66.6% mustard seed meal (*B. juncea*) and 33.3% Neem kernel (*Azadirachia indica*). Oats added as non-brassica control.

Table 5. Inhibition of *in vitro* mycelial growth of *S. minor* by volatile products released from freeze-dried shoot (stem+leaves) tissue of different brassica cultivars and species grown in pots and field plots.

	Mycelial growth (mm)[1]			
	After 5 days of incubation		After 14 days incubation	
Cultivar (species/variety)[3]	**0.25 grams[2]**	**0.50 grams**	**0.25 grams**	**0.50 grams**
Untreated	28.00 a	-	28.00 a	-
Fumafert	6.00 d	0.00 d	7.00 b	0.00 c
Caliente 199 (*B. juncea*)	0.00 d	0.00 d	0.00 c	0.00 c
Caliente 199 (*B. juncea*) - field	7.25 c	1.38 d	28.00 a	7.00 b
Mustclean (*B. juncea*)	13.50 bc	0.00 d	28.00 a	0.00 c
Gladiator (*B. juncea*)	24.25 a	0.00 d	28.00 a	0.00 c
BQ-Mulch (*B. napus/campestris*)	26.38 a	6.38 c	28.00 a	28.00 a
Nemfix (*B. juncea*)	23.75 a	6.75 c	28.00 a	7.00 b
BQ-Mulch – field	15.12 b	4.88 cd	28.00 a	7.00 b
Mustclean (*B. juncea*) - field	25.62 a	22.50 a	28.00 a	28.00 a
Arid (*B. juncea*)	26.75 a	25.12 a	28.00 a	28.00 a
Idagold (*Sinapsis alba*)	26.38 a	27.38 a	28.00 a	28.00 a
Maxima-Plus (*B. napus*)	26.12 a	24.62 a	28.00 a	28.00 a
Addagio (*Raphanus sativus*)	27.00 a	26.00 a	28.00 a	28.00 a
Nemat (*Eruca sativus*)	26.88 a	27.12 a	28.00 a	28.00 a
Oats (*Avena sativa*)[3]	27.12 a	26.75 a	28.00 a	28.00 a

Mean values, within each assessment time, with the same letter are not significantly different according to LSD tests (5%). There was a significant interaction between treatment and amount of residue tested.

[1] PDA plug without mycelial growth after 5 days incubation were transferred to fresh PDA plates to determine if effect was fungistatic or biocidal. Red = biocidal; yellow = did not kill all cultures; green = only inhibitory.

[2] Amount of freeze dried tissue added to each petri dish (split plates).

[3] Brassica plants were grown in large pots in a glasshouse. Fumafert purchased from Organic Crop Protectants (66.6% mustard seed meal (*B. juncea*) and 33.3% Neem kernel (*Azadirachia indica*). Oats added as non-brassica control.

Table 6. Summary i*n-vitro* effect of volatile products released from freeze-dried shoot tissue on mycelial growth of four soilborne pathogens compared to mycelial growth in untreated controls.

		Pathogen[2]			
Treatment[1]	g/plate	*S. minor*	*P. dissotocum*	*F. oxysporum*	*R. solani*
Fumafert	0.25	B	B	B	B
	0.50	B	B	B	B
Caliente 199	0.25	I	I	I	B
	0.50	B	B	B	B
Mustclean	0.25	N	N	N	N
	0.50	N	I	N	N
BQ Mulch	0.25	I	I	I	N
	0.50	I	I	I	I

[1] Three brassica crops were grown in replicated plots at Clyde south.
[2] Isolated from soil and infected plants collected from vegetable farms, *Pythium dissotocum* complex; *Rhizoctonia solani* AG 2.1
B = biocidal; I = inhibitory; N = no effect. Root tissue was ineffective in inhibiting pathogens.

The effect of biofumigant treatments on disease control in pots

Two pot trials investigated the effect of amending soil with fresh whole plant tissue from six brassica treatments on control of *S. minor* and *R. solani* infection on young green bean and broccoli plants, respectively.

S. minor

Soil amended with Nemfix, Gladiator and Mustclean at 5% and 10% (w/w) had the greatest survival of green bean plants, which was statistically comparable to untreated controls 30 days after germination (Table 7). Plant survival was significantly reduced (30-60%) by *S. minor* in other treatments including Fumafert™ and Caliente 199. The exception was the fodder radish Addagio which added to soil at 10% (w/w) had a plant survival level similar to that in untreated controls. Pots treated with oats at 5% had the lowest plant survival (30%) probably due to the ability of *S. minor* to infect this tissue and thus increase inoculum. There was a significant negative interaction between two of less effective treatments and the two levels of tissue tested, except for Addagio. For instance, the percentage of plants that survived in soil treated with Fumafert™ and Caliente 199 was significantly reduced by increasing the rate of tissue (Table 7).

Table 7. Survival of green bean plants in pots after treatment of *S. minor* infested soil (mycelium 1% w/w) with fresh plant tissue from different brassica treatments.

	% survival bean plants per pot[1]	
Cultivar (species/variety)[3]	**5% tissue w/w[2]**	**10% tissue w/w**
Untreated control	100 a	-
Untreated control (grain only)	100 a	-
Nemfix (*B. juncea*)	95 a	80 a
Gladiator (*B. juncea*)	80 ab	85 a
Mustclean (*B. juncea*)	75 ab	80 a
BQ-Mulch (*B. napus/campestris*)	70 b	50 bc
Pathogen control	70 b	-
Fumafert	70 b	40 c
Caliente 199 (*B. juncea*)	70 b	45 c
Addagio (*Raphanus sativus*)	60 bc	90 a
Oats (*Avena sativa*)	40 c	65 bc

Mean values with the same letter are not significantly different according to LSD test (5%). There was no significant interaction between treatments and two levels of tissue tested.

[1] % of plants from total germinated that survived and grew healthy in pots during 30 days.

[2] Amount of fresh macerated whole plant tissue added to each pot with potting mix.

[3] Brassica plants used were grown outdoors in small plots with sandy soil and used when flowering. Fumafert purchased from Organic Crop Protectants (66.6% mustard seed meal (*B. juncea*) and 33.3% Neem kernel (*Azadirachia indica*). Oats added as non-brassica control.

Rhizoctonia

Disease developed slowly and most broccoli seedlings in soil amended with the pathogen had some degree of *Rhizoctonia* infection including stem pruning (damping off) by day 30 after germination. Under these conditions, soil amended with Nemfix at 5% (w/w) and Mustclean at 10% had the greatest survival of broccoli plants which was statistically comparable to the untreated control and the pathogen control (Table 8). In other treatments, plant survival was significantly reduced (35-80%) by *R. solani* compared to untreated and pathogen controls. There was no significant interaction between treatments and tissue levels tested. However, increasing the amount of tissue from 5% to 10% reduced the percentage of plants that survived in soil treated in some of the treatments (Caliente 199, Gladiator, Nemat and oats). It is possible that some of the brassica treatments tested are a host of this isolate of *R. solani* and this increased the amount of inoculum present which increased infection.

Table 8. Survival of broccoli plants in pots after treatment of *R. solani* infested soil (mycelium 1% w/w) with fresh plant tissue from different brassica treatments.

	% survival broccoli plants per pot[1]	
Cultivar (species/variety)[3]	**5% tissue w/w[2]**	**10% tissue w/w**
Untreated control	100 a	-
Pathogen control	75 ab	-
Nemfix (*B. juncea*)	70 ab	60 b
Mustclean (*B. juncea*)	65 b	75 ab
Oats (*Avena sativa*)[3]	65 b	30 c
Nemat (*E. sativus*)	65 b	40 bc
Gladiator (*B. juncea*)	55 bc	25 c
Caliente 199 (*B. juncea*)	40 bc	20 c
Addagio (*Raphanus sativus*)	40 bc	35 c
Arid *(B. juncea)*	40 bc	65 b
Fumafert	35 bc	40 bc
BQ-Mulch (*B. napus/campestris*)	30 c	40 bc

Mean values with the same letter are not significantly different according to LSD test (5%). There was no significant interaction between treatments and levels of tissue tested.
[1] % of plants from total germinated that survived and grew healthy in pots for 30 days.
[2] Amount of fresh macerated whole plant tissue added to each pot with potting mix.
[3] Brassica plants used were grown outdoors in small plots with sandy soil and used when flowering. Fumafert purchased from Organic Crop Protectants (66.6% mustard seed meal (*B. juncea*) and 33.3% Neem kernel (*Azadirachia indica*). Oats added as non-GSL control.

The effect of rotation and biofumigation on inoculum, disease and yield

Isothiocyanate (ITC) release into soil

The concentrations of five ITC compounds were measured in soil four hours after brassica crop incorporation at Lindenow and Clyde south (Table 9). The compounds allyl-ITC, 2-phenylethyl-ITC and 3-butenyl-ITC were detected in soil at the highest concentrations at the two sites. At Lindenow, the crop residue of Mustclean released significantly higher levels of allyl-ITCs in soil than BQ Mulch and Caliente 199 which both produced statistically similar levels of allyl-ITCs. 3-butenyl-ITC was detected only in soil amended with Mustclean and BQ Mulch crop residue, and 4-pentenyl-ITC detected only in soil with BQ Mulch. At Clyde south, Mustclean also released significantly higher levels of allyl-ITCs than BQ Mulch and Caliente 199. 2-phenylethyl-ITC was detected in soil amended with crop residue from all three brassica treatments but levels were only significantly higher in soil amended with Mustclean crop residue. 3-butenyl-ITC was detected only in soil with Mustclean and BQ Mulch residue and 4-pentenyl-ITC detected only in soil with BQ Mulch.

Table 9. Concentrations of ITCs detected four hours after incorporating crop residue of three biofumigant brassica cultivars into soil at two replicated field trials in Victoria.

	ITC concentrations (mg/kg)				
Trial/Cultivar	Allyl-ITC	Benzyl-ITC	2-Phenylethyl-ITC	3-Butenyl-ITC	4-Pentenyl-ITC
Lindenow (alluvial soil)					
Caliente 199	0.194 b	0.000	0.000 b	0.000 b	0.000
Mustclean	0.578 a	0.005	0.038 a	0.092 a	0.000
BQ Mulch	0.160 b	0.000	0.021 ab	0.187 a	0.188
Fallow	0.000 c	0.000	0.000 b	0.000 b	0.000
Clyde south (sandy soil)					
Caliente 199	0.112 b	0.057	0.235 b	0.000	0.000
Mustclean	0.183 a	0.000	0.477 a	0.031	0.000
BQ Mulch	0.063 b	0.000	0.219 b	0.019	0.021
Fallow	0.000 c	0.000	0.000 c	0.000	0.000

Mean values within a column and site with the same letter are not significantly different according to LSD tests (5%).

Effect on inoculum, disease, yield and soil

Four long-term trials have been established in vegetable farms in Victoria to determine the influence of rotation and biofumigation on management of soilborne pathogens, yield and soil condition. Results from the first year of these 2-3 year rotation trials are presented.

Lindenow

This trial was located in a field with poor soil structure and cropped with green beans in a 2-yr rotation with cabbage, sweet corn and other crops. Prior to sowing the green manure treatments, soil had low levels of sclerotia of *S. sclerotiorum* (average 3 sclerotia/kg soil). Culture and DNA tests also revealed that *F. oxysporum*, *Pythium* spp. including *P. clade* f and *R. solani* were also present in this soil (Table 10).

There were no significant differences in fresh weight between green manure crops prior to soil incorporation, probably due to uneven growth of crops at this site with poor soil structure (Table 10). BQ Mulch produced the highest amount of biomass per square metre (7.10 kg/m^2; 71 tonnes/ha) and vetch the lowest (3.26/m^2; 32 tonnes/ha) (see table appendix). The three brassica crops, rye-grass and faba beans were very effective in suppressing weeds during the break period compared to other treatments (Figure 3). A green bean crop was grown in the experimental plots after the break crop treatments were incorporated into soil. The incidence of bean white mould was low and uneven across all plots, ranging from 0 to 2.4% in plots that had only green manure treatments and the fallow, and from 0 to 0.9% in plots where an integrated approach (manure plus Filan™) was tested (data not shown). The incidence of white mould was also low on bean pods (marketable yield), ranging from 0.5% to 2.5% on green manure treatments and from 0.09% to 1.2% on manure plus Filan™ treatments (data not shown). Bean plants in plots treated with Filan™ had slightly lower disease than plants in the respective untreated plots. These results indicated that the majority of infections were caused by airborne ascospores of *S. sclerotiorum*. The results also emphasised the problem in controlling this type of infection with fungicide if a low

volume of water is used (300 L/ha) on a dense crop canopy even though disease pressure is low.

Most bean plants harvested had some degree of root rot infection, most likely caused by a mixture of pathogens including the two detected in soil (Table 10). There were no significant differences in *R. solani* levels across all plots but levels of *P. clade* f were significantly higher in soil that had crop residue of faba bean (Table 10). Green bean plants grown in soil amended with crop residue of Mustclean and faba bean had significantly reduced root rot severity compared to plants from the fallow control, rye-grass and triticale (Figure 4 and Table 10). The root rot ratings for Mustclean and faba bean were statistically similar to those of Caliente 199, BQ Mulch and vetch. There were no significant differences in fresh weights of plants or bean pods. However, plants grown in plots that had residue of Caliente 199, faba beans, triticale and vetch were observed to have higher plant weights than plants in the fallow and other green manure treatments (Figure 5).

Results from this site suggest all brassica treatments provided some degree of soil biofumigation which resulted in reduced root rot infections. From the three mustards, Mustclean gave the highest reduction probably due to the highest levels of allyl-ITCs and 2-phenylethyl-ITCs released into soil (Table 9). Faba beans gave the highest reduction of root infections probably due to pathogen suppression effects from enhanced nitrogen and microbial activity in soil. Amending soil with Caliente 199 also appeared to enhance plant growth. This requires further investigation.

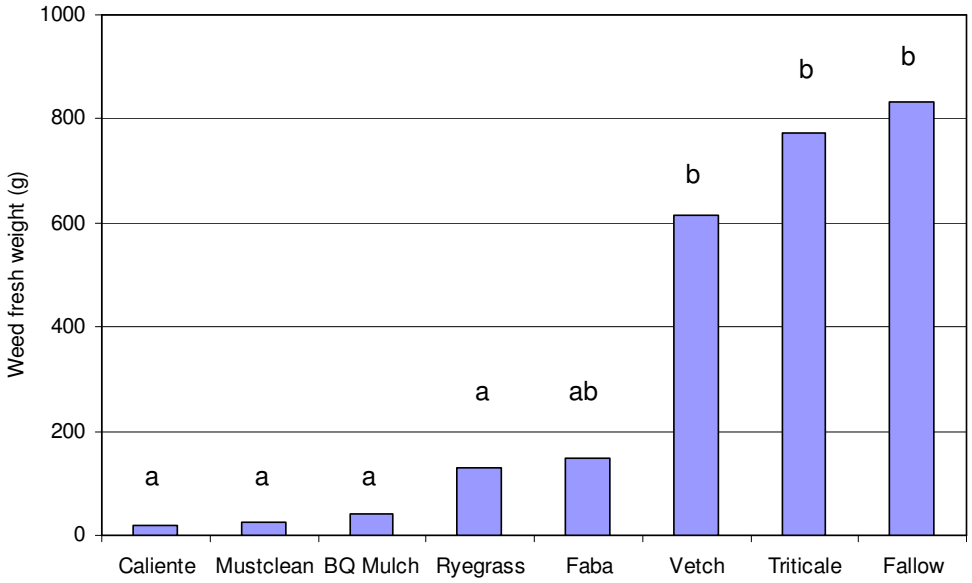

Figure 3. Effect of green manure crops on weed suppression per square meter at Lindenow. Means (bars) with different letters are significantly different at 5%.

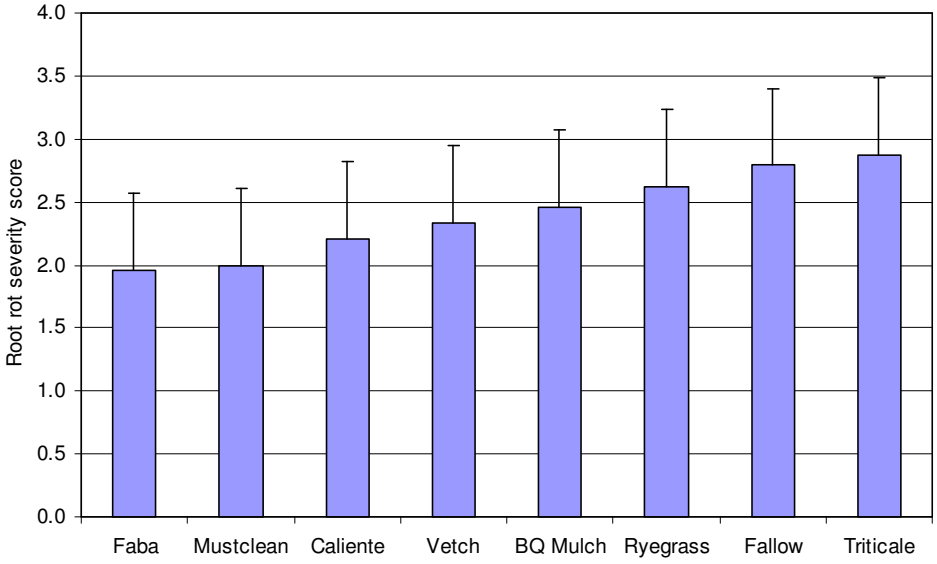

Figure 4. Effect of green manure treatments on root rot severity of green beans at Lindenow. Bars above means (bars) represent lsd values at 5%.

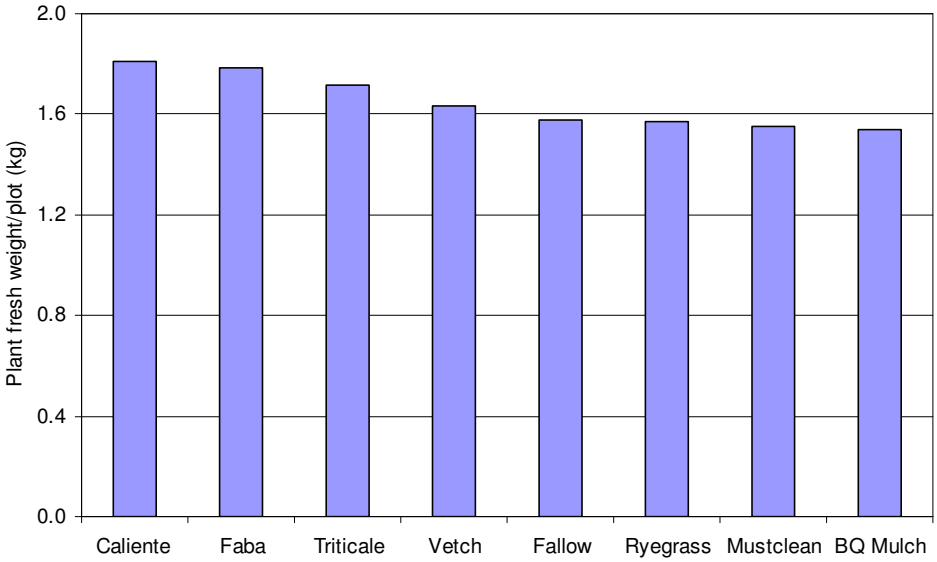

Figure 5. Effect of green manure treatments on mean plant weight (m^2) of green beans at Lindenow.

Analysis of soil showed that there were significant differences in the levels of organic matter, nutrient levels and populations of total fungi among green manure treatments (Table 11, 12 and 13). Plots amended with BQ Mulch and Caliente 199 had the highest levels of organic matter, which was significantly higher than organic matter levels in plots with rye-grass and triticale. This field is always cropped to minimise soil erosion. This may explain the high levels of organic matter in the fallow plots, which also had the highest levels of weeds. In general, biofumigation with brassica treatments did not appear to affect negatively soil communities of actinomycetes, total bacteria and total fungi compared to levels measured in soil from fallow and other treatments (Table 13).

There are several interesting trends between green manure treatments and levels of macro and micro nutrients measured in soil. One is the amount of available nitrogen which was highest in fallow plots (Table 11). Experimental plots with green manure crops were not fertilised and as a result crops utilised ('mopped up') the nitrogen available in the soil to grow. Other relationships will be explored later using multiple linear regression analysis.

Preliminary analysis of penetrometer data also indicated that there was no difference in soil compaction in the top-soil (0-250 mm, kPa resistance penetration) among plots (Table 11). Brassica biofumigant crops (BQ Mulch, Mustclean and Caliente 199) have a high biomass and very strong and deep tap root system and it was expected they would improve soil structure. Measurements of soil resistance (kPa) were high indicating that the soil at this site had poor structure. It is possible that untreated soil from the sub-soil was placed on top of soil amended with residue from green manure treatments during preparation of soil for green beans. Therefore further measurements are warranted to determine changes in soil structure once the ground is prepared again.

Clyde trial

This trial was located in a field cropped with spring onions in short rotation with radish and parsley in raised beds with sandy soil. Two spring onion crops and a radish crop have been grown in the experimental plots after green manure crop treatments were incorporated into soil.

Green manure crops grew evenly across all plots and as expected there were significant differences in fresh weight among green manure crops. BQ Mulch produced significantly more biomass per square meter (11.76 kg/m^2; 117 tonnes/ha) than all other treatments (Table 14). Oats, Mustclean and Caliente 199 produced statistically similar levels of biomass (86.5-95.2 tonnes/ha), while faba beans (seed planted too shallow resulting in reduced germination) and rye-corn had the lowest plant biomass. The three brassica crops were the most effective in suppressing weeds during the break period compared to other treatments (Figure 6). The soil had very low levels of sclerotia of *S. cepivorum* (< 2 sclerotia kg of soil). Consequently very little root rot (<1%) including allium white rot developed on the two spring crops grown in experimental plots during spring 2009 and autumn 2010. An integrated approach combining Filan™ with green manure treatments was also tested at this site for white rot control. There was no difference in disease levels among all plots indicating that the fungicide treatment could be omitted at this site because disease pressure was low.

DNA tests also revealed that presence of *P. clade f* and *R. solani* in soil. However there was no evidence of root infections caused by these pathogens on this particular crop grown during dry and warm periods of spring and autumn. Levels of *P. clade* f were very high at this site and soil amended with crop residue of Mustclean and rye-corn had significantly lower levels of this pathogen and faba beans the highest (Table 14). Previously we have observed outbreaks of root rot caused by *Pythium* sp and

Fusarium sp. on spring onions and parsley at this site when soil is wet for long periods. Therefore, further assessments are warranted at this site.

There were no differences in bunch numbers, but there were significant differences in fresh weights (Table 14, Figure 7). Spring onions are heavily fertilised with two applications of composted poultry manure (topping) and fertiliser to compensate for the low fertility of sandy soil. Despite this, plants grown in plots with faba beans and Caliente 199 had significantly higher fresh weights than plants grown in plots with oats and rye-corn but not on the other treatments (Table 14, Figure 7). It is possible that crop residue from oats and rye-corn were not fully broken down when spring onions were sown delaying germination (phytotoxic). Results with Faba beans, Mustclean and Caliente 199 also suggest some possible biofumigation effect occurred which enhanced plant growth. Mustclean and Caliente 199 released the highest levels of two of the ITCs compounds detected at this site (Table 9). This also requires further investigation.

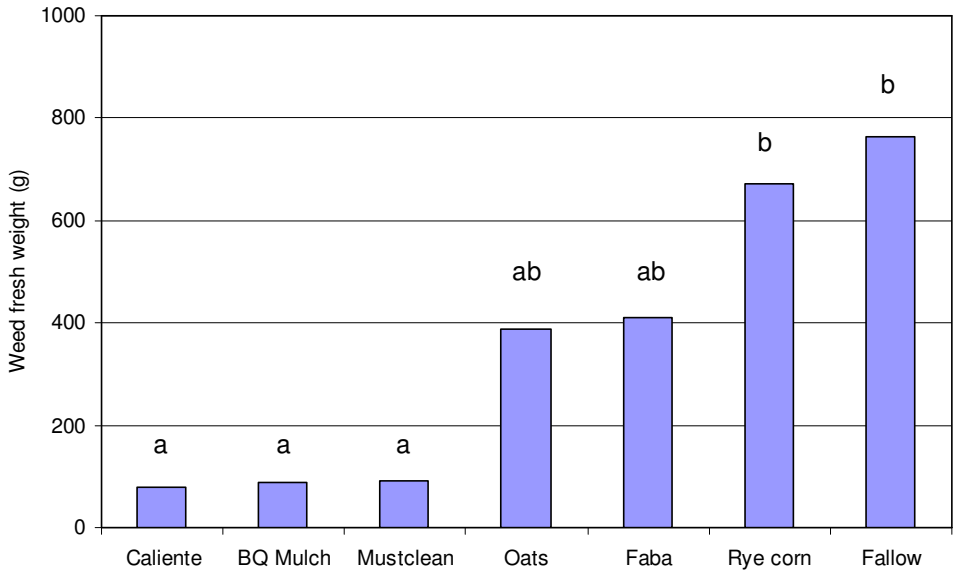

Figure 6. Effect of green manure crops on weed suppression (m^2) at Clyde south. Means (bars) with different letters are significantly different at 5%.

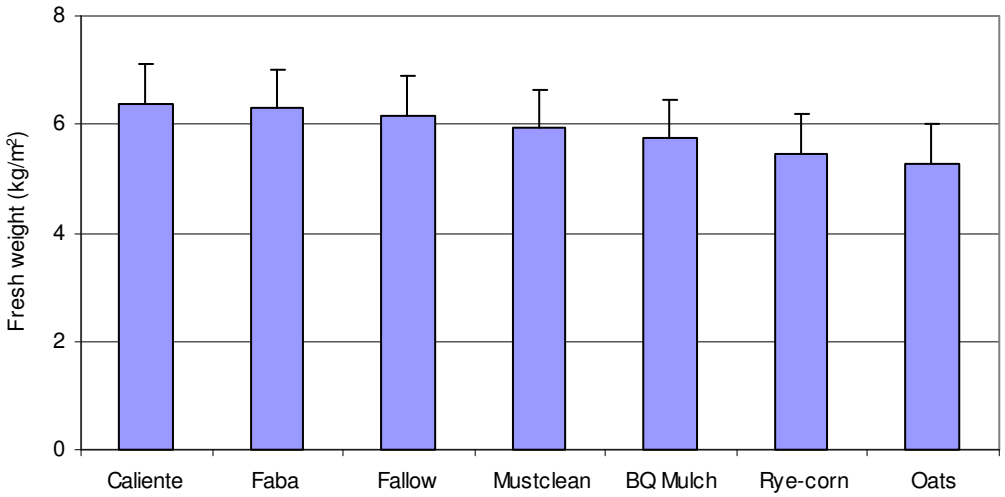

Figure 7. Effect of green manure crops on fresh weight of spring onions (m^2) at Clyde south. Bars above means (bars) represent lsd values at 5%.

Analysis of soil data showed that there were some significant differences in the levels of macro and micro nutrients and populations of bacteria and fungi among green manure treatments (Tables 15-17). Plots amended with BQ Mulch and Caliente 199 had the highest levels of available soil nitrogen, which was significantly higher than nitrogen levels in other treatments. BQ Mulch and Caliente 199 produced the highest levels of biomass and this probably contributed to the high levels of available nitrogen and phosphorus in soil. Other relationships will be explored later using multiple linear regression analysis. Biofumigation with brassica treatments did not affect negatively soil microbial communities in soil (Table 17).

Heatherton

This trial was located in a field routinely cropped with lettuce in short rotation with speciality Asian brassica crops in raised beds with sandy soil. Three commercial crops (Chinese broccoli, lettuce and Pak Choy) have been planted in experimental plots after green manure crop treatments were incorporated into soil.

This site has a history of severe clubroot infection and soil DNA analysis confirmed that soil is heavily infested with the clubroot pathogen. BQ Mulch produced the highest biomass (6.65 kg/m^2; 66.5 tonnes/ha) at this site, which was significantly higher than biomass produced by rye-corn and sudan grass (Table 18). Caliente 199 and Mustclean were severely infected and stunted by the clubroot pathogen. Plants of Chinese broccoli grown in plots amended with crop residue from non-brassica treatments (rye-corn and sudan grass) and BQ Mulch had significantly lower clubroot root infection scores than plants grown in plots amended with Caliente 199 and Mustclean residue (Table 18). Plants in plots with rye-corn and fallow had significantly higher fresh weights than plants in other treatments, except Caliente 199. BQ Mulch, a blend of *B. napus* and *B. campestris,* was less susceptible to clubroot infection although plants of both species exhibited root galls typical of clubroot disease. Mustclean (*B. juncea*) and Caliente 199 (*B. juncea*) were highly susceptible

to clubroot and severe root infections increased the amount of inoculum in soil. This may explain in part the higher levels of infection observed in plants grown in plots that had crop residue from Mustclean and Caliente 199. One of our research priorities is to evaluate biofumigant crops that are non-host of the clubroot pathogen (e.g. radish fodder types) for use in farms with fields infested by clubroot.

DNA soil analysis indicated that *P. clade* f and *R. solani* were also present in this field. Soils amended with residue of BQ Mulch and rye-corn had significantly more *R. solani* inoculum than soil amended with sudan grass. *R. solani* was not detected in other soils. The incidence of lettuce drop, caused by *S. minor*, was too low to allow treatment comparison on the first lettuce crop planted after Chinese broccoli (data not shown). More assessments on lettuce crops are planned in 2010 to determine the effect of green manure treatments on *S. minor* control.

Soil analysis showed that there were significant differences in the levels of some macro and micro nutrients and populations of bacteria and fungi among the manure treatments (Tables 19-21). The data will be analysed later using multiple linear regression analysis to identify useful trends and relationships. Biofumigation with brassica treatments did not appear to negatively affect soil microbial communities (Table 21).

Host susceptibility

A series of pot trials is investigating the susceptibility of the green manure crops evaluated in field trials to infection by *S. minor*, *S. sclerotiorum* and other important soilborne pathogens in vegetable production. The aim of this work is to better understand the factors that influence and drive the infection process on green manure crops so that strategies can be designed to effectively deploy these crops in crop rotation systems.

Commercial seed

Petri dish experiments tested samples of commercial seed used in pot and field trials for the presence of pathogenic soilborne fungi on three selective media (PDA, V8 and WA).

Samples of seed from cereal (oats and triticale) and grass (rye-corn and rye-grass) crops, generally, had significantly more seed infected, both externally and internally (seed surface sterilised), by fungi than seed from brassica and legume crops (Figure 8 and 9). Detailed microscopic examination of infected seed showed that infections were caused by a range of airborne non-pathogenic fungi and a few fungi in the genera that causes foliar diseases (*Cladosporium* sp., *Alternaria* sp. and *Stemphylium* sp.). Seed germination was very high in all samples of cereal and grass seed tested (Figure 10). Brassica seed did not have internal fungal infections and germination was also very high (Figure 8 and 10).

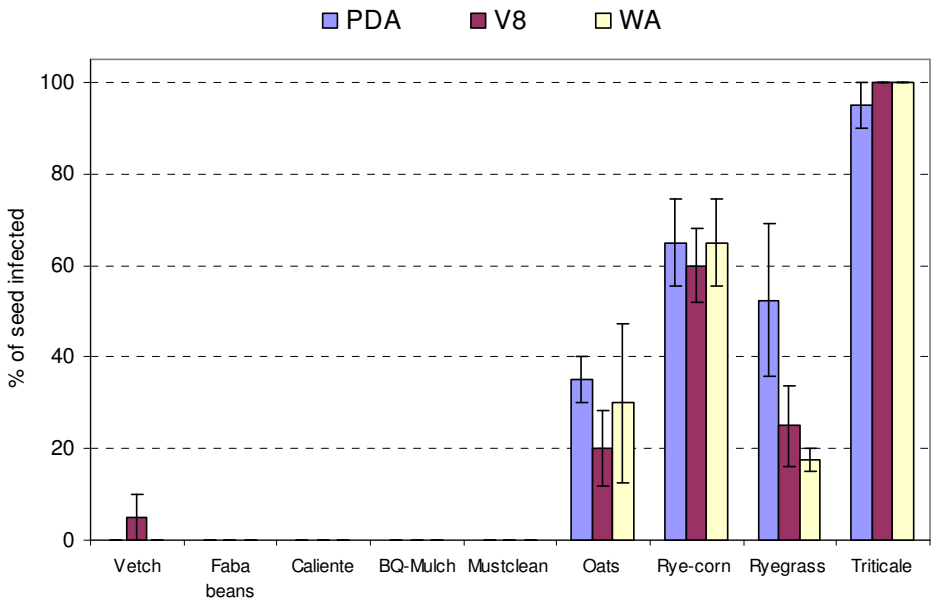

Figure 8. Mean percentage of surface sterilised seed from total germinated in three selective media with fungal infections (internal infections). Bars are standard error of the mean.

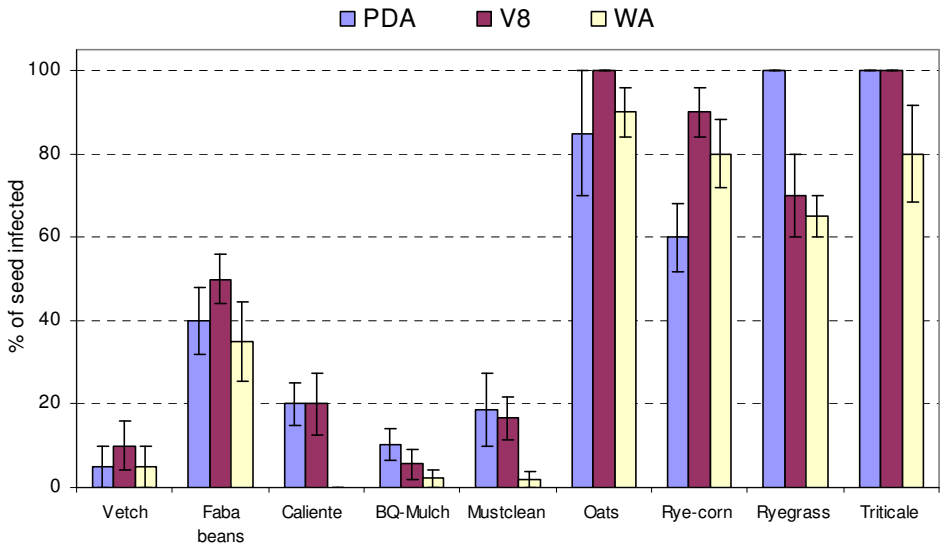

Figure 9. Mean percentage of seed from total germinated in three selective media with fungal infections. Bars are standard error of the mean.

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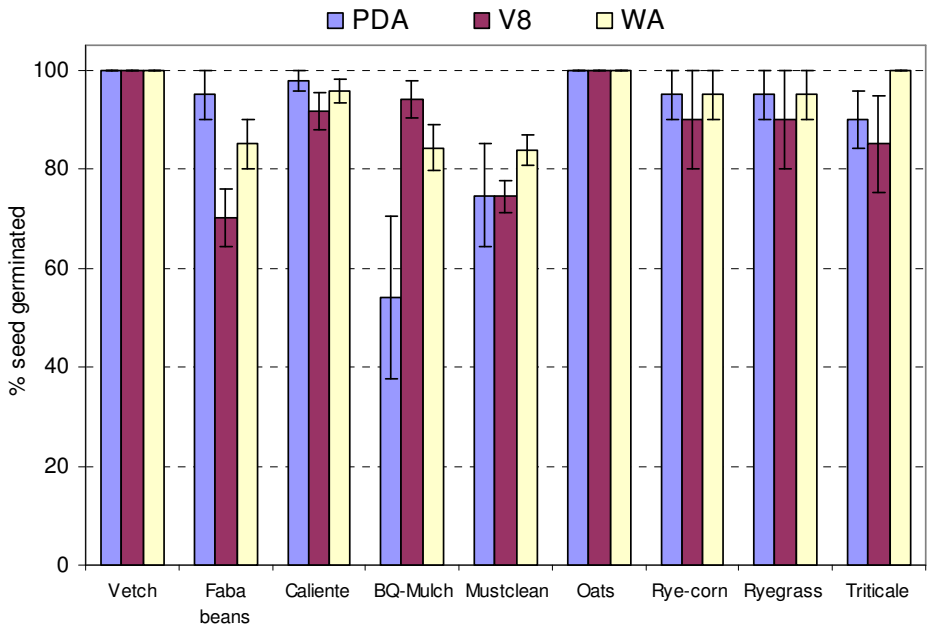

Figure 10. Mean percentage of total seed germinated in three selective media. Bars are standard error of the mean.

Host susceptibility

Many of the host plants tested at the seedling stage (up to 30 days) were highly susceptible to *S. minor* and *S. sclerotiorum* infection in pot soil with relatively high levels (1% w/w of soil) of mycelial inoculum (Tables 22 and 23). As expected, *S. minor* caused significant reductions in plant density (81-97%) on *Phaseolus* and *Vicia species*. *S. sclerotiorum* also caused significant reductions on *P. vulgaris* (90%) and *V. faba* (97.5%) but not on vetch (*Vicia* sp.). The ten brassica crops tested were also highly susceptible to *S. minor* and *S. sclerotiorum* infection at the seedling stage under high inoculum pressure (Tables 22 and 23). All cereal and grass crops were less susceptible to *S. minor* and *S. sclerotiorum* infection. Triticale (*T. secale*) was not infected by the *Sclerotinia* pathogens and oats only infected by *S. minor*. In other cereal and grass crops the two *Sclerotinia* pathogens caused lower but significant reductions (2.5-17.5%) in plant density in pots. Eight of the ten brassica crops tested were also highly susceptible to *R. solani* infection in pot soil with high levels of mycelial inoculum (1% w/w) (Table 22).

In our pot experiments seedlings germinated in soil with abundant mycelium of pathogen. The seedlings that survived appeared to be less susceptible to infection as they matured probably because shoot tissue grew away from inoculum. Levels of mycelial inoculum used in our pot trials are probably unlikely to occur in soils of most commercial vegetable farms in Victoria. For instance, populations of sclerotia of *S. minor* quantified in soils from commercial vegetable farms in Victoria were very low ranging from 1 to 7 sclerotia/kg of soil. *S. minor* and *S. sclerotiorum* survive in soil at lower densities not as mycelium but mostly as sclerotia which germinate in soil in response to exudates emitted by the roots of host plants. Inspection of green

manure crops prior to soil incorporation in our field trials revealed that very few of the host (vetch and brassica crops) were infected by *S. minor* and *S. sclerotiorum.*

Table 22. Reduction in plant density of eight biofumigant brassica cultivars/species in soil inoculated with *S. minor* and *R. solani* (1% w/w) 30 days after germination in pots.

	Plant density reduction (%)[1]	
Cultivar (species/variety)[3]	***Sclerotinia minor***	***Rhizoctonia solani***
Gladiator (*B. juncea*)	57.5	87.5
BQ-Mulch (*B. napus/campestris*)	68.3	86.7
Nemfix (*B. juncea*)	85.4	100.0
Arid (*B. juncea*)	65.0	95.0
Idagold (*Sinapsis alba*)	58.8	100.0
Maxima-Plus (*B. napus*)	65.0	100.0
Addagio (*Raphanus sativus*)	66.2	88.8
Nemat (*Eruca sativus*)	65.0	100.0

[1] Reduction calculated based on the number of plants that germinated and grew healthy in the respective untreated controls.

Table 23. Reduction in plant density of eight green manure break crops, including three brassica biofumigant cultivars, in soil inoculated with two soilborne pathogens 30 days after germination in pots.

Cultivar (species/variety)	***S. minor***	***S. sclerotiorum***
Green beans (*P. vulgaris*)	97.5 a	90.0 ab
Vetch (Vicia sp. Popany)	95.0 a	35.0 c
Caliente 199 (*B. juncea*)	91.7 ab	86.7 ab
BQ-Mulch (*B. napus/campestris*)	89.3 ab	82.0 ab
Faba beans (V. faba)	81.3 b	97.5 a
Mustclean (*B. juncea*)	76.2 b	75.1 b
Oats (*A. sativa*)	10.0 c	0.0 d
Rye-corn (*S. cereale*)	7.5 c	17.5 c
Ryegrass (*L. perenne*)	2.5 c	2.5 cd
Triticale (*T. secale*)	0.0 c	0.0 d

Mean values within columns with the same letter are not significantly different according to LSD test (5%). Reduction calculated based on the number of plants that germinated and grew healthy in the respective untreated controls.

Conclusions and recommendations

This on-going research is investigating the influence of crop rotation and biofumigation on management of soilborne pathogens, yield and soil properties in vegetable production in Victoria. Key outcomes from the first year of laboratory, glasshouse and long-term field trials (first year rotation) are summarised below.

We have identified four new brassica green manure crops with biofumigant activity against four major soilborne pathogens of vegetables. Laboratory experiments showed that volatile compounds (isothiocyanates) released from freeze dried plant tissue of

Caliente 199, Mustclean, Gladiator and Nemfix were inhibitory and/or biocidal to isolates of *S. minor*, *S. sclerotiorum*, *P. dissotocum* f and *R. solani* collected from vegetable farms in Victoria. The biocidal activity appears to be related to amount and quality of tissue and concentration of glucosinolates (eg 2-propenyl-GSL), with the highest levels occurring in shoots. For example shoot tissue of Mustclean grown in pots was more effective in killing *S. minor* mycelium than shoot tissue from field plants. Similarly higher amounts of Mustclean shoot tissue (0.75-1.0 g/plate) were more effective in inhibiting or killing *S. minor* mycelium (unpublished data). Caliente 199, Mustclean, Gladiator and Nemfix, which produced the highest concentrations of volatile GSL, were more effective in inhibiting or killing mycelium of pathogens than another seven brassica treatments evaluated, which produced low or no 2-propenyl-GSL. Caliente 199 and Nemfix were the most biocidal treatments at the lowest rate of tissue tested (0.25g/plate) and were as good as or superior to the standard treatment Fumafert™ and BQ Mulch. Preliminary pot trials confirmed that biofumigant potential of Nemfix, Gladiator, Caliente 199 and Mustclean for controlling *S. minor* infection and Nemfix and Mustclean for controlling *R. solani*.

The four pathogens showed different sensitivity to isothiocyanates (ITC) compounds released from freeze dried tissue in petri plate bioassays, with *P. dissotocum* being most sensitive to allyl-ITCs liberated by the hydrolysis of 2-propenyl-GSL. Results from new experiments confirmed the high sensitivity of *P. dissotocum* to ITCs (unpublished data). This early result would suggest that growers could select biofumigant crops for their paddocks based on their efficacy against particular pathogen problems and agronomic characteristics. In the field, the relative susceptibility of inoculum of different pathogens to biofumigation will depend on the sturdiness of their dormant survival structures. For instance, *S. minor* occurs as melanised sclerotia, *R. solani* as sclerotia and melanised hyphae. These structures may be less vulnerable to the volatile chemicals than fungal hyphae. Therefore our next step is to evaluate the most promising brassica treatments on sclerotia.

Four rotation trials were established in commercial farms in Victoria to determine the feasibility and benefits of incorporating green manure crops in between vegetable crops for the long-term management of soilborne pathogens of vegetables.

Preliminary results from field trials showed that amending soil with crop residues from brassica green manure crops resulted in some positive effects on disease and yield. At Lindenow, for instance, amending soil with residue of Mustclean and Caliente 199 provided significant reductions of root rot infections, caused by *P. clade* f*, R. solani* and probably others on green beans. At Clyde south, amending soil with residue of Caliente 199 significantly increased the fresh weight of spring onions. The effect of Caliente 199 on disease and yield may be related to the relatively high levels of 2-propenyl GSL produced by the foliage of this crop which were converted to high concentrations of allyl-ITCs upon hydrolysis in soil. A gas chromatography method has been successfully developed and validated to quantify the release of ITCs in soil from crop residues of biofumigant brassica crops.

Another beneficial effect provided by rotating with brassica green manure crops is excellent weed suppression.

Preliminary results from field trials also show that crop rotation is critical to the management of soilborne diseases in vegetable production in Victoria. This was clearly shown at the Lindenow site where a minimum of 2-year rotation with green beans has prevented the build up sclerotia of *S. sclerotiorum* in soil and therefore prevented epidemics of white mould even in a crop without fungicide treatment. Short rotation with lettuce and brassica speciality crops at Heatherton has resulted in the build of clubroot inoculum in soil. This has resulted in severe clubroot infections on susceptible crops grown during warmer months of the year. The pathogens *S. minor*, *S. sclerotiorum* and *R. solani* have a wide host range and sometimes occur together in the same field. Controlling these pathogens with crop rotation is therefore difficult. Our research has determined which green manure crops, including biofumigant brassica crops are hosts of soilborne pathogens studied allowing them to grow and multiply and which are poor hosts. For example, a pot study with *S. minor* and *R. solani* showed that the fungi were able to grow and reproduce on fresh tissue of some brassica species. In the Heatherton field trial, brassica biofumigants were hosts of *P. brassicae,* the cause of clubroot. This demonstrates that some green manure crops could be a substrate for these pathogens and thus a poor break crop choice for fields with high levels of these pathogens.

The impact of crop rotation on soil condition may depend on a number of factors which can have a direct or indirect effect on both pathogen inoculum and soil. We have collected a large amount of soil data to determine the potential beneficial effects of rotation on chemical, biological and physical properties of soil. This is currently being analysed by multiple linear regression to identify useful relationships.

Preliminary results from this on-going study indicate that soil biofumigation with brassica green manure crops and crop rotation could be useful tools to manage soilborne pathogens and increase productivity in vegetable production in Victoria. More field data is needed from long-term trials to determine the full effect of rotation with biofumigant and other green manure crops on disease, yield and soil condition. There are other factors, apart from the break crop and biofumigation, which influence disease, yield and soil. Only through on-farm trials with large plots can the impact of all variables be better understood. Our next step is therefore to conduct further assessments at established field trials to continue monitoring changes in inoculum, disease, soil status and other parameters for at least two more years. More trials are also required at new sites with different soils, pathogens and cropping systems to evaluate new rotation strategies. These trials should also evaluate new biofumigant crops coming into the market, especially non-host crops to pathogens like clubroot (i.e fodder rape/radish), and investigate how rotation and biofumigation could be used as a tool to improve soil biology.

In the meantime, we encourage vegetable growers to start trialling the new brassica green manure crops identified by our research to have biofumigant potential against soilborne pathogens tested in our work. During cool periods of year Mustclean should begin flowering 60 days after sowing and Caliente 199 and BQ Mulch 90 days after sowing. A grower in eastern Victoria has begun trialling these crops (eg Mustclean and Gladiator) and reported improvements in soil structure and water infiltration. However, our research indicates growers should avoid growing blends of *Brassica juncea* green manure crops in field infested with clubroot. Insect pest such as cabbage

aphids could be a problem if brassica crops are grown during warmer periods of the year in Victoria.

Our research indicates that there is scope for manipulating rotations to manage soilborne pathogens of vegetables. But for optimum benefit, rotation must be integrated with other IPM practices and tools throughout the rotation. The challenge for researchers is to provide growers with information on suitable green manure crops including biofumigant brassica crops and rotation schedules for optimum disease management and maintenance of soil condition. Our long-term goal is to identify break crops suitable for use on-farm and crop rotation sequences that minimise the build up of populations of soilborne pathogens and weeds. Another goal is to help industry design cropping systems that maintain organic matter, soil structure and re-cycle nutrients.

Acknowledgement

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Table 10. Effects of green manure crop treatments on biomass, soil inoculum, root rot severity and yield of green beans at Lindenow, Victoria.

Treatment	Biomass[1] fresh kg/m^2	*Pythium clade* f (DNA pg/g soil)[2]	*R. solani* (DNA pg/g soil)[2]	Root rot[3] severity	Total plants/plot biomass kg/m^2	Yield pods g/m^2
BQ Mulch	7.10	226 a	706	2.458 ab	1.541	770
Mustclean	5.07	302 a	573	2.000 a	1.555	694
Caliente 199	4.60	260 a	674	2.208 ab	1.813	909
Faba beans	4.83	675 b	981	1.958 a	1.783	890
Vetch	3.26	215 a	401	2.333 ab	1.633	828
Fallow	-	256 a	574	2.792 b	1.578	809
Triticale	5.41	261 a	364	2.875 b	1.718	833
Rye-grass	6.22	178 a	318	2.625 b	1.568	764
F-test	0.106	0.003	0.348	0.024	0.578	0.363

1 Fresh weight (biomass) of green manure crops when incorporated into soil.
[2] DNA analysis conducted by SARDI (qPCR soil testing service).
[3] Root rot severity rating; 0 no disease and 5 all root severely affected by infection.

Table 11. Effects of green manure crop treatments on soil physical and chemical properties (macro nutrients) at Lindenow, Victoria.

Treatment	Biomass[1] kg/m^2	Soil density kPa (top soil 0 – 250 mm)	pH	Org. matter (%)	CEC me/100g	Avail N ppm	Avail P ppm	Avail K ppm
BQ Mulch	7.10	3152	6.483	4.167 a	18.02	37.0	73.5	0.577
Mustclean	5.07	3036	6.483	4.133 ab	17.71	61.3	75.8	0.588
Caliente 199	4.60	2510	6.583	4.233 a	17.79	36.0	85.0	0.637
Faba beans	4.83	2612	6.433	3.783 ab	17.31	31.4	62.7	0.532
Vetch	3.26	2470	6.367	3.800 ab	17.73	45.5	70.6	0.572
Fallow		2657	6.633	4.067 ab	17.07	66.8	71.7	0.540
Triticale	5.41	2971	6.617	3.533 b	17.94	43.3	67.6	0.533
Rye-grass	6.22	3064	6.683	3.317 b	17.47	37.1	64.4	0.473
F-test	0.106	0.184	0.821	0.041	0.865	0.102	0.416	0.875

Table 12. Effects of green manure crop treatments on soil micro nutrients at Lindenow, Victoria.

Treatment	Biomass[1] kg/m^2	Avail B ppm	Avail Ca ppm	Avail Co ppm	Avail Cu ppm	Avail Fe ppm	Avail Mg ppm	Avail Mn ppm	Avail Na ppm	Avail S ppm	Avail Zn ppm
BQ Mulch	7.10	0.517	10.74	1.967	8.58	29.8	2.113	15.83	0.513	3.35	16.9
Mustclean	5.07	0.550	10.81	1.850	7.38	27.0	2.155	15.67	0.517	3.85	26.4
Caliente 199	4.60	0.533	10.25	1.883	6.23	32.2	1.910	16.00	0.593	3.63	17.4
Faba beans	4.83	0.517	10.19	1.883	6.37	33.3	2.007	16.00	0.560	3.82	12.2
Vetch	3.26	0.567	10.38	1.750	5.97	25.3	2.020	17.00	0.640	3.78	16.4
Fallow		0.583	11.06	1.767	6.55	29.2	2.032	17.83	0.645	4.00	23.5
Triticale	5.41	0.517	11.03	1.800	7.02	23.2	2.087	15.67	0.550	3.58	24.6
Rye-grass	6.22	0.500	10.69	1.800	5.83	26.5	2.010	16.17	0.517	3.45	20.5
F-test	0.106	0.438	0.932	0.292	0.151	0.914	0.459	0.831	0.162	0.877	0.851

Table 13. Effects of green manure crop treatments on soil microbial communities at Lindenow, Victoria.

Treatment	Biomass[1] kg/m^2	Actinomycetes CFU/g	Bacteria CFU/g	Fungi MEA CFU/g	Fungi RBA CFU/g	FDA mg fluorescein/3h/g
BQ Mulch	7.10	8.5 x 10^5	5 x 10^7	2.7 x 10^5 a	1.2 x 10^5 a	0.245 a
Mustclean	5.07	3.7 x 10^5	4 x 10^7	1.3 x 10^5 bc	1.7 x 10^5 a	0.231 a
Caliente 199	4.60	5.8 x 10^5	7 x 10^7	2.7 x 10^5 a	3.1 x 10^5 b	0.239 a
Faba beans	4.83	7.2 x 10^5	3 x 10^7	2.2 x 10^5 abd	1.7 x 10^5 a	0.211 a
Vetch	3.26	5.4 x 10^5	3 x 10^7	1.1 x 10^5 cd	1.4 x 10^5 a	0.212 a
Fallow-A		8.9 x 10^5	4 x 10^7	1.7 x 10^5 abc	1.5 x 10^5 a	0.213 a
Fallow-B		5.1 x 10^5	4 x 10^7	1.5 x 10^5 abc	1.1 x 10^5 a	
Triticale	5.41	5.3 x 10^5	5 x 10^7	2.1 x 10^5 abd	1.3 x 10^5 a	0.160 b
Rye-grass	6.22	4.6 x 10^5	5 x 10^7	8.2 x 10^4 c	1.2 x 10^5 a	0.230 a
	0.106					
F-test		0.194	0.062	0.033	0.005	0.006

Table 14. Effects of green manure crop treatments on soil inoculum and yield of spring onions at Clyde south, Victoria.

Treatment	Biomass[1] kg/m^2	*Pythium clade* f (DNA pg/g soil)[2]	*R. solani* (DNA pg/g soil)[2]	Number bunches m^2	Weight bunches[1] kg/m^2
BQ Mulch	11.76 a	1119 b	0.63	24.10	5.743 ab
Mustclean	8.65 bd	788 ab	0.00	23.29	5.921 ab
Caliente 199	9.52 b	1010 b	3.19	23.58	6.393 a
Faba beans	6.83 c	1832 c	0.00	22.87	6.293 a
Fallow		1115 b	0.00	23.47	6.157 ac
Oats	8.91 bd	989 b	0.00	22.87	5.290 b
Rye-corn	8.25 d	949 ab	1.47	23.31	5.456 bc
F-test	<0.001	<0.001	0.611	0.862	0.027

1 Fresh weight (biomass) of green manure crops when incorporated into soil.
2 DNA analysis conducted by SARDI (qPCR soil testing service).

Table 15. Effects of green manure crop treatments on soil physical and chemical properties (macro nutrients) at Clyde south, Victoria.

Treatment	Biomass[1] kg/m^2	pH	Org matter (%)	EC µS/cm	CEC me/100g	Avail N ppm	Avail P ppm	Avail K ppm
BQ Mulch	11.76 a	7.317	2.017	223.7 a	8.55	8.88 a	159.7	0.775 a
Mustclean	8.65 bd	7.383	2.050	176.8 bc	9.12	5.68 bc	163.8	0.688 ab
Caliente 199	9.52 b	7.383	2.167	240.3 a	8.91	8.90 a	148.9	0.770 a
Faba beans	6.83 c	7.550	2.067	196.3 b	8.66	6.70 b	167.4	0.675 b
Fallow		7.433	1.950	179.3 bc	9.31	5.12 bc	151.2	0.682 ab
Oats	8.91 bd	7.417	1.983	172.0 c	8.20	6.23 b	159.7	0.700 ab
Rye-corn	8.25 d	7.417	2.183	169.2 c	9.70	4.93 bc	157.3	0.640 bc
F-test	<0.001	0.053	0.306	<0.001	0.066	<0.001	0.435	<0.001

Table 16. Effects of green manure crop treatments on soil micro nutrients at Clyde south, Victoria.

Treatment	Biomass[1] kg/m^2	Avail B ppm	Avail Ca ppm	Avail Cu ppm	Avail Fe ppm	Avail Mg ppm	Avail Mn ppm	Avail Na ppm	Avail S ppm	Avail Zn ppm
BQ Mulch	11.76 a	0.717	4.758 a	13.17	32.83	1.788	27.00 acd	0.935 a	1.050 a	39.7
Mustclean	8.65 bd	0.733	5.278 ab	13.85	32.00	1.928	26.17 cd	0.688 bc	0.750 bc	48.2
Caliente 199	9.52 b	0.767	5.485 bc	13.02	32.33	2.010	28.00 ac	0.812 ab	1.050 a	47.1
Faba beans	6.83 c	0.783	4.928 ab	13.55	32.50	1.942	31.50 b	0.950 a	0.950 a	47.8
Fallow		0.767	5.508 bc	13.23	31.83	2.007	29.50 ab	0.697 bc	0.900 ab	46.2
Oats	8.91 bd	0.783	4.572 a	13.62	32.17	1.802	28.00 ac	0.755 bd	0.767 bc	48.1
Rye-corn	8.25 d	0.783	5.898 b	13.25	31.67	2.023	24.67 de	0.552 c	0.650 c	44.6
F-test	<0.001	0.483	0.012	0.067	0.669	0.238	<0.001	<0.001	<0.001	0.419

Table 17. Effects of green manure crop treatments on soil microbial communities at Clyde south, Victoria.

Treatment	Biomass[1] fresh kg/m^2	Actinomycetes CFU/g	Bacteria CFU/g	Fungi MEA CFU/g	Fungi RBA CFU/g	FDA mg fluorescein/3h/g
BQ Mulch	11.76 a	3.9 x 10^6	5 x 10^7 a	10.3 x 10^4 a	14.0 x 10^4 a	0.1775 a
Mustclean	8.65 bd	2.2 x 10^6	2 x 10^7 bc	6.2 x 10^4 bc	8.0 x 10^4 bc	0.1605 a
Caliente 199	9.52 b	3.0 x 10^6	3 x 10^7 bd	8.2 x 10^4 ab	8.0 x 10^4 b	0.1763 a
Faba beans	6.83 c	3.4 x 10^6	3 x 10^7 bd	6.8 x 10^4 bc	8.1 x 10^4 b	0.1333 b
Fallow-A		2.7 x 10^6	3 x 10^7 bd	6.2 x 10^4 bc	6.7 x 10^4 bc	0.1742 a
Fallow-B		3.1 x 10^6	1 x 10^7 c	4.8 x 10^4 c	5.5 x 10^4 bc	-
Oats	8.91 bd	2.3 x 10^6	4 x 10^7 ad	7.0 x 10^4 bc	3.2 x 10^4 c	0.1820 a
Rye-corn	8.25 d	3.2 x 10^6	1 x 10^7 c	4.4 x 10^4 c	2.2 x 10^4 c	0.1669 a
F-test		0.316	0.011	0.007	0.001	0.006

Table 18. Effects of green manure crop treatments on soil inoculum, clubroot root severity and yield of Chinese broccoli at Heatherton, Victoria.

Treatment	Biomass[1] kg/m^2	*Pythium clade* f (DNA pg/g soil)[2]	*R. solani* (DNA pg/g soil)[2]	Clubroot root infection severity (1-9)[3]	Yield grams (5 plants)
BQ Mulch	6.65 a	1550	193 a	1.000 a	359.2 a
Sudan grass	2.13 b	2251	5 b	1.000 a	365.7 ac
Rye-corn	3.73 b	2940	14 ab	1.000 a	415.7 bc
Caliente 199	-	2553	0 b	1.555 bc	391.7 abc
Mustclean	-	2101	0 b	1.700 c	369.9 ac
Fallow	-	2279	0 b	1.425 abc	417.5 b
F-test	0.003	0.532	0.003	0.011	0.047

1 Fresh weight (biomass) of green manure crops when incorporated into soil. Caliente 199 and Mustclean were heavily infected and stunted by clubroot resulting in very little biomass available for soil incorporation.

[2] DNA analysis conducted by SARDI (qPCR soil testing service).

[3] Clubroot visually assessed on a scale 1-9 where 1 refers to no clubroot, all roots healthy, 9 refers to all roots galled, no healthy roots present.

Table 19. Effects of green manure crop treatments on soil chemical properties at Heatherton, Victoria.

Treatment	Biomass[1] kg/m^2	pH	Org matter (%)	EC µS/cm	CEC me/100g	Avail N ppm	Avail P ppm	Avail K ppm	Avail Ca ppm
BQ Mulch	6.65 a	6.63	3.200	381	10.77	16.8	149.7	1.177	5.91
Sudan grass	2.13 b	6.45	3.550	474	10.92	24.4	184.2	0.990	7.25
Rye-corn	3.73 b	6.50	3.350	475	8.49	27.7	158.1	0.992	5.68
Caliente 199	-	6.30	3.375	740	9.42	64.2	144.7	0.835	7.36
Mustclean	-	6.13	3.950	712	11.11	64.3	147.7	0.890	7.55
Fallow	-	6.25	3.800	776	9.41	61.4	155.1	0.920	7.65
F-test	0.003	0.061	0.014	0.109	0.078	0.006	0.037	0.001	0.002

Table 20. Effects of green manure crop treatments on soil micro nutrients at Heatherton, Victoria.

Treatment	Biomass[1] kg/m^2	Avail B ppm	Avail Ca ppm	Avail Cu ppm	Avail Fe ppm	Avail Mg ppm	Avail Mn ppm	Avail Na ppm	Avail S ppm	Avail Zn ppm
BQ Mulch	6.65 a	0.925	5.91	7.750	29.2	1.650	11.00	0.873	8.35	34.45
Sudan grass	2.13 b	0.900	7.25	7.925	27.8	1.633	11.75	0.532	5.78	34.83
Rye-corn	3.73 b	0.875	5.68	7.125	28.2	1.363	9.50	0.652	4.65	32.27
Caliente 199	-	1.225	7.36	7.575	40.0	1.225	13.50	0.532	1.27	33.33
Mustclean	-	1.500	7.55	8.200	40.8	1.280	12.00	0.640	2.02	35.50
Fallow	-	1.525	7.65	7.725	50.0	1.350	11.00	0.657	2.30	34.27
F-test	0.003	<0.001	0.002	0.045	<0.001	0.048	0.027	0.154	0.012	0.246

Table 21. Effects of green manure crop treatments on soil microbial communities at Heatherton, Victoria.

Treatment	Biomass[1] kg/m^2	Actinomycetes	Bacteria	Fungi MEA	Fungi RBA
BQ Mulch	6.65 a	1.3×10^6	4×10^7 ab	1.1×10^5 a	9.0×10^4 a
Sudan grass	2.13 b	2.1×10^6	5×10^7 a	1.1×10^5 a	5.2×10^4 b
Rye-corn	3.73 b	1.6×10^6	3×10^7 ab	1.2×10^5 a	7.7×10^4 ab
Caliente199	-	1.5×10^6	4×10^7 ab	0.6×10^5 b	5.3×10^4 b
Mustclean	-	1.4×10^6	10×10^7 c	1.8×10^5 c	5.6×10^4 b
Fallow-A	-	2.3×10^6	1×10^7 b	1.3×10^5 ac	12.6×10^4 ac
Fallow-B	-	1.1×10^6	5×10^7 a	1.3×10^5 ac	14.1×10^4 c
F-test		0.110	0.002	< 0.001	<0.001

CHAPTER 5 - DEVELOPING STRATEGIES FOR MANAGING SCLEROTINIA DISEASES OF VEGETABLES IN QUEENSLAND

John Duff
Agri-Science Queensland, a service of DEEDI

SUMMARY

Trial work conducted by Agri-Science Queensland, a service of DEEDI included:

- In year 1, grower surveys determined the extent of the sclerotinia problem as well as other soilborne plant pathogen problems in the Granite Belt, Lockyer Valley and Gympie regions. The survey also identified current control measures being used by growers.
- In year 1, work in trials set up in the Gympie, and the Granite Belt regions focused on evaluation of pre-planting soil treatments that reduce sclerotia of *S. sclerotiorum* from soils including green manures (biofumigant crops), synthetic and organic soil amendments and biological control agents that destroy sclerotia in soil. A plot trial was established at the Gatton Research Station to look at the short term effectiveness of various control options to include in grower field trials during the 3 years of the project.
- In year 2 & 3, Sclerotinia susceptible crops (lettuce and green beans) were planted in trials. The trials evaluated the potential of crop rotations and/or cultural practices integrated with chemical and non-chemical measures for reducing the incidence/severity of Sclerotinia on susceptible crops (i.e. lettuce, green bean, carrot).
- Also in years 2 and 3, strategic application of fungicides were investigated. This includes different methods, rates (water volumes) and times of fungicide applications in relation to flowering period (beans) and plant canopy structure and transplant method (lettuce).
- In future trials, some of the other strategies to be evaluated include improved irrigation practices, modifying planting density to improve air flow through the crop canopy and incorporation of substrate amendments (i.e. biocontrol agents) into seedlings transplants.
- The trials will also investigate the effect of new strategies identified by national project on control of other soilborne pathogens that may be present in the trials.

GROWER SURVEYS

Two grower meetings were held on the Granite Belt and at Gympie to talk to growers about their experiences with Sclerotinia and how they are managing this disease in vegetable crops. The meeting dates were: Granite Belt 30 January 2008 – 9 attended, Gympie 1 February 2008 – 4 attended

Granite belt growers

Sclerotinia control starts at the seedling stage of transplanted crops. Seedlings are treated with fungicides while still in the nursery but just before they are planted into the field. Filan® is used more and more, however quintozine was mentioned as a fungicide that has been used. Follow up fungicides may include Amistar® and Filan® or even a Folicur® spray at transplanting due to its long with holding period.

Most growers will try and get 2 crops of vegetables from their ground in a year as well as trying to plant a green manure crop. Some typical rotations may include; green beans / green manure crop / cauliflowers, celery / chinese cabbage or lettuce / green manure crop, broccoli / cos lettuce. Other vegetable crops that are grown on the Granite Belt may include onions, capsicums, brussel sprouts and zucchini/squash.

Fertilisers used may include feed lot manure at 20 tonnes/ha at the start of the season. Calcium nitrate or potassium nitrate at planting. Blood and bone can produce toxic levels of phosphorus so this is used with care. Urea is also used but only when needed. Nitrophosca is used by some growers at 400kg/ha but the crop may need a top up of fertilisers with foliar applied products. Simbex a soil microbial activator is being promoted in the region as improving soil health and nutrient availability.

Sclerotinia can cause losses in the order of 5% on beans (more may be found in shipments to market), capsicum 25%, celery 20%, lettuce including mini-cos 10%. Other issues that growers mentioned were *Septoria* on celery.

Gympie region growers

Green beans start being planted from mid February to March. Seed is usually treated with some fungicide eg thiram, Apron®. Most growers would have planted a forage sorghum or other cereal cover crop over summer which is mulched and ploughed into soil to help with soil texture. Some growers will plant broccoli as a rotation. Other crops may include tomatoes, squash or zucchinis.

Standard row spacing was 75cm, but did vary slightly between some growers. Plant spacing was between 5-10 cm depending on the grower. The growers felt it would be too difficult to change either their row spacing to improve air flow or their plant spacing.

Sclerotinia or hot spot (nest) appeared to be worst in areas with high pH values. One grower had a pH of 5.5 with no *Sclerotinia* issues. Infection can be high particularly post harvest where a grower can loose almost all of the crop. Dense plantings are particularly susceptible to *Sclerotinia*. The Gympie pack house that packages most of the beans grown in the area

was particularly interested in the amount of chlorine needed to kill off the mycelium on the pods. Once infection is inside the pod it is impossible to control the infection and when placed into a cool room with healthy beans, the *Sclerotinia* can spread very fast.

Those growers present felt a need for more nutrient testing as it was felt that nitrogen in particular was too high in the soil leading to soft plants and therefore more disease development. One grower used chook manure but found it too high in nitrogen.

The growers realise that the flowering period is the most critical period for disease development and that a shorter flowering period is better for them. However, as the majority of beans are hand harvested in this region, an extended flowering period allows them to pick for longer getting the maximum amount of pods from their plants.

Field trials

Introduction

The objective of the Qld research was to establish three long-term trial sites to evaluate the effectiveness of new management options for Sclerotinia. These sites were on the Granite Belt at Amiens, the Gatton Research Station and Goomboorian (about 20km northeast of Gympie). The grower properties had known infection sites, whereas the Gatton Research Station was artificially inoculated during the first year of the project.

The grower sites looked at the effects of combinations of biofumigants and biocontrol agents on disease incidence in the field and viability of sclerotia in the soil. The biofumigant used on the grower property was one that was readily available, BQ Mulch™ and was applied at 10kg of seed per hectare. The biofumigant was incorporated into the ground at flowering or as close as practical to flowering to allow for breakdown and fumigation, 6 weeks before planting a crop.

The biocontrol agents Contans® and Trich-a-soil® were applied at 2kg/ha and 10kg/ha respectively. The Contans® was applied to the soil through a boom spray where as the Trich-a-soil® was mixed with half a bucket of soil form the individual plots and spread as evenly as possible across the entire plot by hand. The grower would then bed for to incorporate the soil into the soil or cultivate as a final preparation before planting. Biocontrol agents were not used on the Gatton Research Station.

TRIAL GATTON RESEARCH STATION

Introduction

The Gatton Research Station is situated on the southern bank of the Lockyer Creek. On the gentle levee of the Lockyer Creek, soils are light textured clay loams to light clays, while away from Lockyer Creek, heavier dark cracking clays dominate.

The Lockyer Valley has the ability to grow a wide range of horticultural crops which bring with it a wide range of disease issues. *Sclerotinia* has been an on going disease problem for growers for many years during the cooler parts of the year when crops such as lettuce, celery, carrots and brassica crops dominate the cropping landscape. Growers can experience 10% plus losses of their lettuce crops while only isolated cabbages will succumb to the disease. A number of control options have been tested over the years such as fungicides, crop rotations, soil amendments and biocontrol organisms with mixed success across crops and environments. With the exception of fungicides, little work has been undertaken in Queensland under Queensland conditions. This project will look at some of these control options when *Sclerotinia* is most likely to be an issue for growers and on a crop that is known for its susceptibility to this soilborne pathogen.

Materials and Methods

Trial area

The trial site consisted of the heavier dark clay type soil and had a total area of 63m x 48m or 3024m^2. Plot size was 16m x 4.5m (3 beds) with 14 treatments and 3 replications with the treatments listed below. The plot size allowed for 3 beds per plot to be planted to a susceptible crop such as lettuce or green beans.

Inoculum build up

Diseased plants were collected from grower properties and spread across the trial site. A crop of lettuce was also artificially inoculated. Inoculation of plants consisted of placing small pieces of agar impregnated with *S. minor* near the base of leaves on every 5th plant in every 2nd row. Agar inoculated with *S. sclerotiorum* was also placed near the base of leaves on every 5th plant in every other row. Infected plants were then ploughed into the ground for next years trial. Assessment of the effectiveness of this inoculation method consisted of counting the total number of plants planted and the number of infected plants in 10 x 10m length rows for both species of Sclerotinia randomly taken across the trial site.

Table 1. Trial site chronology of activities

Date	**Activity**
January 2008	Culture *Sclerotinia* isolates
May 2008	Plant lettuce seedlings 2 rows per bed.
June 2008	Inoculate lettuce plants
21 August 2008	Assess inoculated lettuce plants
17 February 2009	Prepare isolation area and plant BQ Mulch™ and Mustclean 666
30 April 2009	Plough in biofumigants, Fumifert® and Basamid® and irrigate.
18 May 2009	Apply Perlka®, Urea to plots, plough in and irrigate.
17 June 2009	Collect soil from trial plots for assessing sclerotia numbers.
24 June 2009	Plant lettuce seedlings 3 rows per bed.
27 July 2009	1st assessment of lettuce crop
11 August 2009	2nd assessment of lettuce crop
2 Sept 2009	3rd and last assessment of lettuce crop
7 January 2010	Plant BQ mulch™ seed planted
20 January 2010	Plant Mustclean 666 seed planted
16 March 2010	Plough in biofumigant crops
19 April 2010	Apply Perlka®, Urea, Fumifert® and Basamid® to plots, plough in and irrigate.
May 2010	Plant bean seed and lettuce seedlings

Treatments

- Control plot only standard fertilisers used
- Fungicides (Filan®, Rovral®) (1st Filan® at planting, 2nd Filan® at pre-hearting, Rovral pre-harvest) plus standard fertilizers
- Fungicides (Filan®, Rovral®) (1st Filan® at 5 days after planting, 2nd Filan® at pre-hearting, Rovral pre-harvest) plus standard fertilizers
- Fumigant (Basamid®) (6 weeks prior to planting) plus standard fertilizers
- BQ Mulch™ 10kg/ha (incorporate at 20% flowering and 4-6weeks prior to planting) plus standard fertilizers
- Mustclean 666 8kg/ha (incorporate as for BQ Mulch™) plus standard fertilizers
- Fumifert® 500kg/ha (incorporate as for BQ Mulch™) plus standard fertilizers
- Perlka® 500kg/ha (2-3 weeks prior to planting)
- Urea 500kg/ha (2-3 weeks prior to planting)
- Fumifert® + Perlka® (incorporate as for BQ Mulch™ + 2-3 weeks prior to planting
- Perlka® + Fungicides (2-3 weeks prior to planting)
- BQ Mulch™ + Perlka® (incorporate at 20% flowering and 4-6 weeks prior to planting)
- BQ Mulch™ + Fungicides (incorporate at 20% flowering and 4-6weeks prior to planting) plus standard fertilizers
- BQ Mulch™ + Urea (incorporate at 20% flowering and 4-6weeks prior to planting then 2-3 weeks prior to planting)

These treatments were applied to the same plots during 2009 and 2010. The BQ Mulch™ was planted 2 weeks earlier than the Mustclean 666 in 2010, as the BQ Mulch™ was slower at reaching flowering by about 2 weeks.

Assessment of soil inoculum

Prior to planting soil samples were collected from each plot. These samples consisted of 5 soil cores to 10cm depth from the middle bed using a small trowel. The soil samples were placed in paper bags and marked accordingly. These soil samples were then assessed for presence of sclerotia using the protocols developed by DPI Victoria. Departmental staff were instructed using this protocol by a DPI Victoria laboratory staff member Denise Wite.

Assessment of plants

The middle bed was assessed for the presence of infected plants on three dates with the first assessment on the 27th July (4 weeks after planting), the second assessment on the 11th August (7 weeks after planting) and the final assessment on the 2nd September 2009 (10 weeks after planting). Fourteen metres of bed was assessed from each plot counting the total number of seedlings planted, the number of plants missing due to ducks or poor establishment and the number of plants showing signs of *Sclerotinia* infection. Plants were collected and taken for isolation tests back in the laboratory.

Statistical analysis

The data collected was statistically analysed using the analysis of variance as part of the Genstat 11th Edition program supplied by the Queensland Department of Employment, Economic Development and Innovation.

Results and discussion

Inoculum build-up

Approximately 25% of the plants inoculated by *Sclerotinia minor* became infected. Infection by *S. sclerotiorum* was only about 5% as shown in Figure 1 below. All infected plants were subsequently ploughed back into the trial area for the second year of the project trials.

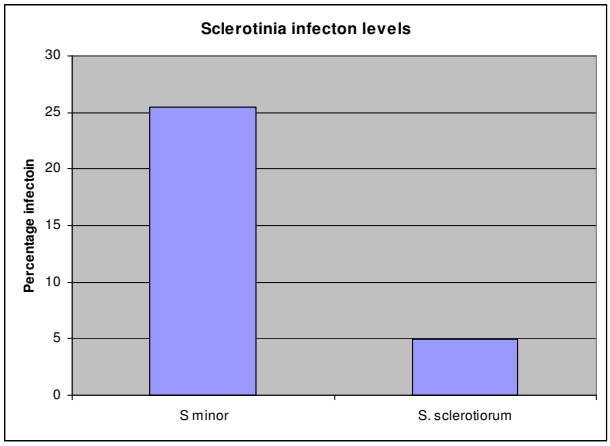

Figure 1. Infection levels for *S. minor* and *S. sclerotiorum* as a result of artificially inoculating lettuce plants in the field.

Assessment of soil inoculum

S. minor was the only *Sclerotinia* isolated from the trial site soil samples. All treatments had at least one viable sclerotia recovered from the soil but not all plots had recovered viable sclerotia. The Basamid ® treatment had the lowest number of viable sclerotia and the control the highest levels of viable sclerotia. No fungicides had been applied at this stage as these were only applied once the crop was planted. Treatments 6, 13 and 14 had similar numbers of sclerotia but different levels of viable sclerotia. Treatments 3 and 10 had variable levels of viable sclerotia even though they only had the one treatment applied before the lettuce seedlings were planted.

Table 2. Recovery of sclerotia from the various treatments prior to planting a crop of lettuce, June 2009.

Treatment	No. Recovered	Viable	% viable
1. Basamid® + Std fertiliser	27	1	3.70
2. BQ Mulch™ + Fungicide + Std fertiliser	18	1	5.56
3. Perlka®	29	4	13.79
4. Fumifert® + Std fertiliser	14	3	21.43
5. Fumifert® + Perlka®	24	6	25.00
6. Std fertiliser + Fungicide @5days	20	5	25.00
7. Urea	20	5	25.00
8. BQ Mulch™ + Std fertiliser	34	10	29.41
9. BQ Mulch™ + Perlka®	36	12	33.33
10. Fungicides + Perlka®	31	11	35.48
11. Mustclean 666 + Std fertiliser	22	8	36.36
12. BQ Mulch™ + Urea	44	17	38.64
13. Control (Std fertiliser)	20	8	40.00
14. Fungicides + Std fertiliser	27	12	44.44

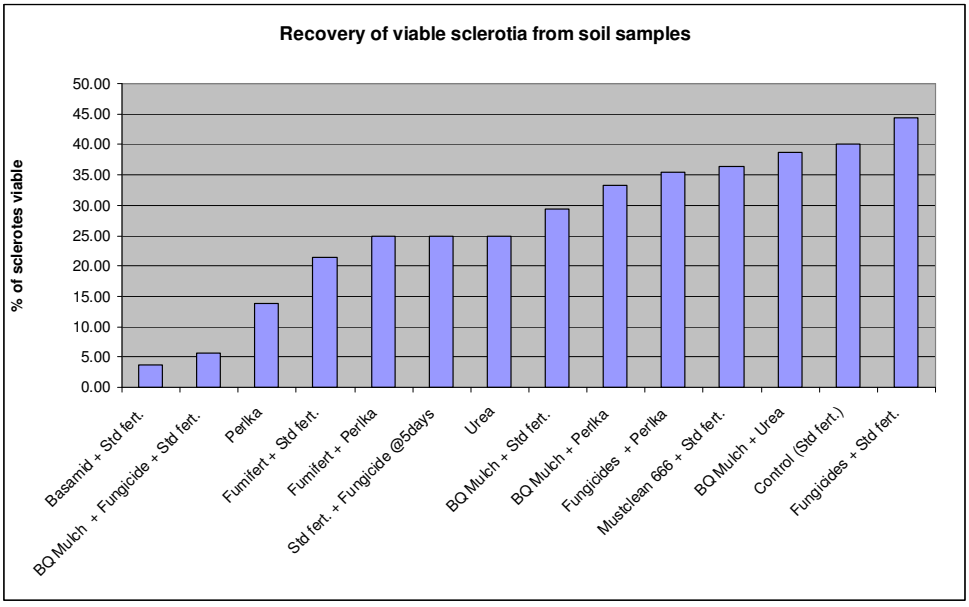

Figure 2. Percentage recovery of sclerotia from the various treatment plots before the lettuce crop was planted in June 2009.

Photo 1 Isolation area being planted to lettuce June 2009.

Assessment of plants

Statistical analysis showed Basamid® to be the most effective treatment at reducing the incidence of *Sclerotinia* rot in lettuce compared with the untreated control (Table 3; Figure 3). BQ Mulch™ plus fungicide applications at transplanting and pre-hearting were significantly more effective than BQ Mulch™ plus Urea or Perlka® in reducing disease. Other treatments were less effective in controlling disease. The use of fungicides at transplanting and pre-hearting also appeared to perform better than applying a fungicide application 5 days after transplanting and again at pre-hearting.

Table 3. Final disease incidnece values taken at harvest on the 2nd September 2009.

Treatments	% Disease
Basamid® + Std fert.	12.98a
BQ Mulch™ + Fungicide + Std fert.	15.58ab
Fumifert® + Perlka®	28.09abc
Fungicides + Perlka	35.40abcd
Fungicides + Std fert.	36.67abcd
Urea	44.08 bcd
Perlka®	44.32 bcd
Std fert. + Fungicide @5days	46.02 cd
Mustclean 666 + Std fert.	47.26 cd
Control (Std fert.)	47.92 cd
BQ Mulch™ + Std fert.	49.45 cd
Fumifert® + Std fert.	52.71 cd
BQ Mulch™ + Urea	56.68 cd
BQ Mulch™ + Perlka®	57.67 d
lsd	29.12

Values in the columns followed by the same letter are not significantly different at the P=0.05 level.

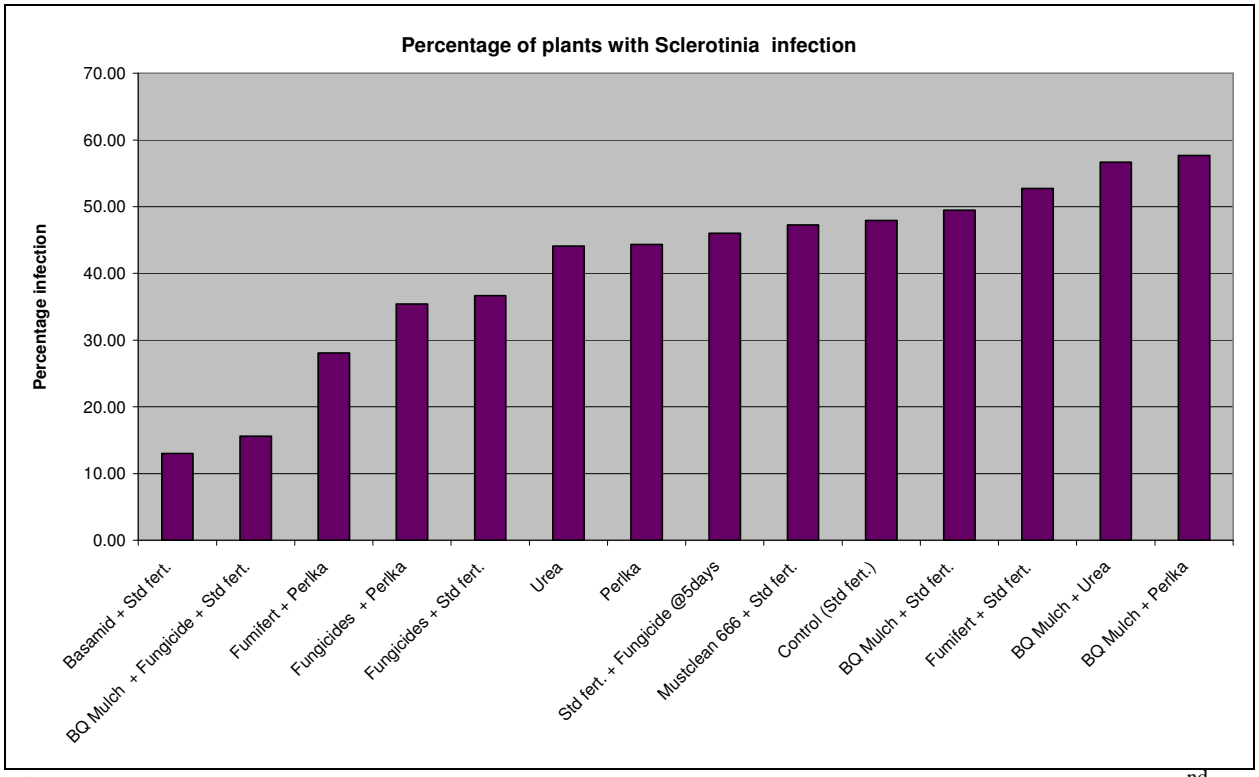

Figure 3. Total percentage of plants with *Sclerotinia* infection recorded on the 2nd September 2009 at the Gatton Research Station.

Conclusion

This site had to be artificially inoculated in the first year of the project to build up inoculum for the field trials. After this, the most effective treatments on lettuce drop control were the soil fumigant Basamid and BQ Mulch integrated with fungicide (Filan). None of the other treatments was effective in controlling disease. This trial also showed differences in flowering times between the two biofumigant crops. The BQ Mulch™ flowered at least 2 weeks later than Mustclean. This trial site has now sufficient inoculum for further trials.

TRIAL GOOMBOORIAN

Introduction

This trial site was situated on a red podzolic soil type gently sloping to the north east. This region can be hot in summer with temperatures over 30ºC but can also experience mild frosts in winter with a mean minimum of 6ºC during July. Rainfall can be in excess of 1000mm per annum with prolonged period of light to moderate rainfall, particularly during the early part of the bean growing season. This site has a history of *Sclerotinia sclerotiorum*, commonly called white mould or nesting, affecting bean crops from late autumn and early winter, due primarily to the environmental conditions, warm days and cool night temperatures, high humidity, showery weather, short day length and increased leaf wetness, coupled with susceptible varieties. The failure of the fungicides registered for use against this disease has left growers with very few options to manage this devastating disease. HAL Project VG03002 looked at plant spacing as a means of control. This in combination with fungicide usage was investigated as part of this previous project, to determine if by reducing the plant spacing within the rows, this would allow for better penetration of fungicide sprays and thus better control of the disease. As a result of this project it was found that there is the possibility of using wider plant spacing to help in the management of *Sclerotinia* rot in green beans but needs additional research on the full benefits attributed to such a change in grower practice. Increasing the plant spacing to twice the grower standard to around 7 cm, there was a trend towards less disease being found in the crop in conjunction with fungicides. Even if this were only carried out during the period of the growing season when *Sclerotinia* rot is most prevalent and combined with the appropriate fungicides in a rotation program, crops losses could be reduced to a more acceptable level for the grower.

This study investigated the use of biofumigant crops and biocontrol agents to reduce the levels of inoculum (*S. sclerotiorum*) in soil and disease incidence. Wider plant spacing in combination with strategic application of fungicides may also offer growers in the Gympie region another option to better manage bean white mould.

Material and Methods

This was a demonstration site, whereby the grower could compare an integrated approach to *Sclerotinia* management, using BQ Mulch™ as a rotation green manure crop and the biocontrol product Contans® incorporated into the soil prior to planting and again at either flowering or when the crop was ploughed into the ground. As a comparison, adjacent bays were managed by the grower using conventional methods of control. The size of the demonstration area consisted of 4 bays each 18m x 70m each with a different variety (Gold rush, Concessa, Green leaf and Parker) to investigate their tolerance to *Sclerotinia*. Treatments were applied across the 4 bays, including BQ Mulch™ and other cover crops. Contans® was applied to half of the bays.

Assessment of sclerotia in the soil

Prior to starting this trial, soil samples were collected from across the area to determine the levels of sclerotia present in soil. These samples consisted of 10 soil cores to a 10cm depth collected using a small trowel. The soil samples were placed in paper bags and labelled. These soil samples were then assessed for the presence of sclerotia using the protocols developed by DPI Victoria. Subsequent soil samples were collect in a similar manner but from each bay or from the adjacent bays managed by the grower.

Assessment of disease

The crop was to be assessed by selecting five 10m sections of row per plot and counting the number of infected plants which would then be converted to disease incidence per plot. All four bean varieties were to be assessed as well as five 10m sections of row from the grower managed sections. This was to be repeated during the second year of the trial site but was dependant on the grower planting beans again next to the trial site.

Table 4. Time line for trial activities at Goomboorian near Gympie.

Date	**Activity**
15 April 2008	Collect soil samples Contans® applied
16 April 2008	Beans planted
6 June 2008	Grower field day and talk
7 June 2008	2nd application of Contans® at flowering
8 July 2008	Assess beans
21 August 2008	Beans ploughed in
September 2008	BQ Mulch™ planted
25 March 2009	Collect soil and spray Contans®
April 2009	Beans planted
22 July 2009	Assess beans
August 2009	Plant broccoli in rotation
7 August 2009	Grower field day and talk
December 2009	Millet as cover crop
15 April 2010	Collect soil samples
18 April 2010	Contans® applied
April 2010	Beans planted

Results and discussion

Sclerotia in soil

The initial recovery of sclerotia is shown in table 5. Only one sclerotium was found to be viable from the initial 2 samples taken from the trial site.

Table 5. Number of sclerotia recovered from the soil at the start of the long term field trial near Gympie, April 2008.

Sample number	No. recovered	No. viable	% viable
1	7	1	14.29
2	5	0	0

Photo 3. Apothecia germinating near base of bean plant.

Assessment of disease

Unfortunately, the grower harvested the trial area before a proper assessment of disease incidence could be conducted. The effects of treatments implemented at this site will be assessed when beans are grown again at this site.

TRIAL GRANITE BELT

Introduction

The Granite Belt region comprises two principal soil types which are highly permeable. They are a granite speckled sandy, grey-black soil (trial site) and a light brownish grey, also speckled. The subsoils are bleached sand passing into clay at depth; while their drainage is good, the need for irrigation is increased. Temperatures on the Granite Belt can reach as high as the mid to high 30ºC to below freezing in winter. It rains during most months of the year but is highest during the summer months with an annual rainfall of around 766mm. Cropping is mostly conducted during the warmer months of the year, September to May when *Sclerotinia* is a problem. *S. minor* and *S. sclerotiorum* are both found on the Granite Belt and affect a wide range of crops including lettuce, celery, green beans and broccoli.

Photo 5. Trial site with BQ Mulch and Rye corn ready to be ploughed into soil.

Materials and Methods

Trial area

The trial site consisted of the sandy grey black soil type covering an area of 1.7 ha. This site was chosen by the grower because *Sclerotinia* is a problem particularly in mini cos lettuce. The six treatments were replicated four times as described below.

Treatments

1. Contans® + BQ Mulch™
2. Contans® + Rye corn
3. Contans® + *Trichoderma* + BQ Mulch™
4. Contans® + *Trichoderma* + Rye corn
5. BQ Mulch™
6. Rye corn

} + Cow manure

The Rye corn and BQ Mulch™ were the 2 cover crops used during the cooler winter months between planting seasons or when sufficient time allowed for their use between crops. Two biocontrol agents were used in different combinations with the cover crops and each other. The only fertiliser used was in the form of cow manure which was applied by spreading it across each bed at the start of the growing season in spring and was applied across all treatments. Plot sizes varied depending on the length of each bay. Treatments were applied to the same plots at the start of each season with the crops planted assessed for *Sclerotinia* incidence during the growing season and close to harvest.

Crops grown during this trial period included the following: Chinese cabbage (pre-trial crop), rye corn, celery (start of long term trial), Chinese cabbage, BQ Mulch™/rye corn, mini-cos lettuce, BQ Mulch™/rye corn, Chinese cabbage.

Table 6. Timetable of activities on the Granite Belt trial site.

Date	**Activity**
May 2008	Plant BQ Mulch™/Rye corn
August 2008	Plough in BQ Mulch™/Rye corn
15 August 2008	Set up trial site
10 September 08	Contans® and *Trichoderma* (Trich-a-soil®) applied to plots and bed formed
Late September 2008	Start planting celery
November/December 2008	Assess celery as they mature
17 March 2009	Collect soil samples
End of March 2009	Start planting chinese cabbage
May/June 2009	Harvest started of chinese cabbage Assess plots as they mature
Late June 2009	Plant BQ Mulch™/Rye corn
Early August 2009	Plough in BQ Mulch™/Rye corn
2 September 2009	Apply Contans® and Trich-a-soil®
21 September 2009	Start planting mini cos lettuce
28 October 2009	Start assessing mini cos lettuce
December 2009	Plant BQ Mulch™/Rye corn
20 January 2010	Plough in BQ Mulch™/Rye corn
15 March 2010	Collect soil samples
22 March 2010	Start planting chinese cabbage
31 May 2010?	Start assessing chinese cabbage

Assessment of sclerotia in the soil

Soil was collected from the trial site before any of the treatments were applied. This provided base line data to compare the efficacy of treatments. Soil samples from each plot were collected on the 17 March 2009 and again on the 15 March 2010 just before planting of the final crop for the growing seasons studied.

Trial area assessed

Each plot consisted of 8 beds. The outside beds were used as buffer beds and so were therefore not used in any assessments. The inner six beds were used to assess the infection levels. Plots were assessed by counting the number of infected plants as well as the total number of plants in a 10m section of bed. Therefore six 10m sections of beds were used in this assessment. Plants were also taken back to the laboratory and examined by plating out to make sure the disease was being correctly identified. The percentage of plants infected was then calculated for each plot.

Statistical analysis

The data collected was analysed using analysis of variance (Genstat 11th Edition program supplied by the Queensland Department of Employment, Economic Development and Innovation.

Results

Sclerotia in the soil

The soil samples taken on the 17th March 2009 and the 15th March 2010 had a number of sclerotia but none of them were viable.

Assessment of disease

Sclerotinia was found in the celery or Chinese cabbage crops grown on this trial site during the 2008/2009 growing season. The mini-cos lettuce planted in September 2009 had infection caused by *S. minor*. *Pythium* was also isolated from dead seedlings. Infection levels were low across all treatments with the average infection levels shown in Table 7 and Figure 5 below. There was significantly less *Pythium* in the BQ Mulch™ treatments compared to the Rye corn and the Rye corn and Contans® treatments, but no significant differences between treatments for *Sclerotinia*.

Table 7. Sclerotinia and Pythium infection levels on mini-cos lettuce at the Granite Belt site, 2009.

Treatments	Average % Sclerotinia	Average % Pythium
BQ mulch™ + cow manure	2.55	0.19 b
BQ mulch™ + cow manure + Contans®	1.99	0.04 b
BQ mulch™ + cow manure + Contans® + Trich-a-soil®	2.60	0.20 b
Ryecorn + cow manure	2.82	0.96 a
Ryecorn + cow manure + Contans®	3.58	1.03 a
Ryecorn + cow manure + Contans® + Trich-a-soil®	3.23	0.33 b
lsd	1.74	0.59

Values in the columns followed by the same letter are not significantly different at the P=0.05 level.

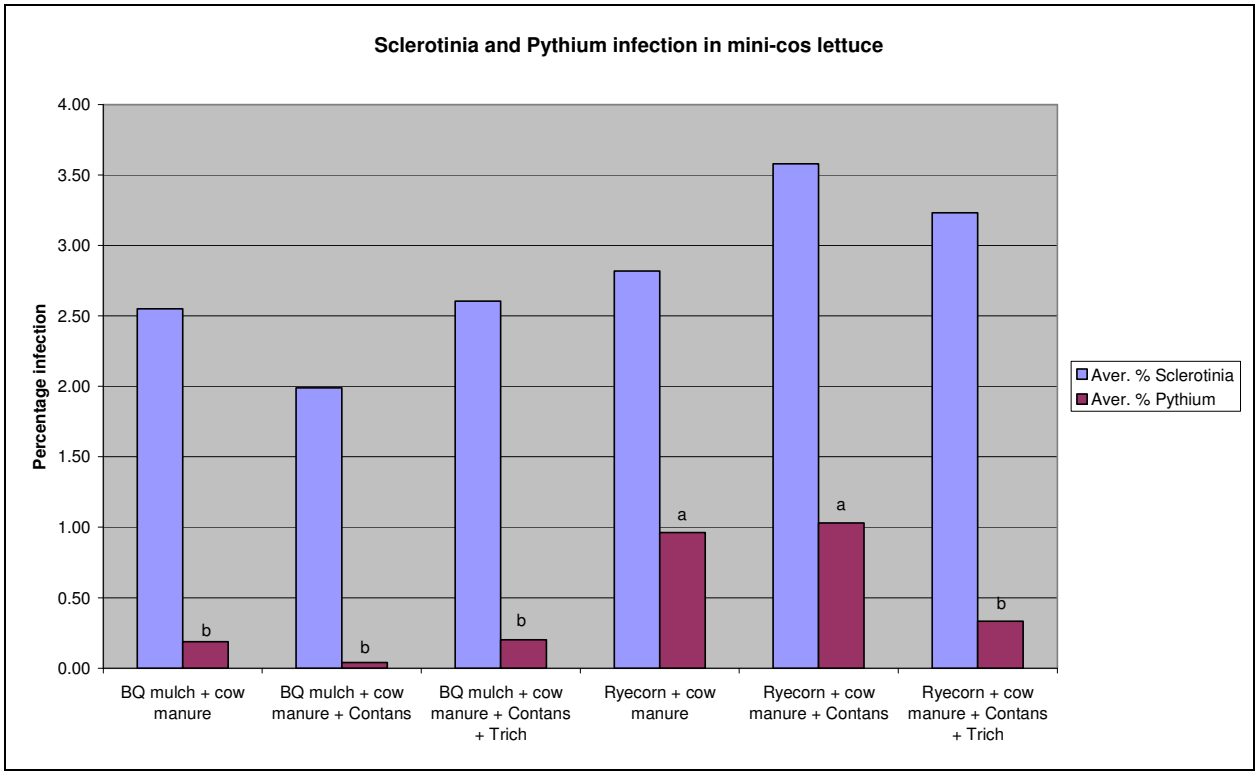

Figure 5. Infection levels of mini-cos lettuce on the Granite Belt during spring 2009.

Discussion

The crops grown on this block were selected by the grower. Mini-Cos lettuce has more upright growth habit so it is more tolerant to infection by S. minor and Chinese cabbage is not susceptible to Sclerotinia. According to Melzer et.al. (1997), Chinese cabbage is not considered a host for *S. minor,* although celery and lettuce are readily attacked by *S. minor*.

With regards to *Pythium* control BQ Mulch® performed better than the Rye corn treatments. Assessemnts must continue to determine the long-term effects of the treatments. It is possible that the biocontrol agents will gradually spread across the trial site, further improving the control of *Sclerotinia*. The grower still insisted on the use of a fungicide

regime (eg Filan® drench of seedlings in the nursery) followed by Amistar® and then Filan® in the field. It would be good in a future project to look at similar treatments but without the addition of fungicides in the field.

GENERAL DISCUSSION

The three trial sites have all started to produce useful data on the effects of a range of tools including fungicides, soil amendments, biofumigants and biocontrol agents on the management of *Sclerotinia*. With three distinctly different soil types and environments to work with, it is hoped that an integrated disease management system can be developed. This would then be offered to growers under Queensland conditions with a degree of confidence that such a system will be better in the longer term, compared to just relying upon fungicides to try and control this disease. This work needs to continue to build on what has been achieved and to show the cumulative effect of the various control options being employed to manage Sclerotinia in vegetable crops.

Alternative biofumigant crops with higher levels of glucosinolates need to be assessed as well as better biocontrol agents. The effects of soil amendments, eg fertilisers, on the activity of biofumigant crops and biocontrol agents need to be investigated to optimise their use for soilborne disease control. The effect of these practices on soil health and hence on disease control also requires investigation under Qld conditions.

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OVERSEAS TRAVEL REPORT

14th International Sclerotinia Workshop

The 14th International Sclerotinia Workshop was held in Wilmington, North Carolina on May 31 – June 4, 2009. The Department of Plant Pathology at North Carolina State University hosted the workshop and Barbara Shew chaired the workshop organizing committee.

The International Sclerotinia Workshop is held every four years and is an important activity of the International Society of Plant Pathology Sclerotinia Committee, James R. Steadman, Organizer.

Seventy researchers, students, extension personnel, and industry members from 11 countries attended the workshop, which was held on the campus of the University of North Carolina-Wilmington. Twenty-three oral reports and 19 posters were presented. Oral session topics and keynote speakers were: population biology, John Clarkson, University of Warwick; host-parasite interactions, Steven Clough, University of Illinois; biocontrol, Dilantha Fernanado, University of Manitoba; disease control, Helene Dillard, Cornell University; host resistance, Thomas Gulya, USDA-ARS, Fargo ND; and Sclerotinia biology, R.C. Venu, The Ohio State University. A *Sclerotinia homeoecarpa* interest group organized by Lane Tredway also met for special sessions during the workshop.

The workshop included a field trip featuring blueberry production in North Carolina, a visit to the American Revolutionary War National Battlefield at Moore's Creek, and a dinner at Wrightsville Beach. The farewell dinner was held near the Cape Fear River waterfront in historic downtown Wilmington.

Images of posters, PowerPoint presentations, workshop participants, and social activities are available at www.cals.ncsu.edu/ sclerotinia_conference.

The next workshop will be held in 2013 at Huazhong Agricultural University, Wuhan, China (www.hzau.edu.cn/en.htm).

What were the outcomes of the travel?

Control options for Sclerotinia using both fungicides and biological control agents

There were a number of fungicides discussed by various researchers that may have promise for Sclerotinia control in Australian vegetable production regions. These need to be looked at in more detail and discussed with the various chemical companies to determine if they will be made available for use in vegetables within Australia. The three fungicides are fluazinam – Shirlan, which is currently being trialled; thiophanate-methyl – Topsin M; boscalid + pyraclostrobin - Pristine. Mixtures of Topsin-M with boscalid were also very effective at Sclerotinia control as were mixtures with iprodione and chlorothalonil. Perhaps we should be looking at such combinations in a range of vegetable crops instead of relying on one particular chemical on its own.

The range of potential biological control agents for Sclerotinia control includes a bacterial agent called Serenade, which is a Bacillus subtilis that was showing control of Sclerotinia in

green beans, a crop with major disease issues in the Gympie region. It performed better as a foliar application rather than a ground based application. There are other Bacillus species also being looked at as control options by various researchers in the USA. Another bacterial biocontrol agent was a Pseudomonas chlororaphis strain PA-23, which is still an experimental product but was showing promise as a control option. This bacterial agent was shown to produce 2 powerful antibiotics which inhibited Sclerotinia growth. The researchers working with this biocontrol are looking at formulations types for application under field conditions.

Contans® (Coniothyrium minitans) was also discussed with sclerotia control as high as 96% when applied as a ground application. Contans® was also evaluated as an aerial application for control of Sclerotinia stem rot of rapeseed, which was also very effective. There was also a study looking at the survival of this biocontrol agent under different soil moisture regimes and temperatures. In short, Contans® can survive for more than 360 days in soil containing between 6.3 and 45.4% water (w/w) and temperatures between 4-28ºC. I assume these are air temperatures, as this was not all that clear in the abstract or the talk. At 30-40ºC survival dropped to between 150 and 120 days at 6.3% water down to a low of 120 to 3 days in soil containing 45.4% water. Temperatures above 45ºC saw survival of Contans® drop to less than a day at similar soil water contents. Under Qld conditions it would appear that Contans® would need to be applied yearly in order to take full advantage of its effectiveness as temperatures do reach above 40ºC at times during the summer months.

How to identify the various Mycelial Compatibility Groups (MCGs) or Biotypes of this plant pathogen

Within Sclerotinia sclerotiorum, researchers have been looking at the variability of Sclerotinia across regions and have been able to identify a number of MCGs from various regions in both the USA and the UK. They have not undertaken any work relating to the variability of aggressiveness of these different groups on various hosts. However I understand that this will happen. This sort of work should be undertaken with the various isolates within Queensland and other parts of Australia with the end result to determine if the different MCGs could explain the variability of the efficacy of certain fungicides in controlling Sclerotinia in green beans and other vegetables. It could be just one of a number of reasons for fungicide failures. A look at S. minor would also be useful across regions. This work had also been supported using molecular tools, looking at DNA fingerprinting, and the use of microsatellites and primers. The molecular tools are a far quicker way to look at the variability between isolates. If laboratory facilities could be set up with the primers being used overseas then isolates of Sclerotinia could be compared between states and regions, perhaps as part of a new project.

Techniques of increasing inoculum for field studies and pot trials

A number of techniques were mentioned by researchers when looking at either resistance of host plants to Sclerotinia or for conducting fungicide efficacy trials to control Sclerotinia. The technique that was most impressive was the use of filter paper impregnated with mycelium. This was then spread over the ground between the plants (eg. lettuce), or placed as small pieces of filter paper to simulate decaying flowers on the leaves of susceptible plants to promote infection. This method was discussed by a researcher from the University of Guelph, Canada. I am trying to get a copy of the procedure for this, which could help with future fungicide trial work.

Green bean resistance to Sclerotinia or white mould

White mould resistance was investigated using traditional plant breeding techniques through sourcing of resistant natural populations of related species in central America gene pools. Potentially resistant lines have been developed using this technique and are undergoing further study. A number of potentially resistant lines were sent to a researcher in Australia a number of years ago who worked with the QDPI&F. Apparently these lines were trialled extensively with little or no use of these lines since. I have since found out that they are currently sitting idle in the seed store at Biloela research station. We should try and access them and find out just what is available and what level of resistance they do have in the field. I was told that studies of these lines in the field, was not carried out in any great depth.

Workshop presentations

A majority of the workshop presentations and posters that were on display, can now be viewed on the following link; http://www.cals.ncsu.edu/sclerotinia_conference/

A range of images taken from the workshop are also available if you are interested in viewing these at the same link.

A seminar has been given to my peers at the QDPI&F of the trip and the major areas that I felt we could be involved in as part of a future project. I will also discuss some of the research from this workshop at 2 future grower meetings in August (at a bean field day I Gympie) and on the Granite Belts in September as part of a grower shed meeting

CHAPTER 6 - MANAGEMENT OF WHITE MOULD DISEASE (*SCLEROTINIA SCLEROTIORUM*) OF BEAN

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MEDIA SUMMARY

White mould disease caused by the fungus *Sclerotinia sclerotiorum*, is one of the most economically damaging diseases of bean (*Phaseolus vulgaris* L). The disease is particularly difficult to control due to the ability of the fungus to survive periods of months to years in soil, its ability to produce large amount of airborne spores from small amounts of initial inoculum, and the lack of resistance to the disease in commercial bean varieties. This project developed more efficient sampling strategies to determine the incidence of disease in crops and undertook a preliminary study which identified some of the main site risk factors for disease development in bean production in Tasmania. The project developed a sensitive PCR assay which successfully detected airborne inoculum of *S. sclerotiorum* in bean fields. A laboratory study of isolates of *S. sclerotiorum* collected from bean fields in Tasmania indicated no evidence of reduced sensitivity to the commonly used fungicide boscalid.

TECHNICAL SUMMARY

White mould disease caused by the fungus *Sclerotinia sclerotiorum*, is one of the most economically damaging diseases of bean *(Phaseolus vulgaris* L). The fungus produces overwintering structures (sclerotia) which allow it to survive in a dormant state within soil for periods of months or years. In bean crops, overwintering sclerotia undergo carpogenic germination to form cup-shaped apothecia on which ascospores are produced. Under favourable environmental conditions, ascospores are released into the airstream and infect senescing petals of flowers. Infected petals fall into the bean canopy and act as a source of nutrient for the fungus, allowing it to grow into, and rot, other plant parts such as bean pods and stems. The disease is difficult to control due to the long-lived sclerotia and the lack of resistance in commercial bean varieties.

A variety of methods of spatial analysis demonstrated that the incidence of pods with white mould disease was characterised by a largely random pattern of disease incidence at the scale of individual plants, with some patches of plants with similar disease levels on pods occurring at a scale of one metre or greater. However, the incidence of plants (versus pods) with white mould tended to be slightly to moderately aggregated. Although processes responsible for patterns cannot be deduced conclusively from this analysis, the small scale aggregation on pods, but greater degree of aggregation on plants observed in the current study, suggests localized spread of ascospores within bean fields. If inoculum is truly dispersed predominantly locally, this suggests that management efforts should be directed at reducing primary inoculum levels within individual fields rather than focusing management efforts on a farm- or regional scale.

The spatial analyses also provided the foundation for developing statistically sound sampling approaches for estimating disease incidence or classifying disease incidence above or below a provisional industry threshold (5% incidence on pods) for crop rejection. The sampling plans developed in this research were not intended to inform decisions regarding application of control measures. Rather, the sampling plans were designed to quantify disease levels on pods near harvest. Given the considerable amount of labour and resources needed to assess disease incidence, these sampling plans will facilitate future research efforts and also assist industry in designing their sampling schemes for deciding which fields should be harvested or not. The sequential estimation sampling plans tend to perform appropriately when disease incidence is at least 4%, which is near the provisional thresholds sometimes used for crop rejection. When only a classification of disease incidence is required, the sequential classification plans developed herein would provide a very useful tool for accurately classifying fields after inspection of relatively few sampling units.

Several risk factors associated with the occurrence of white mould occurrence on bean pods were identified in a preliminary survey of 52 bean fields in 2009 and 57 fields in 2010. Site-risk factors for the occurrence of white mould on pods include high minimum temperatures in the 10 to 30 days before harvest - which may be related to cloud cover, rain, and/or solar radiation, dense planting of beans, planting of the cultivar Flavour Sweet, and the timing of boscalid applications. These risk factors are consistent with the body of knowledge on this disease, and suggest factors for producers and processors to manage to reduce disease risk. Designed experiments are warranted to better quantify the individual and combined

effects of planting density, cultivar susceptibility, and boscalid timing on the occurrence and severity of white mould on pods.

PCR primers were designed using the intergenic spacer region (IGS) of the nuclear ribosomal RNA (rRNA) gene repeat unit and were shown to detect *S. sclerotiorum*, *S. minor* and *S. trifoliorum*, but not the closely related fungus *Botrytis cinerea*. The PCR was highly sensitive and was able to detect DNA concentrations of *S. sclerotiorum* as low as 0.2 ascospores per PCR reaction. DNA from *S. sclerotiorum* was successfully detected even in the presence of 10 mg of soil. Airborne inoculum of *S. sclerotiorum* was detected by PCR from impaction spore traps placed in bean fields. The PCR assay has potential for incorporation into a risk management system for *S. sclerotiorum* in bean crops or for use in disease development studies.

A total of 102 isolates of *S. sclerotiorum* collected from 23 bean crops in the 2008 season and 48 isolates from 32 bean fields in the 2009 season were tested for sensitivity to boscalid and compared with 11 unexposed isolates sourced from pyrethrum crops and culture collections, prior to the use of boscalid. Isolates were tested on potato dextrose agar at concentrations of 0, 0.01, 0.05, 0.5, 5.0 and 50.0 µg/ml boscalid. The frequency distribution of EC_{50} values were unimodal. For 2008 isolates, the mean EC_{50} was 0.166 µg/ml, and ranged from 0.011 to 0.575 µg/ml. For 2009 isolates, the mean EC_{50} was 0.115 µg/ml and ranged from 0.27 to 0.311 µg/ml. For the 11 isolates not previously exposed to boscalid, EC_{50} ranged from 0.017 to 0.159 µg/ml, with a mean of 0.095 µg/ml. There was no evidence of reduced sensitivity of *S. sclerotiorum* from bean in Tasmania to boscalid. This study will provide valuable baseline sensitivity data for monitoring changes in fungicide sensitivity in future years.

Introduction

White mould caused by the fungus *Sclerotinia sclerotiorum*, is one of the most economically damaging diseases of bean *(Phaseolus vulgaris* L). Early symptoms of white mould disease include small irregularly shaped, water-soaked areas on stems, leaves or pods, which develop into soft, pale brown lesions (Koike *et al*. 2007). White cottony mycelium is formed on lesions, and as the tissue senesces, mycelial mounds are formed which mature into hard black sclerotia (approximately 5-10 mm long) which allow the fungus to survive in a dormant state for periods of months or years (Koike *et al*. 2007). Infection of the stem can weaken the stem and cause the plant to lodge. Sclerotia may arise from previously infected crops or may be disseminated among fields in infested and infected seed, irrigation water, manure and soil movement. Sclerotia can undergo two forms of germination (i) myceliogenic and (ii) carpogenic. Myceliogenic germination involves the production of mycelium following germination, which directly infect the base of the plant. Myceliogenic germination is believed to be of minor concern in bean. Sclerotia that are shallowly buried may undergo carpogenic germination. Sclerotia initially produce stalk-like stipes which push to the soil surface and form small, tan cup-shaped apothecia which produce ascospores. Generally up to four apothecia can be formed from each sclerotium, with each apothecium producing 2 x 10^6 ascospores (Schwartz and Steadman 1978). Ascospores are wind-borne and capable of being transported several hundred metres. Ascospores require high humidity and nutrients from senescent or damaged tissue to germinate and initiate infection. On beans, senescing petals are particularly susceptible to infection. Secondary spread occurs when infected petals fall through the canopy and attach to pods, leaves or stems. Mycelium can then grown from the petals and infect healthy plant organs such as stems and pods.

The ability of *S. sclerotiorum* to survive for long periods in soil as sclerotia, the ability of sclerotia to produce large quantities of wind-borne ascospores and the lack of host plant resistance in bean, make white mould disease particularly difficult to control.

This project aimed to develop new approaches for the detection and management of white mould disease in bean. The project aimed to: a) develop efficient sampling methodologies for assessing the level of disease in fields, b) identify the agronomic and environmental factors important in determining disease incidence and severity at particular sites, which could be used to develop a pre-planting decision support tool for predicting the risk of disease occurring at a particular location, c) develop a PCR assay to monitor airborne ascospores of *S. sclerotiorum*, with the potential to provide an early warning of ascospore release in bean fields to trigger the initiation of control measures, and d) monitor for the development of fungicide resistance to the sole fungicide currently registered for control of white mould disease in bean, boscalid.

Plate 1. Symptoms and signs of white mould disease caused by *Sclerotinia sclerotiorum* on bean.

(A) chlorotic and wilted upper foliage

(B) mycelium on stems and pods

(C) mycelium forming on rotting pod

SPATIAL ANALYSIS OF WHITE MOULD DISEASE IN BEAN AND PRELIMINARY DEVELOPMENT OF A SEQUENTIAL SAMPLING PLAN

Introduction

The decision to implement a management strategy based on an action threshold requires a sampling procedure which is capable of accurately estimating disease incidence or classifying incidence as being above or below a specified value (Gent *et al.* 2007 a and b). Sequential sampling methods have been developed and used widely in entomology to minimise time and cost associated with assessing pest density (e.g. Hoffman *et al*. 1996), but have been less used in assessing plant disease. Examples of the latter include common maize rust (Dillard and Seem 1990), stripe rust of wheat (Gaunt and Cole 1992), Phompsis leaf blight of strawberry (Turecheck *et al*. 2001), Botrytis leaf blight of onion (Vincelli and Lorbeer 1987) and powdery mildew of hop (Gent *et al*. 2007a and b). In most cases, the sequential sampling plan has been used to initiate fungicide applications (Vincelli and Lorbeer 1987). However, in the case of white mould disease of bean, the use of a sequential sampling plan for scheduling fungicides for control is of limited value. In this pathosystem, fungicides need to be applied at flowering, some weeks prior to the onset of symptoms on plants. Similarly treatment of pods with fungicides may be less efficacious than treatment of flowers and treatment of pods may lead to issues of inadequate with-holding period prior to harvest. Furthermore, the latent period between infection and disease expression may mean that at the point of detection, the disease has already increased to the point that economic management is not possible (Malloy 1993). However, a sequential sampling plan for white mould disease in bean could be of benefit for (i) research purposes in terms of assessing levels of disease within crops more efficiently, or (ii) for processors in terms of assessing whether disease levels within a crop are too high to warrant harvest of the crop.

The development of a sequential sampling plan requires prior knowledge of the spatial distribution of disease. Sequential sampling can be of two forms (i) sequential estimation in which it is necessary to estimate the level of disease at a particular level of precision, or (ii) sequential classification which merely requires measuring whether disease is above or below a critical value important for disease management. At disease incidences higher or lower than the critical value, disease can be classified with less samples than when the incidence is near the critical value. In general, sequential sampling plans based on classification require less intensive sampling than those based on estimation (Gent *et al.* 2007 a and b).

The purpose of this part of the project was to (i) assess the spatial distribution of white mould disease in bean fields using a variety of statistical techniques, and (ii) use this information to derive a sequential sampling plan which might be used for research purposes or to inform processors of disease incidence just prior to harvest.

Materials and methods

Field sites and data collection

The incidence of white mould on bean pods was quantified in surveys of 18 commercial bean fields in northern Tasmania during 2008 to 2010. A total of 5, 6, and 7 fields were assessed in 2008, 2009, and 2010, respectively. The incidence of diseased pods was assessed using a cluster sampling design. For each field, two to four rows were selected, depending on the size of the field, and a linear transect was established on the selected rows. Each transect was 50 metres long and disease incidence was assessed at 0.5 m intervals (N = 100 sampling units per transect) along the transect by selecting n = 10 pods per sampling unit and assessing each pod for disease signs and symptoms. Each transect was considered a data set. Collectively, these data sets are referred to as the "model development data sets". Disease incidence ($\hat{p}$) was calculated as $\hat{p} = \Sigma x_i / \Sigma n_i$, where x_i is the number of diseased pods and n_i is the number of pods sampled in the i^{th} sampling unit. Disease incidence estimates also were obtained from a cooperating bean processor for a subset of 32 fields. Estimates of disease incidence by the processor (at the factory just before processing) and TIAR personnel (in the field before just before harvest) were compared by linear regression to determine the degree of correspondence between the estimates.

An additional 109 bean fields were assessed for white mould during 2009 and 2010 for the purposes of disease risk analyses (described in section 3). These data sets were considered independent "model validation data sets" and were used to assess the performance of the sequential sampling plans described below. In these assessments, fields were assessed for white mould as close to commercial harvest as practical, which was generally 2 to 10 days before harvest. When assessing disease incidence in the model validation data sets, pods and plants at 64 points in the field were assessed for white mould. The 64 sampling points consisted of four or five transects each with 12 to 16 relatively equally spaced points that spanned the length of the field. At each sampling point, 20 pods were selected arbitrarily from one to two bean plants, depending on the cultivar and number of pods per plant. Additionally, 10 plants at each point were assessed for white mould. Plants were considered diseased if there were disease signs or symptoms on either one or more pods or the stem.

Spatial analyses

Distributional analyses

The beta-binomial and binomial distributions were fit to the incidence of diseased pods or plants using the computer program BBD (Madden and Hughes, 1994). A good fit to the binomial distribution is an indication of a random pattern of diseased pods, whereas a good fit to the beta-binomial distribution is an indication of an aggregated pattern (Madden and Hughes, 1995; Madden et al., 1996). A log-likelihood ratio test statistic was calculated to determine whether the data was a better fit to the beta-binomial distribution or the binomial distribution. The C(α) test was used to test whether aggregation in the distribution of diseased pods could be described adequately by the beta-binomial distribution. Since 20 pods per sampling unit were assessed for white mould in the model validation data sets, 10 pods were selected randomly from among the 20 pods assessed *post hoc* in an Excel spreadsheet (Excel 2003, Microsoft Corp., Redmond, Washington, USA) before conducting distributional analysis on the model validation data sets.

The degree of aggregation of disease incidence was quantified by the heterogeneity parameter θ of the beta-binomial distribution, which provides a measure of variation in disease incidence per sampling unit (Madden and Hughes, 1995; Madden *et al*., 1996). The index of dispersion (*D*) was calculated by dividing the observed variance of diseased pods (v_{obs}) by the theoretical variance for a binomial distribution (v_{bin}) where $v_{obs} = [\sum (x_i - \hat{p}n_i)^2]/(N\text{-}1)$ and $v_{bin} = n\hat{p}(1\text{-}\hat{p})$ and x_i, $\hat{p}$, n and N are as defined previously. When $\theta = 0$ or $D = 1$, the pattern of diseased pods is random, with aggregation indicated when $D > 1$ or $\theta > 0$ and the degree of aggregation directly proportional to the magnitude of the statistic. D has a chi-square distribution, and can be used to test the null hypothesis of a random distribution of disease incidence with $N - 1$ degrees of freedom (Madden and Hughes, 1995).

Binary power law analyses

The binary power law expresses the relationship between the variance between the theoretical variance of binomial (random) pattern of disease incidence and an observed variance (Hughes and Madden, 1982). When a large number of data sets are collected, the relationship between these variances provides a convenient means to characterise aggregation of disease incidence over multiple fields and time (Madden *et al*., 1996). The model was fitted to the observed and binomial variances through the log-transformed relationship

$$\ln(v_{obs}) = \ln(A_x) + b \ln(v_{bin}) \qquad (1)$$

where v_{obs} and v_{bin} are as defined above. When $A_x = 1$ and $b = 1$, equation 1 indicates a random pattern of disease incidence that can be represented by the binomial distribution. When $A_x > 1$ and $b = 1$, disease incidence has an aggregated pattern that is not dependent on p; values of $b > 1$ indicate that aggregation is systematically related to p. Ordinary least squares regression was used to estimate the intercept and slope parameters using PROC REG in SAS version 9.2 (SAS Institute, Cary, NC).

Correlation-based spatial analyses

Two types of correlation-based spatial analyses were conducted: (i) autocorrelation and (ii) runs analyses.

First and second-order autocorrelation statistics were calculated to quantify the degree of similarity of disease incidence between sampling units along a transect. Before autocorrelation coefficients were calculated, the data were transformed using the Haldane transformation $[\ln(y/(1 - y)]$, where $y = (x + 0.5)/(n + 1)$ and x is the number of diseased pods in a sampling unit. This transformation avoids taking the logarithm of 0 values or dividing by 0. Autocorrelation analyses were performed in Minitab version 15 (Minitab Inc., State College, PA).

Ordinary and median runs analyses also were performed to characterize larger scale patterns of diseased pods among samplings units in a transect (Madden *et al*., 1982). For ordinary runs analysis, a sampling unit was assigned a value of "1" if at least one diseased individual was observed in the sampling unit and a "0" otherwise. In median runs analysis, the median incidence of disease was calculated for each data set. Sampling units were then coded as 1 or 0 if the incidence of diseased pods was above or below the median for that data

set, respectively. A run was defined as succession of one or more sampling units with similar disease status (non-diseased or diseased). Runs and associated tests of significance were calculated in Minitab.

Sequential sampling curves

Sequential estimation

A full explanation of the methods and theory for sequential sampling is given in the literature citations below (e.g., Madden *et al.*, 1996), and only a brief explanation is given here. Binary power law parameters estimated from the model development data sets were used in the development of sequential estimation models. Precision was expressed in terms of the coefficient of variation, $C = \text{SE}\ \hat{p}\ /\ \hat{p}$, where SE is the standard error. The SE of $\hat{p}$ was expressed in terms of the binary power law parameters as

$$\sqrt{a[\hat{p}(1-p)]^b / N} \qquad (2)$$

where $a = A_x n^{b-2}$.

In sequential sampling for estimation, the cumulative number of diseased pods over N sampling units, T_N , is tallied after each sampling unit is assessed. Sampling ceases when T_N reaches or exceeds a threshold value, referred to as the stop limit, which is defined by a, b, n, N, and C. Disease incidence, as determined by sequential sampling, is then calculated as $\hat{p} = T_N/nN$. The stop lines can be calculated exactly for a binomial distribution (i.e., $\theta = 0$) or approximated numerically when disease incidence is aggregated (values of b >1) by

$$\gamma_N = T_N^{b-2}(nN-T_N)^b = (C^2/a)n^{2b-2}N^{2b-1} \qquad (3)$$

where γ_N is a function of T_N. A Mathcad (Mathsoft Inc., Cambridge, MA) worksheet was developed to solve equation 3 iteratively for T_N when N = 1 to 500 and C = 0.1, 0.2, and 0.3.

Sequential classification

Statistical methods used for development of the sequential classification models were based on a modified version of Wald's (1947) sequential probability ratio (Madden and Hughes, 1999). For sequential classification, $p_t = (p_0 + p_1)/2$ is some critical value of disease incidence, which in this study was the approximate 5% threshold for processor rejection of beans due to white mould. The parameters p_0 and p_1 represent the lower and upper boundaries of disease incidence such that, when the true incidence of disease, p, is equal to or less than p_0, the field is classified correctly at least $100(1 - \alpha)\%$ of the time; and when the true incidence of disease is equal to or greater than p_1, the field is classified correctly at least $100(1 - \beta)\%$ of the time. In practice, the resulting classifications are interpreted as a test of the null hypothesis H_0: $p \leq p_t$ against the alternative hypothesis H_1: $p > p_t$, respectively. A type I error is made when the true disease incidence, p, is incorrectly classified as greater than the critical value, p_t. A type II error is made when p is incorrectly classified as less than p_t. The rate of these two errors is expressed by the operating characteristic (OC), which is defined as the probability of accepting the null hypothesis given the true value of p. The OC = 1 - (type I error rate) when $p \leq p_t$, and is the type II error rate when $p > p_t$. The OC of a perfect sampling plan is 1 when $p \leq p_t$ and 0 when $p > p_t$, and the steepness of an OC provides an indication of the error rate of a sampling plan. Plots of average sample number (ASN) versus p also are used to evaluate the properties of sequential classification sampling plans. The ASN is the expected number of sampling units that need to be examined in order to accept or reject the null hypothesis for any true value of p. The OC and ASN provide the

expected (average) values over many sampling bouts, and not necessarily the performance of a sampling plan for an individual field. Sampling plans with a steep OC and low ASN are desirable for sequential sampling.

With sequential classification, two stop lines are calculated to represent p_0 and p_1. The exact calculation of stop lines for the SPRT is not possible for data described by the beta-binomial distribution, but approximate formulae are available (Madden and Hughes, 1999). The general formula for stop lines is:

$$i_0 + snN < T_N < i_1 + snN \quad (4)$$

where sn is the common slope of the stop lines, and i_0 are i_1 are intercepts of the lower and upper stop lines, respectively. Intercept terms are defined by a, b, p_0, p_1, and error parameters analogous to type I (α) and type II (β) errors (Madden and Hughes, 1999; Turechek *et al*., 2001).

Sequential classification thresholds were based on three levels of p_t: 0.03 (p_0 = 0.01, p_1 = 0.05); 0.05 (p_0 = 0.03, p_1 = 0.07); and 0.15 (p_0 = 0.088, p_1 = 0.214); and 0.151 (p_0 = 0.088, p_1 = 0.214). These values of p_t were selected based upon processor rejection thresholds for white mould of approximately 5% incidence. The sampling plan with p_t = 0.03 would be a conservative approach to ensuring that most fields would be classified as less than 0.05. The value of p_t= 0.151 was derived from the regression equation of TIAR disease incidence assessments and those of the cooperating processor (Figure 1). In these studies, $\hat{p}$ = 0.151 estimated from the surveys corresponds roughly to $\hat{p}$ = 0.05 as estimated by the processor. Each of these threshold values were evaluated at α and β of 0.05 and 0.10. Thus, six sampling plans were developed and evaluated by plots of OC and ASN versus p. OC and ASN were calculated by Monte-Carlo simulations using a Fortran program developed by J. P. Nyrop and modified by L. V. Madden (Turechek *et al*., 2001).

Sampling plan validation by simulated sampling

Sequential estimation

Sampling plans were validated with 109 independent validation data sets by simulated sampling using a Minitab macro. For a given data set, the sampling units were entered into the macro in the same order they were collected in the field. The macro simulated sampling of diseased pods collected from the sampling units, and tallied the cumulative number of diseased pods until the cumulative number of diseased pods exceeded that of the model T_N calculated from equation 3. Estimates of p, the achieved C, and the achieved N were then calculated. Data sets where incidence of disease was 0 were not used for determining the achieved C. The data were summarised in box plots and the distribution of the median value of the difference between the true and achieved p, achieved C, and N were compared by the nonparametric sign test (Ryan *et al.*, 2005).

Sequential classification

The six sequential classification sampling plans selected after Monte-Carlo simulation were evaluated by simulated sampling of the model validation data sets as described for sequential estimation. Stop lines were calculated based on either the binomial distribution or beta-binomial distribution. To determine if a correct decision for a data set was made, it was assumed that the observed p from all 64 sampling units in a field represented the "true" p for that field. The true value of p for a data set was compared with the hypothesized p_t to

determine whether to reject the H_0 in favor of the H_1. The decision based on results of the simulated sequential classification was compared to the correct decision for the field, in order to calculate type I and type II error rates. A type I error was recorded if mean disease incidence was incorrectly classified as greater than p_t, and a type II error was recorded if mean disease incidence was incorrectly classified as less than p_t.

Sampling plan evaluation by bootstrapping

Sequential estimation

Bootstrapping allows a statistic of interest to be estimated empirically as a frequency distribution from sample data. Evaluation of the sequential sampling plans was conducted by bootstrap evaluation of 12 data sets that encompassed the range of $\hat{p}$ observed among the model development data sets. The 54 model development data sets were classified into four disease incidence categories: $0.02 < \hat{p} < 0.04$, $0.05 \leq \hat{p} < 0.08$, $0.08 \leq \hat{p} < 0.16$, and $\hat{p} \geq 0.16$. Three data sets were selected randomly from each disease incidence class for bootstrap evaluation. For a given bootstrap simulation, sampling units (n = 10 pods from a plant) were sampled randomly one at a time, with replacement, from among all sampling units in a transect. Predicted stop limit curves for sequential estimation were determined according to equation 3 with C = 0.1 and C = 0.2, and binary power law parameters a = 0.194 and b = 1.045. Bootstrap analysis was conducting using a macro executed in Minitab to calculate the achieved C, the difference between the p of the data set (true p) and achieved p using sequential sampling for estimation, and the achieved N for each of the 12 data sets. A minimum of 10 units were collected before sampling ceased to ensure a representative sample was collected. The bootstrap evaluation was conducted 100 times for each data set and specified values of C.

Sequential classification

The six sequential classification sampling plans were evaluated by bootstrap simulation, as described above for sequential estimation. Stop lines were determined using equation 4. Sampling ceased when the cumulative number of diseased pods exceeded the upper or lower stop lines of the model, or the data set was sampled fully. A minimum of 10 sampling units were collected before sampling ceased to ensure a representative sample was collected. The bootstrap simulation was conducted 100 times for each data set and specified values of p_t, p_0, p_1, α, and β, and achieved OC and ASN were then calculated.

Results

Disease incidence

The incidence of pods rated as affected by white mould during field surveys and processor assessments were linearly related (R^2=0.73) (Figure 1). The regression equation explaining the relationship was $y = 0.32x + 0.174$, where y = disease incidence as estimated by the processor and x = disease incidence estimated during field surveys. This indicated that the estimate of disease incidence by TIAR personnel tended to higher than that of the processor. The incidence of pods with white mould determined from the disease surveys ranged from 2.8 to 23.1% among fields assessed, with median of 8.4% (Figure 2A). Among

the model validation data sets, disease incidence ranged from 0 to 14.1% with median of 0.2% (Figure 3A). At the plant level, disease incidence ranged from 0 to 37.03%, with median of 1.1% (Figure 4A).

Figure 1. Association of the incidence of white mould on bean pods as estimated by TIAR personnel and a bean processing company in 32 fields in Tasmania, Australia. The R^2 for the relationship is 0.73.

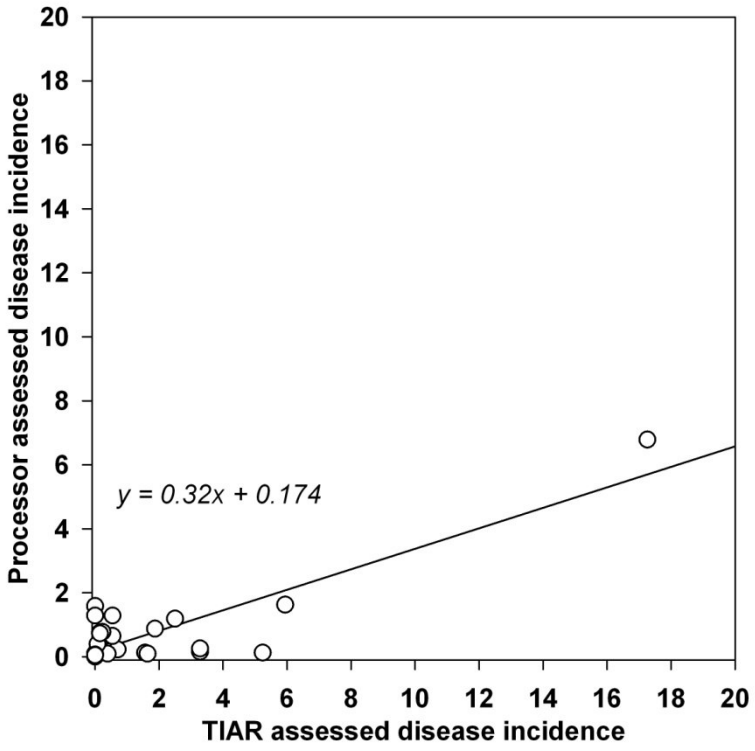
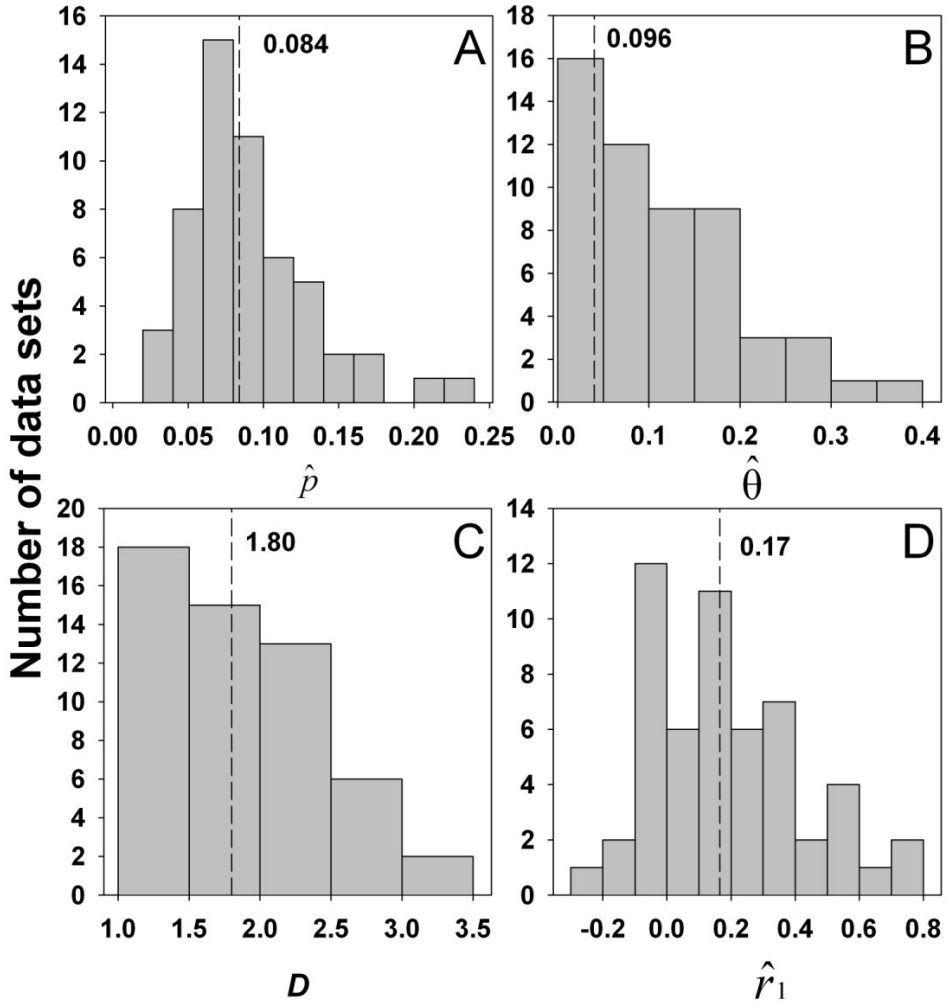


Figure 2. Frequency distribution of the beta-binomial distribution parameters $\hat{p}$ (**A**) and $\hat{\theta}$ (**B**), the index of dispersion *D* (**C**), and the first-order autocorrelation statistic $\hat{r}_1$ (**D**) for the incidence of beans pods with white mould as assessed in 54 transects in 18 commercial bean fields in Tasmania, Australia. The vertical dashed lines are the median value for the indicated statistic, with the numerical value given on each graph.



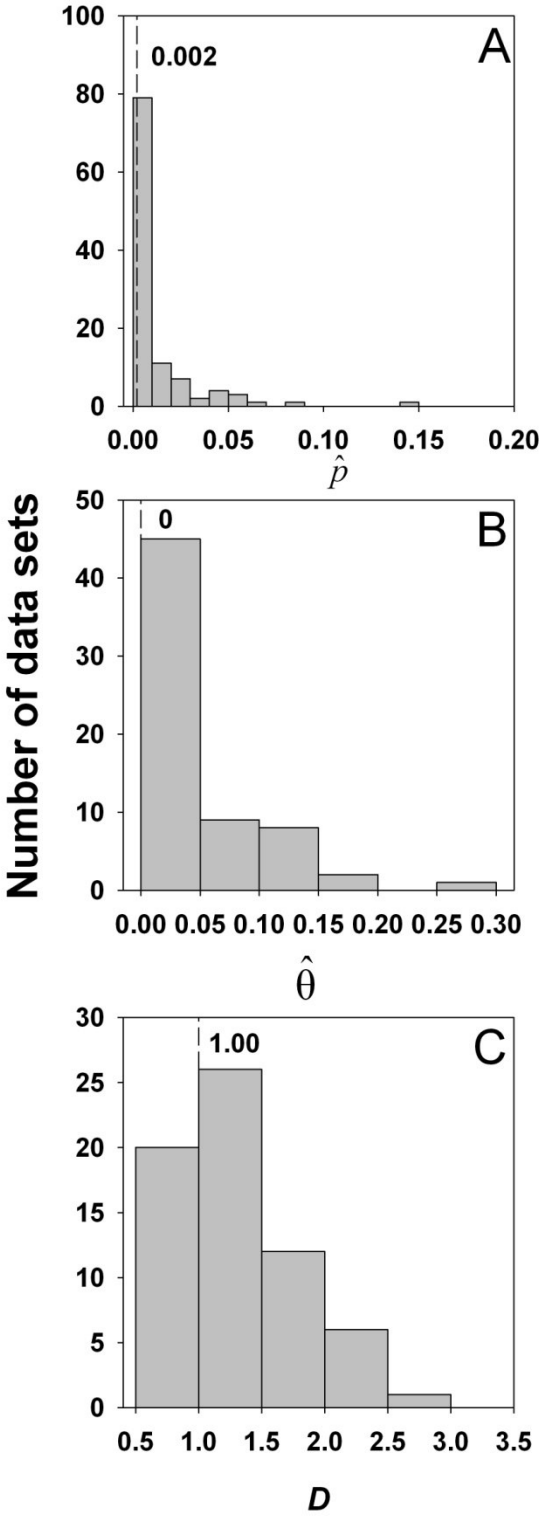


Figure 3. Frequency distribution of the beta-binomial distribution parameters $\hat{p}$ **(A)** and $\hat{\theta}$ **(B),** and the index of dispersion *D* **(C)** for the incidence of bean pods with white mould in the validation data sets. There are 109 data sets (fields) presented in **A; B** and **C** present data from the 65 data sets where $\hat{p} > 0$. The vertical dashed lines are the median value for the indicated statistic, with the numerical value given on each graph.

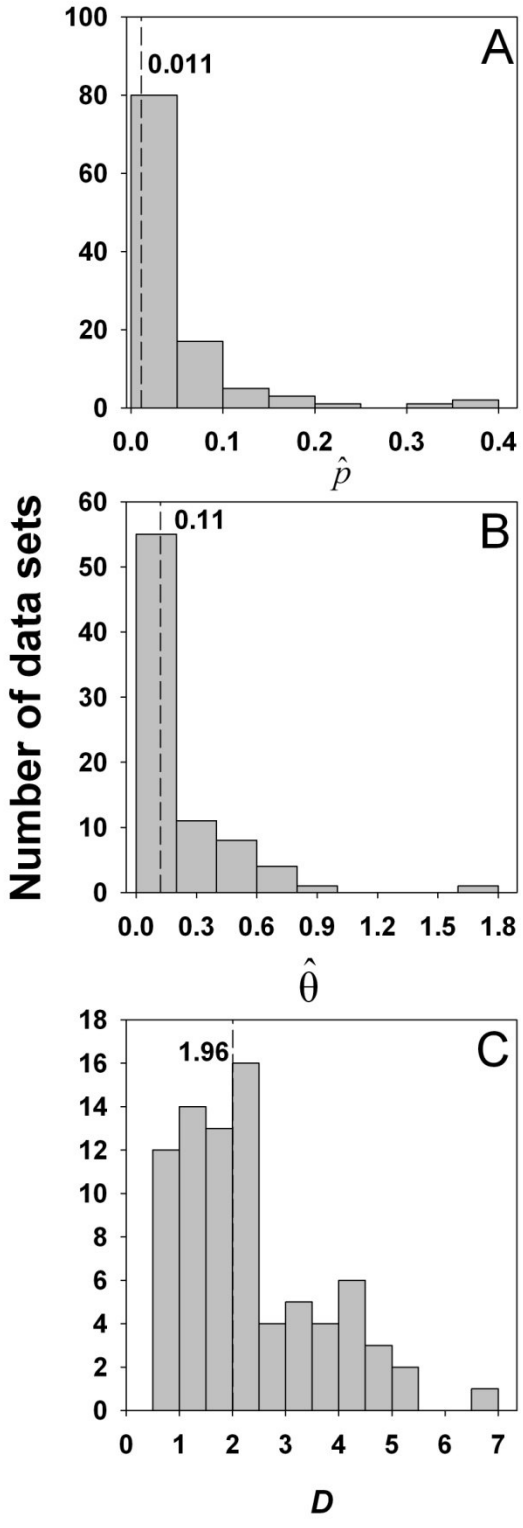


Figure 4. Frequency distribution of the beta-binomial distribution parameters $\hat{p}$ **(A)** and $\hat{\theta}$ **(B),** and the index of dispersion *D* **(C)** for the incidence of bean plants with white mould in the fields used for the validation data sets. There are 109 data sets (fields) presented in **A; B** and **C** present data from the 80 data sets where $\hat{p} > 0$. The vertical dashed lines are the median value for the indicated statistic, with the numerical value given on each graph.

Point pattern analyses

Distributional analyses

All spatial analyses at the scale of individual sampling units indicated that disease incidence was slightly aggregated over all levels of disease incidence observed (Table 1). The log likelihood ratio test was significant for 83% of the data sets, indicating the beta-binomial distribution provided a better fit to the data than the binomial (random) distribution. Similarly, the $C(\alpha)$ test indicated that the beta-binomial distribution provided a better fit than the binomial distribution for 93% of data sets. The frequency distribution of the heterogeneity parameter $\hat{\theta}$ was highly right-skewed and ranged from 0.0042 to 0.37 with median 0.10, which indicated a low degree of aggregation (Figure 2B; Table 1). The frequency distribution of the index of dispersion, D, ranged from 1.05 to 3.48 with median 1.80 (Figure 2C). In 91% of the data sets D was greater than 1 (suggesting significant aggregation) according to a chi-square test.

Disease incidence was randomly distributed on pods within sampling units in the model validation data sets, but was aggregated on plants. On pods, the heterogeneity parameter $\hat{\theta}$ ranged from 0 to 0.27, with median 0 in data sets where $\hat{p} > 0$ (Figure 3B). The index of dispersion ranged from 0.69 to 2.91, with median 1, indicating a random distribution of disease incidence (Figure 3C). On plants, $\hat{\theta}$ varied from 0 to 1.73 with median 0.11 (Figure 4B) and D varied from 0.78 to 6.81 with median 1.96 (Figure 4C).

Binary power law analyses

The binary power law provided a reasonable fit to the data in 2008 and 2009 (R^2 = 0.82 and 0.84), but a poor fit in 2010 (R^2 = 0.45) (Figure 5A). In each year and averaged over all years, the intercept estimates were significantly greater than 0 but the slope parameter estimates was not significantly different than 1 (Table 2). This indicates that the incidence of diseased pods was aggregated (intercept greater than 0), but the degree of heterogeneity was not systematically related to disease incidence (slope equal to 1).

In the model validation data set on pods, disease incidence was significantly more aggregated as compared to the model development data sets. The estimated parameters for the intercept and slope terms were significantly greater than 0 and 1, indicating aggregation that was systematically related to p (Table 2; Figure 5B). On plants, disease incidence was more aggregated than pods (Table 3; Figure 6), as indicated by the greater values for both the intercept and slope terms.

Table 1. Tests of aggregation and spatial pattern of the incidence of bean pods with white mould sampled from commercial bean fields in Tasmania, Australia during 2008 to 2010.

			Variance tests[d]		Median values[e]			Runs Analysis[f]	
Incidence[a]	***T*[b]**	**LRS[c]**	***C*(α)**	***D***	$\hat{\theta}$	***D***	$\hat{r}$	**Median**	**Ordinary**
0.02-0.04	3	1.00	1.00	1.00	0.09	1.79	0.06	0.33	0.33
0.04-0.08	23	0.74	0.91	0.87	0.06	1.54	0.08	0.26	0.26
0.08-0.16	24	0.92	0.96	0.96	0.13	2.07	0.24	0.43	0.46
>0.16	4	0.75	0.75	0.75	0.11	1.86	0.09	1.00	0.25
All	54	0.83	0.93	0.91	0.10	1.80	0.16	0.37	0.35

[a] Incidence of pods with white mould. Disease incidence class ends with the indicated value and classes start with the next highest value above the listed value.

[b] Number of data sets in each disease incidence class.

[c] Proportion of data sets in which the likelihood ratio test statistic was significant ($P \leq 0.05$).

[d] Proportion of data sets in which the $C(\alpha)$ (z-statistic) or D (chi-square) tests were significant ($P \leq 0.05$).

[e] Median estimated value of the beta-binomial distribution parameter $\hat{\theta}$, index of dispersion D, and first-order autocorrelation statistic, $\hat{r}$.

[f] Percentage of data sets in which runs analysis indicated significant aggregation. Median runs analysis was based on 23 or 2 data set in disease incidence classes 0.08 to 0.16 and >0.16, respectively, because runs could not be calculated in some instances since all sampling units had at least one diseased pod.

Table 2. Estimated slope ($\hat{b}$) and intercept parameters (ln [$\hat{A}_x$]) of the binary power law fit to the incidence of bean pods with white mould in commercial bean fields in Tasmania, Australia.

	Model development data sets				Model validation data sets			
Year	**df[a]**	$\hat{b}$ **(SE)[b]**	**ln (** $\hat{A}_x$**) (SE)[b]**	R^2	**df[a]**	$\hat{b}$ **(SE)[b]**	**ln (** $\hat{A}_x$**) (SE)[b]**	R^2
2008	13	1.316 (0.156)	0.555 (0.084)	0.84	…	…	…	…
2009	16	0.947 (0.113)	0.511 (0.059)	0.82	32	1.166 (0.047)	0.649 (0.124)	0.95
2010	19	0.899 (0.226)	0.625 (0.090)	0.45	29	1.090 (0.033)	0.399 (0.095)	0.97
All	52	1.045 (0.094)	0.557 (0.046)	0.70	63	1.127 (0.028)	0.525 (0.078)	0.96

[a] df = degrees of freedom for regression.

[b] SE = standard error of the mean. Parameter estimates for the slope were not significantly different from 1 in any individual year or in the combined analysis ($P > 0.05$) in the model development data sets, but were greater than 1 in the model validation data sets. Intercept parameter estimates for the intercept parameter estimates were greater than 0 in all years and in the combined analyses ($P < 0.0001$).

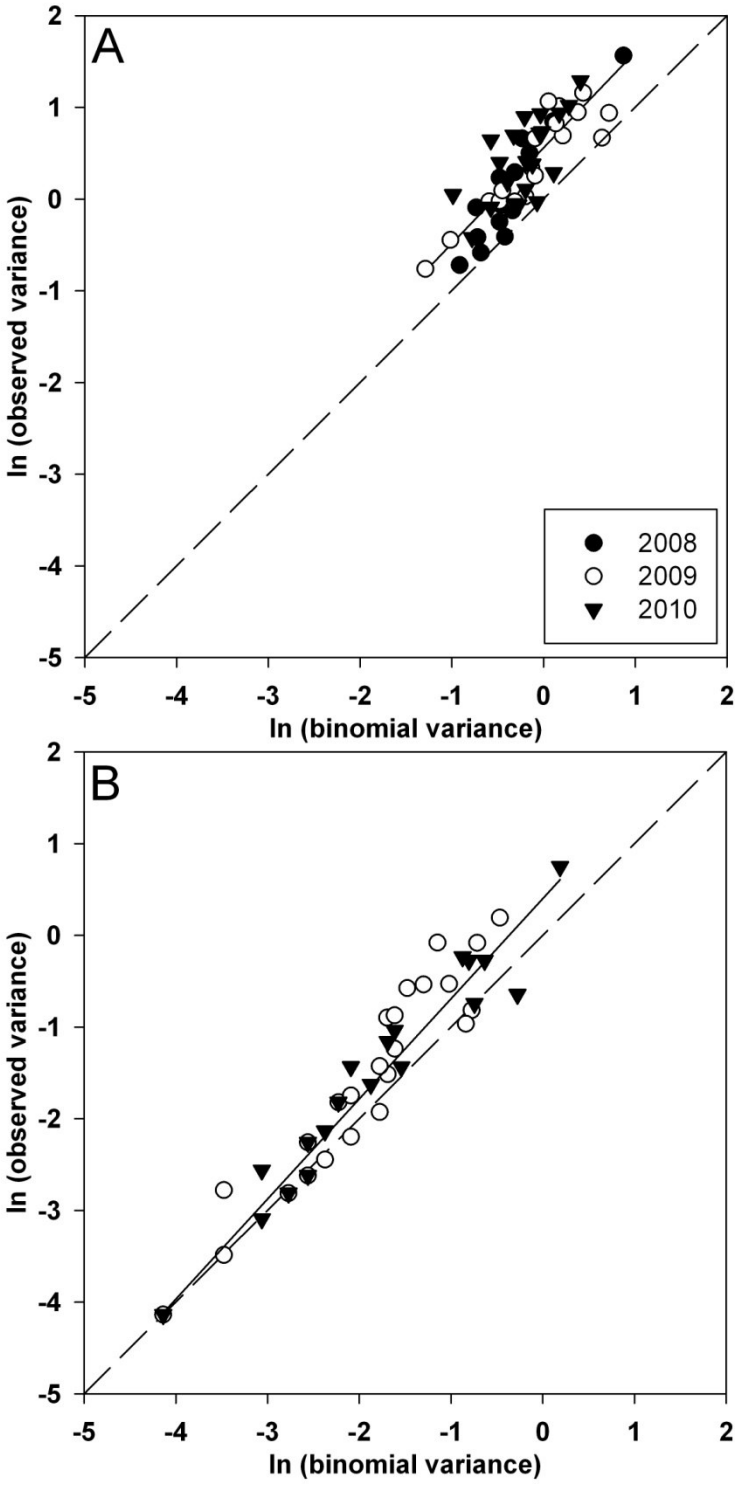


Fig. 5. Relationship between the logarithms of the observed variance and binomial variance of the incidence of bean pods with white mould in Tasmania, Australia in the model development data sets **(A)** and validation data sets **(B)**. The solid line is the least squares regression fit to data from 2008 (solid circles), 2009 (open circles), and 2010 (triangles), and the dashed line represents for a binomial (random) distribution of disease incidence. Slope and intercept parameter estimates are given in Table 2.

Figure 6. Relationship between the logarithms of the observed variance and binomial variance of the incidence of bean plants with white mould in Tasmania, Australia in fields where model validation data sets were collected. The solid line is the least squares regression fit to data from 2009 (open circles) and 2010 (triangles), and the dashed line represents a binomial (random) distribution of disease incidence. Slope and intercept parameter estimates are given in Table 3

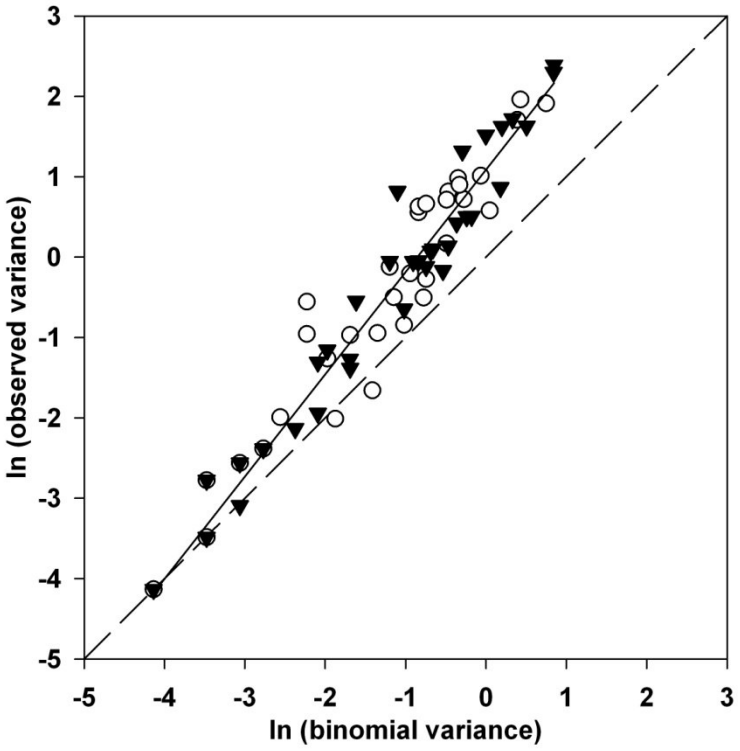


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Table 3. Estimated slope ($\hat{b}$) and intercept parameters (ln [$\hat{A}_x$]) of the binary power law fit to the incidence of bean plants with white mould in commercial bean fields in Tasmania, Australia.

Year	df[a]	$\hat{b}$ (SE)[b]	ln ($\hat{A}_x$) (SE)[b]	R^2
2009	36	1.235 (0.051)	1.085 (0.108)	0.94
2010	40	1.272 (0.040)	1.086 (0.082)	0.96
All	78	1.254 (0.032)	1.086 (0.064)	0.95

[a] df = degrees of freedom for regression.
[b] SE = standard error of the mean. Parameter estimates for the slope and intercept terms were 1 and 0 respectively, in all years and in the combined analysis ($P < 0.0001$).

Correlation-based spatial analyses

Autocorrelation

Significant first-order autocorrelation, $\hat{r}_1$, was detected in 50% of the data sets, with significant second-order autocorrelation in 44% of data sets (Table 2). The frequency distribution of $\hat{r}_1$ ranged from -0.23 to 0.78 with median 0.16 (Figure 2D). At a lag = 2, median $\hat{r}_2$ was 0.15. Together, this indicates a low but significant level of aggregation of disease incidence in patches of about 1 metre or slightly greater.

Runs analyses

Significant aggregation was detected in 37% of the data sets by median runs analysis and 35% of the data sets where more than 1 run was present (Table 1), indicating patches of disease extending beyond individual sampling units. There was a tendency for median runs analysis to detect greater aggregation as disease incidence increased.

Sequential estimation

Sequential estimation stop limits for estimating mean incidence of pods with white mould and the corresponding disease incidence at T_N is shown in Figure 7A and 7B, respectively, for C = 0.1, 0.2, and 0.3. The achieved level of precision increased as the true level of disease incidence decreased, or the number of diseased pods that needed to be sampled in order to end the sequential sampling increased.

Simulated sampling validation

Estimates of disease incidence obtained by sequential sampling were very similar to the true disease incidence (Figure 8A and 8B). However, the data sets were fully sampled (all 64 sampling units) and thus $\hat{p} = p$. Due to small values of p, achieving the pre-specified level of precision was not possible in any of the 65 data sets where $p > 0$ when C was set to 0.1 (Figure 8C), and only 6 of the 65 data sets when $C = 0.2$ (Figure 8D). At $C = 0.1$, all 64 sampling units were evaluated in all of the data sets, while all but 3 of the data sets were completely sampled when $C = 0.2$.

Figure 7. Sequential-estimation stop limits for estimating the mean incidence of bean pods with white mould with a coefficient of variation (C) of 0.1, 0.2, and 0.3 indicated numerically on the graph. **A**, Cumulative number of diseased pods (T_N) versus the total number of sampling units (N). **B**, Mean disease incidence at critical T_N (the point where the observed cumulative number of diseased pods crosses the model T_N curve) in relation to N. Sampling ceases when the cumulative number of diseased pods crossed the critical T_N in **A**, at which point mean disease incidence is then calculated as T_N/nN.

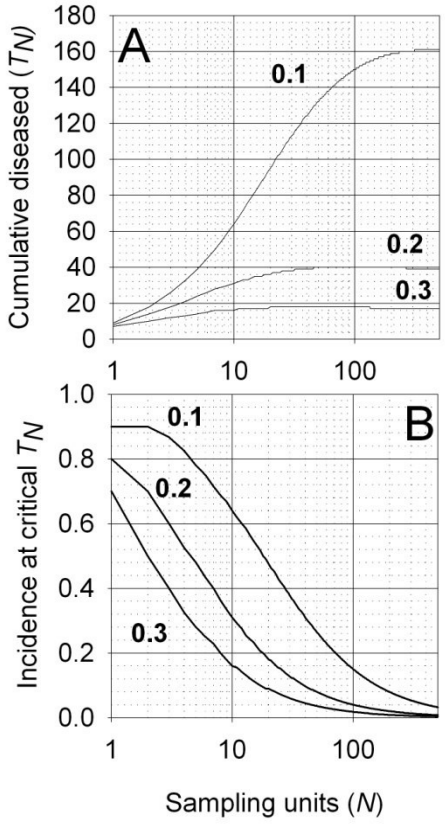
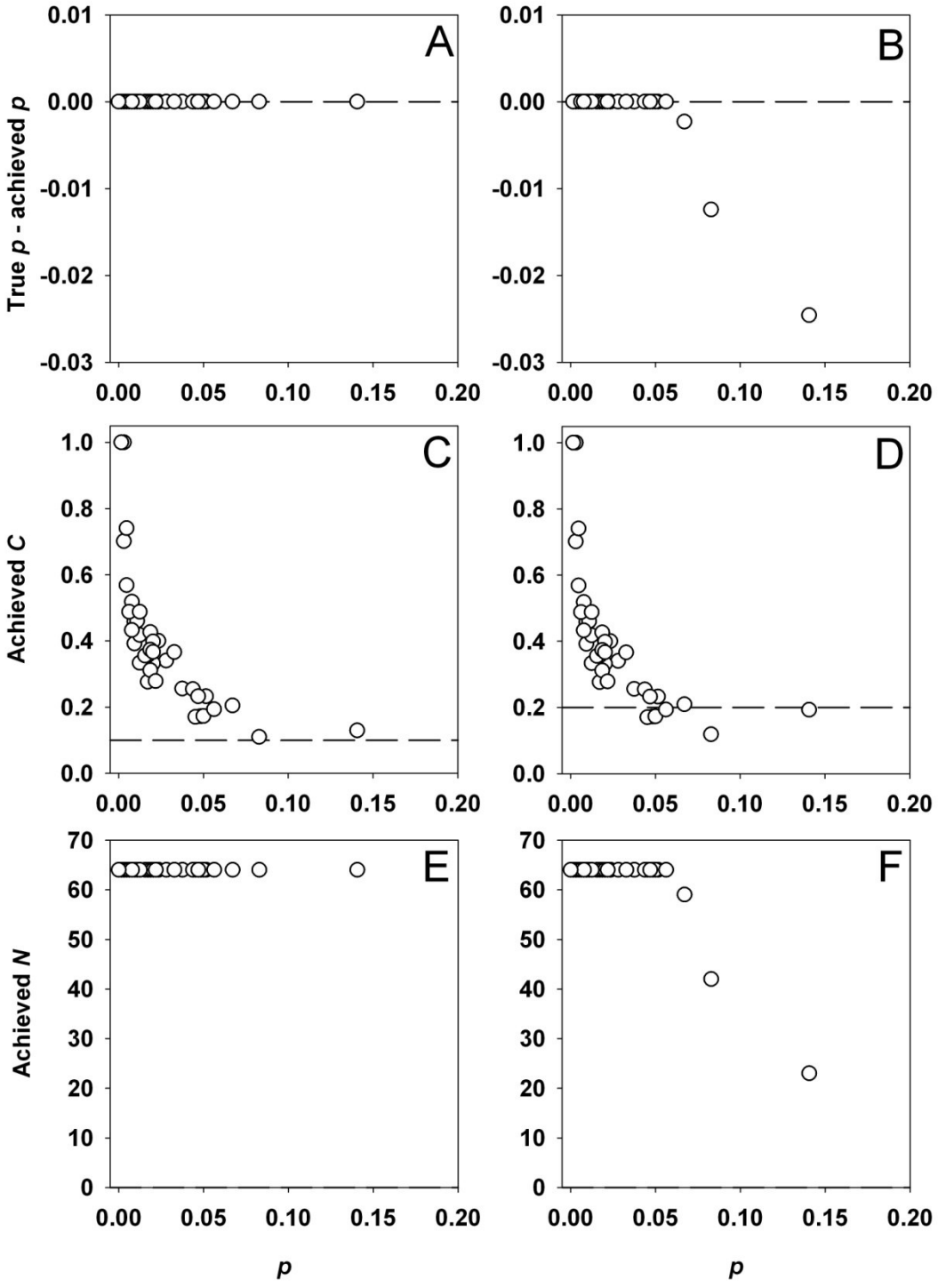


Figure 8. Validation of sequential sampling plans for estimating the incidence of bean pods with white mould in 109 commercial bean fields in Tasmania, Australia. **A**, Relationship between true *p* and estimated disease incidence from a sequential sample. **B**, Relationship between true disease incidence (*p*) and the achieved coefficient of variation (*C*). **C**, Relationship between true *p* and number of sampling units (*N*) collected. **A, C, and E** present data for *C* = 0.1 and **B, D, and F** present data for *C* = 0.2. The dashed lines in **C and D** are the pre-specified values of *C* = 0.1 **(C)** and 0.2 **(D)**. Data sets contained 64 sampling units located along four to five transects.



Bootstrapping evaluation

Disease incidence estimates were imprecise when $\hat{p}$ was low. The achieved C was greater than the pre-specified C for data sets where $\hat{p} \leq 0.16$ when $C = 0.1$ (Figure 9A) and $\hat{p} \leq 0.04$ when $C = 0.2$. The achieved C approached the pre-specified C as $\hat{p}$ increased, and the median achieved C was less than the pre-specified C for 2 data sets when $C = 0.1$, and 6 data sets when $C = 0.2$, due to the minimum sampling rule.

Although the pre-specified precision was not attained in some instances, the estimates of $\hat{p} - p$ included zero for all data sets at both $C = 0.1$ and 0.2 (Figure 9C and 9D), indicating that $\hat{p}$ was close to p for these data sets. Confidence intervals generally increased with increasing p because the sample size (N) decreased with increasing p for both levels of C (Figure 9E and 9F). For $C = 0.2$, sample sizes were greatest for data sets where $p \leq 0.04$ (data sets 1 to 3) and in these data sets the estimated $\hat{p} \approx p$.

Sequential classification.

Sequential classification stop lines for the six sequential classification plans are shown (Figure 10). Increasing α and β slightly reduced the distance between the stop lines (Figure 10A and 10B); the OC curves also were similar between error rates of 0.05 and 0.1 (Figure 10C and 10D). That is, a higher correct decision rate was achieved when $p > p_t$. The OC was 0 for all combinations of α and β at $p \geq 0.10$. Varying α and β had a relatively large effect on the ASN curves, which was most evident when p was near p_t. The ASN (rounded up to the nearest integer) at p_t 0.03, 0.05, and 0.151 was 35, 48, and 21 sampling units for $\alpha = \beta = 0.05$, and 24, 31, and 16 sampling units for $\alpha = \beta = 0.10$, respectively (Figure 10E and 10F). The ASN was near or identical to the minimum sample size for all sampling plans when p was far (≥ 0.10) from p_t.

Figure 9. Box plots of the achieved coefficient of variation (*C*) **(A and B);** the difference between the true incidence of pods with white mould, *p*, and the estimated disease incidence, $\hat{p}$, based on sequential samples **(C and D);** and the achieved sample size (*N*) **(E and F)** from 100 bootstrap samplings of 12 data sets used for model evaluation. Pre-selected values of *C* are 0.1 **(A, C, and E)** and 0.2 **(B, D, and F),** and are indicated by dashed lines in **A and B**. Sequential estimation stop lines were generated with estimate binary power law parameters $a = 0.194$ and $b = 1.045$. The model evaluation data sets were chosen by selecting three data sets randomly from each of four disease incidence classes ($0.01 < \hat{p} < 0.02$, $0.02 \leq \hat{p} < 0.04$, $0.04 \leq \hat{p} < 0.08$, and $\hat{p} > 0.08$) from among the 54 model construction data sets. The data sets are arranged in ascending order of $\hat{p}$. Data sets contained 100 sampling units selected along a single transect. Box plots show the median (line in open boxes), middle 50% of the data (open box), 95% confidence interval for the median based on the non-parametric sign-test (solid bar inside box), extremes of the data points (whiskers), and outliers (solid circles).

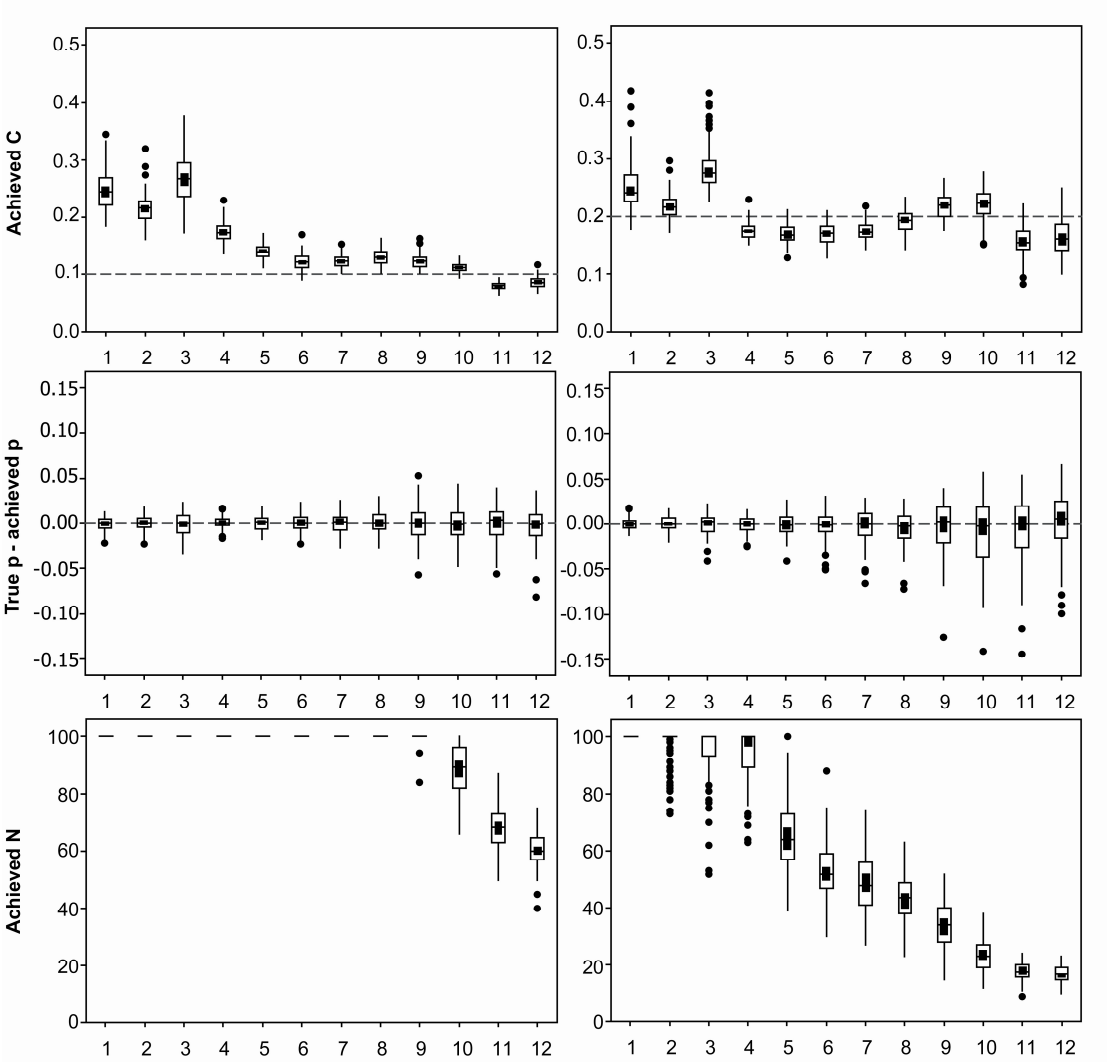
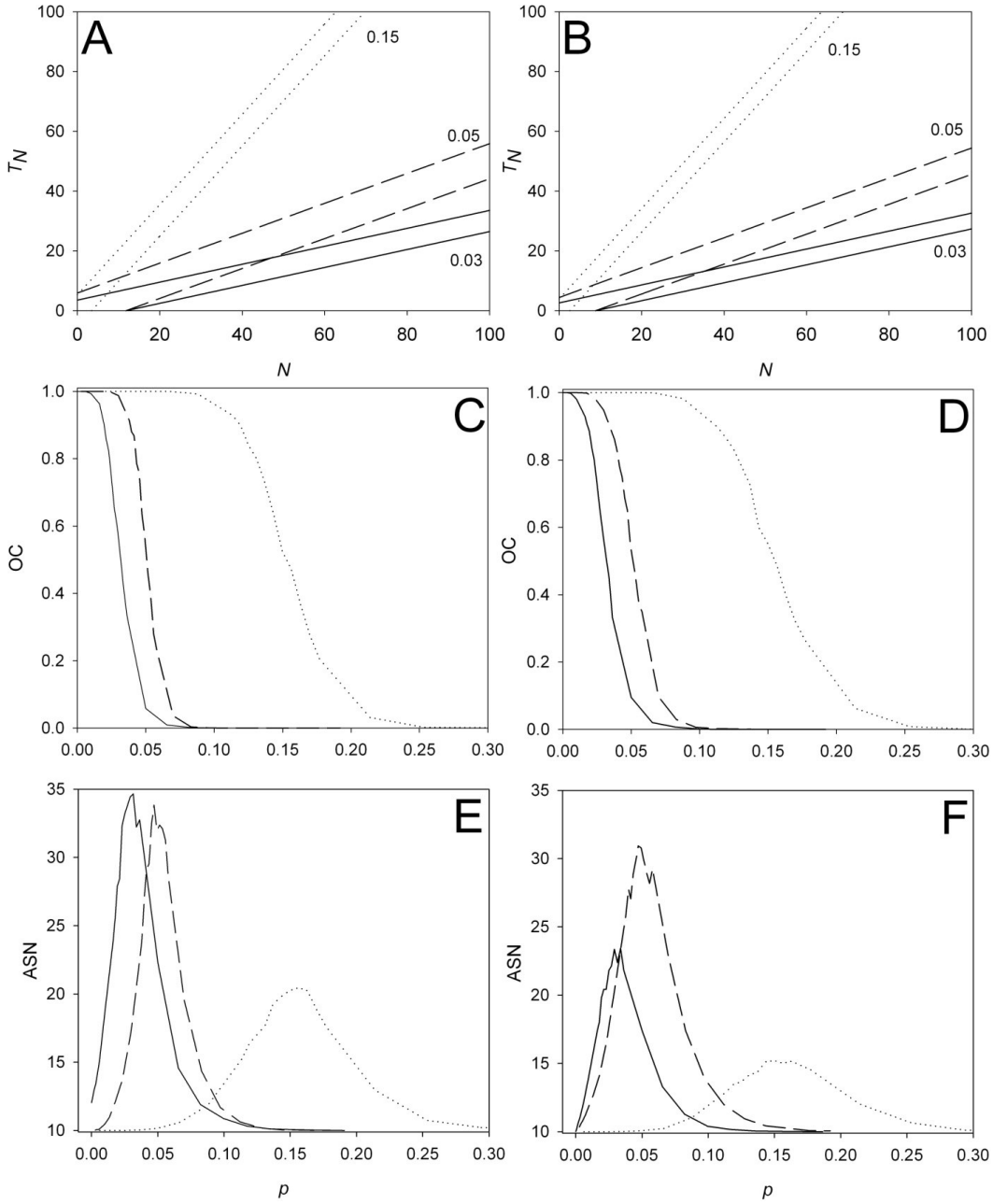


Figure 10. Stop lines for classifying the incidence of bean pods with white mould as above or below a critical threshold, p_t, from a cluster sample with $n = 10$ pods per sampling unit. **A and B**, Stop lines based on Wald's sequence probability ratio test with $p_0 = 0.01$ and $p_1 = 0.05$ ($p_t = 0.03$), with $p_0 = 0.03$ and $p_1 = 0.07$ ($p_t = 0.05$), and with $p_0 = 0.088$ and $p_1 = 0.214$ ($p_t = 0.15$). **C and D**, Operating characteristic (OC) curves, and **E and F,** average sample number (ASN) curves for sequential classification stop lines defined in **A and B.** OC and ASN curves were determined by 1000 Monte Carlo simulations with the heterogeneity parameter θ as a function of disease incidence according to the binary power law where $a = 0.194$ and $b = 1.045$. Type I (α) and type II (β) error rates were set at $\alpha = \beta = 0.05$ in **A, C, and E** and $\alpha = \beta = 0.10$ in **B, D, and F.**



Simulated sampling validation

At the three values of p_t and two combinations of error rates evaluated, correct decisions regarding classification of p were made at least 93% of the time when stop lines were generated assuming a random (binomial) distribution of disease incidence (Table 4). Fewer misclassification errors occurred at the higher values of p_t. On average, 10 to 11.27 sampling units had to be evaluated to classify a data set.

Correct decisions were made more often when the stop lines were generated assuming a beta-binomial (aggregated) distribution of disease incidence (Table 4). However, the slightly higher correct decision rate came at the cost of assessing more sampling units, on average 10.06 to 15.03 depending on p_t.

Bootstrap evaluation

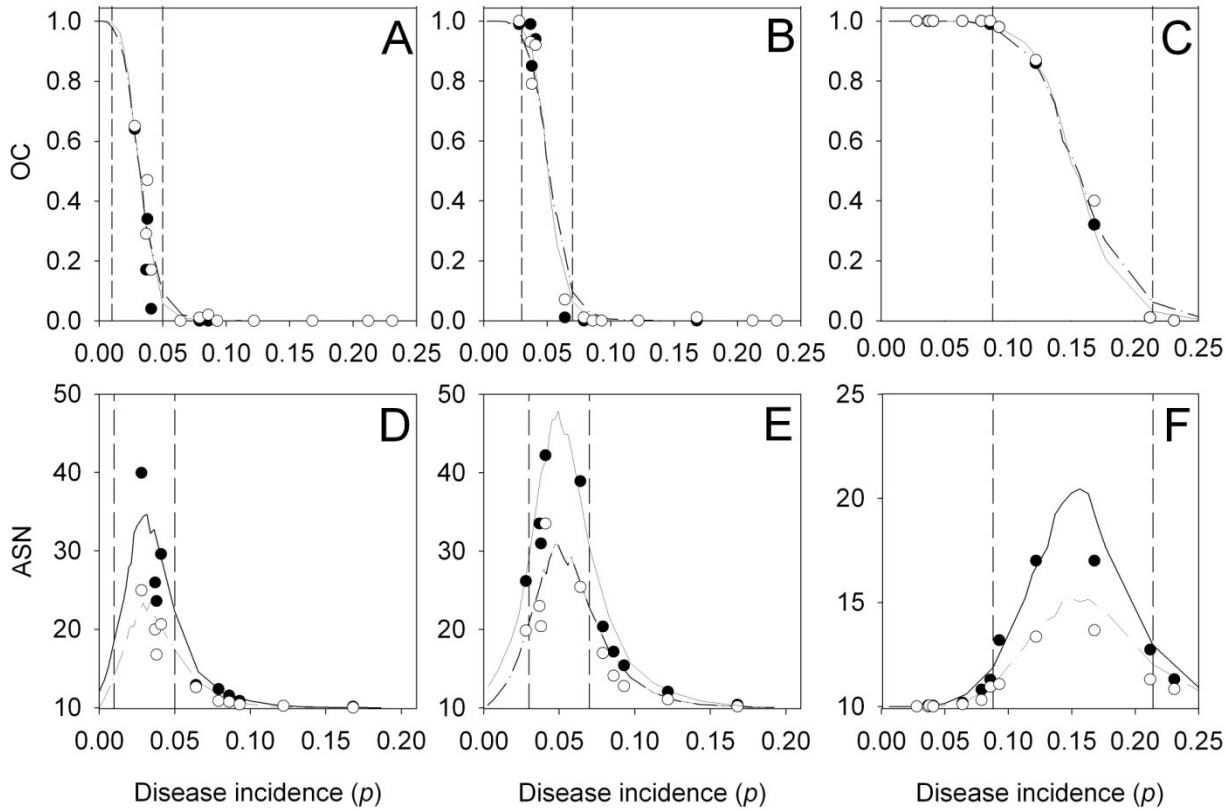
Changing p_t had a greater effect on the OC and ASN curves than did altering α and β. Increasing p_t flattened the OC curve and decreased the ASN, indicating an overall increase in the incorrect decision related to collection of fewer samples (Figure 10C-F). Bootstrap evaluation of the sampling plans indicated that the achieved OC and ASN were similar to the OC and ASN curves obtained by Monte Carlo simulation for the 12 data sets evaluated (Figure 11).

Table 4. Correct decision and error rates (proportion) of sequential classification plans for classifying the incidence of bean pods with white mould above or below varying disease incidence thresholds (p_t)

	$p_t = 0.03$[a]		$p_t = 0.05$		$p_t = 0.15$	
	α = 0.05	**β = 0.1**	**α = 0.05**	**β = 0.1**	**α = 0.05**	**β = 0.1**
Binomial						
Correct decision	0.93	0.93	0.96	0.95	1	1
Type I error	0.05	0.05	0.01	0.02	0	0
Type II error	0.03	0.03	0.03	0.03	0	0
Type I or II error	0.07	0.07	0.04	0.05	0	0
Mean *N*	11.27	10.40	11.26	10.53	10.06	10
Median *N*	10	10	10	10	10	10
Beta-binomial						
Correct decision	0.95	0.95	0.98	0.98	0.99	1
Type I error	0.02	0.02	0.00	0.00	0.01	0
Type II error	0.03	0.03	0.02	0.02	0	0
Type I or II error	0.05	0.05	0.02	0.02	0.01	0
Mean *N*	15.03	12.02	14.86	11.95	10.21	10.06
Median *N*	12	10	12	10	10	10

[a] Type I error indicates that mean disease incidence was incorrectly classified as $> p_t$. Type II error indicates that mean disease incidence was incorrectly classified as $< p_t$, where p_t is a critical value of disease incidence. Mean and median *N* were calculated from all 109 model validation data sets. The parameters α and β were specified to control type I and type II error rates, respectively.

Figure 11 (overleaf). **A** and **B**, Operating characteristic (OC), and **C** and **D**, average sample number (ASN) curves for sequential classification sampling plans as determined by Wald's sequential probability ratio test for the incidence of pods with white mould. Threshold values are, **A and D,** $p_0 = 0.01$, $p_1 = 0.05$, and $p_t = 0.03$; **B and E,** $p_0 = 0.03$, $p_1 = 0.07$, and $p_t = 0.05$; and **C and F,** $p_0 = 0.088$, $p_1 = 0.214$, and $p_t = 0.15$. OC and ASN curves were determined by 1000 Monte Carlo simulations with the parameter θ a function of the mean disease incidence according to the binary power law, where $a = 0.194$ and $b = 1.045$. Curves with error probabilities of $\alpha = \beta = 0.05$ (solid line) and $\alpha = \beta = 0.10$ (dot-dashed line) are shown. Circles are the achieved OC and ASN from 100 bootstrap simulations of sequential sampling for classification of 12 model development data sets; open circles are simulations where $\alpha = \beta = 0.05$, and solid circles are simulations where $\alpha = \beta = 0.10$. The 12 data sets were chosen by selecting three data sets randomly from each of four disease incidence classes ($0.02 < \hat{p} < 0.04$, $0.04 < \hat{p} < 0.08$, $0.08 < \hat{p} < 0.16$, and $\hat{p} > 0.16$) from among the 54 model development data sets.



Discussion

The incidence of pods with white mould was characterized by a largely random pattern of disease incidence at the scale of individual plants, with some patches of plants with similar disease levels on pods occurring at a scale of 1 metre or greater. However, the incidence of plants (versus pods) with white mould tended to be slightly to moderately aggregated. Although processes responsible for patterns cannot be deduced conclusively from this analysis, the small scale aggregation on pods but greater degree of aggregation on plants observed in the current study suggests localized spread of ascospores within bean fields. If inoculum is truly dispersed predominantly locally, this suggest that management efforts should be directed at reducing primary inoculum levels within individual fields rather than focusing management efforts on a farm- or regional scale.

The spatial analyses also provided the foundation for developing statistically sound sampling approaches for estimating disease incidence or classifying disease incidence above or below the provisional industry threshold for crop rejection. The sampling plans developed in this research were not intended to inform decisions regarding application of control measures. Rather, the sampling plans were designed to quantify disease levels on pods near harvest. The sequential estimation sampling plans tended to perform appropriately when disease incidence was at least 4%, which is near the provisional thresholds sometimes used for crop rejection. To achieve a highly precise estimate of disease incidence ($C = 0.1$), greater than 100 plants—with 10 pods sampled on each plant—need to be evaluated when disease incidence is relatively low (less than 8%). When precision requirements can be relaxed ($C = 0.2$), the number of plants that need to be evaluated decreases with increasing disease incidence to as few as 10.

For practical pest management, often only a classification of disease or pest status above or below some critical value for decision making is needed (Binns and Nyrop, 1992; Binns *et al.*, 2000). When only a classification of disease incidence is required, the sequential classification plans developed herein appeared to provide a very useful tool for accurately classifying fields after inspection of relatively few sampling units. The sequential classification plans enabled disease incidence on pods to be classified correctly in at least 95% of fields after sampling only 10 to 15 plants, with 10 pods evaluated on each plant. Given the considerable amount of labour and resources needed to assess disease incidence, these sampling plans will facilitate future research efforts and also assist industry in designing their sampling schemes for deciding which fields should be harvested or not.

The incidence of white mould on bean pods as estimated in the field by TIAR personnel tended to be an over-estimate of that assessed by the bean processing company in 32 fields in Tasmania. This may have been due to differences in sampling (i.e., from across the field compared to from truckloads), or from a proportion of bean pods infected by *S. sclerotiorum* being less robust (rotten) and broken up and left in the field during the harvesting process. Nonetheless, the sequential sampling plan for classifying disease incidence based on an estimate of the processor disease rating performed well. Correct decisions on disease status above or below the processor threshold were made at least 99% of the time after evaluation of, on average, only 10 plants.

SITE SPECIFIC RISK FACTORS OF WHITE MOULD DISEASE IN BEAN

Introduction

Forecasting of disease risk can be undertaken through developing a decision support system based on an understanding of the relative importance of particular cultural and environmental factors on disease development (section 3 of this report), or through the monitoring of inoculum within the crop (section 4 of this report), or a combination of both.

Most research in the development of forecasting risk of diseases caused by *Sclerotina* spp. has been undertaken for Sclerotinia stem rot (SSR) of oilseed rape. This disease can reportedly cause losses of up to 50% in the U.K. (Pope *et al*. 1989) and up to 20-30% in Germany (Dunker and von Tiedemann 2004). Control of SSR relies predominately on one fungicide application at flowering to control infection of petals by ascospores (Koch *et al*. 2007). However, analysis of numerous field experiments in Germany, conducted over several seasons, demonstrated that less than 33% of fungicides at flowering were cost effective (Dunker 2006, Dunker and von Tiedemann 2004, Wahmhoff 2000). Koch *et al*. (2007) summarised the development of decision support systems for SSR which were based on site-specific factors and weather conditions during flowering (Ahlers 1986, 1989) linked with the timing of the apothecia emergence (Krüger 1975, Nordin *et al.* 1992) or spore trapping (Ahlers 1989). Ahlers and Hindorf (1987) developed a system for predicting disease incidence which incorporated site specific risk factors. This system was used to schedule fungicide applications and had a predictive accuracy of approximately 60%. Later a threshold of 10% disease incidence at growth stage 61 to 67 was proposed for cost effective fungicide use (Ahlers and Hindorf 1987). A checklist of risk points was developed in Sweden to support the decision for a fungicide spray in spring sown canola based on information collected from some 800 fields over a period of 10 years. The decision support was based on field specific factors including precipitation, the short term weather forecast, previous disease levels, frequency of oilseed rape in rotation, crop density and the development of apothecia (Twengström *et al*. 1998). This decision model predicted mild Sclerotinia epidemics (<25% disease incidence), for which spraying was not recommended, with the accumulation of <40 points, with spraying recommended at ≥ 50 points (>25% disease incidence). Using this system in canola in Canada, the need for fungicide application was correctly predicted in 75% of fields which required fungicides, i.e. those fields in which actual disease incidence was > 25% (Thomas 2003). However, the system also predicted the need for fungicide application in 16% of fields for which no fungicide was required, i.e. disease incidence was < 25% (Thomas 2003).

In Germany the agrometeorological model SKLERO was developed to estimate the likelihood of infection in oilseed rape (Friesland 1998, Friesland 2000), with no attempt to predict disease levels or crop loss. Koch *et al.* (2007) expanded this work to develop the forecasting model SkleroPro, intended to provide 'an accurate and easy-to-handle, fully computerized decision support system based on weather and field-site-specific data and made accessible to growers and advisors via the Internet'. Historical field data over multiple years was used to assess the impact of agronomic factors on SSR incidence. Shorter rotations of every two years in comparison to every four years were shown to significantly increase disease risk, while other factors such as plant density, nitrogen rate and cultivation method had no effect on disease incidence. SkleroPro was based on a two-tiered system based on:

(i) a regional assessment of disease risk based on hourly estimates of temperature and relative humidity in the crop canopy which are used to calculate 'infection hours'. Infection is

assumed to have occurred following weather conditions suitable for a continuous infection period exceeding 23 hours, after the crop has passed growth stage 58.

(ii) a field-site-specific, cost/benefit recommendation based on cost of fungicide application, expected yield and price of rapeseed which is used to calculate the number of infection hours corresponding to the disease incidence at the economic damage threshold.

The SkleroPro forecasting system was shown to provide economically correct decisions in 70-81% of cases, corresponding to savings in fungicides of 39-81% and increased net returns for the grower of €23-45/ha (Koch *et al.* 2007). The study suggested that in regions of abundant inoculum, SSR in oilseed rape could be predicted from conditions of stem infection during late bud or flowering, without the added and laborious assessment of apothecial development and ascospore dispersal.

Attempts have been made to assess risk factors to white mould in other crops. The prevalence of Sclerotinia stem rot of soybean was associated with cultural practices that maximize yield, such as high plant density, narrow row widths, and lush plant growth from high levels of irrigation and fertilization (Mila *et al*. 2004).

The purpose of this study was to conduct an analysis of survey data collected from Tasmanian bean fields to identify environmental and agronomic factors associated or disassociated with risk of white mould disease, as a precursor to developing a decision support system which could be used to inform growers of the risk of disease in particular crops prior to planting.

Materials and methods

Field sites and data collection

Commercial bean crops in Tasmania were surveyed to identify agronomic, site and farm practices that influence infection and development of white mould. A total of 52 and 57 fields were surveyed in 2009 and 2010 respectively, from February to early April. Fields in all commercial bean production districts were assessed including Cressy, Longford, Hagley, Mersey Lea, Sassafras, Wesley Vale, East Devonport, Barrington, Wynyard, and Forest. Fields were situated within a 230 km range in northern Tasmania between the western boundary of Forest (40° 51' 29" S, 145° 12' 24" E) and the eastern boundary of Bishopbourne (41° 41' 26" S, 147° 02' 43" E), and were up to a maximum of 100 km inland from the north coast.

Disease incidence

Fields were assessed for disease incidence as close to commercial harvest as practical, which was generally 2 to 10 days before harvest. In each field, pods and plants at 64 points were examined for symptoms of white mould. This sample size was selected based on fixed sampling curves with precision (coefficient of variation) set to $C = 0.3$ for disease incidence equal to 1% incidence of disease pods, considering spatial aggregation of disease incidence. The 64 sampling points were obtained by sampling at 12 to 16 relatively equally spaced points that spanned the length of the field, along four or five transects that spanned the width of the field. Latitude, longitude and elevation were recorded at each sampling point with a hand-held global positioning system (GPS eTrex Vista® HCx; Garmin International inc., Olathe, Kansas, USA). At each sampling point, 10 plants and 20 pods were assessed. The 20 pods were selected arbitrarily from one to two bean plants, depending on the cultivar and

number of pods per plant. Plants were considered diseased if there were white mould symptoms on either the stem or on one or more pods. The presence or absence of sclerotia and apothecia were also recorded.

Agronomic data

Agronomic production records were obtained from cooperating growers and/or the contracting processing companies (Simplot Australia and McCain Foods). The information obtained generally included planting date, harvest date, dates of herbicide and fungicide (boscalid) applications, bean variety, inputs of major nutrients, previous cropping history, and yield. Elevation, aspect, soil type, irrigation method, crop planting density, weed density, and irrigation type were determined during visits to each site. From these variables, we also calculated degree-days (base 4.5°C) from planting to the first fungicide application.

Weather data

Estimated weather data for each field site was obtained from the Queensland Department of Natural Resources and Mines DataDrill algorithm (Hutchinson 1995) accessed on the internet at http://www.dnr.qld.gov.au/silo.

Weather variables evaluated included cumulative rainfall, number of days with measurable rain, evapotranspiration, solar radiation, and daily mean, average, and minimum temperatures. The precision of interpolated temperature and rainfall data was 0.5°C and 1 mm respectively at a spatial resolution of 5 km^2 (Hutchinson 1995, Jeffrey *et al*. 2001, Stone *et al.* 1996). Weather variables were selected based on considerations of the disease biology and cropping cycle, and calculated for the first 30 days after planting and the last 30, 14, or 10 days before the disease assessment date, which was within a few days of crop harvest. It was assumed that sclerotial germination and subsequent apothecial emergence would be best predicted by weather during the last 30 days of the cropping cycle in sync with crop development (i.e., flowering and canopy closure).

Statistical analysis

Data analysis was based on a total of 109 fields surveyed during 2009 and 2010. As a preliminary means to identify risk factors for white mould occurrence on pods, scatterplots of continuous variables were made to examine their relationships to the incidence of disease pods. Correlations were quantified with Spearman's nonparametric rank correlation (*S*). The Kolmogorov-Smirnov (K-S) test also was conducted to identify variables associated with severe white mould. The K-S test examines the distributions of data in two categories and statistically tests whether the data were derived from the same distribution. Monte Carlo estimation was used to derive exact *P*-values.

To generate two groups for testing, fields were classified into "controls" and "cases", where controls were defined as fields where white mould was not observed on pods and cases were fields where white mould was observed on pods. Commercially, 5% incidence of white mould on pods is an approximate threshold for crop rejection that could be used to classify fields for identification of risk factors for severe white mould occurrence. This threshold was not used because only 6 of the 109 fields had disease incidence that exceeded 5%. Therefore,

we considered the occurrence of white mould on pods as the classification criteria to generate an overall data set with sufficient cases and controls to identify disease risk factors.

After univariate statistical analyses to identify presumptive risk factors, subsets of potential risk factors were combined and expressed in both parametric and non-parametric discriminant analysis models and logistic regression models. A number of preliminary models were examined and evaluated based on model accuracy, sensitivity, and specificity, the number of variables in the model, and biological considerations. Models with the least number of independent predictor variables were preferred to avoid over-specification.

Results

Disease incidence

For the 2009 season, white mould disease was not detected on either pods or plants in 16 of the 52 fields surveyed. Fifty fields had less than 5% disease incidence on the pods, and 38 fields had less than 5% diseased plants. For the 2010 season, white mould disease was not detected on plants or pods in 13 of 57 fields surveyed. Fifty three fields had less than 5% disease on pods and 41 fields had less than 5% diseased plants. Mean disease incidence on pods across all fields was 1.05 and 1.08 % for the 2009 and 2010 seasons respectively, and on plants was 3.80 and 5.61 % for the 2009 and 2010 seasons respectively. Disease incidence for individual sites is summarised in appendix 1.

Site specific risk factors

Among the continuous variables investigated, associations were found for variables relating to inoculum density, temperature, and solar radiation. Significant correlations ($P < 0.05$) between the incidence of diseased pods was found with the number of apothecia ($S = 0.33$; $P = 0.0004$), the incidence of plants with white mould ($S = 0.91$; $P < 0.0001$), degree-days (base 4.5C) between planting and the first boscalid application ($S = 0.20$; $P = 0.04$) or the number of days between planting and the first boscalid application ($S = 0.20$; $P = 0.03$), average temperature in the 10 days before harvest ($S = 0.19$; $P = 0.04$) and mean minimum temperature in the 10 days before harvest ($S = 0.22$; $P = 0.02$). Few variables were significant (at $P < 0.05$) based on the K-S test. Using the occurrence of white mould on pods as the classification criteria, the number of apothecia per sampling point ($P = 0.004$) was significantly different between fields with and without white mould on pods. There also was a trend for mean minimum temperature in the 10 days preceding harvest to be different between fields with and without disease occurrence on the pods ($P = 0.08$). When the analysis was conducted considering the distribution of the incidence of diseased pods (instead of simply occurrence or not), the distributions of diseased pods was significantly different between plant density categories (low versus medium-to-high; $P = 0.04$) and cultivar Flavour Sweet versus the other cultivars ($P = 0.04$).

A number of combinations of these and other relevant potential predictor variables were evaluated in logistic regression and discriminant analysis models. The model with the best predictive ability was a nonparametric discriminant model with five variables (Table 5; Figure 12), which included mean minimum temperature in the 10 and 30 days before harvest,

cultivar (Flavour Sweet versus others), plant density (low versus medium-high density), and a categorical variable for boscalid application timing. Inspection of scatterplots suggested that disease risk was greater when the first application of boscalid occurred before 450 degree-days or after 600 degree-days from planting. A categorical predictor was developed that if the first application occurred between 450 to 600 degree-days after planting the field was coded '1' and '0' otherwise. In fields where boscalid was not applied (14 fields), this variable was set to '0'. A model containing these predictors correctly classified 77% of the data sets into the correct disease risk category, with 84% sensitivity and 65% specificity in cross validation (Table 6).

Table 5. Spearman's correlation (*S*) and significance of the Kolmogorov-Smirnov (K-S) test for variables associated with the incidence or occurrence of white mould in commercial bean fields.

	2009 and 2010 data sets		
Predictor variable	***S***	***P*-value**	**K-S[a]**
Mean minimum temperature in past 30 days	0.10	0.32	0.47
Mean minimum temperature in past 10 days	0.22	0.02	0.08
Degree-days from planting to first application of boscalid	-0.22	0.04	0.04
Cultivar Flavour Sweet[b]	---	---	0.04
Planting density[b]	---	---	0.01

[a] K-S = Kolmogorov-Smirnov test that variables from fields with white mould belong to the same distribution. Monte Carlo estimation was used to derive exact *P*-values.

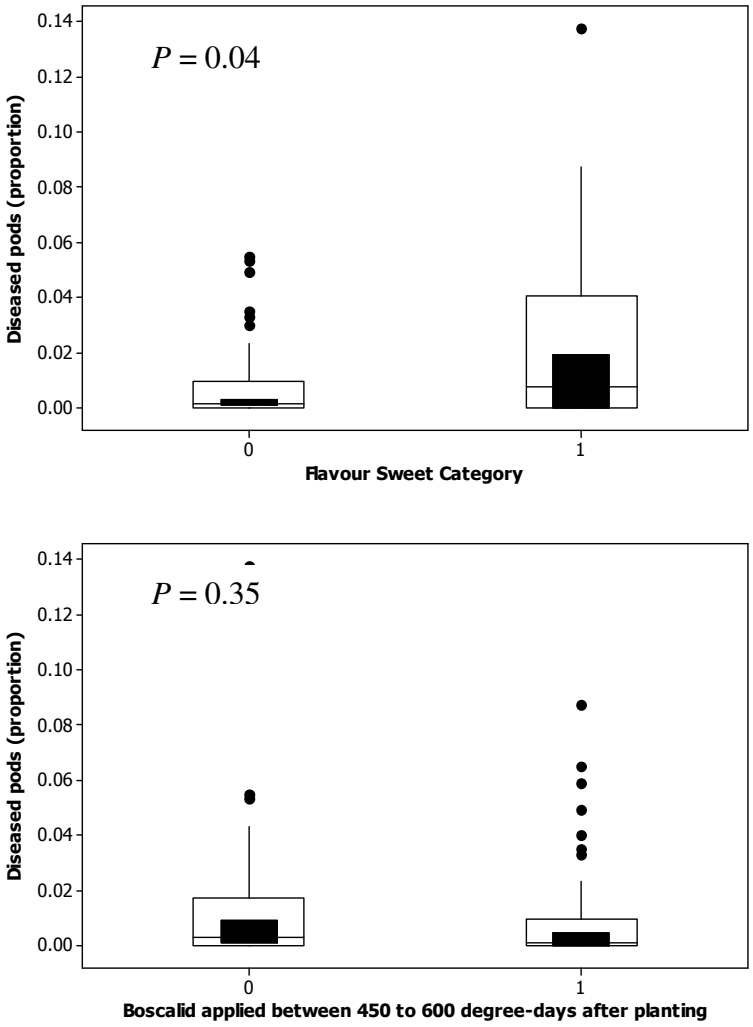
[b] Values for Spearman's rank correlation coefficient are not presented for Cultivar Flavour Sweet and Planting Density because these variables are categorical. The K-S test for these variables was conducted using the distribution of the incidence of pods with white mould in each category (i.e., Flavour Sweet "0" or "1", and Planting density low versus medium to high)

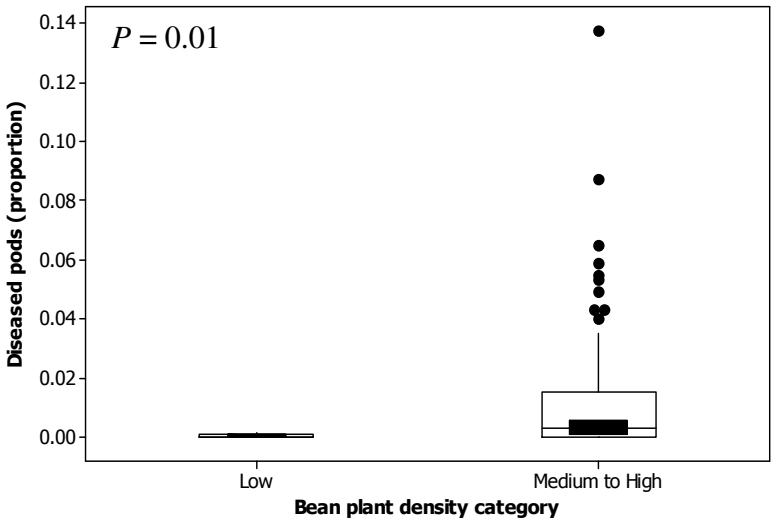
Table 6. Characteristics and performance of a nonparametric discriminant model for classifying fields as having white mould occur on pods

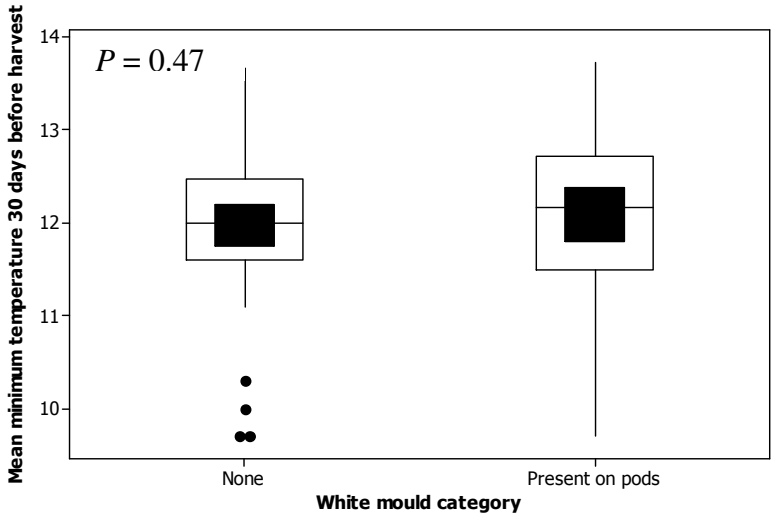
Model	**Cases[a]**	**Controls[b]**	**Accuracy[c]**	**Sensitivity[d]**	**Specificity[e]**
Model 1	69	40	0.77	0.84	0.65

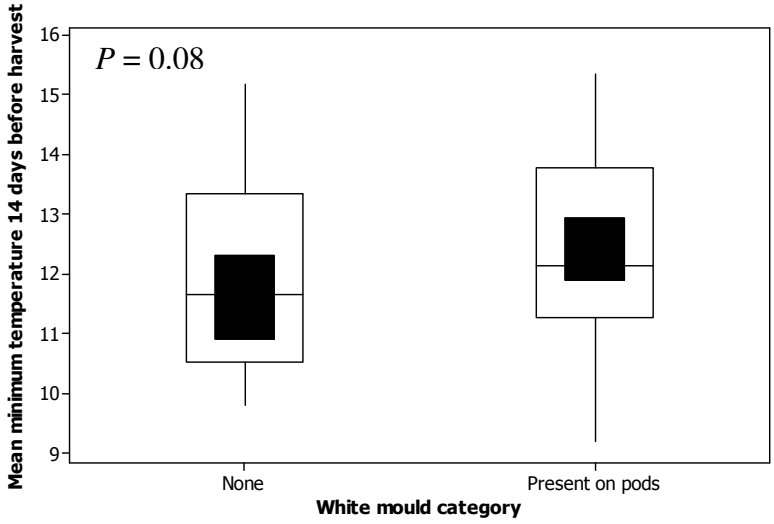
[a] Number of data sets where white mould occurred on pods.
[b] Number of data sets where white mould did not occur on pods.
[c] Proportion of total data sets classified correctly in cross validation.
[d] Proportion of cases classified correctly in cross validation.
[e] Proportion of controls classified correctly in cross validation.

Figure 12. Box plots showing the distribution of the incidence of pods with white mould in relation to discriminant model predictor variables. *P*-values indicate the significance of the K-S test that tests whether the data have a similar distribution between the two categories.




P = 0.01
Diseased pods (proportion)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Low
Medium to High
Bean plant density category


P = 0.47
Mean minimum temperature 30 days before harvest
14
13
12
11
10
None
Present on pods
White mould category


P = 0.08
Mean minimum temperature 14 days before harvest
16
15
14
13
12
11
10
9
None
Present on pods
White mould category

Discussion

Interpolated weather data estimated from the Data Drill algorithm was used as the main source of meteorological data for this study due to the lack of on-ground weather stations in northern Tasmania, and the prohibitive expense associated with locating sufficient data loggers at each of the sites sampled in this study. Pethybridge *et al*. (2009) showed relatively good Pearson correlation coefficients between on ground measurements and interpolated estimates from the Data Drill algorithm for daily rainfall ($r = 0.51$, $P < 0.001$), and daily mean temperature ($r = 0.85$, $P < 0.001$) at 12 sites across northern Tasmania. Their study reported that the mean error and mean absolute error for daily mean temperature were -0.17°C, (range -4.26 below to 3.91°C above the interpolated measurement), and 0.96°C (range 0 to 3.91 above the interpolated measurement), respectively. For daily rainfall, the mean error and mean absolute error between the measured and interpolated data were 0.49 mm (range -23.7 to 53.1) and 1.79 mm (range 0 to 53.1), respectively. Although the range of error for rain was large on some days, daily mean errors and mean absolute errors were relatively small. Pethybridge *et al*. (2009) were able to derive a number of site risk factors, including environmental, for ray blight disease of pyrethrum, by collecting information from a total of 105 fields over a three year period. Other studies examining the role of weather in site-risk have utilised information from on-ground weather stations located up to 20 km or more from particular fields (e.g. Mila *et al*. 2004).

This analysis has identified several risk factors associated with the occurrence of white mould occurrence on bean pods. Site-risk factors for the occurrence of white mould on pods include high minimum temperatures in the 10 to 30 days before harvest - which may be related to cloud cover, rain, and/or solar radiation, dense planting of beans, planting of the cultivar Flavour Sweet, and the timing of boscalid applications. These risk factors are consistent with the body of knowledge on this disease, and immediately suggest factors for producers and processors to manage to reduce disease risk. The association of mean minimum temperature to disease occurrence and incidence may be indirect. Days with higher minimum temperature tend to be characterised by cloudy conditions during the evening and morning hours, with or without rain. Indeed, solar radiation during the 10 to 30 days before harvest also was associated with the incidence of diseased pods but the strength of the correlation was weaker than minimum temperature. In other pathosystems, white mould is favoured by wet, rainy conditions. However, rain is one of the most difficult meteorological variables to forecast or estimate in interpolation algorithms due to its localized occurrence. Mean minimum temperature would tend to be less variable spatially, and thus may be serving as a proxy for generally rainy or cloudy conditions that may favour white mould outbreaks.

Designed experiments are warranted to better quantify the individual and combined effects of planting density, cultivar susceptibility, and boscalid timing on the occurrence and severity of white mould on pods.

DEVELOPMENT OF A PCR ASSAY FOR DETECTION OF *SCLEROTINIA SCLEROTIORUM* ASCOSPORES IN BEAN FIELDS.

Introduction

The primary infection mode of *S. sclerotiorum* on beans is through infection of senescent flowers by airborne ascospores. Infected flowers fall and adhere to plant stems and pods, and then the fungus spreads into healthy plant tissues. Hence fungicide applications usually target the flowering stage of the bean plant and aim to prevent the flowers becoming infected before they senesce and drop onto other plant parts. Currently boscalid (Filan®) is the only product registered for use on bean crops, with a maximum of three applications per crop recommended.
Identification of periods of airborne *S. sclerotiorum* ascospores may provide growers with an early warning of the risk of disease development. The ability to detect ascospore liberation could also reduce the number of fungicide applications by more closely targeting applications to when they are required, thereby reducing production costs and delaying the onset of fungicide resistance.

Disease forecasting systems have been developed for several plant diseases based upon the time at which inoculum of the pathogen is detected. However, most of these forecasting systems have not been adopted because the time and labour required for monitoring of inoculum, such as airborne spores. Ascospore release has been clearly associated with disease incidence and distribution of disease at harvest in crops such as oilseed rape (McCartney and Lacey 1991, 1992, 1999; McCartney et al. 1999). The prevalence and timing of ascospore release can be modelled in comparison to crop and disease development, and inclusion of a measurement of inoculum availability in forecasting models developed in Canada and the USA has significantly improved prediction of diseases caused by *S. sclerotiorum*.

Various strategies have been employed for assessing inoculum (e.g., ascospores) of *S. sclerotiorum*. Assessment of the level of petal infestation at various stages of flowering has been utilized for forecasting risk of Sclerotinia stem rot in spring-sown canola in Canada (Gugel and Morall 1986, Thomas 2003). Petals were collected from the field during flowering and analysed for the presence of *S. sclerotiorum*. Assessment of petal infection combined with leaf area index, light penetration and plant height predicted 55-98% of the variability in disease incidence at harvest (Turkington and Morall 1993). Others have attempted to model the relationship between weather and spore dispersal, and used weather monitoring as a means of warning growers of potential infection periods. For example, Clarkson *et al*. (2004) used laboratory and field experiments to derive relationships between temperature and soil water potential and carpogenic germination of sclerotia of *Sclerotina sclerotiorum* in an attempt to predict Sclerotinia leaf drop of lettuce. Thermal time analysis of buried sclerotia was shown to be a good predictor of the first appearance of apothecia in the field in each of two seasons. However, other unidentified factors were shown to influence germination of sclerotia at particular times during the season (Clarkson *et al*. 2004, Young *et*

al. 2005). Zi-Qing (2003) conducted a preliminary study of white mould disease in bean in Tasmania, which related periods of peak ascospore release to particular weather events. Although some relationships were evident, insufficient data was collected to develop a robust prediction system.

Other studies have shown a relationship between apothecial numbers and subsequent disease. However the small size of apothecia, make such assessment impractical as a means of forecasting disease. In many previous studies, ascospore monitoring has been conducted via techniques such as semiselective microbiological media (Ben-Yephet and Bitton 1985, Gutierrez and Shew 1998), trap plants, and various types of spore traps. Common spore traps include the Burkard (Burkard Manufacturing Co. Limited, Hertfordshire UK) 7-day volumetric trap (Hirst-type) and the Rotorod (Model 20 Rotorod Sampler, Multidata, St. Louis Park, Minnesota, USA) rotating arm sampler, which impact particles present in the air onto a thin layer of adhesive on a transparent tape, or glass rod respectively. The tape or rod is then traditionally observed under a microscope for the presence of spores. However, ascospores of *S. sclerotiorum* are difficult to differentiate visually from spores of some other fungi and microscopic examination is time consuming and laborious, and not practical over large production regions. However, recently developed, rapid molecular techniques now afford the ability to monitor ascospore release in near real time. This approach has been used successfully to time the initial fungicide application for control of powdery mildew of cherry and grape (McDonald and Boland 2004). In these systems, fungicide spray programs initiated 3 to 7 days (cherry) or 14 days (grape) after the first detection of airborne spores resulted in disease control equal to that achieved by regular calendar-based applications, but with two to three less sprays per season. This monitoring program could also help growers time fungicide applications later in the season because applications may not be necessary if airborne spores of the pathogen are absent. Similar approaches have recently been developed for *Sclerotinia sclerotiorum*. Freeman *et al*. (2002) conducted a preliminary study using a similar approach to white mould management in oilseed rape. PCR primers and methodologies were developed for detection of *Sclerotinia sclerotiorum* ascospores on adhesive tape from a Burkard volumetric spore trap placed in crops. The technique also detected *S. sclerotiorum* in petal samples, more rapidly than with conventional culturing techniques. Although the primers did not differentiate *S. sclerotiorum*, *S. minor* or *S. trifoliorum*, differences in the lifecycle and host range of these fungi suggested that it was unlikely that ascospores other than *S. sclerotiorum* would be detected in oilseed rape fields (Freeman *et al*. 2002). More recently, Rogers *et al*. (2009) have reported the development of a quantitative PCR for *S. sclerotiorum* and methodology for quantifying ascospores collected in spore traps in fields of oilseed rape in the U.K.

Identification of periods of airborne *S. sclerotiorum* ascospores may provide growers with an early warning of the risk of disease development. This would enable growers to apply fungicides only during high disease-risk periods, especially those that coincide with the vulnerable flowering period. The aim of this work was to develop a polymerase chain reaction (PCR) based assay to detect airborne ascospores of *S. sclerotiorum,* and to monitor ascospore release in bean crops.

Materials and methods

Collection of air samples in bean fields

Spore traps were placed in commercial bean crops in north-western Tasmania to monitor airborne ascospores of *S. sclerotiorum*. Two types of spore traps were used in the 2007-2008 and 2008-2009 seasons, (i) a 7 day Burkard volumetric spore trap (Burkard Manufacturing Co. Limited, Hertfordshire UK); and 2) a rotation arm impactor (Rotorod) sampler (Model 20 Rotorod Sampler, Multidata, St. Louis Park, Minnesota, USA). The Burkard trap sucks air through a slit orifice at 10 l/min. and impacts airborne particles including spores onto an Melinex tape treated with adhesive and mounted on a rotating drum. The Rotorod sampler model 20 is a rotating arm impactor that collects spores on two rapidly rotating (2400 RPM) rods, which provide a volumetric sample of approximately 22 l/min.

The traps were positioned approximately 40 cm above the ground near the centre of the fields. Traps were placed in one field in the Wesley Vale area in the 2007-2008 bean season and in four fields in 2008-2009 (two fields at Wesley Vale, one at Mersey Lea and one at Don). Only rotorod samplers were used in the 2009-2010 bean season, in two crops at Wesley Vale and two crops at Mersey Lea. The spore monitoring period in each crop was from pre-flowering to a few days before bean harvest, a period of approximately four to six weeks in each crop. The sampling periods were from 7 January to 8 February 2008, 29 December 2008 to 18 March 2009 and 12 January to 18 March 2010. Tapes were collected from the Burkard traps every seven days. Rods from Rotorod samplers were collected every three to four days. The two rods from each sampling period were stored separately in plastic screw-top containers at – 20°C.

PCR assay and primer design

Two sets of primers were used in this study. The primers of Freeman *et al*. (2002) (SSFWD/SSREV) which amplify a region of rDNA containing ITS regions were initially used. Freeman *et al*. (2002) reported these to detect *S. sclerotiorum*, *S. minor* and *S. trifoliorum*, but not other fungi including the closely related *B. cinerea*. However, in our study problems were encountered with lack of specificity (see below). Subsequently, new primers were designed (SIG2598F and SIG2767R) which targeted a region with more differentiation between these species, the intergenic spacer region (IGS) of the nuclear ribosomal RNA (rRNA) gene repeat unit.

Assay for sensitivity and specificity of PCR from S. sclerotiorum *ascospore concentrations*

DNA was extracted from spore suspensions and rotorod rods collected from the field with a Mo Bio UltraClean Soil DNA isolation kit (Mo Bio Laboratories, Inc Carlsbad, CA) according to the manufacturer's protocol.

PCR conditions were optimised on a gradient thermocycler (Bio-Rad C-1000, Bio-Rad Laboratories, Inc., Hercules, CA). Primers SIG2598F (ATCAGGGTGGTCCAGTTTTG) and SIGS2767R (TCGCATTCATAGAACGCTTG) were used to amplify a 170 bp region. Amplifications were performed in a total reaction volume of 20 µl. The PCR reaction mixture consisted of 1 × Qiagen PCR buffer with 1.5 mM $MgCl_2$ (Applied Biosystems Inc., Foster City, CA), 200 µM dNTPs, 0.2 µM each primer, 0.5 units *Taq* polymerase and DNA (20 to 40

ng from mycelium, various amounts from spore samples and rotorods). PCR reactions were performed on a GeneAmp PCR Systems 2400 thermocycler (Perkin Elmer, Norwalk, CT, USA). Cycling conditions consisted of initial denaturation for 10 min at 94ºC, followed by 40 cycles of 30 s denaturation at 94ºC, 30 annealing at 60ºC, 1 min extension at 72ºC, and a final 10 min extension step at 72ºC to complete the reaction. PCR products were visualised on a 1.25% agarose gel post stained with GelRed (Biotium Inc., Hayward, CA).

The PCR was optimised to improve sensitivity and minimise the risk of false negatives due to low ascospore quantities or through inhibition from debris that is likely to adhere to the rotorods in the field. The sensitivity of the PCR was tested with a range of ascospore concentrations (0, 10, 25, 50, 100 and 500 ascospores per DNA extraction). These ascospore concentrations were tested alone and with 10 milligrams of soil added to the DNA extraction. To further minimise the risk of false negatives, samples that tested negative with 1 µl DNA added to the PCR reaction were tested again with 3 µl DNA added to the PCR reaction.

The specificity of the PCR was tested with other *Sclerotinia* spp., *B. cinerea* and several other fungal species (*Alternaria alternata*, *Cladosporium* sp., *Fusarium* sp., *Microsphaeropsis tanaceti*, *Phoma ligulicola* var. *inoxydablis and Phoma exigua* var. *exigua*).

Detection of airborne S. sclerotiorum *from field samples*

Rotorods from the nine bean fields that were sampled (one from 2008, four from 2009 and four from 2010) were tested with this PCR assay. DNA was extracted from one of the two rods from each sampling period (three to four days) and used for the PCR assay. Samples that tested negative with the initial 1 µl DNA were tested a second time (as described above), to minimise the chance of a false negative result.

Selected tapes from Burkard traps at Sites 2 and 3 (Table 8) were examined for *S. sclerotiorum* ascospores. The tapes were cut into 24 h sections, mounted on microscope slides with a 1 hour increment transparency template and stained with aniline blue. Tapes were examined along three equidistant traverses across the long axis (across the 24 h) under 400 × magnification. Spores were only counted as ascospores of *S. sclerotiorum* if they were of similar shape, size and pigmentation as reference slides. The aim was to identify ascospores to validate detection by PCR, not to accurately quantify spore numbers.

Weather stations (Watchdog 450, Spectrum Technologies) were installed at each field to monitor ambient temperature, relative humidity, leaf wetness, soil moisture and temperature.

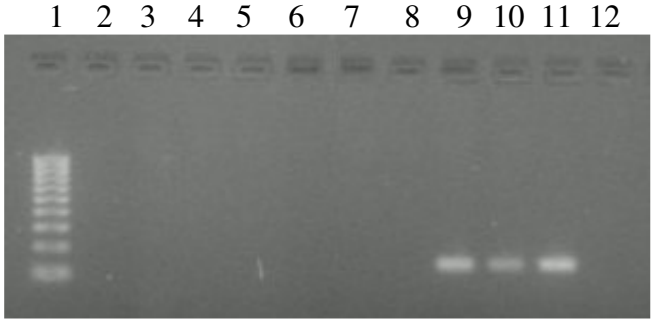
Results

Testing of specificity of the SSFWD/SSREV primers (Freeman *et al*. 2002) demonstrated that these primers also detected *B. cinerea* and amplified a fragment the same size as *S. sclerotiorum* (278 base pairs). Attempts to adjust PCR conditions to improve

specificity were unsuccessful (data not shown). Comparison of sequences on GenBank showed that the regions where these primers anneal were almost identical between *S. sclerotiorum* and *B. cinerea.* As *B. cinerea* is a common airborne fungus, and frequently found in bean crops, these primers were considered to be unsuitable for aerobiological monitoring of *S. sclerotiorum* ascospores in this study.

The primers designed in this study amplified a 170 bp fragment, and were specific to *S. sclerotiorum*, *S, trifoliorum* and *S. minor* but did not amplify several other fungal species including *B. cinerea* (Plate 2).

Plate 2. PCR amplification using *S. sclerotiorum* specific primers SIG2598F and SIG2767R to amplify 170 bp of DNA prepared from mycelium. Lane 1, 100 bp DNA ladder; Lane 2, *Alternaria alternata*; Lane 3, *Cladosporium* sp. Lane 4, *Fusarium* sp.; Lane 5, *Microsphaeropsis tanaceti*; Lane 6, *Phoma ligulicola* var. *inoxydablis*; Lane 7, *Phoma exigua* var. *exigua*; Lane 8, *B. cinerea*; Lane 9, *S. minor*; Lane 10, *S. trifolium*; Lane 11, *S. sclerotiorum*; Lane 12, no DNA control.



The PCR method detected DNA of *S. sclerotiorum* as low as 10 spores per DNA extraction (0.2 ascospores per PCR) (Table 8). Detection was not affected by the presence of soil for ascospore concentrations of 50, 100 and 500 ascospores per DNA extraction. Soil reduced PCR sensitivity at 25 and 10 ascospores per DNA extraction by 10 % and 30 % respectively (Table 7).

Table 7. The number of positive PCR tests/total number of PCR samples for each ascospore concentration with and without 10 mg soil added to the DNA extraction.

Ascospores per DNA extraction	Ascospores per PCR with 1 µl DNA (3 µl DNA) added to reaction	Number of positive tests/total number of samples	
		Ascospores only	Ascospores + 10 mg soil
0	0	0/10	0/10
10	0.2 (0.6)	10/10 (1)[a]	7/10 (2)[a]
25	0.5 (1.5)	10/10 (1)[a]	9/10 (0)[a]
50	1	10/10	10/10
100	2	10/10	10/10
500	10	10/10	10/10

[a] number of samples that were negative when tested with 1 µl DNA but positive when tested with 3 µl DNA

Table 8. Detection of *S. sclerotiorum* air samples, collected on a rotorod spore trap, by PCR amplification using primers SIG2598F and SIG2767R. Each sampling period represents three or four days.

Site no.	**Location**	**Total period monitored**	**Sampling period**											
			1	2	3	4	5	6	7	8	9	10	11	12
1	Wesley Vale	08-Jan-08 to 04-Feb-08	+	×	×	+	-	×	×					
2	Wesley Vale	29-Dec-08 to 05-Feb-09	-	×	-	-	+	×	×	×	×	×	×	
3	Mersey Lea	13-Jan-09 to 17-Feb-09	-	-	-	×	-	+	×	×	+			
4	Wesley Vale	12-Feb-09 to 18-Mar-09	×	×	×	×	+	-	-	+	×	×		
5	Don	23-Feb-09 to 18-Mar-09	+	+	-	×	-	+	-					
6	Wesley Vale	12-Jan-10 to 05-Feb-10	+	-	-	+	+	-	-	-				
7	Mersey Lea	12-Jan-10 to 19-Feb-10	-	-	+	-	-	+	-	-	-	+	-	-
8	Mersey Lea	12-Feb-10 to 09-Mar-10	-	+	-	-	×	×	-	-				
9	Wesley Vale	08-Feb-10 to 18-Mar-10	+	-	-	+	-	+	+	-	+	+	+	

+ = positive with 1 µl DNA added to PCR; × = positive with 3 µl DNA added to PCR; - = negative result; blank spaces not sampled.

DNA of airborne inoculum of *S. sclerotiorum* was detected by PCR on rods of rotorod spore traps obtained from all nine fields sampled. Ascospores were detected in the air during most sampling periods in 2009, but occurred more intermittently in the 2010 season (Table 8). *Sclerotinia sclerotiorum* ascospores were not found on the Burkard tapes in periods that corresponded to negative PCR results, and very few ascospores were conclusively identified in periods that coincided with PCR detection.

While there were insufficient data points to statistically test relationships between weather variables and ascospore detection, *S. sclerotiorum* ascospores were generally detected within a day or two of rainfall (Appendix 2). PCR detection periods start before rainfall occurrence in some instances because the PCR monitoring periods were three or four days duration. Selected weather data for the sites monitored with spore traps are summarised in Appendix 3.

Discussion

Because of the similarities between Sclerotinia spp. and *B. cinerea* in the ITS region of rDNA, Freeman *et al*. (2002) developed a touch-down PCR protocol to avoid co-detection of *B. cinerea*. However, using this method we were unable to avoid detection of *B. cinerea*, even when more stringent annealing temperatures were utilized. Therefore primers which targeted a region of greater differentiation (IGS) were developed. The IGS primers were designed specifically to detect *S. sclerotiorum*, but also detected *S. trifoliorum* and *S. minor*, similar to those of Freeman *et al*. (2002). All three species have potential to produce ascospores but detection of *S. minor* was not considered to be problematic for aeriobiological monitoring in bean crops because apothecia production of *S. minor* has rarely been observed in the field in Australia (Ekins *et al.* 2002). Furthermore, *S. minor* was not detected in any of the crops monitored with spore traps during the surveys of 64 points throughout each field just prior to harvest and all isolates collected from these sites were identified morphologically as *S. sclerotiorum*. *Sclerotinia minor* initiates infection through mycelia that arise from sclerotia and infect the host directly while airborne ascospores are the principal infection means for *S. sclerotiorum* (Wong 1978). The apparent rareness of *S. minor* apothecia compared to *S. sclerotiorum* could also be partially due to a more stringent temperature range required for *S. minor*. *Sclerotina sclerotiorum* produced apothecia over a temperature range from 4°C (Smith and Boland 1989) to 30°C (Huang and Kozub 1993). The temperature range for apothecia development for *S. minor* was from 11 to 17°C (optimum 15°C) and a few hours at 20°C or higher was sufficient to inhibit stipe initiation (Hawthorne 1976). Hence apothecia production is likely to occur only in cooler seasons of spring and autumn *S. minor.* The temperature range for apothecial development is thought to be similar for *S. trifoliorum* as it has been observed almost exclusively in autumn (Williams and Western 1965). For this reason, and because *S. trifoliorum* is confined to infection of forage legume crops (Willetts *et al.* 1980), PCR detection of this species in Tasmanian bean crops was considered to be unlikely. Freeman *et al*. (2002) also suggested that the biology of the *Sclerotinia* spp. was

sufficiently different to suggest that detection of ascospores of species other than *S. sclerotiorum* in fields of oilseed rape was unlikely.

Apothecia development and ascospore release are reported to occur most readily after a cool (10 to 20°C) moist period of several days (Wong 1978) and generally after canopy closure (Boland and Hall 1987). PCR detection of airborne *S. sclerotiorum* inoculum in Tasmanian bean crops showed that ascospores were present in the pre-flowering stage and before row closure. This agrees with the findings of HAL project VG00020 (Zi-Qing 2003), where ascospores were observed from pre-planting to harvest of bean crops, and demonstrates that apothecia develop and release ascospores under a wide range of conditions.

The presence of low numbers of spores over a long period may be sufficient to cause an epidemic (Rogers *et al.* 2009). Hence detection of low numbers of spores at the time when plants are most vulnerable to infection is important. The PCR method developed in this study allowed very low amounts (1 ascospore per PCR reaction) of airborne *S. sclerotiorum* inoculum to be detected consistently, even in the presence of soil. This method was more sensitive than PCR diagnostic methods reported by Freeman *et al.* (2002) and Rogers *et al.* (2009) where detection limits were 10 and <1.4 ascospores per PCR respectively.

Burkard traps were included in this study to provide a means of validating the PCR results. However ascospores were difficult to conclusively identify, even during periods that coincided with positive PCR results. This may be partially explained by the greater volume of air sampled by the rotorod sampler (22 l/min) compared to the Burkard trap (10 l/min). Additionally the PCR was able to detect very low numbers of ascospores (<1 ascospore per PCR reaction or 10 per rotorod sample). By comparison, Rogers *et al.* (2009) found that it was usually not possible to detect less than 30 to 40 ascospores per day on Burkard traps unless the entire tape was examined. This was not a feasible in practice because examination of two 2 mm traverses of 48 mm tape, which represents only approximately 2.9% of a 24 h period, can take more than 2 hours (Rogers *et al.* 2009). Another possible explanation for the lack of *S. sclerotiorum* ascospore detection on the Burkard tapes is that the spores were masked by soil and debris, or by masses of pigmented spores and hyphal fragments of other fungal species similar to that reported by Rogers *et al*. (2009).

Further work is required to determine relationships between climatic and environmental factors and timing of spore release in Tasmanian bean crops. The small primer set developed in this study (SIG2598F and SIG2767R) could potentially be used for quantitative PCR (qPCR) and enable quantification of ascospores over a given period of time. Daily monitoring of samples by qPCR, combined with environment data, may provide detailed information on the factors that promote *S. sclerotiorum* ascospore release. This information could be used to develop a predictive model for the risk of disease development and enable growers to target high risk periods with fungicide applications. Testing over a wide geographical range and over a number of seasons would be needed to confirm that airborne inoculum is a reliable indicator of white mould disease risk.

SENSITIVITY OF *SCLEROTINIA SCLEROTIORUM* TO THE FUNGICIDE BOSCALID

Introduction

Management of white mould disease in bean relies mainly upon the use of fungicides at the flowering stage to prevent infection of the senescing petals. Boscalid was first registered for use on Tasmanian bean crops in December 2004, following the withdrawal of Sumisclex (procymidone). Boscalid (Filan®, BASF Ltd.) is the only fungicide currently registered for control of *S. sclerotiorum* on bean crops. Current recommendations are to apply 800 to 1000 grams of product (Filan 500 g/kg boscalid) per hectare, with the first application at early flowering and subsequent applications at 7 to 10 day intervals. A maximum of three applications per crop is recommended to reduce the potential for the development of resistance in the *S. sclerotiorum* population. Boscalid is an inhibitor of succinate dehydrogenase and is classified in group 7 (FRAC 2009). Resistance to boscalid has been reported in several fungi including *Alternaria* spp. in apple, pistachio and potato, *B. cinerea* in strawberry and *Didymella* spp. in cucurbits, watermelon and chickpea (FRAC 2009). In Tasmania, boscalid is also used for control of *S. sclerotiorum* and other fungal diseases on vegetable and horticultural crops including potatoes, onions, carrots, lettuce, pyrethrum, brassica and brassica leafy vegetables. These crops are often included in rotations with beans. Hence *S. sclerotiorum* fungal populations are potentially exposed to numerous applications of boscalid over time and may develop reduced sensitivity. The aim of this study was to determine the baseline sensitivity to boscalid of *S. sclerotiorum* isolates from bean crops and to determine if there is currently any evidence of reduced sensitivity in the fungal population.

Materials and methods

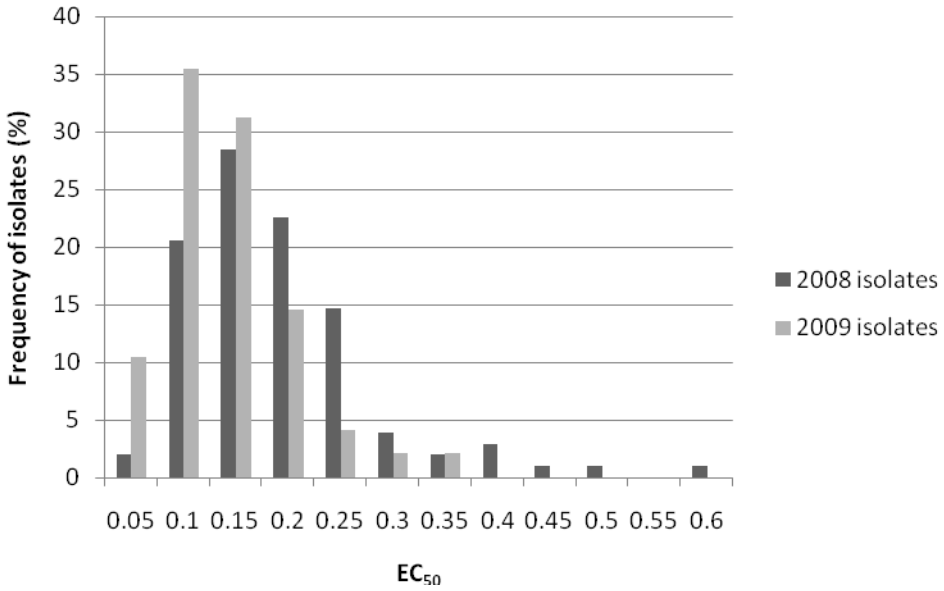
One hundred and two *S. sclerotiorum* isolates collected from 23 bean crops in the 2008 season and 48 isolates from 32 bean fields in the 2009 season were tested for sensitivity to boscalid. For comparison with unexposed isolates, 11 *S. sclerotiorum* isolates sourced from pyrethrum and other crops prior to the introduction of boscalid, and maintained in culture collections, were also tested. Stock solutions of fungicide of varying concentration were made by dissolving the required amounts of Filan (BASF Limited, Research Triangle Park, NC) fungicide (500 g/kg boscalid) in sterile distilled water and making up to volume with ethanol to obtain a sterile 70% solution of ethanol. An appropriate aliquot of stock solution was then mixed with 400 ml potato dextrose agar (PDA) which had been autoclaved and cooled to 60°C to obtain concentrations of 0, 0.01, 0.05, 0.5, 5.0 and 50.0 µg/ml. PDA was poured into 9 cm diameter polystyrene petri dishes in a laminar flow cabinet and allowed to dry overnight. For each isolate, three replicates of each concentration were inoculated with a 4-mm plug of mycelium taken from the edge of a colony (< 7 days old) grown on PDA at 20°C. Mycelial growth was measured after 48 hours incubation in darkness at 20°C. The sensitivity of each isolate to boscalid was determined by probit analysis, which calculated the effective concentration of active ingredient required to cause 50% inhibition (EC_{50}) of mycelial growth rate. Inhibition was calculated as (=1 – (mean growth rate on amended media

divided by the mean growth rate on unamended media)). Probit analyses were done using Statistical Analysis System (Version 9.0) software and a generalised form of the macro (Hsiang *et al.* 1997).

Results

The mean EC_{50} for the 2008 isolates was 0.166 µg/ml, and ranged from 0.011 to 0.575 µg/ml. The mean EC_{50} for the 2009 isolates was 0.115 µg/ml, and ranged from 0.27 to 0.311 µg/ml. Frequency distributions of the EC_{50} values for each of the two seasons showed a unimodal distribution (Figure 13). For the 11 isolates not previously exposed to boscalid, the EC_{50} values ranged from 0.017 to 0.159 µg/ml, with a mean of 0.095 µg/ml.

Figure 13. Frequency distribution of effective concentrations of boscalid to inhibit 50% of mycelial growth (EC_{50}) for *S. sclerotiorum* isolates collected from bean crops in 2008 (*n* = 102) and 2009 (*n* = 48).



Discussion

Results suggested that the isolates tested were sensitive to boscalid based on (i) a unimodal frequency distribution of EC_{50} values, (ii) similarity between the EC_{50} values of *S. sclerotiorum* isolates collected from bean and those collected from pyrethrum prior to the use of boscalid, and (iii) similarity to baseline sensitivity of *S. sclerotiorum* in other studies. Furthermore, there was no evidence of reduced sensitivity in isolates collected in 2009 in comparison to those collected in 2008. Among the 102 *S. sclerotiorum* isolates collected in 2008, 75 had EC_{50} values lower than 0.2 µg/ml. For isolates collected in 2009, 46 of the 48 isolates tested were below this EC_{50} level. EC_{50} values recorded in our study were similar to those reported overseas. Liu *et al*. (2009) reported baseline sensitivity of 161 isolates of *S. sclerotiorum* from rapeseed to boscalid of 0.002 to 0.391 µg/ml with a mean of 0.042 µg/ml. Three isolates from the 2008 season had slightly higher EC_{50} values (0.4 to 0.6 µg/ml), but this may reflect natural variability rather than reduced sensitivity. By comparison much higher EC_{50} values have been recorded for fungi in which resistance to boscalid has been suspected. For example 38 out of 46 *Alternaria alternata* isolates from Californian pistachio orchards were highly insensitive to boscalid and had EC_{50} values >500 µg/ml (Avenot *et al.* 2008). Control failures had not been observed in these orchards, but boscalid had been applied two or three times per season for up to four years. Our results indicate that poor control or control failures in Tasmanian bean fields were unlikely to be due to reduced sensitivity to boscalid. Other factors such as high disease pressure or poor efficiency of applications due to suboptimal environmental conditions or application technique may result in poor efficacy of fungicide.

Filan® is currently registered for use on green beans until 2013 and is currently the only fungicide used for control of *S. sclerotiorum*. Hence monitoring in forthcoming seasons is needed to assess the on-going efficacy of boscalid. The data generated in this study provided baseline sensitivity data the will enable boscalid sensitivity to be monitored in the future.

GENERAL DISCUSSION

This project has provided valuable baseline information required for the development of methods of site risk assessment, detection of ascospores of *S. sclerotiorum* in bean fields, assessment of disease incidence within fields, and current status of fungicide resistance to boscalid in the *S. sclerotiorum* population.

White mould disease was prevalent in bean fields in northern Tasmania in both the 2008/2009 and 2009/2010 seasons. For the 2008/2009 season, white mould disease was detected on either pods or plants in 36 of the 52 fields surveyed. However, incidence was generally low with 50 fields with less than 5% disease incidence on the pods, and 38 fields with less than 5% diseased plants. For the 2009/2010 season, white mould disease was detected on plants or pods in 44 of 57 fields surveyed. Fifty three fields had less than 5% disease on pods and 41 fields had less than 5% disease on the plants. Mean disease incidence on pods across all fields was 1.05 and 1.08 % for the 2008/2009 and 2009/2010 seasons respectively, and on plants was 3.80 and 5.61 % for the 2008/20099 and 2009/2010 seasons respectively.

A variety of methods of spatial analysis demonstrated that the incidence of pods with white mould disease was characterized by a largely random pattern of disease incidence at the scale of individual plants, with some patches of plants with similar disease levels on pods occurring at a scale of one metre or greater. However, the incidence of plants (versus pods) with white mould tended to be slightly to moderately aggregated. Although processes responsible for patterns cannot be deduced conclusively from this analysis, the small scale aggregation on pods but greater degree of aggregation on plants observed in the current study suggests localized spread of ascospores within bean fields. If inoculum is truly dispersed predominantly locally, this suggest that management efforts should be directed at reducing primary inoculum levels within individual fields rather than focusing management efforts on a farm- or regional scale.

The spatial analyses also provided the foundation for developing statistically sound sampling approaches for estimating disease incidence or classifying disease incidence above or below the provisional industry threshold for crop rejection. The sampling plans developed in this research were not intended to inform decisions regarding application of control measures. Rather, the sampling plans were designed to quantify disease levels on pods near harvest. Given the considerable amount of labor and resources needed to assess disease incidence, these sampling plans will facilitate future research efforts and also assist industry in designing their sampling schemes for deciding which fields should be harvested or not. The sequential estimation sampling plans tend to perform appropriately when disease incidence is at least 4%, which is near the provisional thresholds sometimes used for crop rejection. When only a classification of disease incidence is required, the sequential classification plans developed herein would provide a very useful tool for accurately classifying fields after inspection of relatively few sampling units.

Analysis of survey data collected from 52 bean fields in the 2008/2009 season and 57 fields in the 2009/2010 season has identified several risk factors associated with the

occurrence of white mould occurrence on bean pods. Site-risk factors for the occurrence of white mould on pods included high minimum temperatures in the 10 to 30 days before harvest - which may be related to cloud cover, rain, and/or solar radiation, dense planting of beans, planting of the cultivar Flavour Sweet, and the timing of boscalid applications. These risk factors are consistent with the body of knowledge on this disease, and suggest factors for producers and processors to manage to reduce disease risk. Designed experiments are warranted to better quantify the individual and combined effects of planting density, cultivar susceptibility, and boscalid timing on the occurrence and severity of white mould on pods.

PCR primers were designed using the intergenic spacer region (IGS) of the nuclear ribosomal RNA (rRNA) gene repeat unit and were shown to detect *S. sclerotiorum*, *S. minor* and *S. trifoliorum*, but not the closely related fungus *Botrytis cinerea*. The PCR was highly sensitive and was able to detect DNA concentrations of *S. sclerotiorum* as low as 0.2 ascospores per PCR reaction. DNA from *S. sclerotiorum* was successfully detected even in the presence of 10 mg of soil, suggesting the technique was robust enough to be used for monitoring for ascospores in fields. Subsequently, airborne inoculum of *S. sclerotiorum* was detected periodically during the season in bean fields by PCR from rotorod spore traps. The PCR assay has potential for incorporation into a risk management system for *S. sclerotiorum* in bean crops or for use in disease development studies. Subsequently, Rogers *et al*. (2009) have reported a quantitative PCR for specific detection of *S. sclerotiorum* which, although it requires equipment which is considerably more expensive than the methods described in our study, does enable quantification of ascospore numbers.

A total of 102 isolates of *S. sclerotiorum* collected from 23 bean crops in the 2008 season and 48 isolates from 32 bean fields in the 2009 season were tested for sensitivity to boscalid and compared with 11 unexposed isolates sourced from pyrethrum crops and culture collections, prior to the use of boscalid. Isolates were tested on potato dextrose agar at concentrations of 0, 0.01, 0.05, 0.5, 5.0 and 50.0 µg/ml boscalid. The frequency distribution of EC_{50} values were unimodal. For 2008 isolates, the mean EC_{50} was 0.166 µg/ml, and ranged from 0.011 to 0.575 µg/ml. For 2009 isolates, the mean EC_{50} was 0.115 µg/ml and ranged from 0.27 to 0.311 µg/ml. For the 11 isolates not previously exposed to boscalid, EC_{50} ranged from 0.017 to 0.159 µg/ml, with a mean of 0.095 µg/ml. There was no evidence of reduced sensitivity of *S. sclerotiorum* from bean in Tasmania to boscalid. This study will provide valuable baseline sensitivity data for monitoring for fungicide resistance in future years.

Further survey work is required to improve the accuracy of the site specific risk assessment, and to develop this into a format which would be of use to growers. Similarly, the PCR techniques developed in this project could be used in further research to more clearly derive an association between environmental conditions and ascospore release. This information could then be combined to provide growers with a disease risk prediction system where site risk may be quantified and forecasted. Furthermore this would provide a management decision support system where sites more prone to disease development may be avoided or managed differently, while those of low risk may require less chemical input and be managed less stringently to control white mould.

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Appendix 1. Summary of disease incidence for **(A)** 2008/2009 and **(B)** 2009/2010 bean seasons.

(A) Disease incidence (%) in 52 fields surveyed in 2008/2009 Tasmanian bean season.

Site no.	Location	% diseased pods/1280 pods	% diseased plants/640 plants
1	Moriarty	0.00	0.31
2	Sassafrass	3.52	6.56
3	Thirlstane	0.47	0.63
4	Cressy	0.31	1.41
5	Moriarty	0.00	0.00
6	Sassafrass	0.00	0.16
7	Sassafrass	0.00	0.00
8	Rocky Cape	4.92	34.53
9	Rocky Cape	0.31	0.31
10	Boat Harbour	0.00	0.00
11	Boat Harbour	0.23	1.09
12	Forest	1.17	3.75
13	Hagley	1.88	10.47
14	Hagley	0.47	3.13
15	Moriarty	0.00	0.00
16	Kimberly	0.00	0.00
17	Bishopbourne	0.00	0.00
18	Mersey Lea	0.00	0.00
19	Mersey Lea	0.00	0.00
20	Forest	1.88	5.00
21	Cressy	0.00	0.00
22	Cressy	0.00	0.00
23	Moriarty	0.94	0.47
24	Hagley	1.17	3.28
25	Hagley	0.31	0.78
26	Mersey Lea	0.00	0.00
27	Forest	2.27	11.88
28	Thirlstane	0.16	0.31
29	Hagley	1.09	6.56
30	Forest	0.00	0.00
31	Forest	0.47	1.09
32	Thirlstane	0.63	0.16
33	Longford	0.00	0.00
34	Sassafras	0.70	6.72
35	Wesley Vale	2.34	9.06
36	Don	0.08	0.31
37	Cressy	2.97	11.09

Site no.	Location	% diseased pods/1280 pods	% diseased plants/640 plants
38	Wesley Vale	5.47	4.53
39	Cressy	0.16	2.66
40	Penguin	0.00	0.00
41	Forth	1.88	7.81
42	Forth	3.28	10.16
43	Don	0.00	0.00
44	Mersey Lea	0.00	1.56
45	Mersey Lea	6.48	21.72
46	Sassafrass	0.00	0.31
47	East Devonport	1.56	5.00
48	Wesley Vale	4.30	4.06
49	Sassafrass	1.88	17.97
50	Wesley Vale	0.00	0.00
51	Squeaking Point	0.08	0.16
52	Bishopbourne	1.41	2.50

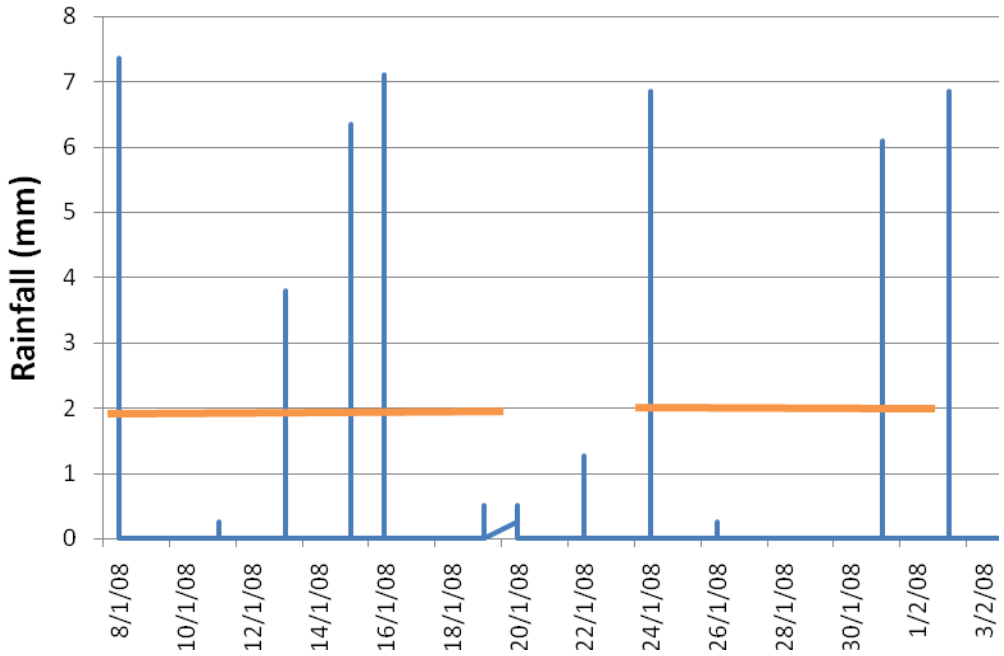
(B) Disease incidence (% diseased pods and plants) in 57 fields surveyed in 2009/2010 Tasmanian bean season.

Site No.	Location	% diseased pods/1280 pods	% diseased plants/640 plants
1	Rocky Cape	1.80	9.22
2	Forest	0.86	4.53
3	Forest	1.72	7.50
4	Forest	0.16	3.75
5	Forest	0.00	0.31
6	Thirlstane	0.00	0.00
7	Hagley	0.16	0.31
8	Forth	0.08	0.31
9	Sassafrass	3.98	14.22
10	Northdown	0.00	0.00
11	Northdown	0.00	0.47
12	Harford	0.00	0.00
13	Barrington	1.80	4.84
14	Barrington	0.08	1.88
15	Barrington	0.08	0.63
16	Barrington	0.08	0.94
17	Sassafrass	0.00	0.47
18	Cressy	5.31	13.91

19	Cressy	0.55	1.88
20	Flowerdale	0.00	0.47
21	Westbury	0.55	5.00
22	Westbury	0.00	1.41
23	Flowerdale	0.16	0.94
24	Flowerdale	0.47	1.41
25	Mersey Lea	0.16	0.31
26	Mersey Lea	8.75	73.59
27	Hagley	0.31	6.72
28	Sassafrass	0.08	0.31
29	Forth/Don	0.16	1.88
30	Sassafrass	0.00	0.00
31	Don	0.08	2.03
32	Sassafrass	4.30	18.13
33	Forth/Don	1.17	5.31
34	East Devonport	0.08	0.47
35	East Devonport	0.00	0.00
36	East Devonport	0.00	0.16
37	Westbury	2.03	8.13
38	Mersey Lea	1.02	5.47
39	Cressy	0.00	0.00
40	Thirlstane	1.95	16.88
41	Northdown	0.47	4.22
42	Cressy	0.08	1.25
43	Northdown	0.78	6.25
44	Mersey Lea	5.86	30.78
45	Mersey Lea	13.75	46.56
46	Harford	0.00	0.00
47	Flowerdale	0.16	3.44
48	Wynyard	0.00	0.00
49	Cressy	0.00	0.00
50	Bishopbourne	0.00	0.31
51	Cressy	0.00	0.00
52	Sassafrass	0.00	0.00
53	Sassafrass	0.55	1.25
54	Sassafrass	0.00	0.00
55	Wesley Vale	0.94	8.59
56	Wesley Vale	1.33	3.13
57	Forth	0.00	0.00

Appendix 2. Daily rainfall and PCR detection of *S. sclerotiorum* ascospores at **(A)** Wesley Vale Site 1, 2008; **(B)** Wesley Vale Site 2, 2009; **(C)** Wesley Vale Site 4, 2009; **(D)** Don Site 5, 2009; **(E)** Wesley Vale Site 6, 2010; **(F)** Mersey Lea Site 7, 2010 **(G)** Mersey Lea Site 8, 2010 and **(H)** Wesley Vale Site 9, 2010. The horizontal orange lines are periods when ascospores were detected by PCR.

A



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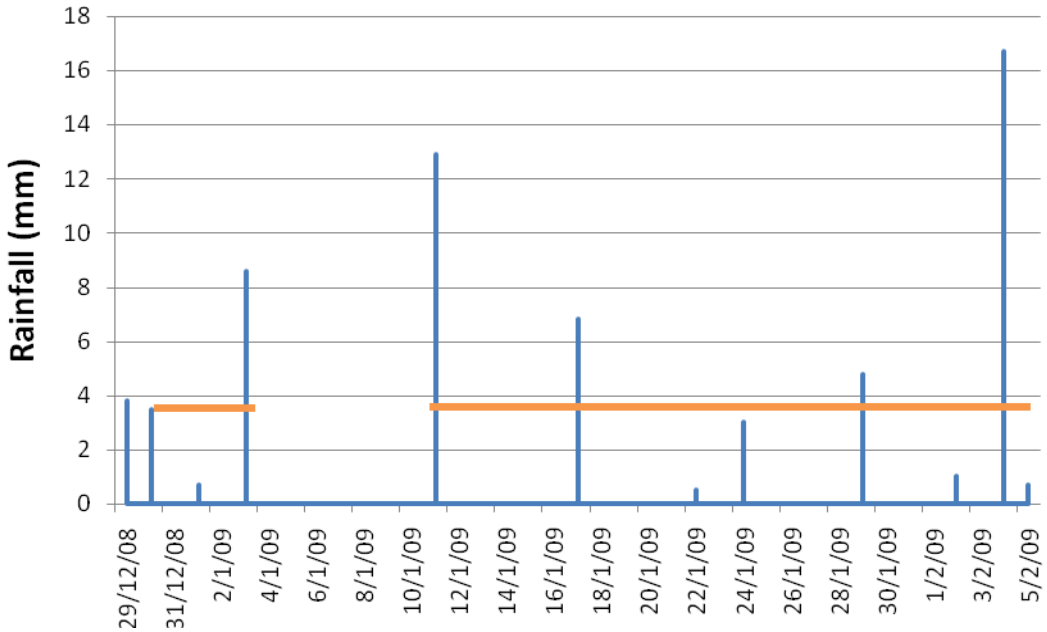

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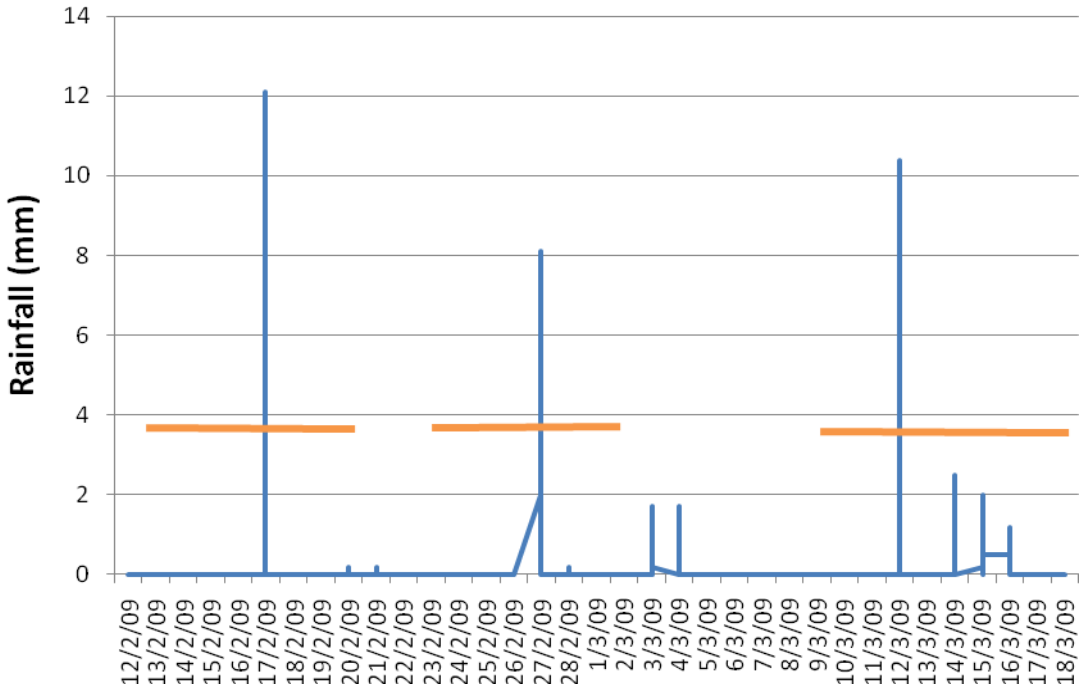
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10
8
6
4
2
0
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13/2/09
14/2/09
15/2/09
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17/2/09
18/2/09
19/2/09
20/2/09
21/2/09
22/2/09
23/2/09
24/2/09
25/2/09
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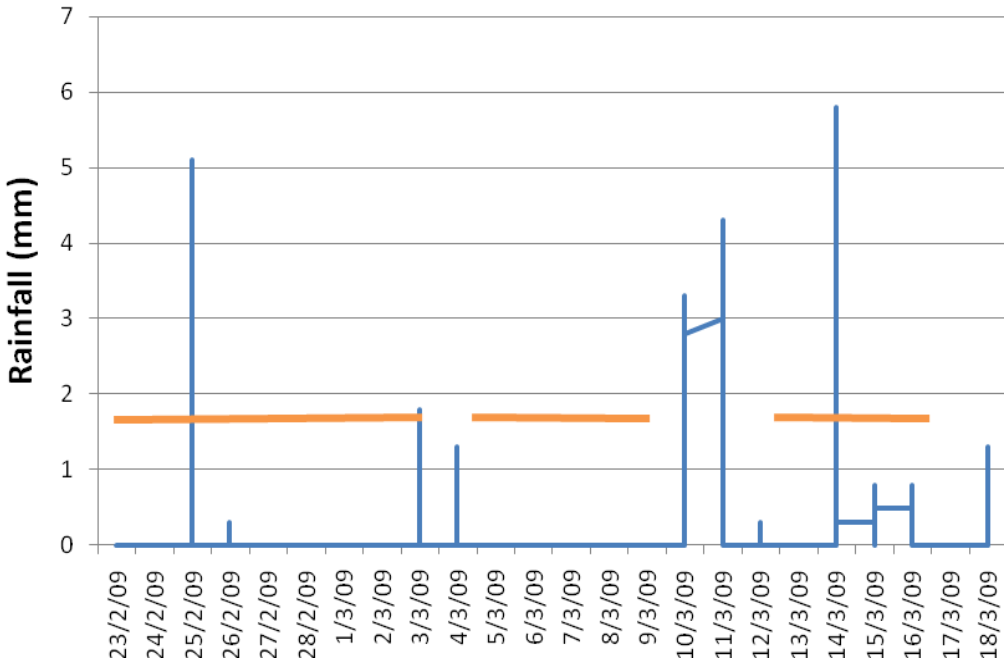
17/3/09
18/3/09

D


Rainfall (mm)
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6
5
4
3
2
1
0
23/2/09
24/2/09
25/2/09
26/2/09
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28/2/09
1/3/09
2/3/09
3/3/09
4/3/09
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9/3/09
10/3/09
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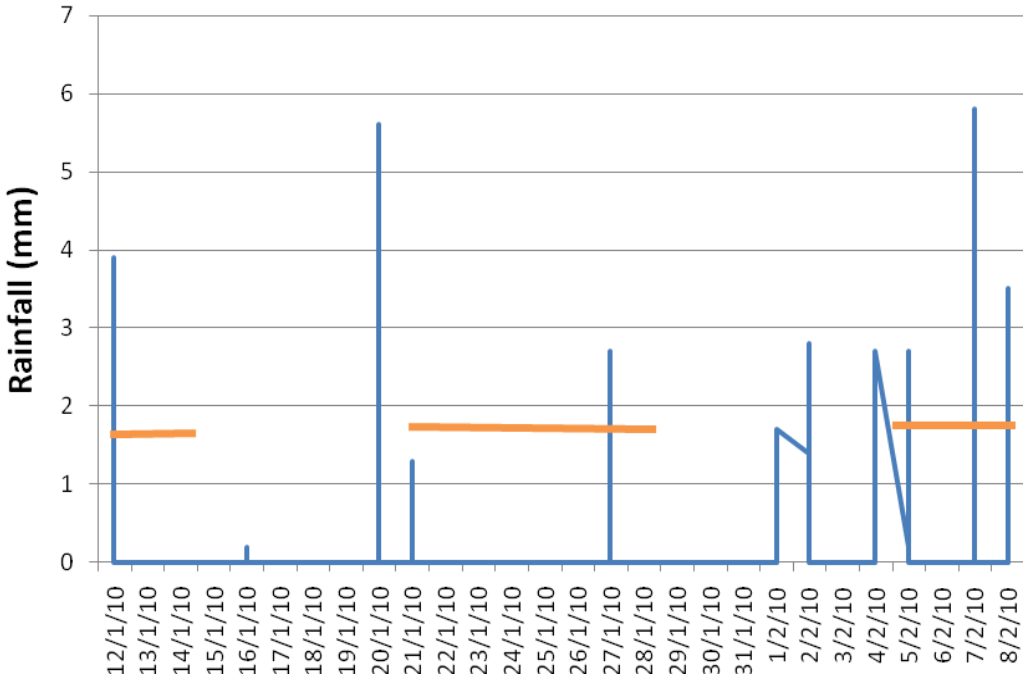
14/3/09
15/3/09
16/3/09
17/3/09
18/3/09

E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1
2
3
4
5
6
7
12/1/10
13/1/10
14/1/10
15/1/10
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17/1/10
18/1/10
19/1/10
20/1/10
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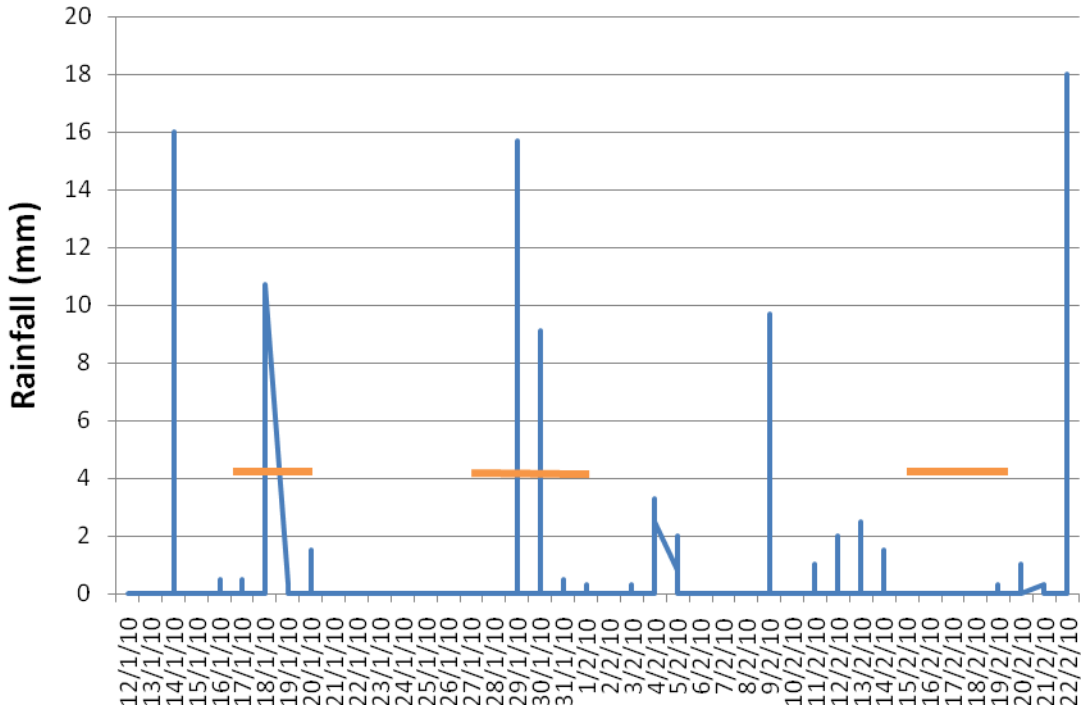
7/2/10
8/2/10

F


Rainfall (mm)
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2
4
6
8
10
12
14
16
18
20
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13/1/10
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17/1/10
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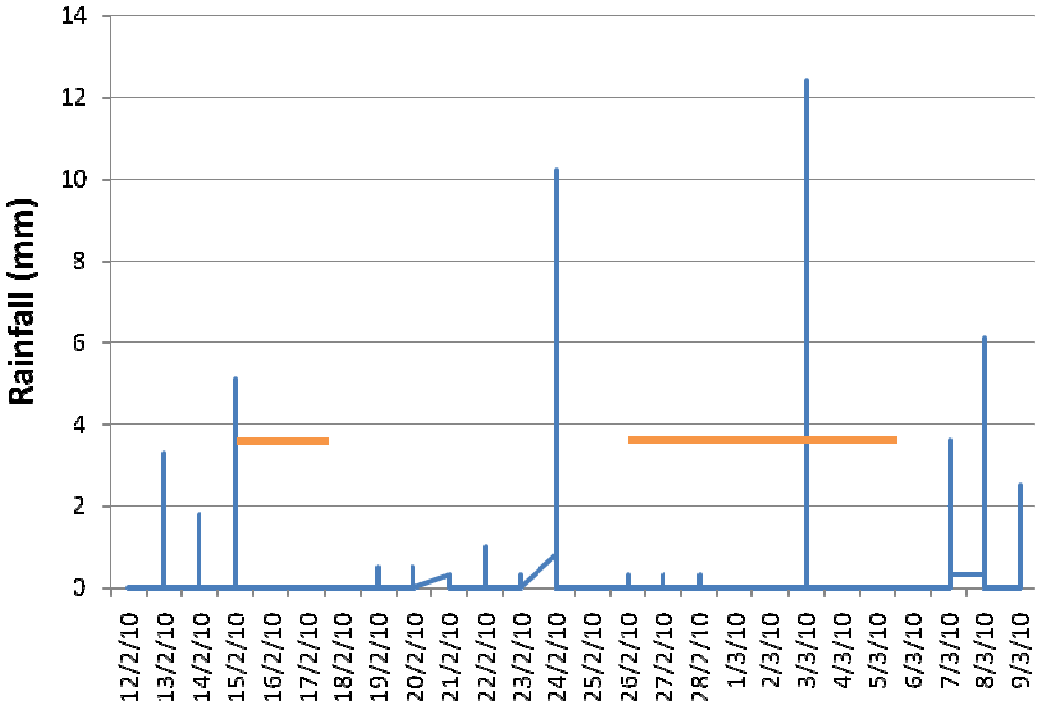
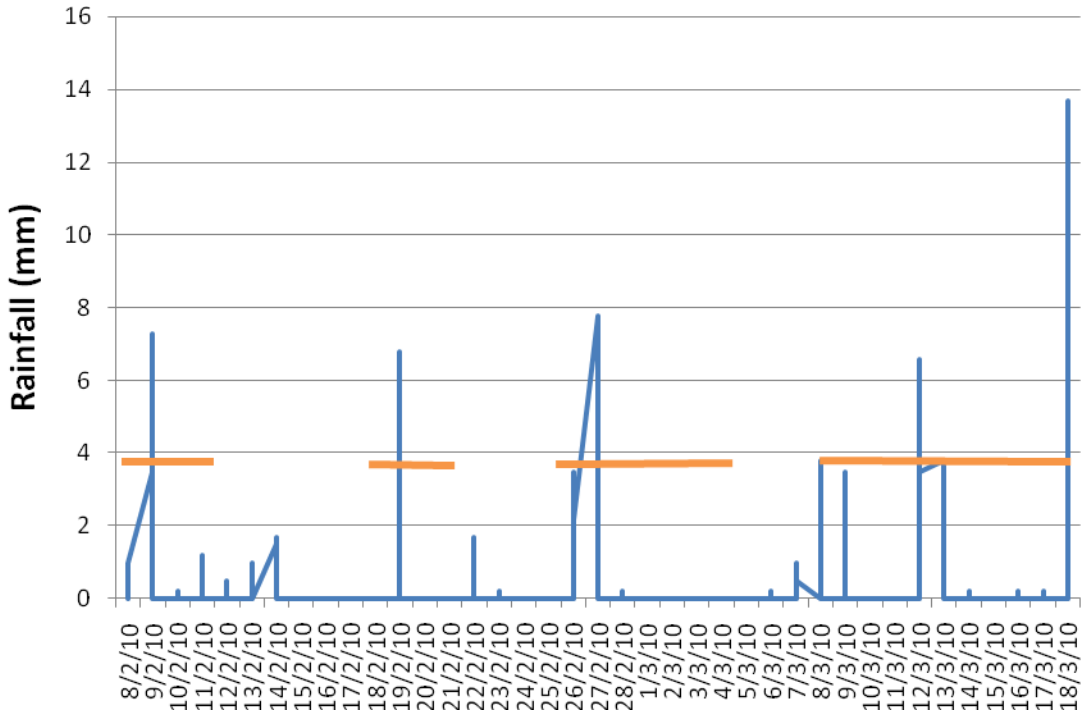
22/2/10

G



H



Appendix 3. Summary of weather data collected at Tasmanian bean crops monitored with spore traps during the 2007/2008, 2008/2009 and 2009/2001 growing seasons. (n.b. weather data not obtained from site 3 at Mersey Lea due to malfunction of datalogger).

Site 1 Wesley Vale	Week ending: 14/01/2008	21/01/2008	28/01/2008	04/02/2008	Total
Rainfall (total mm)	18.28	31.16	12.69	27.69	98.2
Air temperature					
Average (°C)	18.06	16.69	17.67	18.07	
Minimum (°C)	5.30	6.90	7.30	10.50	
Maximum (°C)	32.40	27.20	27.20	26.80	
Soil temperature					
Average (°C)	20.59	17.99	19.17	19.28	
Minimum (°C)	12.90	12.10	14.40	14.80	
Maximum (°C)	27.20	22.90	24.80	25.20	

Site 2 Wesley Vale	Week ending: 05/01/2009	12/01/2009	19/01/2009	26/1/2009	01/02/2009	Total
Rainfall (total mm)	50.20	36.20	44.40	9.40	31.50	171.7
Air temperature						
Average (°C)	13.07	14.82	15.58	15.56	20.11	
Minimum (°C)	3.50	3.20	4.60	5.20	9.30	
Maximum (°C)	20.10	21.70	27.30	24.60	31.00	
Soil temperature						
Average (°C)	16.87	18.46	18.03	16.77	18.20	
Minimum (°C)	14.40	16.60	13.90	14.70	16.00	
Maximum (°C)	19.30	20.80	21.60	18.90	20.20	

Site 4 Wesley Vale	Week ending: 18/02/2009	25/02/2009	04/03/2009	11/03/2009	18/03/2009	Total
Rainfall (total mm)	28.9	0.4	44.5	0	55.7	129.5
Air temperature						
Average (°C)	16.0	16.9	16.5	13.9	15.2	
Minimum (°C)	6.8	9.6	6.9	2.4	6.7	
Maximum (°C)	22.8	24.4	23.3	22.8	24.3	
Soil temperature						
Average (°C)	19.2	18.8	18.0	15.6	16.1	
Minimum (°C)	18.3	17.8	16.9	15.1	15.3	
Maximum (°C)	20.2	19.7	18.7	16.9	16.9	

Site 5 Don	Week ending: 2/03/2009	9/03/2009	16/03/2009	Total
Rainfall (total mm)	40.3	22.4	55.7	118.4
Air temperature				
Average (°C)	16.9	13.3	15.8	
Minimum (°C)	9.4	2.7	6.9	
Maximum (°C)	27.2	22.1	23.7	
Soil temperature				
Average (°C)	18.3	15.2	16.3	
Minimum (°C)	13.7	9.4	12.1	
Maximum (°C)	25.6	21.7	23.7	

Site 6 Wesley Vale	Week ending: 18/01/2010	25/01/2010	01/02/2010	08/02/2010	Total
Rainfall (total mm)	9.8	19.5	16.4	66.6	112.3
Air temperature	16.8	15.3	17.4	18.9	

Average (°C)				
Minimum (°C)	5.9	4.0	8.5	10.6
Maximum (°C)	25.2	23.3	26.1	26.7
Soil temperature				
Average (°C)	19.3	16.4	17.2	18.2
Minimum (°C)	13.9	12.3	12.5	14.6
Maximum (°C)	28.0	21.5	22.0	21.6

Site 7 Mersey Lea	Week ending:					
	23/01/2010	30/01/2010	06/02/2010	13/02/2010	20/02/2010	Total
Rainfall (total mm)	20.4	35.0	16.2	36.0	8.6	166.2
Air temperature						
Average (°C)	15.3	17.6	19.5	19.6	19.1	
Minimum (°C)	4.9	7.3	10.1	7.7	12.1	
Maximum (°C)	26.4	28.0	29.5	32.0	29.5	
Soil temperature						
Average (°C)	18.6	21.1	19.8	20.0	18.8	
Minimum (°C)	12.1	15.6	16.0	14.4	16.4	
Maximum (°C)	28.7	29.1	25.6	26.4	22.1	

Site 8 Mersey Lea	Week ending:			
	19/02/2010	26/02/2010	05/03/2010	Total
Rainfall (total mm)	24.7	29.2	48.4	102.3
Air temperature				
Average (°C)	18.8	16.9	16.8	
Minimum (°C)	12.1	5.7	0.6	
Maximum (°C)	27.2	28.0	29.9	
Soil temperature				
Average (°C)	no data	no data	17.8	
Minimum (°C)	no data	no data	12.9	
Maximum (°C)	no data	no data	21.0	

Site 9 Wesley Vale	Week ending:					
	15/02/2010	22/02/2010	01/03/2010	08/03/2010	15/03/2010	Total
Rainfall (total mm)	64.0	55.6	51.8	13.5	60.3	245.2
Air temperature						
Average (°C)	18.8	18.5	15.7	18.7	14.8	
Minimum (°C)	13.9	8.5	2.6	6.0	7.2	
Maximum (°C)	25.8	25.4	24.5	26.7	22.6	
Soil temperature						
Average (°C)	20.5	20.0	17.3	18.3	16.1	
Minimum (°C)	17.9	17.1	13.9	14.2	13.7	
Maximum (°C)	23.7	22.1	19.8	20.6	19.3	

CHAPTER 7 - ECONOMIC ANALYSIS OF NEW STRATEGIES EVALUATED FOR MANAGING SCLEROTINIA AND OTHER SOILBORNE DISEASES IN VEGETABLES

O. Villalta[1] and L. Trapnell[2]

Research team: O. Villalta, C. Donald[1], C. Scoble[1], D. Riches[1], D. Wite[1], S. Mattner[1], I. J. Porter[1,] H. Pung[3]

[1] Department of Primary Industries, Knoxfield Victoria
[2] Farmanomics Research and Consulting, Benalla, Victoria
[3] Peracto Pty Ltd, Tasmania

Summary

Research showed that Filan™, Shirlan™, Switch™ and AEC656948 were the most effective fungicide treatments for control of *Sclerotinia minor* lettuce drop in field trials in Victoria and Tasmania (Chapters 2 and 3). Economic analyses show these treatments can increase lettuce yields by 5 - 13.5% (cartons/ha) and profits by $2,510 - 6,828 per ha and therefore can be cost-effective for lettuce growers. Filan™, Shirlan™ and Switch™ were also the most effective treatments for control of bean white mould (*S. sclerotiorum*) (Chapters 2 and 3). These treatments can increase green bean yield by 2.8 - 4.0 t/ha and profits by $1,336 - 1,654 per ha and therefore can be cost-effective for bean growers. The use of these treatments alone or in alternation with Filan™ and as part of an integrated control strategy has the potential to substantially reduce crop losses while maintaining profitability and minimising the use of Filan™.

Research also showed that Caliente 199, a brassica biofumigant crop, and Faba beans may have advantages over other green manure crops evaluated for improving disease and yield management (Chapter 4). Economic analysis indicates that incorporating residue of Caliente 199 and Faba beans into alluvial soil before growing green beans has the potential to increase profits by $1,152 and $1,878 per ha, respectively, compared to fallow. Incorporating residue of Caliente 199 and BQ Mulch into sandy soil before a spring onion crop also has the potential to increase profits by $3,964 and $4,862 per ha, respectively. Additional research is required to determine if these green manure crops can improve yields in other cropping systems, and to better understand the mechanisms involved in disease suppression and yield improvement.

Studying the effects of changing cropping practices is a long-term process. For this reason, we have constructed an Excel-based model to study the economic consequences of changing management practices (e.g. crop rotation and biofumigation) on disease, yield and soil. Data currently available from long-term trials is insufficient to complete calibration and validation of the model. If future funding and data is limited, then the next-best option will be to use simulation modelling to study the potential economic benefits of using alternative

management practices. The model was developed as a research tool to help researchers identify the most practical and profitable disease management options for the vegetable industry.

Introduction

Management of soilborne diseases of vegetables is best achieved by using a number of methods that include beneficial cultural practices (e.g. site selection, rotation, cultivar resistance) and chemical (fungicides) and non-chemical (biofumigation, biocontrol agents) control. Projects VG07125 and VG07126 have evaluated a number of new management options (e.g. fungicides, rotation, biofumigation) to improve the control of soilborne diseases including Sclerotinia. The long-term objective of our research is to identify sustainable disease management practices that result in integrated control of soilborne diseases in vegetable production in Australia. Another objective is to develop an alternative systems-based approach with multiple economic and agro-ecological goals for managing soilborne diseases.

Disease management decisions at the farm level are largely driven by economics. While many control strategies can lead to significant reductions in crop losses, growers are unlikely to adopt strategies that are not profitable. Therefore, before new control strategies are recommended to growers it is essential to determine if they are suitable and profitable for on-farm use. This means that a new control option must be effective and economical; the value of the improved yield must exceed the cost of control.

This chapter looks at the cost-effectiveness of new management strategies being investigated by projects VG07125 (Best Practices IPM for control of soilborne diseases) and VG07126. We analysed the potential profitability of new disease control materials (e.g. fungicides) for Sclerotinia control using gross margin analysis. We also used gross margin analysis to begin examining the potential economic benefits of using green manure and biofumigant crops in between cash crops to reduce disease carry-over (inoculum) and increase yield and soil quality using preliminary data from long-term trials. In addition, we constructed an Excel model using whole of farm financial analysis to study the economic consequences of changing farm practices (e.g. rotation, biofumigation, etc) to manage soilborne diseases.

Materials and Methods

Data used in economic analysis

Several fungicide treatments were evaluated in Victorian and Tasmanian field trials for their capacity to reduce the incidence of lettuce drop (*S. minor)* and bean white mould (*S. sclerotiorum).* Results of these trials, including efficacy and statistical analysis, are reported in detail in Chapters 2 and 3. Economic analysis was conducted only on trials where treatments showed useful efficacy against Sclerotinia. Several experiments evaluating novel soil and disease control treatments were omitted from the economic analysis either because they were too preliminary, or were not effective in controlling disease. The effects of rotation with biofumigant and other green manure crops on disease suppression and yield were investigated in long-term trials in Victoria (Chapter 4). Preliminary results for the first

vegetable (cash) crops grown in plots amended with residue of green manure treatments were analysed for profitability.

Gross margin analysis

We conducted economic analyses for the fungicide treatments evaluated in lettuce and green bean trials in Victoria and Tasmania to determine their cost-effectiveness. We also conducted economic analyses for the green manure treatments using green beans (Lindenow) and bunching onions (Clyde) data. The gross margin per hectare for a particular treatment depended on the difference in yield between treated and non-treated plots, the farm-gate price of the crops (lettuce, green beans and spring onions), and the variable costs (cultural practices, production inputs, harvest, freight) for these crops. Production costs and sales figures were obtained from farms where the trials were located (Victoria and Tasmania) and from Qld. The economic analysis was based on gross margins (return above cash costs) per ha and approximates the return to management and investment.

Modelling economic consequences of changing management practices

The effects of changing farm practices (e.g. rotation, biofumigation, etc) on the management of soilborne pathogens, yield and soil quality must be evaluated for a minimum of 2-3 years to fully determine the potential economic benefits for growers. For this reason, the project constructed a whole of farm financial model (Excel-based) to study the economic consequences of changing practices for controlling soilborne diseases. The model consists of farm budgets for conventional (current grower practice) and integrated (new practices) cropping systems with costs calculated based on the operations and inputs used in the field study each year. The budgets also include calculations for harvest, management, overhead costs, interest on operating capital and prices and yields. The two systems can be economically compared by using net returns and costs for the year of the trials and modelling using cash-flow analysis and annuity net present values (NPVs). In other words, it will examine whether or not growers would have the resources or positive benefits year by year to invest in changing a current management practice. At this stage, there is insufficient data from the long-term trials to complete calibration and validation of the financial model. Therefore we only briefly describe the modelling approach to be used.

Results

Cost-effectiveness of new fungicide treatments

Lettuce drop (S. minor)

Lettuce drop incidence was similar in untreated and plots treated with the most effective fungicide treatments evaluated in four trials in Victoria (Table 1). We decided therefore to conduct the economic analysis using the averages of these treatments. Table 1 shows estimated crop losses to Sclerotinia, income, variable costs and gross margins per ha for the different fungicide treatments. In all trials, the best control was obtained with Shirlan™, Switch™ and Filan™ and as a result these treatments provided the highest profits per ha. These three treatments increased yield by 5% (cartons/ha) and profit by $2,510 - 2,872 per ha

compared to the untreated control when disease pressure was low. Folicur™ gave variable disease control and therefore gave less income.

Table 1. Potential profitability for fungicide treatments evaluated in low disease pressure field trials with lettuce in Melbourne metro region, Victoria.

Treatment[1]	Disease incidence (%)[2]	Plants ha[3]	Cartons ha[4]	Income ha[4]	Variable costs ha[5]	Gross margin ha	Rank[6]
Untreated control	7.0 (6.0 - 7.6)	55800	4650	$55800	$27770.00	$28030.00	5
Folicur 0.35 L/ha	5.0 (2.2 - 7.1)	57000	4750	$57000	$27792.58	$29207.42	4
Filan 1 kg/ha	2.1 (1.3 - 3.4)	58740	4895	$58740	$28070.00	$30670.00	2
Switch 1 kg/ha	2.2 (1.3 - 3.2)	58680	4890	$58680	$28139.80	$30540.20	3
Shirlan 0.35 L/ha	2.0 (1.3 - 2.9)	58800	4900	$58800	$27897.44	$30902.56	1

[1] Two applications/crop.
[2] Average incidence of lettuce drop recorded in four lettuce trials (Cos and Iceberg); disease range in parenthesis.
[3] Number of harvestable plants after calculating crop loss to *Sclerotinia minor* based on lettuce heads per ha.
[4] Twelve lettuce heads per carton estimated at $12/carton for both Cos and Iceberg lettuce (price range $0.80-1.5/head).
[5] Variable costs includes harvesting and freight costs for Melbourne metro region.
[6] 1 = best return to management/investment.

Table 2 shows lettuce yields and gross margins for fungicide treatments evaluated in a trial in Tasmania. Best control was obtained with the new fungicide treatment AEC656948 (fluropyram, Bayer) at 0.3 and 0.5 L/ha. These treatments increased yields by 12.6 - 13.5% (cartons/ha) and profits by $6,288 -6,828 per ha, respectively, compared to the untreated control. Filan™ also increased yield by 8% and profit by $3,696. Combining Contans™ (a biological control product) with Filan™ did not result in a higher return to that provided by Filan™ alone. In another trial in Tasmania, Shirlan™, Switch™, Filan™ and AEC656948 did not provide acceptable lettuce drop control when disease pressure was too high (data not shown; 64% incidence in untreated plots). This indicated that a single strategy (eg fungicides alone) will not provide acceptable control of Sclerotinia when disease pressure is too high.

Table 2. Potential profitability for fungicide and biological treatments evaluated in a high disese pressure field trial with lettuce in northern Tasmania.

Treatment[1]	Disease incidence (%)[2]	Plants ha[3]	Cartons ha[4]	Income ha[4]	Variable costs ha[5]	Gross margin ha	Rank[6]
Untreated control	15.3	45738	3811.50	$45738	$26047.00	$19691.00	5
Filan 1 kg/ha	7.9	49734	4144.50	$49734	$26347.00	$23387.00	3
Contans + Filan	9.2	49032	4086.00	$49032	$26502.20	$22529.80	4
AEC656948 0.3L/ha	3.1	52326	4360.50	$52326	$26347.00	$25979.00	2
AEC656948 0.5L/ha	2.1	52866	4405.50	$52866	$26347.00	$26519.00	1

[1] Two fungicide applications/crop. One application of Contans in combination with two applications of Filan.
[2] Lettuce drop incidence on an Iceberg lettuce crop.
[3] Number of harvestable plants after calculating crop loss to *Sclerotinia minor* based on lettuce heads per ha.
[4] Twelve lettuce heads per carton estimated at $12/carton (price range $10-15/carton).
[5] Variable costs includes harvesting and freight costs for northern Tasmania; AEC656948 costed at Filan's price and Contans at $38.80/kg.
[6] 1 = best return to management.

Bean white mould (S. sclerotiorum)

Table 3 shows green bean yields (fresh market) and gross margins for fungicide treatments evaluated in a trial in Lindenow, Victoria. The three fungicide treatments were equally effective in reducing white mould infection on bean pods under low disease pressure. Filan™ at 1 kg/ha ($151/kg) gave the best control but Folicur™ at 0.35 L/ha ($32.26/L) returned a higher gross margin because it is cheaper that Filan™. All treatments increased yields by 7 -

9% (cartons/ha) but profits only by $433 - 640 per ha. A key outcome from this trial is that combining Filan™ with Folicur™ gave good disease control, suggesting this strategy could be used to reduce the number of applications of Filan™ to one per crop if disease pressure is known to be low. Another economic consideration is that even though the return to investment was very low, the use of a fungicide strategy is still essential to prevent the build up of disease/inoculum.

Table 3. Potential profitability for fungicide treatments evaluated in a field trial with green beans (fresh market) in Lindenow, Victoria.

Treatment[1]	Disease incidence (%)[2]	t/ha[3]	Cartons ha[4]	Income ha[4]	Variable costs ha[5]	Gross margin ha	Rank[6]
Untreated control	9.5	7.69	641.04	$7692.50	$4754.15	$2938.35	4
Filan 1 kg/ha	0.4	8.47	705.50	$8466.00	$5054.15	$3411.85	2
Folicur 0.35 L/ha	1.7	8.36	696.29	$8355.50	$4776.73	$3578.77	1
Folicur + Filan	2.5	8.29	690.62	$8287.50	$4915.44	$3372.06	3

[1] Two applications/crop.
[2] Incidence of white mould on pods.
[3] Yield t/ha after calculating crop loss to *Sclerotinia sclerotiorum* based on yield (bean pods) measured at the site.
[4] Twelve kgs/carton at $12/carton (price range $10-15/carton).
[5] Variable costs includes harvesting costs but not freight.
[6] 1 = best return to management.

Table 4 and 5 show green bean yields (processing) and gross margins for fungicide treatments evaluated in two trials in Tasmania. In the first trial, Switch™ and Filan™ were equally effective, followed by Shirlan™ (0.25 L/ha), in reducing white mould severity under high disease pressure (Table 4). As a result Filan™ and Switch™ provided the highest yield increases (about 4 t/ha) and gross margins, increasing profitability by $1,654 - 1,496 per ha, respectively, followed by Shirlan™ ($1,336), compared to no treatment. Under lower disease pressure, combining Shirlan™ with Filan™ gave complete disease control, increasing yield by 1.3 t/ha and profit by $312 per ha (Table 5). Folicur™ alone was not profitable in both trials (Tables 4 and 5).

Table 4. Potential profitability for fungicide treatments evaluated in a field trial with green beans (slicing) in Tasmania.

Treatment[1]	Disease severity (%)[2]	t/ha[3]	Income ha[4]	Variable costs ha[5]	Gross margin ha	Rank[6]
Untreated control	48.0	5.2	$2735.20	$2421.75	$313.45	5
Folicur 0.35 L/ha	41.0	5.9	$3103.40	$2455.62	$647.78	4
Shirlan 0.25 L/ha	20.0	8.0	$4208.00	$2558.30	$1649.70	3
Switch 1 kg/ha	9.0	9.1	$4786.60	$2976.45	$1810.15	2
Filan 1 kg/ha	8.0	9.2	$4839.20	$2871.75	$1967.45	1

[1] Three applications per crop.
[2] Disease severity: plants heavily infected by white mould.
[3] Yield t/ha of pods after estimating crop loss to *Sclerotinia sclerotiorum* based on average yield measured in the region (10 t/ha).
[4] $526/t.
[5] Variable costs do not include freight to factory.
[6] 1 = best return to management.

Table 5. Potential profitability for fungicide treatments evaluated in a low disease pressure field trial with green beans (slicing) in Tasmania.

Treatment[1]	Disease severity (%)[2]	t/ha[3]	Income ha[4]	Variable costs ha[5]	Gross margin ha	Rank[6]
Untreated control	12.5	8.7	$4602.50	$2421.75	$2180.75	5
Folicur 1 L/ha	13.5	8.6	$4549.90	$2454.01	$2095.89	6
Shirlan 0.25 L/ha	6.6	9.3	$4912.84	$2467.27	$2445.57	2
Filan 1 kg/ha	5.6	9.4	$4965.44	$2721.75	$2243.69	4
Folicur + Filan	2.8	9.7	$5112.72	$2754.01	$2358.71	3
Shirlan + Filan	0.0	10.0	$5260.00	$2767.26	$2492.74	1

[1] One application of Shirlan or Folicur alone or in combination with two applications of Filan per crop.
[2] Disease severity: plants heavily infected by white mould.
[3] Yield t/ha pods after estimating crop loss to *Sclerotinia sclerotiorum* based on average yield measured in the region (10t/ha).
[4] $526/t.
[5] Variable costs do not include freight to factory.
[6] 1 = best return to management.

Economic benefits of rotation with biofumigant and green manure crops

Table 6 shows the gross margins estimated for a green bean crop grown in alluvial soil plots amended with residue of eight green manure treatments including three biofumigant brassica crops and fallow at Lindenow, Victoria. White mould (*S. sclerotiorum*) and root infections caused by *Pythium* spp and other soilborne pathogens affected the bean crop grown at this site. White mould incidence was too low to determine the effects of treatments on *Sclerotinia* suppression. On the other hand, statistical analysis showed that root infection severity was significantly lower on bean plants grown in soil amended with Faba beans, Mustclean and Caliente 199 when compared to the fallow treatment. Plots amended with residue of Caliente 199 and Faba beans had the highest weights of marketable pods/m^2 and estimated yields (t/ha) and therefore the highest profits per ha. These treatments increased profits by $1,878 - 1,152 per ha compared to fallow. Other green manure treatments were less profitable ($125 - 898/ha).

Table 6. Profitability for biofumigant and green manure treatments incorporated into soil before growing green beans at a low disease pressure site at Lindenow, Victoria.

Treatment	Disease[1]	Pods grs/m^2	t/ha	No. cartons ha[2]	Income ha[2]	Variable costs ha[3]	Gross margin ha	Rank[4]
Fallow	1.61 (2.91)	647	6.47	539.17	$6470	$4754.15	$1715.85	8
Mustclean	2.46 (1.91)	718	7.18	598.33	$7180	$5339.03	$1840.97	7
BQ Mulch	0.70 (2.25)	794	7.94	661.67	$7940	$5337.03	$2602.97	5
Caliente 199	1.04 (2.08)	894	8.94	745.00	$8940	$5345.43	$3594.57	1
Vetch	1.24 (2.16)	809	8.09	674.17	$8090	$5221.95	$2868.05	3
Faba bean	0.50 (1.83)	839	8.39	699.17	$8390	$5092.95	$3297.05	2
Triticale	0.77 (2.83)	760	7.60	633.33	$7600	$5073.95	$2526.05	6
Ryegrass	0.91 (2.91)	774	7.74	645.00	$7740	$5126.45	$2613.55	4

[1] Sclerotinia white mould on pods (and root infection severity caused by other soilborne pathogens).
[2] Twelve kgs/carton at $12/carton (price range $0.80-1.50 kg).
[3] Variable costs include harvesting and freight costs.
[6] 1 = best return to management.

Table 7 shows the gross margins estimated for a spring onion crop grown in sandy soil plots amended with residue of seven green manure treatments including three biofumigant crops and fallow at Clyde, Victoria. White rot (*S. cepivorum*) levels were too low to determine the effects of treatments on disease suppression (data not shown). Plants grown in soil amended

with Caliente 199 and Faba beans had the highest plant fresh weights, which were significantly higher than weights of plants in plots amended with oats and rye-corn. Spring onions are sold in bunches (10 bunches/deck). Although there were no significant differences in bunch numbers among treatments, Caliente 199 and BQ Mulch had the highest yields (no. marketable bunches/m^2) and therefore the highest income per ha. These two treatments increased profits by $4,862 - 3,964 per ha compared to the fallow. Oats increased profit by $1,337 but the other treatments were slightly less profitable than the fallow. Faba bean plant density at this site was lower that that at Lindenow due to uneven planting and bee hives were not used to assist with pollination during flowering. This reduced plant biomass available for soil incorporation and potential profitability.

Table 7. Profitability for biofumigant and green manure treatments evaluated over one season in a field trial before growing bunching (spring) onions, Victoria 2009. Low root rot infections recorded.

Treatment	bunches/m^2	bunches/ha	decks/ha[1]	Income[2]	Var. costs[3]	gross margin[4]	Rank[4]
fallow	22.78	227800.00	22780.00	$102510	$9860.44	$92649.56	5
Mustclean	22.87	228700.00	22870.00	$102915	$10173.84	$92741.16	4
BQ Mulch	23.73	237300.00	23730.00	$106785	$10171.84	$96613.16	2
Caliente 199	23.93	239300.00	23930.00	$107685	$10173.84	$97511.16	1
Faba bean	22.60	226000.00	22600.00	$101700	$10199.24	$91500.76	7
Oats	23.15	231500.00	23150.00	$104175	$10188.24	$93986.76	3
Rye-corn	22.63	226300.00	22630.00	$101835	$10163.99	$91671.01	6

[1] Ten bunches (each 10 plants) of spring onions per deck.
[2] Price range $4-6/deck (calculated at $4.50/deck for this site and time of year).
[3] Variable costs do not include freight to market costs.
[4] Field used for trial one of the best field for growing spring onions in farm; return above region average ($40,000-$60,000 gross margin).

Whole farm financial analysis

An Excel-based model using a whole of farm financial analysis was constructed to study the economic consequences of changing cropping practices to manage soilborne diseases.

Model structure

This economic analysis uses a whole of farm approach that captures the dynamic and stochastic nature of the vegetable growing businesses (Malcolm *et al.* 2005). A hypothetical, but realistic, farm system was devised for modelling the long-term effect of cropping practices (e.g. rotation and biofumigation) on disease and yield. The system can be modified to study vegetable crops (e.g. lettuce, green bean, spring onions, etc) being investigated in long-term trials. The farm system has two rotational cropping systems (conventional and integrated). New management practices are studied in the integrated system. Each cropping system has 17 fields where the crops investigated are rotated over five years. For each farm system, the net benefits of the new management strategy are compared with those for the conventional system using economic modelling.

Economic modelling

The economic modelling is conducted in two parts. The first part is a gross margin analyses taking into account the additional costs for new management practices included in the integrated system. Cash budgets were constructed using Excel® spreadsheets. Values in the gross margin budgets were derived from 'Gross Margins for Vegetable Crops in the River

Valleys of North East Victoria', 2000, 'NSW Farm Enterprise Budgets' and 'Seven Veg Report' compiled by Natural Resources and Environment and Ernst and Young, 2001 (Faour and Singh 2001, Trapnell 2000). The values for production costs, yields and prices can be adjusted to current market values. The values for benefits and costs are initially expressed as real or present day values. Initial capital costs are added as well as real values for overhead costs and costs for managerial labour and management. Real benefits and costs are summed, then inflated to become nominal values for the various years of the analyses. Rates of inflation from 1986 to 2009 were sourced from the Australian Bureau of Statistics (Australian Bureau of Statistics, 2005; Year Book of Australia, 2006 to 2009). Net nominal benefits for each year are calculated by subtracting nominal costs from nominal benefits. Annual net nominal benefits are discounted by a risk free nominal rate that represented the average yield on 10 year government bonds from 1986 to 2009 (Australian Bureau of Statistics 1986 to 2009).

The second part is a cash flow analysis using Monte Carlo Simulation (Excel®, Crystal Ball™) over five years. Excel® spreadsheets were linked to Monte Carlo Simulation Models, which allowed probability distribution functions (pdf) to be applied to variables in the analyses such as yields and prices. The analysis uses cost-benefit analysis (CBA) to compare the Net Present Values (NPV's) of the two rotational systems (Mishan 1972; Sinden and Thamampillai 1995). The main focus is on identifying profitability, opportunities and constraints of adoption.

Examples of model outputs

Table 8 shows an example of a gross margin budget for a green bean crop, with the option to include a biocontrol treatment (Contans™) for Sclerotinia control.

Table 8. Example of gross margin budget for green beans with the option to include a biocontrol treatment.

Expected yield				1,400	cartons/ha	(10 kg per carton)		
Expected farm gate price per carton				$10.00				
							$/ha	**$/ha**
Income								$14,000
Variable costs								
Seedlings								
		30,800	@	$0.04	per seeding		1,232	
Machinery costs								
		19 hours per ha.	@	$45.00	per hour		855	
Irrigation								
Water		5 ML per ha.	@	$17.36	per ML	87		
Pumping costs		5 ML per ha.	@	$30.00	per ML	150	237	
Fertiliser								
Single super		200 kg per ha.	@	$0.24	per kg	48		
Starter fertilizer		250 kg per ha.	@	$0.45	per kg	113		
Ammonium nitrate		350 kg per ha.	@	$0.41	per kg	144	304	
Pest control								
Fungicides	2	2.5 kg per ha.	@	$7.45	per kg	37		
	1	2.2 kg per ha.	@	$8.30	per kg	18		
Insecticide	2	0.5 L per ha.	@	$46.00	per L	46		
	1	2.0 L per ha.	@	$10.00	per L	20		
	2	0.8 L per ha.	@	$8.75	per L	14	136	
Weed control								
Pre-emergent	1	2.0 L per ha.	@	$10.25	per L	21		
Post-emergent	1	1.0 L per ha.	@	$63.70	per L	64	84	
Apply Contans on crop residue								
Contans		2.0 kg per ha.	@	$38.80	per kg	78		
Application						15	93	
Casual labour								
Transplanting	3	6.0 hours per ha.	@	$20.00	per hour	360		
Chipping	1	10.0 hours per ha.	@	$21.00	per hour	210	570	
Harvesting and packaging								
Harvest labour		93 hours per ha.	@	$20.00	per hour	1,867		
Trailer pickup		12 hours per ha.	@	$35.00	per hour	408		
Octabins		187 octabins per ha.	@	$14.00	per octabin	2,613		
Cooling		187 octabins per ha.	@	$1.00	per octabin	187	5,075	
Total variable costs per hectare								**$8,585**
Gross margin per hectare								**$5,415**

Table 8 and 9 is an example from a simulation run showing the whole farm financial analysis and NPV's for cropping systems that included a new management practice (e.g. green manure crop) in the rotation. In the example, the NPVs for the rotation system with the green manure crop are greater than the NPVs for the control system and less risky because they have a lower co-efficient of variability (Table 10). In principle, it would be in the farm financial interest to adopt a new management practice if the NPV of the incremental returns from

switching from one farming practice to another were positive (NPV >0) (Cary and Wilkinson 1997, Pannell 1999).

Table 9 Economic and financial analyses for a hypothetical farm.

Item	Year					
	0	1	2	3	4	5
	$	$	$	$	$	$
1. Economic analysis						
Annual real benefits						
Paddock 1		242,603	242,603	222,353	328,765	135,794
Paddock 2		0	222,353	328,765	135,794	242,603
Paddock 3		222,353	328,765	135,794	242,603	0
Paddock 4		328,765	135,794	242,603	0	222,353
Paddock 5		135,794	242,603	0	222,353	135,794
Paddock 6		242,603	0	222,353	135,794	176,294
Paddock 7		0	222,353	135,794	176,294	0
Paddock 8		222,353	135,794	176,294	0	135,794
Paddock 9		135,794	176,294	0	135,794	242,603
Paddock 10		176,294	0	135,794	242,603	328,765
Paddock 11		0	135,794	242,603	328,765	222,353
Paddock 12		135,794	242,603	0	222,353	135,794
Paddock 13		242,603	328,765	222,353	135,794	0
Paddock 14		328,765	222,353	135,794	0	242,603
Paddock 15		222,353	135,794	0	242,603	328,765
Paddock 16		135,794	0	242,603	328,765	222,353
Paddock 17		0	242,603	328,765	222,353	328,765
Trade in value machinery						
Self propelled machinery						
Complex non-powered						
Simple non-powered						
Salvage value land & improvements						7,000,000
Salvage value machinery						
Self Powered						202,500
Complex non-powered						60,000
Simple non-powered						35,100
Total farm annual real benefits		2,771,868	3,014,471	2,771,868	3,100,632	10,398,232
Annual inflation of benefits		0.0374	0.0374	0.0374	0.0374	0.0374
Total farm annual nominal benefits		2,875,535	3,244,170	3,094,648	3,591,164	12,493,690

Table 9 (continuation).

Annual real costs						
Variable costs						
Paddock 1		130,741	129,271	134,881	218,054	106,261
Paddock 2		7,460	134,881	218,054	106,261	129,271
Paddock 3		136,352	218,054	106,261	129,271	1,733
Paddock 4		218,054	106,261	129,271	1,733	134,881
Paddock 5		106,261	130,741	1,733	134,881	106,261
Paddock 6		129,271	7,460	134,881	106,261	116,127
Paddock 7		1,733	136,352	106,261	116,127	2,180
Paddock 8		134,881	106,261	116,127	2,180	106,261
Paddock 9		106,261	116,127	2,180	106,261	129,271
Paddock 10		116,127	2,180	106,261	129,271	218,054
Paddock 11		2,180	106,261	130,741	218,054	134,881
Paddock 12		106,261	129,271	7,460	136,352	106,261
Paddock 13		129,271	218,054	136,352	106,261	1,733
Paddock 14		218,054	134,881	106,261	1,733	129,271
Paddock 15		134,881	106,261	1,733	129,271	218,054
Paddock 16		106,261	1,733	129,271	218,054	134,881
Paddock 17		1,733	129,271	218,054	134,881	218,054
Overhead costs		70,000	70,000	70,000	70,000	70,000
Owner operator's labour and management		80,000	80,000	80,000	80,000	80,000
Land purchase	7,000,000					
Machinery Investment						
Self Powered	300,000					
Complex non-powered	80,000					
Simple non-powered	45,000					
Total farm annual real costs	7,425,000	1,935,780	2,063,318	1,935,780	2,144,902	2,143,432
Annual inflation of costs		0.0374	0.0374	0.0374	0.0374	0.0374
Total farm annual nominal costs	7,425,000	2,008,178	2,220,540	2,161,199	2,484,234	2,575,377
Annual net benefits before tax	-7,425,000	867,358	1,023,630	933,449	1,106,930	9,918,313
Tax payable		266,430	318,372	303,319	395,438	439,668
Annual net benefits after tax	-7,425,000	600,928	705,258	630,130	711,492	9,478,645
Discount rate		0.082	0.082	0.082	0.082	0.082
Discounted annual net benefits	-7,425,000	555,386	602,412	497,449	519,112	6,391,605
Net Present Value	**1,140,964**					
Difference	**328,392**					

Table 9 (continuation).

2. **Financial analysis**					
Cumulative net cash flow after tax	-1,626,572	-1,122,597	-649,918	-43,327	619,016
Interest on cash deficit or surplus	-201,283	-157,451	-104,902	-48,085	19,324
Cumulative net cash flow after interest	-1,827,855	-1,280,048	-754,820	-91,413	638,339
3. Taxations schedule					
Tax relevant real benefits	2,771,868	3,014,471	2,771,868	3,100,632	3,100,632
Tax relevant nominal benefits	2,875,535	3,244,170	3,094,648	3,591,164	3,725,474
Tax relevant real costs	1,935,780	2,063,318	1,935,780	2,144,902	2,143,432
Tax relevant nominal costs	2,008,178	2,220,540	2,161,199	2,484,234	2,575,377
Machinery depreciation					
Self powered machinery @ 18% pa		54,000	54,000	54,000	54,000
Complex non-powered machinery @ 13% pa		10,400	10,400	10,400	10,400
Simple non-powered machinery @ 13% pa		5,850	5,850	5,850	5,850
Total tax relevant nominal costs	2,008,178	2,290,790	2,231,449	2,554,484	2,645,627
Net taxable income/deficit before interest	867,358	953,380	863,199	1,036,680	1,079,846
Interest:					
Interest received	21,467	25,335	23,103	27,397	28,465
interest paid on annual cash flow before tax	222,750	0	0	0	0
interest paid on cumulative deficit or surplus	0	182,786	128,005	75,482	9,141
Taxable income	666,075	795,929	758,297	988,595	1,099,170
Marginal tax rate	0.40				
Tax payable	266,430	318,372	303,319	395,438	439,668
4. Interest schedule					
Interest paid on money borrowed during the year	-222,750	0	0	0	0
Interest paid on cumulative cash deficit		-182,786	-128,005	-75,482	-9,141
Interest received on annual cash surplus	21,467	25,335	23,103	27,397	28,465
Interest received on cumulative cash surplus		0	0	0	0
Interest paid/received on annual cash deficits/surpluses	-201,283	-157,451	-104,902	-48,085	19,324
Interest paid on overdraft	0.1000	0.1000	0.1000	0.1000	0.1000
Interest received on cash surpluses	0.0450	0.0450	0.0450	0.0450	0.0450

Table 10 Net present values (NPVs) and co-efficient of variability for rotational system with green manure treatment compared with the control.

Item	Rotation with treatments	Control
Mean NPV	$1.15 million	$0.82 million
Maximum NPV	$2.98 million	$2.61 million
Minimum NPV	- $0.31 million [a]	- $0.57 million [b]
Co-efficient of variability in NPV	0.366	0.500

[a] Probability of NPV being < 0 = 0.0023, [b] Probability of NPV being < 0 = 0.027

Figures 1 and 2 show probability distribution functions generated by the simulation for NPV's in the two rotational systems. Figure 3 shows a comparison of cumulative distribution functions. In this example, the rotational system with new management practice has First Degree Stochastic Dominance over the rotation system for the control. This means that system with the new management practice has a smaller probability than the control farm for each net benefit displayed on the x axis and would be therefore the optimally preferred investment option (Vose 2000).

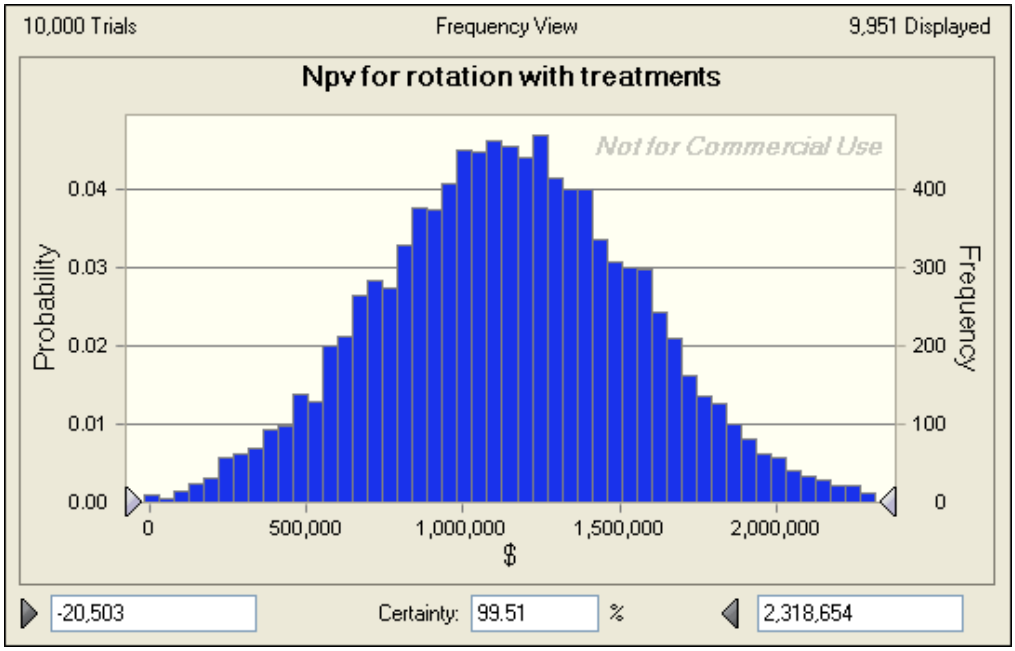


Figure 1 Example of probability distribution function of values for NPV's in the system with new practice (eg green manure crop) to control Sclerotinia on green beans.

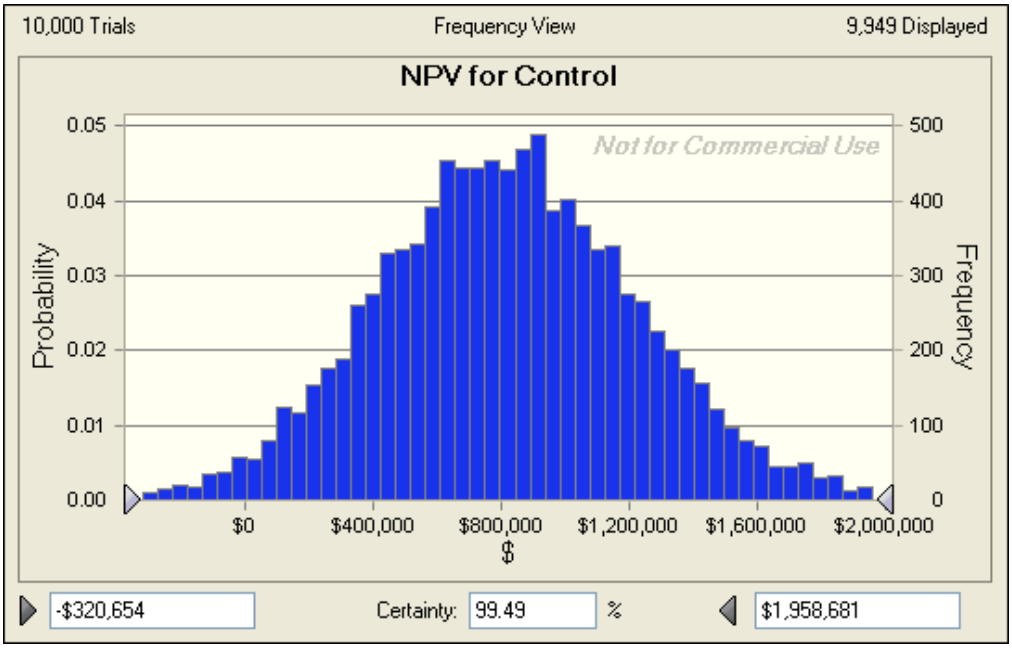


Figure 2 Example of probability distribution function of values for NPV's for the standard practice.

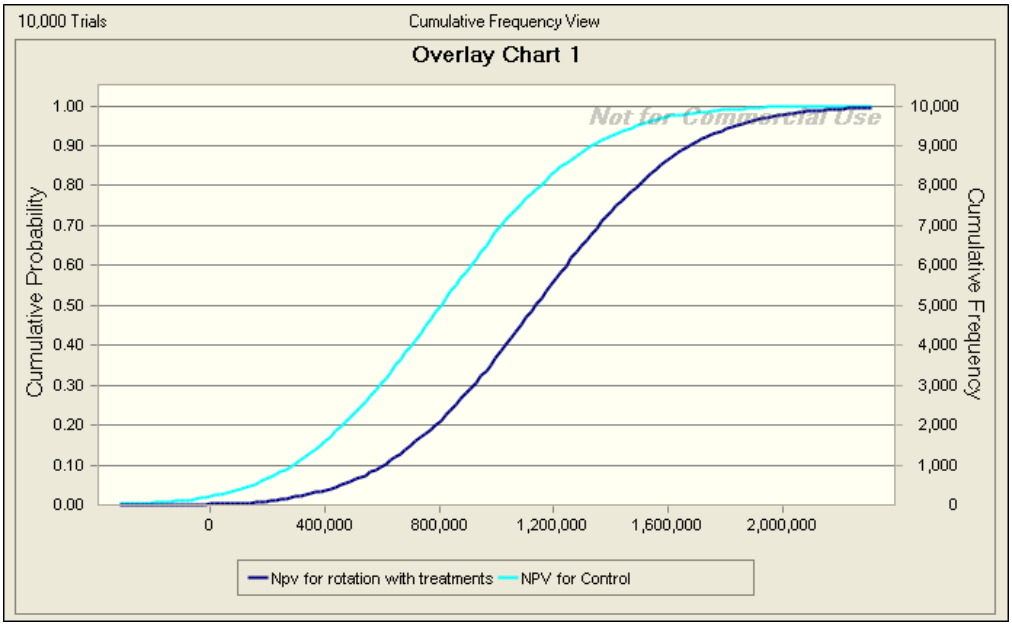


Figure 3 Comparison of cumulative distribution functions for rotational systems with and without a new farming practice for managing Sclerotinia.

Discussion and conclusions

This economic analysis showed that the most efficacious fungicide treatments evaluated in lettuce trials (Filan™, Shirlan™, Switch™ and AEC656948) can increase yields by 5 - 13.5% (cartons/ha) and hence profits by $2,510 - 6,828 per ha compared to untreated controls. These treatments are therefore cost-effective for the control of lettuce drop (*S. minor*) under low-to-moderate disease pressures (7 - 15% disease incidence). Shirlan™ ($182/L) and Switch™ ($184/kg) are slightly more expensive than the standard fungicide Filan ($151/kg). AEC656948 (fluropyram, Bayer) will be registered for control of various diseases including Sclerotinia in the USA/European market in 2010-2011.

The analysis also showed Filan™, Shirlan™ and Switch™ can increase processing green bean yields by 2.8 - 4 t and profits by $1,336 - 1,654 per ha under high disease pressure. These treatments are also cost-effective for the control of white mould (*S. sclerotiorum*) on green beans. Under low disease pressure (9.5% incidence), Filan™ at 1 kg/ha ($151/kg) and Folicur™ at 0.35 L/ha ($32.26/L) were equally effective in controlling bean white mould. However, Folicur™ was slightly more profitable because it is cheaper that Filan™. Results from field trials (efficacy data) and economic analysis indicate that Filan™, Shirlan™ and Switch™ can be recommended for the integrated control of bean white mould and lettuce drop. Folicur™ was ineffective in controlling white mould under high disease pressure. However, it may have a role in the integrated control of Sclerotinia, for example used in alternation with Filan™ if disease pressure is known to be low. Application rates and methods are discussed in Chapters 2 and 3. Efficacy data has been given to AgAware (Peter DalSanto) to develop minor use permits as required.

Crop rotation and biofumigation are key cultural practices to improve the management of soilborne diseases. Preliminary results from long-term trials indicate that Caliente 199 and Faba beans may have advantages over other green manure crops for disease and yield management. The economic analysis indicates that amending alluvial soil with residue of Caliente 199 and Faba beans before growing green beans has the potential to increase gross margins by $1,152 and $1,878 per ha, respectively, compared to a fallow system. Similarly, amending sandy soil with residue of Caliente 199 and BQ Mulch before growing spring onions has the potential to increase gross margins by $3,964 and $4,862 per ha, respectively. In general, the economic analysis indicates that all of the green manure treatments can be cost-effective, and some profitable, for spring onion and green bean cropping systems. We need to examine other economic indicators (e.g. break-even costs and economic profit) to determine the profits required for growers to change from one practice (e.g. fallow or cash crop) to another (e.g. green manure break crop). Other potential benefits of rotation with biofumigant and green manure crops may include improvements in soil conservation and quality and this should also be considered.

Results reported here must be treated with caution until additional long-term trial data is collected from different soil types and cropping practices. This is because there are many soil/crop/climatic factors that can influence the ability of break crops to suppress soilborne disease and/or enhance yield. For instance at Lindenow, the

positive effects of Caliente 199 and Faba beans on disease suppression and yield improvement in beans were probably due to the biofumigant (e.g. isothiocyanates) and nitrogen compounds released into soil, respectively. At Clyde, Caliente 199 also enhanced yield, but not Faba beans, probably due to issues discussed previously. The mechanisms involved in disease suppression and yield improvement need to be further investigated to identify the most suitable green manure or biofumigant crop for a particular soil and cropping system.

Within the timeframe of this study, we aimed to construct a framework to study the economic consequences of changing cropping practices for managing soilborne diseases. Studying the effects of changing farming practices (e.g. crop rotation and biofumigation) on disease, yield and soil is a long process and therefore must be evaluated over several years to fully determine the economic benefits for growers. In this report we described the approach taken to develop an Excel-based model for the proposed economic analysis. At the time of writing this report we did not have sufficient field data to calibrate and validate the model. If funding and data in the future is limited, then the next-best option will be to use modelling to identify the potential economic benefits of the new management practices being investigated. Information generated by this model could be used by researchers for decision-making and policy development.

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CHAPTER 8 - GENERAL DISCUSSION

Before this project began there were limited options available to growers for control of Sclerotinia diseases.

For the last twenty years growers have relied mainly on fungicides to reduce crop losses to Sclerotinia diseases, especially on highly susceptible crops such as green beans and lettuces. After losing procymidone, growers had to rely solely on the fungicide boscalid (Filan™) to manage Sclerotinia because there was limited information available on the efficacy of alternative fungicide treatments. Boscalid is safe to beneficial insects such as bees and predatory mites and has a low mammalian toxicity. However, it binds to soil particles and can persist in certain soil types long enough to affect MRLs of other vegetable crops.

A comprehensive laboratory study conducted by this project found no evidence of resistance to boscalid in populations of *S. sclerotiorum* from bean fields in Tasmania. This study has provided valuable baseline sensitivity data for monitoring boscalid resistance in the future. Nevertheless, the use of boscalid has to be carefully managed to ensure it remains available to vegetable growers for a long time. Other fungicides with minor use permits for Sclerotinia control (iprodione, tebuconazole and azoxystrobin) are not as effective as boscalid under high disease pressure. The lack of fungicides that are effective under high disease pressure is a major problem for vegetable growers. Therefore there was an urgent need for us to provide the industry with more fungicide treatments and new management options which are effective, economical and IPM compatible.

New fungicide treatments to protect against infection

A major outcome from this project was the identification of new fungicide treatments for the control of *S. minor* lettuce drop and *S. sclerotiorum* bean white mould.

Field trial work demonstrated the potential of plant applications and soil drenches of Shirlan™ (fluazinam) and Switch™ (cyprodinil + fludioxonil) to control lettuce drop and white mould in both low and high disease conditions. Switch™ provided control equivalent to Filan™ (boscalid). Fluazinam (Shirlan™) was moderately effective in controlling white mould in high disease but was very effective in controlling lettuce drop in low disease. Tebuconazole (Folicur™) had little or no effect for Sclerotinia control on vegetables. AE C656948 (fluopyram, Bayer), a new fungicide, was found to be highly effective for lettuce drop control. New application tactics were also developed. Filan™ and Shirlan™, applied to the soil surface before row-canopy closure, reduced white mould incidence by 72% and 55%, respectively. Shirlan™ applied before row closure followed by Filan™ applied at flowering reduced white mould by 91%. This work demonstrated the potential of integrating applications of new treatments (Shirlan™) before row closure with foliar applications of Filan™ for controlling white mould in high disease situations.

New strategies to reduce inoculum carry-over

In eastern Australia, lettuce and green bean are major crops sown in rotations with other horticutural crops including brassica, carrot and pea. Most of these crops are susceptible to the Sclerotinia pathogens. Unfortunately, these crops are sown in short rotation due to market and financial pressures on farms. This intensive crop production system has resulted in gradual increases of inoculum (sclerotia) of Sclerotinia in soil to levels where fungicides alone sometimes do not provide satisfactory disease control, leading to decline in yields, soil and farm productivity. Fungicides have no effect on inoculum carry-over in soil. Therefore the use of integrated control strategies that include soil treatments or other cultural practices that reduce or prevent inoculum build up are desirable for sustainable disease control. Eradication of sclerotia from soils is difficult due the large volume of soil that needs to be treated. Chemical treatments such as metham sodium and newer soil fumigants can be very effective in reducing the levels of inoculum in soil but these treatments are too costly for vegetable production and harmful to the soil and environment.

New soil treatments

This project has provided valuable efficacy and application data for two new plant-derived soil treatments which have potential for use in integrated control programs for Sclerotinia and other soilborne diseases of vegetables.

In laboratory trials, Voom at commercial rates was biocidal to sclerotia of *S. minor*, *S. sclerotiorum* and *S. cepivorum*. Voom and Vigor® also at commercial rates were also biocidal to mycelium of Sclerotinia and other important pathogens (*S. cepivorum*, *S. rolfsii*) of vegetables. Field trial work demonstrated the potential of Voom for reducing soil inoculum which reduced the severity of root infections caused by *R. solani* and *P. clyde* f on beans. These two plant-derived products can be cost-effective, IPM compatible will be available in the market in the near future. We recommend these treatments to be considered as IPM tools for managing Sclerotinia and other soilborne diseases of vegetables.

Rotation and biofumigation

This project has conducted the first major study of the effects of rotation and biofumigation practices on disease management in vegetable production.

Long breaks between Sclerotinia susceptible crops are highly desirable (5 to 10 years) to prevent inoculum build up but this practice is no longer economically feasible in modern vegetable production. Other approaches are therefore required such as green manuring, biofumigation and improvements to soil health for the sustainable management of Sclerotinia. Previous research conducted by this team (VG00048) showed that amending soil with residue of the brassica biofumigant crop BQ Mulch reduced *S. minor* infections on lettuce in Tasmania. Before this research began, BQ Mulch was the only biofumigant crop available to growers.

New biofumigant crops

Our research has evaluated eleven new biofumigant crops in the field and another six in the laboratory and glasshouse. The new varieties evaluated in the field were Indian mustard (*B. juncea* – Mustclean, Caliente 199, Gladiator), white mustard (*Sinapis alba* - Architect™, Abraham™, Attack™), forage rape (*B. napus* - Greenland™), oilseed radish (*Raphanus sativus* - Adios™, Arena™ and Doublet™) and Ethiopian mustard (*B. carinata*). Four of these new biofumigant crops (Mustclean, Caliente 199, Gladiator and Nemfix) have been rated as having excellent 'biofumigation' potential for management of Sclerotinia and three other important soilborne pathogens in vegetable production. Therefore these four crops are recommended for on-farm use.

When to grow these crops

Growers have now valuable information on the agronomic performance of the new brassica and other green manure break crops to suit different soils and seasons.

New biofumigant crops grow slow during the cool months of year (3-5 months) and fast in spring and autumn (2-3 months). The white mustards, Architect and Abraham can be susceptible to frost damage, whereas Attack is tolerant. Mustclean and Indian mustard were moderately tolerant. All the oilseed radish varieties, Adios, Arena and Doublet, and BQ Mulch were highly tolerant. Therefore, the oilseed radish varieties and BQ Mulch are more suitable for autumn/winter sowing, when frost conditions occur in Tasmania. All the varieties are suitable for spring sowing. Additional information will be provided to growers through extension materials.

Other benefits of green manure crops

This project has also provided a valuable insight into other benefits of crop rotation and biofumigation from a number of production areas in Victoria, Tasmania and Queensland.

In addition to the biofumigant effect on soil, brasicas crops offer other benefits such as increased organic matter and reduced soil compaction due to their strong tap root systems. The depletion of organic matter causes soil degradation, compaction, reduce fertility and water retention. The depletion of organic matter and carbon also reduces beneficial microbial activity in soil which favours proliferation of soilborne plant pathogens. Green manure crops produced different levels of biomass. In Tasmania and Victoria, the plant biomass of all the biofumigant varieties sown in spring was 3 to 6 times higher than that of the standard break crops. Poor soil structure affected the growth of brassica crops in one site in Victoria. In soil with good fertility, however, brassica crops produced 90-100 t of biomass per hectare in Victoria, with corresponding increases in organic matter and available nitrogen. To compensate for crop, soil and climatic variability, the use of blends of two or three brassica green manure varieties is recommended to European growers. Thus the recommendation for on-farm use will depend on the specific needs of each field including availability, disease and soil problems. Growers will be informed of the agronomic characteristics of each brassica crop through extension materials.

New approaches for detection and quantification of Sclerotinia

This project has developed new approaches for the detection and assessment of white mould on bean.

A PCR assay developed is highly sensitive and able to detect DNA concentrations of *S. sclerotiorum* as low as 0.2 ascospores per PCR reaction. DNA can be detected even in the presence of soil, indicating the technique is robust for monitoring ascospores in bean fields.

In bean fields, detection of more than 5-10% diseased plants may result in rejection of the whole crop by processors. Spatial analyses of large sets of survey data has provided the foundation for developing statistically robust sampling methods for measuring disease incidence at provisional industry thresholds for crop rejection. This information is crucial to industry also for identifying fields that require additional management such as biofumigation to reduce disease pressure.

Analysis of large sets of survey data has also provided valuable information for assessing and identifying site-specific risk factors that drive disease development. Further survey work and validation is required to develop this assessment method into a format which could be given to growers to quantify and forecast disease risk.

After commercial validation, these methods have the potential to become important decision-support tools to help researchers and industry develop better management systems for Sclerotinia. Our long-term goal is to integrate all these new tools into a management decision support system to help growers improve the management of Sclerotinia.

RECOMMENDATIONS

This national project has successfully developed several new management options to improve the control *S. minor* lettuce drop and *S. sclerotiorum* white mould and farm productivity in vegetable production in Australia.

1) New fungicide treatments
The project provided valuable efficacy and application data to support minor use permits for three new fungicide treatments (Switch™, Shirlan™, AE C656948) in lettuce and green beans. Economic analysis indicated that these new treatments are cost-effective for growers. Further work is required to validate their efficacy in different regions of Australia to ensure high adoption by industry. This includes evaluating their effect on other vegetable crops. Alternative non-chemical controls including nutrients were also evaluated but failed to control disease at commercial rates used for nutritional purposes. Further research is required with these products to identify concentrations that prevent infection and optimise their use for integrated control of Sclerotinia.

2) New soil treatments
Efficacy and application data was also generated for two new plant-derived soil treatments (Voom and Vigor®). These products can be cost-effective, IPM compatible and will be available soon in the market. These treatments have the potential, as new IPM tools, to reduce disease carry-over in soil. Further work is required to evaluate their efficacy on different soils to increase adoption. A variety of other novel plant-derived products were also evaluated and concentrations that kill soilborne pathogens of vegetables identified. These require further development before they can be recommended to growers.

3) New biofumigant crops
Rotation and biofumigation with brassica and other green manure crops can provide considerable improvements in disease management and crop productivity. Mustclean, Caliente 199, Gladiator and Nemfix were rated as having excellent 'biofumigation' potential for managing Sclerotinia and three other important soilborne pathogens of vegetables. BQ Mulch, Caliente 199 and Faba beans also improved the yields of green beans and spring onions. Economic analysis indicated that these green manure treatments are cost-effective and can increase profits for growers. Therefore these green manure crops are highly recommended for use in rotations with crops susceptible to Sclerotinia and other soilborne pathogens. Further work is required, however, to study the long-term impact of crop rotation with these green manure crops on diseases, yield and soil quality. The industry needs to consider the long-term resourcing of a new project to ensure growers get the full benefit of these practices for sustainable vegetable production. An Excel-based model was constructed with the aim of studying the economic consequences of changing cropping practices for the management of soilborne diseases using data from long-term trials.

4) New decision-support tools
Three methods were devised to predict infection risk during flowering, to quantify disease before harvest and to identify site-specific risk factors that drive disease development in bean fields. After further (commercial) validation, these tools should

be integrated into a decision support system to assist growers with disease risk management. It is suggested that future projects should complete development of these tools.

5) New areas of research

In the long-term, sustainable management of soilborne diseases will be achieved by using beneficial farming practices that minimise the build up of inoculum in soils and thus the occurrence of disease outbreaks. Research into this area is seriously hampered by the lack of knowledge of the effects of practices such as crop rotation and biofumigation on disease suppression, yield and the biology of soils in vegetable cropping systems. Therefore it is imperative that future research includes studies of soil biology to better understand how soil can be changed to destroy long-lived sclerotia. This is essential for developing effective integrated disease management strategies. New techniques are now available for the study of these two areas.

Finally, this national collaboration between plant pathologists has resulted in much better coordination of research activities to address industry priorities and thus develop new and practical strategies for Sclerotinia control. Hence, it is imperative that HAL continues funding this national program to ensure growers get the full benefit of research on soilborne pathology for sustainable vegetable production.

TECHNOLOGY TRANSFER

Communication with growers and industry

Results of this project have been presented to vegetable growers and industry across Australia through workshops, seminars and field days. Details of many of the presentations are listed below. The final outcomes and recommendations of the project were published in a brochure distributed during five national workshops in Victoria, Tasmania and Qld during August 2010. More workshops are planned in other states in late 2010 and more brochures will be distributed to growers through extension specialists and grower organisations.

Communication with growers and industry

First National Sclerotinia Workshop, Davonport, Tasmania 28 and 29 November 2007.

First National Vegetable Pathology Program Workshop, Melbourne, 6 -7 September 2007.

Met with representatives of Simplot Australia, December 2007, to outline project aims and plan surveys of fields.

'Soilborne Diseases Workshops', Stanthorpe and Gatton, Queensland, 21-22 May 2008.

Industry 'Soilborne Diseases Workshop', Amtel Club, Cranbourne Victoria, 23 May 2008.

Project update at the 'Research on Bean Diseases Workshop' Bellfield Tasmania, 14 March 2008.

Second National Vegetable Pathology Program Workshop, Melbourne 27th and 28th November 2008.

Field day at Heatherton trial. Local growers invited to walk the plots of fungicide and soil amendment trials on 8/12/08.

Project update presented at meeting with bean growers in Gympie, Queensland, 6 June 2008.

Sclerotinia project update at the Vegetable Pathology Workshop in Sydney, 21st May 2008.

Meetings with Simplot and McCain representatives to update on project progress and findings during 2009.

Sclerotinia Project. R&D showcase on Vegetables. Presentation and poster at Vegetable Industry Conference, Melbourne, 7-6 May 2009.

Sclerotinia Project in 'Vegetable IPM Diseases Program Handbook' published and distributed at Vegetable Industry Conference and Werribee Expo, 4-7 May 2009, Melbourne.

John Duff overseas travel report '14th International Sclerotinia Workshop' North Carolina, USA 31/5 – 4/6 2009.

Field day (10/06/09) to demonstrate proper incorporation of biofumigant crops into soil, Lindenow, Victoria.

Field day (19/06/09) to demonstrate proper incorporation of biofumigant crops into soil, Clyde, Victoria.

Grower meetings on the Granite Belt and at the Gympie Pack house in first year of the project to discuss what the project was about.

Green bean grower field day – Gympie 6 June 2008. Sclerotinia talk and project objectives and outcomes presented.

Sclerotinia articles in the Gympie Times from the grower field day (19 June 2008) and in Hort happenings – Lockyer Valley grower newsletter (July 2009).

Green bean grower field day – Gympie 6 August 2009. Project update presented.

IPM soilborne diseases and soil health national workshop, Devonport, Tasmania, 4 August 2010.

IPM soilborne diseases and soil health national workshop, Gympie and Lockyer Valley, Qld 11 and 12 August 2010.

IPM soilborne diseases and soil health national workshop, Lindenow and Cranbourne, Victoria 11 and 12 August 2010.

Managing Sclerotinia Diseases in Vegetables. Project Brochure included in "Vegetable Disease Program Notes' package published and distributed at grower workshops, August 2010.

Publications arising from this project

Referred

Villalta, O., Wite, D., Hunt, J., Stewart, A. and Porter, I.J. (2010). Biological control of *Sclerotinia minor* on lettuce using *Trichoderma* and *Coniothyrium* species. Acta Horticulturea (accepted April 2010).

McLean, K.L., Hunt, J.S., Stewart, A., Wite, D., Porter, I.J. and Villalta, O.N. (2010). Compatibility of a *Trichoderma atroviride* biocontrol agent with management practices of *Allium* crops. (submitted to biological control).

A PCR technique for detection of ascospores of *Sclerotinia sclerotiorum* in bean (*Phaseolus vulgaris*). (in preparation to be submitted to Plant Disease by end 2010).

Spatial analysis of white mould disease (*Sclerotinia sclerotiorum)* in bean (*Phaseolus vulgaris*). (in preparation to be submitted to Plant Disease by early 2011).

Development of a sequential sampling plan for estimation of the incidence of white mould disease (*Sclerotinia sclerotiorum)* in bean (*Phaseolus vulgaris*) fields. (in preparation to be submitted to Plant Disease by mid 2011).

Effect of crop rotation with biofumigant and other green manure crops on management of soilborne diseases and yield. (in preparation to be submitted to APPJ by mid 2011).

Other

Villalta, O., Wite, D., Porter, I.J., Ames, A. and Imsic, M. (2008). Evaluation of biofumigant amendments for the management of diseases caused by sclerotial pathogens in vegetable crops. 3rd International Biofumigation Symposium, Canberra, 21-25 July 2008, p66.

Progress toward integrated management of Sclerotinia in lettuce production in Victoria, Australia. Vegetable Production, Quality and Process Standardization in Chain: a worldwide perspective. Beijing, China, October 14-17, 2008, pp 28-29.

Villalta, O. (2009). 'Smart strategy beats cabbage-patch killer'. Vegetables Australia, vol 4.6 May / June 2009, pp42-43.

Wite, D., Villalta, O. and I. J. Porter (2009). Evaluation of plant extracts for control of Sclerotinia pathogens of vegetable crops. Australasian Plant Pathology Society Conference, Newcastle, NSW, September 2009, p221.

Sclerotinia Research. Primary Producer Section, Advocate Newspaper, Tasmania, 28 January 2010.

Plant Pathology seminar – July 2009. Talk (J. Duff) at the 14th International Sclerotinia Workshop, USA.

Article 'Outcomes National Sclerotia Project. Vegetables Australia, Sep / October 2010- in press.