Know-how for Horticulture™

Development of biological controls for Sclerotinia diseases of horticultural crops in Australasia

Dr. Ian Porter
VIC Department of Primary Industries

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Development of Biological Controls for Sclerotinia Diseases of Horticultural Crops

Final Report VG00048

Horticulture Australia Project

(June 2004)

Villalta *et al.*

Primary Industry Research Victoria, Knoxfield Centre
Serve-Ag Research, Tasmania
Lincoln University, Canterbury, New Zealand

HORTICULTURE AUSTRALIA PROJECT VG 00048 – Development of Biological Controls for Sclerotinia Diseases of Horticultural Crops

This is the final report for project VG 00048. It covers research into the biological and chemical control of Sclerotinia lettuce drop (SLD) and the use of soil amendment and cultural strategies for the integrated control of Sclerotinia diseases.

June 2004

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1 Media Summary

Sclerotinia diseases are a major cause of crop loss in horticultural crops (eg. lettuce, beans, carrots, crucifers, peas and others) despite the widespread use of fungicide sprays. In some regions of Victoria and Tasmania, where lettuce are sown every year, high losses to Sclerotinia lettuce drop (SLD) ranging from 10 to 45% were reported by growers despite the use of a regular fungicide spray program. Inconsistent chemical control and increasing public concerns over fungicide residue levels were major issues of concern to the industry and therefore this project was funded to find alternatives which gave more sustainable control of Sclerotinia diseases.

This project examined the effectiveness of well developed biological control agents for reducing inoculum and controlling disease, and compared a range of application methods to improve their efficacy using lettuce as a model system. The project evaluated strategic application of fungicide treatments for control of sclerotinia disease on lettuce and beans. Soil amendment strategies were also evaluated in combination with the use of registered chemicals and cultural strategies examined for their potential to reduce disease pressure and improve SLD control. The overall aim of the project was to provide growers with new options for the integrated control of Sclerotinia diseases and better inform them of the most appropriate use of fungicide treatments for disease control in their farms.

The main outcomes of the project were:

- Control of SLD was improved in farms by 80 to 96% with the use of new strategic application of procymidone (sold as Sumisclex™ or Fortress™) sprays. Improved application can improve its efficacy and therefore growers are advised to modify their fungicide application methods for maximum disease control.
- The new fungicide BAS 510 (BASF – boscalid) was shown to be as effective as procymidone in controlling SLD and bean white mould and therefore it has the potential to replace procymidone or for use in alternation with procymidone.
- The treatment of seedling growing mixes in nurseries was the most effective method for delivering *Trichoderma* biocontrol agents into a seedling transplant system. Two commercial composted pine bark mixes were identified as suitable substrates to incorporate *Trichoderma*.
- In the field, biocontrol treatments did not give the same consistent and effective control of SLD provided by fungicide treatments and were not effective in reducing inoculum in soil. This was due to the inability of biocontrol agents (BCAs) to establish in soil at levels considered optimal for effective biocontrol. A better understanding of the compatibility of BCAs with farm practices (eg fertilisers, pesticides) and soil characteristics (eg organic matter) conducive for maximum growth of BCAs is required to improve the field efficacy of biological treatments.
- BQ-Mulch (green manure crop) used in rotation with lettuce in a Tasmanian soil reduced Sclerotinia disease by suppressing infection and improved soil characteristics.
- Seedlings of onions, beets and spinach, which have upright foliage, were less susceptible to Sclerotinia infection and therefore can be selected for crop rotations in high Sclerotinia pressure sites.

An integrated disease management strategy has been devised for the control of SLD. This will be summarised in the brochure ‘Integrated Control of Sclerotinia Lettuce Drop’, which will be distributed to nurseries and vegetable growers.

2 Technical Summary

Sclerotinia diseases are a major cause of crop loss in horticultural crops (eg. lettuce, beans, carrots, crucifers, peas and others) across Australian farms despite the widespread use of fungicide sprays. This is because on these farms intensive cropping and the use of Sclerotinia susceptible crops in rotations has led to a build up of disease and inoculum in soil. In some regions of Victoria and Tasmania, where lettuce are grown every year, high losses from *Sclerotinia minor* ranging from 10 to 45% were reported by growers despite the use of a regular fungicide (procymidone) spray program. Inconsistent chemical control and increasing public concerns over fungicide residues were two major issues of concern to the industry and therefore this project was funded to find alternatives for a more sustainable control of Sclerotinia diseases. Another market issue for Australian lettuce producers were crop losses to Sclerotinia lettuce drop (SLD). Consequently, as lettuce is the most susceptible crop to Sclerotinia, lettuce crops were used as a model to evaluate biological and fungicide alternatives and other strategies for a more sustainable control of SLD disease.

Two fungal biological control agents (BCAs) were selected from the biocontrol program at Lincoln University (New Zealand) for evaluation in the Australian trials. These two BCAs (*T. hamatum* 6Sr4 and *C. minitans* A69), which have good antagonistic activity against *S. minor* and are being considered for commercialisation, were evaluated for their potential to reduce inoculum in soil and control SLD. Several other biocontrol products developed in Australasia and overseas were also evaluated. These included products containing a fungal mycoparasite (*C. minitans* - Contans™) of *S. minor*, one isolate of *Bacillus subtilis* (Companion™), six isolates of *Trichoderma* spp and two products containing a mixture of *Trichoderma* spp and bacterial isolates (sections 5.4, 5.6, 6.3, 6.4). Laboratory experiments (Lincoln University) compared methods to produce inoculum (spores) of *T. hamatum* 6Sr4 and *C. minitans* A69 (section 5.2). *T. hamatum* 6Sr4 was a fast growing fungus and therefore was easy to produce in bulk and formulation technology was available which provided a product with good viability and shelf-life. In contrast, *C. minitans* was a slow growing fungus and none of the methods evaluated gave sufficient spores to enable commercial bulk production of its inoculum.

Different methods for delivering BCAs into a lettuce cropping system were examined in glasshouse, nursery and field experiments (sections 5.2, 5.3, 5.4, 6.3). The incorporation of BCAs into seedling growing mixes in nurseries was the most effective method for delivering *Trichoderma* spp BCAs into a seedling transplant system. Two commercial mixes were identified as suitable substrates to incorporate *Trichoderma* spp. A maize-perlite method of inoculum incorporation (soil incorporation at planting) was also effective in delivering *T. hamatum* 6Sr4 and *C. minitans* A69 into soil. The effectiveness of several biocontrol treatments for reducing *S. minor* inoculum in soil and controlling SLD was investigated in twelve field trials conducted in Victoria (8) and Tasmania (4) over several growing seasons at sites with different disease pressures (sections 5.4, 5.6, 6.3, 6.4). Biocontrol treatments were not effective in reducing sclerotial levels in soil. Despite some biocontrol treatments showing some potential to reduce disease (50-76% disease reduction) at 2 low disease pressure sites (≤7% disease), biocontrol treatments did not give the same consistent and effective control of SLD provided by the standard fungicide procymidone. Further field trials showed an integrated approach using biocontrol treatments combined with a single fungicide application did not offer potential for enhanced control of SLD (section 5.4). The low efficacy of biocontrol treatments was due to the inability of biocontrol agents (BCAs) to establish in soil at levels considered optimal for effective biocontrol. A better understanding of the compatibility of BCAs with farm practices (eg fertilisers, pestcides) and soil characteristics (eg organic matter) conducive for survival and maximum growth of BCAs is required to improve the field efficacy of biological treatments.

The effectiveness of strategic application of fungicides for improving SLD control was investigated. Results from fourteen field trials in commercial farms in Victoria and Tasmania showed that consistent and effective control of SLD (80-96% disease reduction) can be obtained with strategic (plant-targeted) applications of procymidone (sold as Sumisclex™ and Fortress™) sprays (sections 5.4, 5.5, 6.3, 6.4, 6.7, 6.8). In four of the fourteen field trials, the effectiveness of the new fungicide BAS 510 (BASF, boscalid) for controlling SLD was also investigated. Two applications of the new

fungicide BAS 510 provided as good SLD control (90-97% disease reduction) as two procymidone sprays on lettuce and beans in (sections 5.4, 5.5, 6.8). Higher rates of BAS 510 (ei. 500 and 800 g a.i./ha) were more effective in controlling SLD. BAS 510 has the potential to replace procymidone and for use in alternation with procymidone. Experiments in Tasmania also investigated an integrated approach using procymidone sprays combined with chemicals that enhance the plant natural defence systems for improving control of SLD (sections 6.3, 6.7 and 6.8). In the field, Agri-Fos™ (plant activator) and MicroGyp™ (fertiliser), when applied in mixtures with Sumisclex™, tended to consistently lower Sclerotinia disease incidences (lettuce and beans) compared to the use of use of Sumisclex™ alone in four field trials.

The effectiveness of soil amendments strategies for reducing inoculum and disease pressure was investigated (sections 5.6, 6.6). Cold pressed mustard meal (biofumigation) and the fertilisers Urea™, Perlka™ and blood and bone meal™ were effective in reducing sclerotia of *S. minor* in soil when used as pre-planting treatments using high rates of product/ha. In Tasmanian soils, the mustard meal and Perlka™ suppressed disease at the early lettuce crop stage, when applied one day before planting. In Victorian soils, however, Perlka™ and Urea™ were toxic to plants when applied 7-14 days prior to transplanting. Soil amendment treatments require further research to optimise their use for Sclerotinia control. The effectiveness of a green manure crop for reducing inoculum and disease pressure was also investigated in a trial in Tasmania (section 6.5). BQ-Mulch (fodder), which produces high levels of isothiocyanates (ITCs) in its roots, was more effective in suppressing SLD infection than Fumus (mustard), which produces high levels of ITCs in its foliage. These two brassica green manure crops did not reduce *S. minor* inoculum in soil, and short-term suppression of disease is believed to be mode of action of BQ-Mulch against SLD. Other benefits of the high biomass and deep tap root systems of two green manure crops were soil improvements such as improved infiltration and increased organic matter.

The susceptibility of crops used in rotation with lettuce was investigated (6.9). Pot experiments in Tasmania confirmed that the susceptibility of seedlings of brassica green manure crops (BQ-Mulch and Fumus) and rotation crops (broccoli, onion, broad beans) to Sclerotinia infection was closely associated with plant architecture. Young seedlings of onions, beets and spinach, which have upright foliage, were less susceptible to Sclerotinia infection and therefore these plants can be selected for crop rotations, particularly in ground that has high Sclerotinia pressure.

There was a positive correlation between the concentration of sclerotia of *S. minor* in soil and disease incidence at field sites in Victoria and Tasmania (sections 5.4, 6.9). In Victorian sites, however, environmental factors greatly influenced the development of disease compared to Tasmanian sites where conditions for Sclerotinia disease were more consistent (cool and wet weather) from season from season. Consequently, counts of sclerotia (eg. wet sieving method) will be more useful in Tasmania than in Victoria for identifying high disease pressure sites.

This project has shown that control of *S. minor* (SLD) in farms can be improved by:

- improved application and timing of the standard fungicide procymidone,
- use of rotation crops (green manure crops) suppresive to disease,
- selecting crops less susceptible to Sclerotinia infection for rotations with lettuce in high Sclerotinia pressure sites (stop build up of inoculum in soil) and
- use of the new fungicide BAS 510 (once registered).

Nurseries using *Trichoderma* to improve the growth of seedling transplants should consider using, when possible, mixes with low levels of naturally occuring microorganisms (eg composted pine bark mixes) because these mixes have more space and available food sources for *Trichoderma* spp to grow.

3 Recommendations

Recommendations arising from this research have been summarised in the brochure 'Integrated Control of Sclerotinia Lettuce Drop', which will be distributed to nurseries and vegetable growers nationally. A copy will be inserted in this report at a later date.

This report provides a comparison of application methods and effectiveness of biocontrol treatments for controlling Sclerotinia lettuce drop (SLD) in different pressure sites, seasons and agricultural soils in Victoria and Tasmania. The methods evaluated for incorporating biocontrol treatments into a lettuce transplant system were effective in delivering biological control agents (BCAs) into the region of roots of lettuce seedling transplants, where they are needed for effective biocontrol. However, the levels of BCAs into the region of roots of seedling transplant and plants in the field, especially at the base and under the leaves of plants, were considered sub-optimal to suppress *Sclerotinia minor* infection in the Australian trials. Under these conditions, it was difficult to verify that BCAs agents, with reported good antagonistic activity against sclerotial pathogens in other countries, have the potential to control early season and late season (spread of disease from infected plants) infection by *S. minor* in Australian soils. In one trial in a Tasmania soil, *C. minitans* (Contans™) showed some potential for reducing *S. minor* sclerotia in soil (56% disese reduction) when applied 6 months prior to planting at a high rate of 10 kg/ha. This product has been registered in Europe and USA for the control of Sclerotinia diseases and based on the results obtained in this project it warrants further evaluation. More research is required to further understand the factors (eg. compatibility of BCAs with common nursery and farm practices such as fertilisers, fungicides) which influence the ability of BCAs to colonise the region of roots of field plants at levels considered suitable for effective biocontrol. This research is needed to ensure that these alternative treatments are developed for the vegetable industry in Australia.

Since the mid 1990s, procymidone (sold as Fortress or Sumisclex™) has been the standard and predominant fungicide for Sclerotinia control in south-eastern Australia. Field trials conducted in this project and other work indicated that many growers may be applying this product ineffectively. Effective fungicide application methods are essential for good disease control, with appropriate spray volume and timing in different types of horticultural crops. Improved application can improved its efficacy. Drenching of plants at transplanting is critical for effective control of SLD. The applications of procymidone at the early crop stage before canopy closure, approximately 2 and 4 weeks after lettuce transplanting, appeared to leave good residual levels for effective control of late season infection. This is critical for controlling mycelial growth and preventing lower leaf infections, especially with crisphead lettuce. Irrigating after application of procymidone sprays can help to drench the fungicide into the plant base and top soil.

Most other registered fungicides, such as benomyl (Marvel™ WA only) and iprodione (Rovral™) and new fungicides such as azoxystrobin, fluazinam, fludioxinol and tebuconazole, were found to be less effective than procymidone under high disease pressure conditions in this and other projects (Pung and O'Brien 2000). The reliance on procymidone for Sclerotinia control can result in the loss of product effectiveness with overuse. Although field trials and *in vitro* tests showed that there was no evidence of fungal resistance or enhanced degradation of procymidone, it is still good practice to use no more than two applications of procymidone in a crop or alternate its use with a fungicide from a different chemical group.

This project also demonstrated the potential of the new fungicide BAS 510 (BASF - boscalid) to control Sclerotinia diseases, caused by *S. minor* and *S. sclerotiorum*, on lettuce and beans. Under high disease pressure conditions, the consistency and efficacy of BAS 510 was similar to procymidone and different rates of the product evaluated indicated that higher rates of BAS 510 (eg 500 and 800g/ha) were more adequate for Sclerotinia control. This fungicide can be used as a potential replacement for procymidone or used in alternation with procymidone. Procymidone is a dicarboximide fungicide, while BAS 510 belongs to the new chemical group benzanilide. BAS 510 has recently been registered in Australia for the control of powdery mildew of grapes (Filan™) and based on the results obtained in

this project it warrants further development as a plan treatment for control of Sclerotinia diseases of vegetables. This project has collected efficacy data (non-GLP) on this product. The supplier in Australia (BASF - NuFarm) has shown some interest in developing the fungicide for the Australian market.

This project showed the potential of using soil amendment (nitrogenous products) and cultural strategies (biofumigation green manure crop) to reduce inoculum levels and improve control of SLD. The integration of these strategies into Sclerotinia management programs is crucial to reduce disease pressure in fields with high levels of sclerotia. A reduction in disease pressure will contribute to reductions in fungicide sprays and expenses, ensuring that they are only used when required, thus prolonging the life of these chemicals. More research is required to optimise the use of soil amendment and green manure crops for inoculum reduction in soil.

There is an increasing pressure to produce vegetable crops with minimum to zero pesticide input. Work done in this project demonstrated the potential of using soil amendment and cultural strategies for reducing inoculum levels and thus disease pressure. Integration of these treatments with reduced fungicide applications can minimise the expense of fungicides, ensuring that they are only used when required, thus prolonging the life of these chemicals and providing growers with an effective means of risk management. Although the biocontrols treatments evaluated in this project were not effective in controlling SLD, these and new biocontrol treatments should be further evaluated under Australian conditions to determine the factors that influence their survival in different soils. This new research should also focus on finding soil conditions and organic matter levels which assist BCAs to colonise soils to improve their biocontrol activity. There has been other work done in overseas countries, demonstrating the efficacy of other treatments, for example, the use of new natural soil fumigants (eg. DMDS) for eradication of sclerotial pathogens in soil. In the future, integration of a number of treatments such as BCAs, rotation crops and soil amendments with reduced fungicide applications for control of Sclerotinia diseases will be essential to the sustainability of industry.

4 Technology Transfer

Field days/walks

- Visit to Australian field trials (Werribee, Tasmania) by NZ researchers and presentation of results to grower groups at Werribee and Cranbourne (20-25 October 2001).
- Tasmania – Field day attended by growers and industry representatives to examine the Brassica green manure crops (biofumigation crops) field trial at Cuprona (06/11/02).
- Victoria – Field walk attended by local growers to inspect the biocontrol field trial at Werribee (12/12/03).

Extension activities

- Lettuce Growers Conference Hay, NSW (2001).
- Tasmanian Vegetable Extension Day, Devonport (15/8/1).
- Integrated management of Sclerotinia lettuce drop, Brisbane (May 2002).
- Biocontrol of *Sclerotinia minor*. 2nd Australian Lettuce Industry Conference Gatton Qld – May 5-8 2002.
- Industry Forum – Brassica green manure and biofumigation. Devonport Tasmania (24/2/03).
- Biocontrols and soil amendments for Sclerotinia diseases, Lettuce Industry Focus Meeting, Werribee (24/2/3).
- Integrated Control of Lettuce Drop. National Lettuce Industry Field Day, Robinvale Vic (25/7/3)

Extension articles

- Sclerotinia Lettuce Drop, Vegetable Matters-of-Facts Publication, DPI (VegCheque). No. 7 August 2003.
- Managing Lettuce Drop - Article in Lettuce Leaflet, Best Management Options Newsletter Issue No. 10, June 2003.
- Managing Lettuce Drop - Article in Lettuce Leaflet, Best Management Options Newsletter Issue No. 8, August 2002.
- Brochure 'Integrated Control of Sclerotinia Lettuce Drop'. pending

Conference abstracts and papers

Villalta, O., Porter, I, Wite, D., Czerniakowski, B., Pung, H., Stewart, A. and Hunt, H. (2004). Integrated Control of *Sclerotinia minor* in lettuce. Proceedings of the 3rd Australasian Soilborne Diseases Symposium. Ophel Keller, K.M. and Hall, B.H. (Eds). pp 139-140.

Rabeendran, N., Jones, E.E., Hunt, J., Moot, D. and Stewart, A. (2004) Biological control of Sclerotinia species in lettuce using *Trichoderma hamatum*. Proceedings of the 3rd Australasian Soilborne Diseases Symposium. Ophel Keller, K.M. and Hall, B.H. (Eds). pages 63-64.

Pung, H. and Cross, S. (2003). The use of biofumigant crops for soilborne disease management in lettuce crops in Tasmania. 8th International Congress of Plant Pathology, Christchurch New Zealand, pp 138.

Pung, H. and Cross, S. (2003). Integrating materials that enhance plant defence systems with procymidone, for improved Sclerotinia minor control. 8th International Congress of Plant Pathology, Christchurch New Zealand, pp 180.

Porter, I,J., Pung, H., Villalta, O., Crnor, R., Stewart, A. (2002) Development of biological controls for Sclerotinia diseases of horticultural crops in Australasia. 2nd Australasian Lettuce Industry Conference, 5-8 May, University of Queensland Gatton Campus.

Stewart, A., Rabeendran, N., Porter, I., Launonen, T., Hunt, J. (2001) Biological control of Sclerotinia diseases of vegetables using *Coniothyrium minitans* A69. XIth International Sclerotinia Workshop, York, UK 8-12 July, pp145-146.

Stewart, A., Rabeendran, N., Porter, I., Launonen, T., Hunt, J. (2001) Biological control of Sclerotinia diseases of vegetables using *Coniothyrium minitans* A69. 13th Biennial Australasian Plant Pathology Conference, Cairns, 24-27th September, abstract p337.

T.M. Launonen, I. J. Porter, A. Stewart, H. Pung and P.J. Keane (2001). Relative efficacy of biological and chemical products for control of *Sclerotinia minor* in lettuce: preliminary results of a field study. Proceedings of the 2nd Australasian Soilborne Diseases Symposium (insert).

5 TECHNICAL REPORT PART A – Evaluation of biocontrol, chemical and soil amendment treatments for control of Sclerotinia lettuce drop – Victorian and New Zealand Studies

5.1 Introduction

Sclerotinia diseases are a major cause of crop loss in horticultural crops (eg. lettuce, beans, carrots, brassicas, peas and others) across Australian farms despite the widespread use of fungicide sprays. This is because on these farms intensive cropping and the use of Sclerotinia susceptible crops in rotations has led to a build up of disease and inoculum (fungal sclerotia) in soil. As a result, fungicide spray programs alone may not give adequate control of disease in highly susceptible horticultural crops. In some areas of Victoria and Tasmania, where lettuce are sown every year, high losses to Sclerotinia lettuce drop (SLD) ranging from 10 to 45% have been reported by growers despite the use of a regular fungicide (procymidone) spray program. Inconsistent chemical control and increasing public concerns over fungicide residues were two major issues of concern to the industry and therefore this project was funded to find alternatives for a more sustainable control of Sclerotinia diseases.

The vegetable industries rely on the use of fungicides to control Sclerotinia diseases with variable success and additional costs to crop production. Poor control of Sclerotinia can lead to heavy losses in yield and quality. The over reliance on fungicides for disease control could also result in reduced fungicide efficacy, development of fungicide resistance and enhanced degradation of fungicide in soil. Fungicide alternatives (eg. biocontrols) and cultural control strategies (crop rotation and soil amendments) are not widely used within the lettuce and other industry because it has not been demonstrated they can provide effective (or enhance) disease control in a variety of crop and soil systems. Inconsistent chemical control and increasing public concerns over repeated fungicide use indicates that an integrated disease control approach reducing reliance on chemical control is desirable for a more sustainable control of Sclerotinia diseases and production.

Soil microorganisms antagonistic to Sclerotial pathogens (eg. *Coniothyrium minitans* and *Trichoderma* species) are reported to have potential for biocontrol of Sclerotinia diseases. Biological control, which aims to reduce inoculum levels in soil (mycoparasites of sclerotia) or protect the region of roots of plants from infection (root colonising biocontrol agents), could be an effective alternative control measure for the control of Sclerotinia diseases if biocontrol can be demonstrated to be effective over a wide range of cropping systems, climatic conditions and soil types. The integration of soil treatments that reduce inoculum levels in soil (eg. biocontrols, soil amendments, biofumigation crops) with reduced fungicide applications offers another possibility to minimise the use and expense of fungicides, ensuring that fungicides are only used when required, thus prolonging the life of these chemicals and providing growers with an effective means of risk management.

The aims of the studies in Victoria were to evaluate the most promising biological control agents, developed in Australasia and overseas, for their ability to kill sclerotia in the soil and control SLD, and compare methods for their application into a lettuce cropping system in Australia. The project also aimed to evaluate the use of biocontrol treatments in combination with fungicides and strategic application methods for fungicide (procymidone) to maximise disease control. The project also aimed to examine the effectiveness of soil amendment strategies for controlling SLD and investigate the relationship between concentrations of sclerotia in soil and disease incidence with a view to develop a simple method to predict crop loss and recommendations for application of strategies for SLD control. As lettuce is the most susceptible crop to Sclerotinia, lettuce crops were used as a model to evaluate the effectiveness of the different treatments for controlling SLD.

5.2 A COMPARISON OF METHODS FOR CULTURING, OPTIMISING SPORE PRODUCTION AND INCORPORATING INOCULUM OF BIOLOGICAL CONTROL AGENTS

Alison Stewart, Nimal Rabeendran, Kirstin McLean and John Hunt
Lincoln University and Agrimm Technologies Pty Ltd New Zealand

Includes results of one experiment (5.2.3.4) conducted by Oscar Villalta and Rosa Crnov, DPI Vic.

5.2.1 Summary

Three experiments were conducted at Lincoln University (New Zealand) to compare methods for culturing and optimising spore production of two biological control agents (*T. hamatum* 6Sr4 and *C. minitans* A69), and compare methods for incorporating inoculum of both biocontrol agents into a lettuce transplant system. There was no significant difference between liquid culture and solid medium agar culturing methods for production of spores of *C. minitans* A69. Agar plate culturing has the advantage of being less likely to get contamination compared to liquid culture. However, it is more time consuming to collect spores off plates. A commercial scale production system requires $10^7/10^8$ spores/mL to be efficient and neither plate or liquid culturing gave sufficient spores to enable bulk production of inoculum.

Medium, inoculum source and temperature all influenced the sporulation capabilities of *T. hamatum* (6Sr4). *T. hamatum* (6Sr4) sporulated best in culture when inoculated as spores onto PDA plates and incubated at 20°C for 10 days in a 12h light/ dark cycle. Sporulation was greater and faster when agar plates were inoculated with a spore suspension compared with a mycelial plug and incubated at 20°C rather than 26°C. The growth medium influenced spore production, with PDA increasing the ability of *T. hamatum* (6Sr4) to sporulate compared with PMA, MEA, OMA and PCA. The medium that promoted maximum growth and sporulation of *T. hamatum* 6Sr4 was OMA. The maize-perlite method for incorporating both biocontrols (*T. hamatum* 6Sr4 and *C. minitans* A69) into soil gave significantly greater cfu recovery in soil throughout the 56 day trial period compared with the untreated control.

One field trial was conducted at Canterbury New Zealand to test the ability of the two biocontrol agents from New Zealand (*T. hamatum* 6Sr4 and *C. minitans* A69) to reduce disease in lettuce caused by *S. minor*. Level of disease achieved in the field trial was low and there was, therefore, no significant difference between the treatments and the untreated control. The two biocontrol treatments produced higher quality lettuce heads, evident after 12 days storage.

An *in vitro* experiment was conducted at DPI Knoxfield to examine the effect of temperature on mycelial growth and spore production of the two biocontrol agents from New Zealand, an isolate of *C. minitans* (Contans™) from Europe, and an Australian isolate of *T. harzianum* (Trich-A-Soil™). The optimum temperature for mycelial growth of *T. hamatum* (6Sr4) and *T. harzianum* (Trich-A-Soil™) was at 20 and 25ºC, which was recorded at days 5 and 8 of incubation. The optimum temperature for mycelial growth of *C. minitans* (A69) and *C. minitans* (Contans™) was at 20ºC, which was recorded at day 8 of incubation. The greatest sporulation occurred at 20 and 25ºC for all four biocontrol agents. The two *Trichoderma* isolates grew faster and produced more spores than the two *Coniothyrium* isolates at 20 and 25ºC.

5.2.2 Introduction

One of the aims of this project was to test the most promising biological control agents developed in Australasia and overseas for their ability to kill sclerotial pathogens and prevent Sclerotinia disease in vegetables and to identify strategic application methods that maximise disease control by these biocontrols. Research at Lincoln University (New Zealand) has concentrated on the biocontrol of sclerotial pathogens of fruit and vegetable crops (Jones and Stewart 2000, McLean and Stewart 2000, Ridgway *et al.* 2001). In this research, *Trichoderma hamatum* isolate 6Sr4 and *Coniothyrium minitans* isolate A69 were identified as potential biological control agents for *Sclerotinia minor*, the cause of lettuce drop. Research field trials conducted at Lincoln University showed that the two biocontrol agents were able to parasitise sclerotia and mycelium of *S. minor*, grow well in soil around the root of lettuce plants and protect the plants from *S. minor* infection (50-80% disease control) (Jones and Stewart 2000, Ridgway *et al.* 2001, Stewart *et al*. 2001). Hence, these two biocontrol agents were selected to test their relative efficacy against *S. minor* on lettuce under Australian conditions.
The use of promising biocontrol agents for the biocontrol of Sclerotinia diseases in commercial crops would rely on the ability to produce and formulate biocontrol agents cheaply in large quantities and develop appropriate

delivery methods to ensure colonisation of the region of roots of plants can occur at levels considered optimum for effective biocontrol. Consequently in this project, three experiments were conducted at Lincoln University to compare methods for culturing and optimising spore production of *T. hamatum* 6Sr4 and *C. minitans* A69, and compare methods for incorporating inoculum of both biocontrols into a lettuce transplant system. One experiment was conducted at Canterbury New Zealand to test the ability of the two biocontrols from New Zealand to reduce disease in lettuce caused by *S. minor*. Another experiment was conducted at DPI Knoxfield to examine the effect of temperature on mycelial growth and spore production of the two biocontrols from New Zealand, a German isolate of *C. minitans* (Contans™) and an Australian isolate of *T. harzianum* (Trich-A-Soil™).

The specific aims of the five experiments were:

- The first experiment was conducted to determine the best culturing method and optimise sporulation for the production of *C. minitans* isolate A69.
- The second experiment was conducted to optimise the sporulation capabilities of *T. hamatum* isolate 6Sr4 in culture by determining the best inoculum source, temperature and growth medium.
- The third experiment compared methods for incorporating viable inoculum of the two biocontrol agents antagonistic to *S. minor* (*T. hamatum* 6Sr4, *C. minitans* A69) into a lettuce transplant system to determine which method best promoted proliferation of the biocontrol agents.
- A field trial was conducted to evaluate the ability of *C. minitans* A69 and *T. hamatum* 6Sr4 to reduce disease in lettuce caused by the fungal pathogen *S. minor* in Canterbury, New Zealand.
- An *in vitro* experiment was conducted at DPI Knoxfield to examine the effect of temperature and time of incubation on mycelial growth and spore production of four biocontrol agents (*T. hamatum* 6Sr4, *C. minitans* A69, *T. harzianum* isolate Trich-A-Soil™ and *C. minitans* isolate Contans™).

5.2.3 Materials and methods

5.2.3.1 Optimise sporulation of *C. minitans* A69

Comparison of culturing methods

This *in vitro* experiment was conducted at Lincoln University. The following culturing methods were evaluated:
a) Nutrient source
-concentration of Potato extract broth (PEB)
-concentration of carbohydrate in the form of Sucrose
b) Aeration of liquid cultures
c) Volume of liquid medium used
d) Liquid culture vs solid medium culture.
To determine the optimum nutrient source for maximum sporulation, the potato extract broth (PEB) concentrate (Appendix) was diluted with distilled water before use. Two concentrations of PEB were investigated, 20% and 40% (v/v), and three concentrations of sucrose 0g/L, 2g/L or 5g/L using the concentration of PEB that yielded the most growth. Aliquots of 100 mL of the relevant nutrient broths were transferred to 250 ml flasks that were subsequently capped with cotton wool bungs and tinfoil before being autoclaved for 15 min at 121^{o}C. Once cooled, flasks were inoculated with 1 mL A69 spore suspension (10^7 spores/mL) harvested from 10 day old PDA plates. Level of sporulation was determined after 12 and 30 days.

Aeration of cultures

Aliquots of 100 mL of 20% (v/v) PEB plus 2g/L sucrose was transferred to 250 mL smooth walled flasks that were subsequently capped with cotton wool bungs and tinfoil before being autoclaved for 15 min at 121^{o}C. Once cooled, flasks were inoculated with 1 mL A69 spore suspension (10^7 spores/ml) harvested from 10 day old PDA plates. Some flasks were aerated by shaking on a rotary shaker at 120 rpm, while others were non-aerated by leaving them to stand on the bench. All flasks were incubated at room temp in the dark. Spore concentrations were determined after 12 and 30 days using a haemocytometer.

Liquid broth vs agar plate cultures

The aeration experiment was repeated, but this time using 200mL of 20% PEB (v/v) plus 2 g sucrose/L in a 1 L flask and inoculating with 3 mL spore suspension at 1.9 x 10^6 spores/ml. Flasks were shaken on a large Gallenkamp shaker in the dark at 120 rpm at 20-25^{o}C. Potato dextrose agar plates were inoculated with 1 µL of

the same spore suspension and incubated in the dark at 20°C. Spore production was determined at days 9 and 12. After 9 days, there were 2.5 x 10^7 spores/mL in the flasks and this meant a total of 5 x 10^9 spores in the 200 mL of liquid. In contrast, there was only 6.6 x 10^8 spores in 10 mL harvested from PDA plate cultures. After 12 days, there was no change in spore numbers for either medium. Spores were filtered through lens tissue to remove any hyphal fragments and then air dried before their dry weight was determined.

5.2.3.2 Optimise sporulation of *Trichoderma hamatum* 6Sr4

Inoculum source

This *in vitro* experiment was conducted at Lincoln University. Both mycelia plugs and spore suspensions were investigated as potential inoculum sources. Mycelial plugs (5 mm diameter) were taken from the actively growing edge of a 5 d old culture grown on Potato dextrose agar (PDA) at 20°C in a 12 h light/dark cycle. Spore suspensions were prepared from 12 d old PDA cultures grown at 20°C in a 12 h light/dark cycle. Spores were suspended in Tween 80 by adding 5 mL to the top of the plate culture and dislodging them with a sterile glass hockey stick. The resultant spore suspension was diluted with an additional 5 ml Tween 80 and filtered through lens tissue 4x to remove any hyphal fragments. Spore concentration was determined with a haemocytometer and adjusted to give a final concentration of 1 x 10^7 spores/mL.

Medium

The following five media were investigated for the influence of nutrient source on the level of sporulation of *T. hamatum* 6Sr4 in culture (See appendix for recipes):
Potato dextrose agar (PDA)
Potato molasses agar (PMA)
Malt extract agar (MEA)
Oatmeal agar (OMA)
Potato carrot agar (PCA)

Assessment

Four plates of each medium/ inoculum source combination were incubated at 20, 26 and 35°C in a 12 h light/dark cycle. Plates were inoculated by placing either a mycelial plug or 0.25 mL spore suspension in their centre. Plates were examined after 5 and 10 days, colony growth was noted and the level of sporulation determined by dislodging spores in 5 mL of Tween 80 per plate using a glass rod, pooling the resultant suspensions from the four plates and counting the spores/mL using a haemocytometer. The average number of spores/mL was calculated from five counts for each of the temperature/medium variations for both inoculum sources. Results were analysed using analysis of variance (ANOVA), with inoculum source, temperature and media as the factors. Where ANOVA indicated a significant treatment effect, this was further explored using a Fisher's Least Significant Difference (LSD) test.

5.2.3.3 Comparison of methods for incorporating viable inoculum of 2 biocontrol agents (*T. hamatum* 6Sr4, *C. minitans* A69) into a lettuce transplant system

Production of spore inoculum

This experiment was conducted at Lincoln University. Petri plates of different media were inoculated centrally with a 5 mm diam. mycelial disc of the fungus and incubated at 20˚C under a 12 h light/dark cycle. Media investigated were: Oatmeal Agar (OMA), Potato dextrose agar (PDA), V8 juice agar (V8A) and Czapek-dox agar (CZA). Rates of mycelial growth and time taken for maximum sporulation were recorded for each antagonist. The medium that promoted maximum growth and sporulation of *Trichoderma hamatum* 6Sr4 was OMA, whereas PDA was the best for isolate *Coniothyrium minitans* A69. Spores were obtained for each antagonist fungi by flooding these culture plates with sterile distilled water (SDW) and dislodging spores with a sterile laboratory hockey stick. Resultant spore suspensions were filtered through a sterile double layer of lens tissue and the concentration of spores adjusted to 10^8 spores/mL using a haemocytometer. Viable spore counts (colony forming units, cfu per mL) for each isolate suspension were determined. A ten fold dilution series was made down to 10 spores/mL. Aliquots (0.25 mL) taken from the 10^1 –10^3 spores/mL dilutions were spread onto PDA plates amended with Triton-X-100 (2mL/L) and incubated at 20˚C in the dark for 3 days. The number of resultant colonies was counted from five plates of each dilution and percentage spore viability calculated to be approximately 85% for both antagonists.

Maize-perlite inoculum

Inocula of both isolates were prepared in a maize-perlite substrate for soil incorporation. Spore suspensions of *C. minitans* A69 and *T. hamatum* 6Sr4 were produced as described above and adjusted to 10^6 viable spores/mL. To prepare inocula, livestock maize was ground with a commercial Waring blender for 5 min and mixed with perlite at a ratio of 15:85 (v/v). To prepare *C. minitans* A69 inoculum, 1.5 L of the maize-perlite mix was placed into autoclavable Sterilin bags (300 x 660 mm) and moistened with 300 mL of tap water. A metal ring (50 mm diam) was inserted into the mouth of the Sterilin bag and plugged with non-absorbent cotton wool wrapped with aluminium foil. The mixture was autoclaved twice at 15 psi for 30 min over 2 consecutive days. Once cooled, the substrate was inoculated with 75 mL of the A69 spore suspension and bags were incubated in the dark at 20 ˚C for 42 days. The bags were shaken periodically throughout incubation to distribute the mycelial growth evenly throughout the mix. For preparation of *T. hamatum* 6Sr4 inoculum, 1L conical flasks were filled with 500 mL of maize:perlite, inoculated with 25 mL of 6Sr4 spore suspension and incubated under a 12 h light/dark cycle for 42 days. Following incubation, both forms of inoculum were stored at 4 ˚C for 7-14 days before use.

Monitoring of populations of biocontrol agents in the soil

Maize-perlite preparations of both biocontrol agents were prepared as outlined in section 2 above. Inoculum was applied to the soil in field plots at Lincoln University at a rate of $0.8L/m^2$ and the cfu's of each fungus monitored over a 56 day period.

5.2.3.4 Effect of temperature and incubation time on growth and sporulation of four biocontrols

This experiment was conducted at DPI Knoxfield. The biocontrols tested in this experiment were *T. hamatum* (6Sr4, Lincoln University, NZ), *T. harzianum* (Trich-A-Soil™, Organic Crop Protectants Pty Ltd, NSW), *C. minitans* (Contans™, Prophyta Germany) and *C. minitans* (A69, Lincoln University NZ). Inoculum was isolated from experimental and commercial biological products.

One gram of each product formulation was placed in a falcon tube with 10 mL of sterile distilled water and shaken in a vortex. The spores dispersed in water were collected with an inoculation sterile needle and streaked on standard PDA plates amended with antibiotic. Microscopic examination of mycelial and spore morphology was conducted to confirm that each isolate culture was the fungi tested. A 2-week old culture of each isolate on PDA plates was used in the experiment. A sterile steel borer of 8 mm diameter was used to cut out mycelial discs from the plates. Single discs were aseptically transferred to the centre of standard PDA agar plates amended with antibiotic. Two replicate plates were prepared for each treatment. The plates with the discs were incubated at 7, 10, 14, 20 and 25 ±2 °C under diffuse light in controlled temperature cabinets. Measurements of fungal colony growth and spore production were made 2, 5 and 8 days after the plates were incubated in the respective temperatures.

Two measurements of fungal colony growth (radial growth), one at right angles to the other, were taken from each plate until the colony growth had reached the edge of the plate. The average linear growth for each set of two plates was then calculated. Two streaks across the diameter of the colony, one at right angles to the other, were made with an inoculation sterile needle. The spores collected were dispersed in 1 ml of distilled water in an eppendorf tube. The number of spores per ml of suspension was counted using a Neubauer-Improved haemacytometer. The average for each set of two plates was then calculated.

Results were analysed using analysis of variance (ANOVA), with temperature and incubation time as the factors. Spore data were transformed to $\log_{10}$ values before analysis to stabilise the variance. Where ANOVA indicated a significant treatment effect, this was further explored using a Fisher's Least Significant Difference (LSD) test.

5.2.3.5 Evaluation of *T. hamatum* 6Sr4 and *C. minitans* A69 in a NZ field trial.

Professor Stewart and Dr Nimal Rabeendran contributed to the setting up and assessment of this trial which was run by Agrimm Technologies Ltd. The results of this trial are presented here for completeness but remain the property of Agrimm Technologies Ltd.

Amendment of transplant potting mix

The biocontrol capabilities of the two antagonists *C. minitans* A69 and *T. hamatum* 6Sr4 were assessed as commercial dry formulations incorporated into transplant potting mix prior to the sowing of seeds, applied

according to the manufacturers recommendations (Agrimm Technologies Ltd.). Seedling germination trays, each consisting of 198 cells of 16 mL volume each, were filled with the relevant inoculated potting mix. Non-infested potting mix served as the control treatment. Pelleted lettuce seeds (cv. Casino, Yates NZ Ltd.) were mechanically sown into these trays at a commercial nursery (Seedling Transplants, Kaiapoi, New Zealand) and grown for 42 days (including 14 days of hardening period in the shade house) prior to planting in the field.

Field design and management

A field site (147 m x 7.5 m) naturally infested with *S. minor* and located in Marshlands, Christchurch, was chosen for planting. The soil in the site was classified as organic soil, as it contained up to 70% organic matter with a low bulk density in the range of 0.03-0.4 g/cm^3 (Hewitt, 1992). The field site had previously been planted with cauliflower (*Brassica oleracea* var. botrytis L.) and an adjacent field site had exhibited *S. minor* disease at a level of 28.5% in the previous year. The site was prepared into five beds each of 147 m x 1m. Beds were fertilized with fowl manure (5 tons ha^{-1}) and Nitrophosphoska blue TE (12:15:14:4, N:P:K:Mg) (750 kg ha-1) prior to planting.

Planting

Lettuce seedlings growing in treated and untreated transplant potting mixes were transferred into the field site by planting evenly along each of the beds. Lettuce seedlings were planted in three rows 32 cm apart, one row per treatment, using a transplanting machine to give a final density of 12 plants/m^2. Weeding was carried out using one hand hoeing at 10-14 days after planting, before the development of root spread. Irrigation was provided when necessary, according to the grower's management practice. Plants were harvested after 2 months growth in the field site. A seedling survival count was taken 15 days after planting and four replication plots of 50 plants each were randomly assigned within each of the treatment rows.

Yield

For each treatment, 40 lettuce heads were harvested, washed with tap water and weighed individually after 2 months growth in the field (ie 40 per treatment = 4 replicates x 10 heads/ replicate). After weighing, lettuce heads were labelled at the bottom and stored upside down in a plastic crate at 5°C. Visual observations were made after 12 days storage. The quality of lettuce was assessed for eight randomly selected heads per treatment by visually observing the level of discolouration in the middle stem after cutting them into two halves. Level of discolouration was categorised into three classes: 1) good (no colour change), 2) moderate (1-8 mm diameter stem discolouration) 3) poor (>8 mm diameter discolouration).

Analysis

Percentage disease and mean weight of harvestable heads was compared between treatments and analysed using ANOVA. For significant results ($P<0.05$), means of treatments were compared using FLSD.

5.2.4 Results and discussion

Optimisation of A69 sporulation

Potato extract broth concentration: It was observed that most hyphal growth occurred in the more dilute PEB (20% v/v). The 2 g sucrose/L and 12 days incubation gave the greatest concentration of spores at 5.5 x 10^6 spores/mL (Table 5.1).

Shaken vrs non-shaken: There was no significant difference in the level of sporulation achieved between shaken and non-shaken cultures. Of the shaken cultures, 12 days incubation gave the best results yielding 5.5 x 10^6 spores/mL. In contrast, the highest level of sporulation was achieved after 30 days for non-shaken cultures, yielding 6.9 x 10^6 spores/mL (Table 5.2). However, many of the 30 day spores looked unhealthy compared to the corresponding 12 day spores, suggesting they may not be viable. There was no significant difference in the level of sporulation achieved between the shakers used although it was observed that there was a lot of black crust around the sides of the flasks that had been shaken and this was not present in non-shaken flasks.

Liquid broth vs agar plate cultures: There was no significant difference between liquid culture and solid medium culturing for production of spores (Table 5.3).

Table 5.1 Effect of sucrose concentration on sporulation of A69 in 100 mL of 20% (w/v) potato extract broth.

Sucrose g/L	Spores recovered (per mL)	
	12 days	30 days
0	1.2×10^5	1.2×10^5
2	5.5×10^6	-
5	2×10^6	2.7×10^6

Table 5.2 Sporulation of A69 for shaken and non-shaken treatments in100 ml of 20% (v/v) potato extract broth plus 2g/L sucrose.

Flask	Spores recovered (per mL)	
treatment	12 days	30 days
Shaken	5.5×10^6	7.2×10^5
Non-shaken	2.0×10^5	6.9×10^6

Table 5.3 Dry weight spore recovery from liquid and agar plate cultures of A69.

Growth medium	Spore dry weight recovered per flask or plate
200 mL PEB	44 mg
1 PDA plate	40 mg

Optimisation of 6Sr4 sporulation

20°C: After 5 days at 20°C, the growth of both mycelial plug and spore suspension inoculated plates had reached the edge for all media, although hyphal growth was more dense on plates inoculated with mycelial plugs compared with the spore inoculation plates. There was no sporulation on any of the plates inoculated with mycelial plugs after 5 days (Table 5.4). This was also true for all but one of the medium treatments when plates were inoculated with spores. The exception was the spore inoculated PDA plates that produced an average of 4.3 x 10^6 spores/plate after 5 days. Examination of plates after 10 days incubation at 20°C revealed sporulation had occurred on all plates irrespective of inoculum source or medium. Spore inoculation of PDA plates promoted sporulation the most as there were significantly more spores (1.1 x 10^9 spores/plate) on these plates compared with all other treatments (Table 5.4).

26°C: As with the 20°C treatment, after 5 days at 26°C, colonies had reached the plate edge irrespective of medium or inoculum source. In contrast to the 20°C incubation treatments, sporulation had occurred on plates of both mycelial and spore inoculations. However, the presence of sporulation was inconsistent between replicate plates of a single medium when inoculated with mycelial plugs (Table 5.4). Sporulation was evident on three replicate plates of PDA, two plates of PMA and MEA and one replicate plate of the remaining media.
Again, as with the 20°C inoculation results, sporulation was significantly greater on PDA when inoculated with a spore suspension (3.2 x 10^8 spores/plate) compared with all other treatments. PDA inoculated with a mycelial plug produced (9.8 x 10^7 spores/plate), which was significantly less than PDA inoculated with a spore suspension but significantly greater than all other medium/inoculum source combinations. After 10 days at 26°C, sporulation remained inconsistent between replicate plates of the same treatment. PDA inoculated with both a spore suspension and a mycelial plug produced a significantly greater number of spores than all other medium/inoculum source combinations, although, PDA inoculated with a spore suspension (8.4 x 10^8 spores/plate) gave a significantly higher number of spores than a mycelial plug (5.9 x 10^8 spores/plate) (Table 5.4). Inoculating PMA with both spores and mycelium produced a significantly higher number of spores (2.2 x 10^8 and 2.8 x 10^7 spores/plate, respectively) compared with the other media, these concentrations were significantly less than those achieved with PDA.

35°C: No fungal growth occurred at 35°C, irrespective of inoculum source or medium. Inconsistent sporulation between replicate plates of the same treatment was observed. Consistent sporulation was achieved by placing the plates in a single layer on a shelf within an incubator to ensure each plate received an even amount of light rather than placing the replicate plates on top of one another.

Effect of temperature and incubation time on biocontrol growth

The ability of the four biocontrols to grow on standard PDA media during 2, 5 and 8 days at a range of temperatures is shown in Table 5.5 and Figure 5.1. There were significant differences in radial growth (mm) between the two *Trichoderma* and two *Coniothyrium* isolates in all temperatures and times of assessments, except at day two for temperatures at 7 and 10ºC. The optimum temperature for mycelial growth for the two *Trichoderma* isolates was at 20 and 25ºC which was recorded at days 5 and 8 of incubation. For *C. minitans*, the optimum temperature for mycelial growth for the two isolates was at 20ºC which was recorded at day 8 of incubation. The least growth for *Trichoderma* isolates was recorded at 7ºC and for *C. minitans* at 4 and 10ºC.

The ability of the four biocontrols to produce spores on standard PDA media during 2, 5 and 8 days at a range of temperatures is shown in Table 5.6 and Figure 5.2. There were significant differences in spore production levels between the two *Trichoderma* isolates and the two *C. minitans* isolates at temperatures of 20 and 25ºC in all assessment dates and at temperatures of 7 and 10ºC at 5 days of incubation. The greatest sporulation occurred at 20 and 25ºC for all isolates.

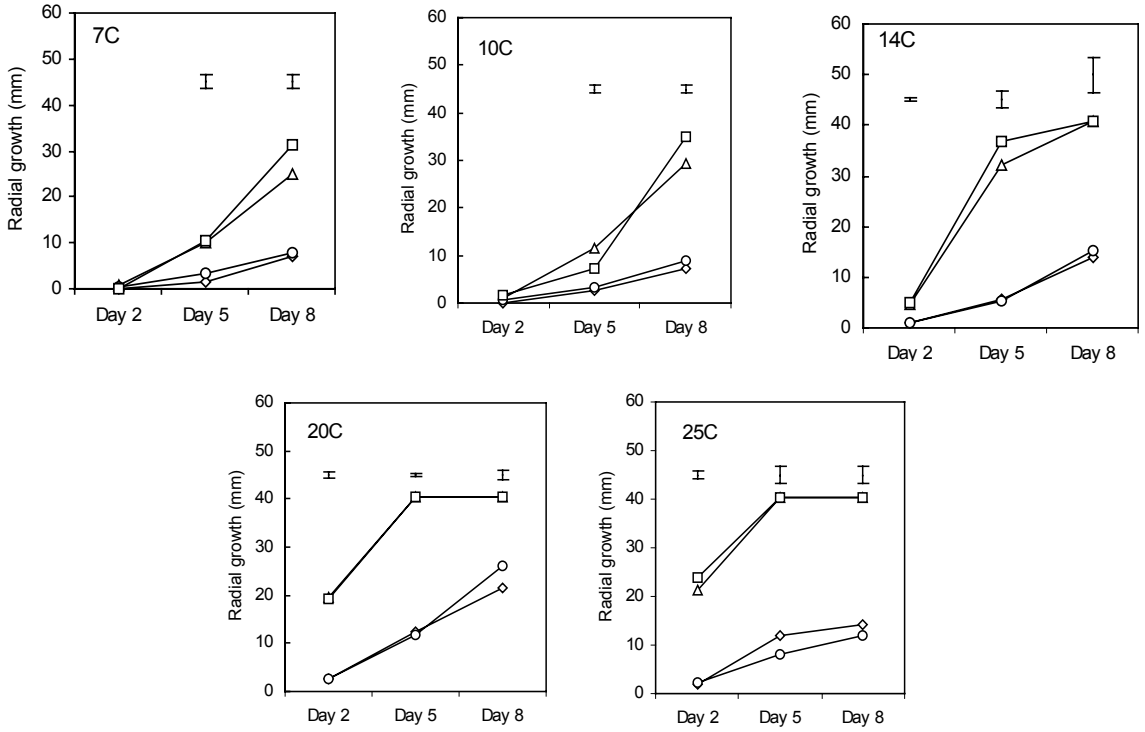


Figure 5.1 Effect of five temperatures on radial growth of four biocontrol agents incubated in PDA plates and assessed 2, 5 and 8 days after plated out. Bars above the lines represent the lsd at P≤0.05. Symbols □ = *T. harzianum* Trich-A-Soil™; ◊ = *C. minitans* A69; ○ = *C. minitans* Contans™; Δ = *T. hamatum* 6Sr4.

Table 5.4 Mean number of *Trichoderma hamatum* (6Sr4) spores produced per plate after 5 and 10 days incubation at both 20 and 26°C on selective media.

			Mean number of spores[1]									
Inoculum source	Temp (°C)	medium	5 days		10 days		Temp (°C)	medium	5 days		10 days	
Mycelial plug	20	PDA	0	C	1.8×10^7	ef	26	PDA	9.8×10^7	b	5.9×10^8	c
		PMA	0	C	2.3×10^6	f		PMA	1.7×10^5	c	2.8×10^7	e
		MEA	0	C	3.4×10^5	f		MEA	8.9×10^4	c	1.6×10^6	f
		OMA	0	C	5.6×10^2	f		OMA	2.3×10^5	c	1.8×10^6	f
		PCA	0	C	1.2×10^2	f		PCA	0	C	1.2×10^3	f
Spores		PDA	4.3×10^6	c	1.1×10^9	a		PDA	3.2×10^8	a	8.4×10^8	b
		PMA	0	C	4.7×10^6	f		PMA	2.9×10^7	c	2.2×10^8	d
		MEA	0	C	2.6×10^5	f		MEA	3.6×10^3	c	1.7×10^6	f
		OMA	0	C	5.6×10^5	f		OMA	2.1×10^2	c	1.2×10^6	f
		PCA	0	C	4.6×10^4	f		PCA	1.3×10^2	c	4.6×10^4	f

1 Mean number of spores per plate (n = 4) followed by the same letter do not differ significantly from one another within either day 5 or day 10 assessments ($P \leq 0.05$, Fisher's LSD test). PDA = potato dextrose agar, PMA = potato molasses agar, OMA= oatmeal agar, PCA= potato carrot agar.

2

Table 5.5 Mean radial growth (mm) of biocontrol agents incubated in PDA Petri dishes at five temperatures and assessed 2, 5 and 8 days after plated out.

	Incubation Temperature (ºC)					
Assessment/Biological agent	**7**	**10**	**14**	**20**	**25**	lsd (P=0.05)[A]
2 days						0.9
C. minitans (A69™)	0	0	1.0	2.6	2.0	
C. minitans (Contans™)	0.5	0.5	1.0	2.5	2.2	
T. hamatum (6Sr4™)	0.8	1.0	4.6	19.5	21.4	
T. harzianum (T-A-Soil™)	0	1.5	5.1	19.3	24.0	
lsd (P=0.05)[B]	ns	ns	0.5	1.5	1.4	
5 days						1.6
C. minitans (A69™)	1.6	2.5	5.6	12.5	11.9	
C. minitans (Contans™)	3.2	3.4	5.3	11.9	8.0	
T. hamatum (6Sr4™)	10.1	11.6	32.0	40.5	40.3	
T. harzianum (T-A-Soil™)	10.5	17.1	36.9	40.5	40.4	
lsd (P=0.05)	2.8	1.9	3.4	0.7	3.5	
8 days						2.4
C. minitans (A69™)	7.0	7.3	14.0	21.4	14.1	
C. minitans (Contans™)	7.9	8.7	15.1	26.1	12.0	
T. hamatum (6Sr4™)	24.9	29.4	40.9	40.5	40.3	
T. harzianum (T-A-Soil™)	31.4	34.9	40.9	40.5	40.4	
lsd (P=0.05)	3.5	1.8	7.0	1.6	3.5	

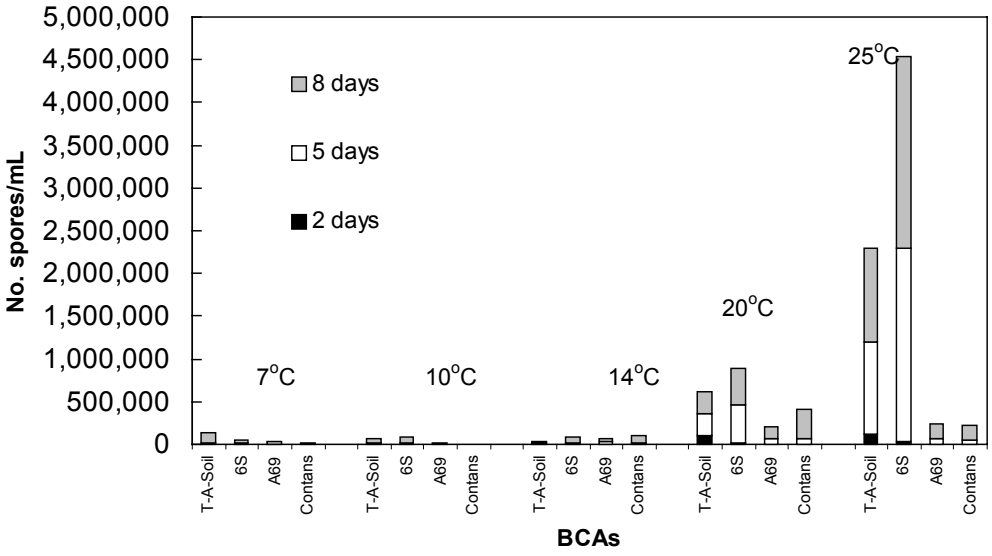
[A] Least significant difference (lsd) for biocontrol agents x all temperatures for each assessment date.
[B] Least significant difference (lsd) for biocontrol agents x each temperature within each assessment date.
There were significant interactions (F-test ≤0.001) between biocontrol agent x temperature and biological agent x temperature x date of assessment.

Table 5.6 Mean spore production (spores/mL - log_{10}) of biocontrol agents incubated in PDA Petri dishes at five temperatures and assessed 2, 5 and 8 days after plated out.

	Incubation Temperature (ºC)					
Assessment/Biological agent	**7**	**10**	**14**	**20**	**25**	lsd (P=0.05)[A]
2 days						0.13
C. minitans (A69™)	0	0	0	0	0	
C. minitans (Contans™)	0	0	0	0	0	
T. hamatum (6Sr4™)	0	0	0	4.4	4.6	
T. harzianum (T-A-Soil™)	0	0	0	4.9	5.0	
lsd (P=0.05)[B]	ns	ns	ns	0.2	0.4	
5 days						0.18
C. minitans (A69™)	0	0	4.4	4.7	4.7	
C. minitans (Contans™)	0	0	4.2	4.8	4.7	
T .hamatum (6Sr4™)	4.2	4.4	4.2	6.6	7.4	
T. harzianum (T-A-Soil™)	4.0	4.2	4.4	5.4	6.0	
lsd (P=0.05)	0.3	0.3	ns	0.2	0.3	
8 days						2.0
C. minitans (A69™)	4.5	2.2	4.6	5.1	5.3	
C. minitans (Contans™)	4.2	2.0	4.9	5.5	5.2	
T. hamatum (6Sr4™)	4.5	4.7	4.9	6.6	7.4	
T. harzianum (T-A-Soil™)	5.1	4.7	4.1	5.4	6.0	
lsd (P=0.05)	ns	ns	ns	0.6	0.5	

[A] Least significant difference (lsd) for biocontrol agents x all temperatures for each assessment date.
[B] Least significant difference (lsd) for biocontrol agents x each temperature within each assessment date.
There were significant interactions (F-test ≤0.001) between biocontrol agent x temperature and biological agent x temperature x date of assessment.

Figure 5.2 Effect of five temperatures on spore production of four biocontrol agents incubated in PDA plates and assessed 2, 5 and 8 days after plated out.



Comparison of methods to incorporate biocontrol agents into soil

Recovery of *C. minitans* A69 from soil: There was a significant difference (P<0.01) between the method of application and recovery of *C. minitans* from the soil. Samples from untreated soil showed a background level of C. minitans in soil ranging form 10^2 –10^3 cfu per gram (Table 5.7). Colonies of *C. minitans* recovered from the untreated plots were morphologically categorised into three types when they were subcultured onto PDA and grown at 20˚C under a 12 h light/dark cycle for 21 days. Commonly occurring colony types of *C. minitans* were 1) fluffy olive green colonies with sparse sporulation (irregularly arranged), 2) fluffy olive green concentric colonies with sparse sporulation and 3) amber concentric colonies, with sparse sporulation. None of these colony types were indicative of *C. minitans* A69. Colonies of *C. minitans* were recovered at a significantly higher level in the *C. minitans* A69 maize-perlite plots compared with the untreated soil at all sampling times (Table 5.7). Commonly occurring colony types (when plated onto PDA as previously described) were light yellowish brown concentric colonies with high levels of sporulation and yellowish brown colonies with sporulation in ropes. Both of these colony morphologies are indicative of introduced isolate *C. minitans* A69.

Recovery of *T. hamatum 6Sr4* from soil: The initial level of *Trichoderma* in untreated soil was 3.6 x10^3 cfu/g at the time of planting. *Trichoderma* recovery from the application of *T. hamatum* 6Sr4 incorporated into a maize-perlite soil amendment was significantly higher (P<0.05) (1.9-5.1 x 10^6 cfu/g) than the untreated soil and this remained constant for the period of the trial (Table 5.8).

Table 5.7 Recovery of *C. minitans* from soil (cfu/g) after different methods of application

Treatment	**Days after planting**			
	14	**28**	**42**	**56**
Untreated control	1.6 x 10^3 b	2.7 x 10^2 cd	1.3 x 10^2 c	2.4 x 10^3 a
Maize-perlite	4.5 x 10^5 a	4.2 x10^4 a	9.5 x 10^4 a	3.4 x 10^4 b

Means followed by the same letter are not significantly different (P<0.05) (FLSD)

Table 5.8 Recovery of *Trichoderma hamatum* 6Sr4 from soil (cfu/g)

Treatment	Days after planting			
	14	**28**	**42**	**56**
Untreated control	6.5×10^3 a	6.1×10^3 a	5.1×10^3 a	9.7×10^3 a
Maize-perlite	5.1×10^6 b	3.7×10^6 b	1.9×10^6 b	1.9×10^6 b

Means followed by the same letter are not significantly different (P<0.05) (FLSD)

Commercial scale evaluation of biocontrol agents

Infection: Plants showed infection 28 days after planting but at a very low level with no significant difference between treatments (Table 5.9). Level of disease achieved for the trial was low and there was, therefore, no significant difference observed between the treatments and the untreated control. The two biocontrol agent treatments produced higher quality lettuce heads, evident after 12 days storage.

Table 5.9 Cumulative disease (%) and mean harvestable yield of lettuce

Treatment	% disease 7 days after planting	Mean wt of harvestable head[1] (kg)	Proportion of high quality heads[2]
Untreated	2.7 a	1.49 a	2/8
T. hamatum 6Sr4	1.8 a	1.54 a	5/8
C. minitans A69	2.4 a	1.62 a	6/8

[1] n=4

[2] Quality was assessed in 8 randomly selected lettuce heads after 12 days storage.

5.3 SUITABILITY OF COMMERCIAL SEEDLING RAISING MIXES FOR INCORPORATION OF BIOLOGICAL CONTROL AGENTS

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5.3.1 Summary

A series of glasshouse (4) and nursery (1) experiments were conducted to evaluate the effect of commercial mix type on the ability of several biological control agents (BCAs) to colonise the region of roots of seedling transplants at levels considered optimal for effective biocontrol of *S. minor* on lettuce. The experiments also examined the effect of biocontrol treatments on seedling growth and control of Sclerotinia lettuce drop. Six commercial mixes were evaluated, which were prepared with different levels of composted pine bark, Kiwi peat, Canadian pet and a mixture of the composted pine bark and peat.

The experiments showed that the mix and roots of seedling transplants produced in composted pine bark mixes (CPBM) had consistently higher cfu counts (10^4-10^6 cfu g-^{1} mix) of *Trichoderma* BCAs than seedling transplants produced in mixes prepared with peat. CPBM mixes were colonised by lower levels of naturally occuring non-pathogenic microorganisms (eg total fungi), when compared to peat mixes, and this probably allowed *Trichoderma* BCAs to colonise CPBM mixes at higher levels. *T. hamatum* 6Sr4 (Trichodry™ and Trichoflow™) and *T. viride* and *T. harziamun* (Trich-A-Soil™), for example, in two experiments colonised the region of roots of lettuce seedling transplants in CPBM at levels considered optimal (10^5 – 10^6 cfu/g-^{1} mix) for effective biocontrol of *S. minor* on lettuce.

Despite the high levels of the *Bacillus subtilis* GB03 (Companion™ 5.5 x 10^9 cfu mL) applied as a drench (x2) to mixes and seedling transplants, this treatment did not consistently increase the levels of total culturable aerobic bacteria (including *B. subtilis* GB03) in the region of roots of seedling transplants produced in the different mixes. All untreated mixes were colonised by high levels and diversity of naturally occuring bacteria (indigenous bacteria). This probably prevented *B. subtilis* GB03 from colonising and/or increasing the levels of bacteria in the different mixes. Despite the small increases in bacterial levels measured in the different mixes, in most experiments seedlings produced in mixes treated with *B. subtilis* GB03 had bigger sizes and higher fresh and dry weights than seedlings grown in untreated mixes and mixes treated with *Trichoderma* treatments. In contrast, seedlings produced in mixes treated with *Trichoderma* treatments had larger roots than seedlings produced in untreated mixes and in mixes treated with *B. subtilis* GB03.

In two pot experiments, levels of *B. subtilis* GB03 (10^6 – 10^7 cfu/g-^{1} mix) and *Trichoderma* BCAs (10^2 –10^5 cfu/g-^{1} mix), measured in seedling transplants treated with two applications of biological treatments prior to potting up, did not suppress *S. minor* infection on butterhead and iceberg lettuce under conditions of high disease pressure. The levels of BCAs were sub-optimal for effective biocontrol of Sclerotinia lettuce drop.

5.3.2 Introduction

Field trials conducted by Lincoln University in New Zealand showed that the biocontrol agents *Coniothyrium minitans* A69 and *Trichoderma hamatum* 6Sr4 provided good control of Sclerotinia lettuce drop disease (60-85%), when biocontrol applications coincided with planting of lettuce (incorporated into seedling transplant) and population levels of both biocontrols were maintained in the region of roots of plants at 10^5-10^6 cfu/g-^{1} soil (Ridgway *et al.* 2001, Stewart and Rabeendran 2000). These results indicated that the incorporation of biocontrol agents into the transplant medium (10^6 spores/g mix) was an effective method of delivering the biocontrol treatments into the region of roots of lettuce seedlings transplants prior to transplanting in the field. Efficient use of fungal and bacterial biocontrol agents relies on their ability to colonise potting mixes and soil ecosystems at optimum levels for effective biocontrol of soil borne diseases. Commercial potting and transplant mixes have different levels of microorganisms that have naturally colonised the different components of the mix or the mix during the preparation/composting process. Potting mixes prepared with composted pine bark (low in microbial levels), for example, were found to support higher population levels of the biocontrol agent *T. hamatum* 382 than potting mixes prepared with decomposed Sphagnum peat (Krause *et al.* 2001).

Aims

Five experiments were conducted in Victoria to evaluate the effect of commercial seedling growing mixes on the ability of the biocontrol agent *T. hamatum* 6Sr4 (antagonistic to *S. minor*) to colonise the region of roots of lettuce seedling transplants at levels considered optimal for effective biocontrol of *S. minor* on lettuce. Four other commercial biocontrol products, recommended for the management of various soil borne diseases, were also tested. The experiments also examined the effect of biological treatments on seedling/plant growth and control of *S. minor* on lettuce.

The specific aims of 5 the experiments were:

- A seedling tray experiment was conducted to evaluate the effect of three commercial mixes on the ability of two biocontrol agents (*T. hamatum* 6Sr4 and *B. subtilis* GB03) to colonise the region of roots of lettuce seedling transplants.
- Three pot experiments were conducted to evaluate the effect of two commercial composted pine bark mixes on the ability of several biocontrol agents, formulated in five biocontrol products, to colonise the region of roots of lettuce seedling transplants, and compare the relatively efficacy of these biocontrol treatments for the control of *S. minor* on lettuce.
- A nursery experiment was conducted to evaluate the effect of four commercial mixes on the ability of the two biocontrol agents *T. hamatum* 6Sr4 and *B. subtilis* GB03 to colonise the region of roots of lettuce seedling transplants, and compare the relatively efficacy of the two biocontrol treatments for the control of *S. minor* on lettuce in the field.

5.3.3 Materials and methods

5.3.3.1 Seedling mixes

Six commercial seedling-growing mixes were evaluated in the five experiments. The first mix type, a low Kiwi peat mix, was prepared by mixing 60% Kiwi peat™, 30% Vermicullite, 10% Perlite and macro and micronutrients (Velisha Bros™, Werribee). The second mix type, a high Kiwi peat mix, was prepared by mixing 75% Kiwi peat, 25% river sand and macro and micronutrients (Marshall nursery™ NZ). The third mix type, a composted pine bark mix, was prepared by mixing 85% composted pine bark, 15% Kiwi peat and macro and micronutrients (Boomaroo nursery - HortiPine™). The fourth mix type, another composted pine bark mix, was prepared by mixing 90% composted pine bark, 10% peat and macro and micronutrients (Debco - Plugger™ starter 222). The fifth mix, another high peat mix, was prepared by mixing 80% Canadian peat moss, 10% Perlite, 10% Vermicullite and macro and micronutrients (T&M Marketing - TH-2™ mix). All mixes did not contain fungicide and were not sterilised. The pH of all the mixes ranged from 4.5 to 5.7. The moisture content of all mixes was approximately 40-60% on the total weight basis.

5.3.3.2 Inoculation of mixes

Table 5.10 describes the different biocontrol agents formulated in commercial products used in the experiments and describes the suppliers, target pathogen(s), application and mode of action. Products were obtained from manufacturers and store at 4°C until used. Products were used as recommended by manufacturers. Trichodry™ and Trichoflow™ are commercial formulations containing the biocontrol agent *T. hamatum* 6Sr4. This biocontrol agent has been shown to parasitise sclerotia and mycelium of *S. minor* and grow well in the soil region around lettuce roots and protect the plant from infection (Ridgway *et al*. 2001, Stewart and Rabeendran 2000). The four other biocontrol products contained one bacterial biocontrol agent (Companion™), a mixture of *Trichoderma* spp biocontrol agents (Trich-A-Soil™, TRI-D25™) or a mixture of *Trichoderma* spp and bacterial biocontrols (Trichoshield™). All these biocontrol products are recommended for use in field and vegetable crops to improve the growth of plants and management of pathogenic soil fungi. The main mode of action of these products is claimed to be competition, and some antibiosis, making these biocontrol products potential treatments to protect the region of roots of lettuce plants against *S. minor* infection.

Table 5.11 describes the amount of product used in the experiments and application methods. The viability of biocontrol agents in products was tested *in vitro* prior to application using dilution planting and selective medium. *Trichoderma* spp were cultured in PDA medium plus antibiotic and Triton x100 (Agrimm Technologies recipe) and *B. subtilis* and other bacteria in TSA medium. Concentration of biological agents in products (eg cfu g^{-1}) was within

label specifications. Inoculum (spores) of *T. hamatum* 6Sr4 was formulated in a dry commercial formulation suitable for blending with the transplant mix (Trichodry™ – 1 x 10^6 cfu g^{-1}) and suitable for drenching plants/soil (Trichoflow™ – 2.0 x 10^8 cfu g^{-1}). Inoculum of the other biocontrol products, except *B. subtilis*, was formulated in dry commercial formulations suitable for drenching plants/soil. *B. subtilis* was formulated as a liquid formulation also suitable for drenching plants/soil.

Trichodry™ was blended with growing mix at the rate of 2 kg of product per cubic metre of mix. Seedling trays were filled with Trichodry™ treated or untreated mix and seeded with pelleted lettuce seed. Trays with untreated mix were treated (drench) with solutions from the other biocontrol treatments by mixing the corresponding amount of products in distilled water (dry and liquid formulations) and later drenching the seeded trays. Before application, each product was mixed in distilled water and allowed to stand for 1-2 hrs to stimulate spore growth. Trays were drenched with the different solutions until saturation point. Control mixtures were made with the same mixes but were not blended or drenched with biological treatments.

Table 5.11 Biocontrol and chemical treatments and rates of product used in glasshouse and nursery experiments.

Treatment/product	Product rate (kg or l/ha)	Active rate (cfu/g or g/kg)	Rate for nursery plants
Nil			water only
Sumisclex™	2 L/ha	500 g procymidone	Seedling plug drench
Trichodry™ 6Sr4	2 kg/cubic metre mix	1.0 x 10^6 cfu/g	2 kg/cubic metre mix
Trichoflow™ 6Sr4	100g/100L water	2.0 x 10^8 cfu/g	100g/100L water drench
Trich-A-soil™	1.25 kg/200 L/ha	2.0 x 10^8 cfu/g	100g/50 L water-drench
T-D25™	1 kg/200 L/ha	5.0 x 10^7 cfu/g	100g/50 L water-drench
Companion™	1 L/800 L/ha	5.5 x 10^{10} cfu/mL	1 L/800 L water-drench
T-Shield™	1 kg/100-200 L/ha	1.0 x 10^8 cfu/g*	200g/50L water-drench

* Product contains *Trichoderma* spp and bacteria (eg *B. subtilis* and *P. aureous*)

5.3.3.3 Effect of mix type on biocontrol colonisation and seedling growth

The effect of mix type on the capacity of two biocontrol agents to colonise the region of roots of lettuce seedling transplants was determined in a seedling tray experiment during June-July 2003, using the biocontrols T. hamatum 6Sr4 and B. subtilis GB03. The three mixes evaluated were a low (Velisha Bros™) and high Kiwi peat mixes (Marshall NZ™) and a composted pine bark mix (HortiPine™).

One batch of *T. hamatum* 6Sr4 (Trichodry™) treated mix and two batches of untreated mixes were prepared for each type of mix. Four seedling trays were filled with *T. hamatum* 6Sr4 treated mix and 8 with untreated mix for each type of mix. The trays were sown manually with pelleted lettuce seed (Lactuca sativa cv Great Lakes). Four trays with untreated mix were drenched with a solution containing *B. subtilis* GB03 (Companion™). There were four replicate trays for each of the three mixes and the two biocontrol/mix treatment combinations. Each tray was 35cm x 29cm with 100 conical cells (3 x 2.5 x 4.5 cm depth) but only 36 cells in the centre were used. Cell volume was approximately 15ml of mix. Trays were randomized on benches (9 x 4 rows) using a fully randomised design in a controlled temperature and RH glasshouse, with 12-h of natural/artificial light. Temperature was monitored in the seedling plug with a temperature probe (Tinytag™ Gemini data logger). Watering was applied by overhead sprinkler irrigation.

The population density of microbial communities (total fungi and total culturable bacteria) was measured on untreated mixes using dilution plating and selective medium. Total fungi was cultured on MEA medium and total culturable bacteria on TSA medium. Five weeks after biocontrol application, populations of *T. hamatum* 6Sr4 and *B. subtilis* GB03 were measured in seedling transplants using Trichoderma-selective medium (Alison Stewart recipe) and total culturable bacteria medium (TSA), respectively. Numbers of colony forming units (cfu) were counted, with colonies identified from key colony characteristics from product dilutions. Phialides produced by *Trichoderma* species were examined under a compound microscope. Two seedling transplants (root/soil) were removed from each of the 4 replicate trays per treatment and mixed well in a plastic bag. Ten g (wet weight) of mix/roots was blended with 90 mL of 0.01% sterilised water agar using an orbital shaker for 1 hr. From this composite sample, 3 sub-samples were used for serial dilutions (n = 3) to 10^7. Colonies (cfu/g mix) of total fungi and bacteria were counted after 3-4 days and *Trichoderma* after 7-10 days of incubation at room temperature. Seedling growth (fresh and dry weight, shoot and root length) was measured 5 weeks after sowing.

Analysis of variance (ANOVA) was used to determine the effect of seedling mix type on transformed counts (log_{10}) of biocontrol agents in seedling transplants and seedling growth data. An LSD was calculated when differences were significant (P = 0.05) to compare treatment means.

5.3.3.4 Effects of biological treatments on plant growth and disease control

Three pot experiments were conducted to evaluate the effect of five biocontrol treatments on lettuce seedling/plant growth and control of *S. minor* on lettuce. The pot experiments were conducted during September-December 2003.

Lettuce seedlings in the 3 experiments were grown in two composted pine bark mixes. Seedlings in the first two experiments were grown in HortiPine™ and Debco Plugger™ mixes, respectively, and in the third experiment grown in a HortiPine™ mix. In each experiment, one batch of mix was treated with *T. hamatum* 6Sr4 (Trichodry™) prior to sowing. The other biological treatments were applied to seeded trays with untreated mix immediately after sowing as previously described. All treatments were repeated 3-4 weeks after the first application, with *T. hamatum* 6Sr4 (Trichoflow™) applied as a drench in the second application. Tables 5.10 and 3.11 describe the biocontrol agents formulated in the different biocontrol treatments, rate of product and application method used in the experiments. Pelleted lettuce seed were planted with a pneumatic seeder using butterhead lettuce cv 'Nadine' (experiment 1 and 3) and crisphead lettuce cv 'Green harvest' (experiment 2). Each seedling tray had 144 conical cells (3 x 2.5 x 4.5 cm depth). There were two seedling trays for each of the seven treatments. Trays were randomized on glasshouse benches using a complete block design. Seedlings in all trays were grown for 4-5 weeks and after this period, 10 plants from each treatment were planted singly in pots. All biological treatments were compared to a fungicide (procymidone) treatment. This was applied as a drench to seedling transplants (soil/roots) in trays 24-hrs before transplanting into pots at the recommended rate of 2 L/ha in 1000 L of water.

The soil used in the pots was a clay loam soil collected from a lettuce field in Werribee, Victoria. The pH was 7.5. The soil contained naturally occurring sclerotia of S. minor at the rate of 70 sclerotia/kg of dry soil. In addition to field sclerotia in the first two experiments, three blocks of agar (1 square cm) containing actively growing mycelium of S. minor were placed in close proximity (2-3 cm) to the seedling plug at transplanting. In the third experiment, field and sclerotia produced in the laboratory were mixed in the soil at the rate of 10 sclerotia/100 g of soil. Sclerotia of S. minor were collected from a field site in Werribee Victoria, surface-disinfected in 0.5 sodium hypochloride for 5 min, rinsed in sterile distilled water and placed on potato-dextrose agar to determine their viability. Cultures of S. minor were produced by centrally inoculating Petri dishes containing potato dextrose agar (PDA, Oxoid) with a 8 mm diam agar plug from the growing edge of a PDA plate culture. Actively growing mycelium from 20 isolate cultures was subcultured and used to produce sclerotia on PDA medium plus antibiotic. After sclerotia had formed, sclerotia were harvested by washing in two sieves (2 mm and 500 micron), dried in an airflow cabinet overnight before being mixed with the field soil to obtain a theoretical concentration of 10 sclerotia per 100 g of soil. Laboratory produced sclerotia were conditioned in soil for 1 month during spring before being mixed evenly in the soil.

Each treatment was replicated 10 times (one lettuce plant per pot). Pots were randomised on benches according to a randomised complete block design in a controlled temperature and RH glasshouse, with 12-h of natura/artificial light. Temperature in soil was monitored with a temperature probe (Tinytag™ Gemini data logger). Pots were watered with overhead sprinklers and kept wet at all times to induce *S. minor* infection. Four to five weeks after two applications of each of the five biocontrol treatments were applied to seeded trays and prior to seedling transplanting into pots, the populations levels of *Trichoderma* species and total bacteria were determined in seedling transplants using Trichoderma-selective medium (Alison Stewart recipe –appendix) and total culturable bacteria medium (TSA), respectively. Serial dilutions and cfu counts were conducted as described previously. Seedling growth (fresh and dry weight, shoot and root length) was measured prior to seedling transplanting into pots. Disease incidence was recorded daily and plant weight determined 6 weeks after transplanting.

Analysis of variance (ANOVA) was used to determine the effects of biocontrol treatments on seedling growth and lettuce drop incidence. An LSD was calculated when differences were significant (P = 0.05) to compare treatment means. Counts of microorganisms were transformed to log_{10} values before analysis when required to stabilise the variance.

5.3.3.5 Effects of mixes and biocontrol treatments on plant growth and disease control

The effect of four commercial seedling growing mixes on the ability of two biocontrols (*T. hamatum* 6Sr4 and *B. subtilis* GB300) to colonise the region of roots of lettuce transplants was determined in a nursery experiment. The

experiment also examined the effects of biocontrol treatments on seedling growth (nursery) and control of *S. minor* on lettuce in the field. The nursery experiment was conducted during July-September 2003 and the field trial during October-December 2003.

The mixes evaluated were two composted pine bark mixes (HortiPine™ and Debco Plugger™ starter 222) and two peat mixes (Kiwi peat Velisha Bros™ and Canadian peat TH-2™). One batch of T. hamatum 6Sr4 (Trichodry™) treated mix and two batches of untreated mixes were prepared for each type of mix. Two seedling trays were filled with *T. hamatum* 6Sr4 treated mix and another 4 with untreated mix for each type of mix. The seed was planted into trays with a pneumatic seeder using butterhead lettuce cv 'Nadine' (HortiPine mix only) and crisphead lettuce cv 'Green harvest' (Debco, Velisha Bros and Canadian peat mixes). Two trays with each untreated mix were drenched with a solution of B. subtilis GB03 (Companion™) as previously described and the other two trays left untreated. The biocontrol treatments were repeated (drenches) 6 weeks after the first application. Tables 5.10 and 5.11 describe the biocontrol agents in commercial products and rates and method of application used in the experiment.

Lettuce seedlings were grown in a nursery (Velisha Bros farm) at Werribee, Victoria. There were two replicate trays for each of the three treatments (*Trichoderma*, *B. subtilis* and untreated). Each tray had 144 conical cells. Trays were placed on nursery benches in an open plastic house. Trays were randomized to benches according to a randomized block design and separated from each other to prevent cross-contamination. Trays were watered by an overhead irrigation system until the mix was saturated and received all foliar fungicide and nutrient sprays. Temperatures in the seedling plug averaged 10ºC (range 5-15ºC) during the 7 weeks seedling trays were in the nursery. Seven weeks after the application of two biocontrol treatments and prior to transplanting seedlings into the field, Trichoderma spp and bacterial populations were measured in seedling transplants (soi/roots) using Trichoderma-selective medium and total culturable bacteria medium (TSA), respectively. The white-pigmented colony morphology of B. subtilis allowed it to be easily separated from other bacterial colonies in TSA medium. Four seedling transplants were removed from each replicate tray and mixed in a plastic bag to provide a composite sample. Serial dilutions and cfu counts were conducted as described previously.

Seedling growth (fresh and dry weight, shoot length) was measured on four seedling transplants per replicate tray. Butterhead seedlings were measured and analysed separately due to varietal differences. Crisphead lettuce seedlings in the different treatments were used in a field trial. The trial was located in a commercial lettuce field at Werribee (Velisha Bros farm) Victoria. The soil was a clay loam soil and the pH was 7.5. The soil contained naturally occurring sclerotia at the rate of 70 sclerotia/kg of dry soil. Seedling transplants were planted in the field using a 4-row seedling planter. There were two replicate plots for each of the three treatments (*Trichoderma, B. subtilis* and untreated) applied to each of the 3 mix types. Plots had 80 plants each spaced approximately 25 cm in 4 rows. Disease incidence was recorded every week and a plant survival count conducted 1 week after field transplanting. Plant fresh weights (10 plants per plot) were measured 11 weeks after seedlings were planted in the field.

Analysis of variance (ANOVA) was used to determine the effects of mix type on biocontrol agent colonisation of seedling transplants, and the effect of biocontrol treatments on seedling/plant growth and disease control. An LSD was calculated when differences were significant (P = 0.05) to compare treatment means. Data was transformed when required prior to analysis to stabilise the variance.

5.3.4 Results and discussion

Effect of mix type on biocontrol colonisation of seedling transplants

The mean colony forming units (cfu) counts of microorganisms that naturally colonised the mixes and counts of two biological control agents incorporated into seedling transplants grown in 3 commercial seedling growing mixes are presented in Table 5.12.

In untreated mixes, the mean population density (cfu g^{-1} mix) of total fungi was significantly higher in the two mixes prepared with Kiwi peat than in a mix prepared with a composted pine bark and Kiwi peat. There was no significant difference in total fungi levels between the two mixes prepared with two levels of Kiwi peat. In contrast, the mean cfu counts of total culturable bacteria was significantly higher in the mix prepared with composted pine bark than in the two mixes prepared with Kiwi peat. After application of the biocontrol agent *B. subtilis* GB03, the mean cfu counts of total culturable bacteria was significantly higher in the seedling transplants grown in the composted pine bark mix than in seedling transplants grown in the two Kiwi peat mixes. There was no significant difference in total culturable bacteria levels (after biocontrol application) in seedling transplants grown in the two Kiwi peat mixes. A comparison of cfu counts in seedling transplants grown in untreated and treated mixes (for each mix type) showed no

significant differences in total culturable bacteria levels. Only small increases in total culturable bacteria, including *B. subtilis* GB03, were observed in seedling transplants grown in the three commercial mixes treated with Companion™. This suggests that the level and diversity of indigenous bacteria colonising the commercial mixes affected the ability of *B. subtlis* GB03 to colonise the mix at high levels.

Trichoderma species were not detected in the three untreated mixes (data not shown). In mixes treated with Trichodry™, the cfu counts of T. hamatum 6Sr4 were significantly higher in the seedling transplants grown in the composted pine bark mix than in seedling transplants in the two mixes prepared with Kiwi peat. The Trichoderma levels measured (1.3×10^6) in the region of roots of seedling transplants grown in the composted pine bark were within the levels considered optimal for effective biocontrol of *S. minor* on lettuce. There was no significant difference in *Trichoderma* levels in seedling transplants grown in the two Kiwi peat mixes. The levels measured (10^4 cfu/g^{-1}) in the two Kiwi peat mixes were probably too low for effective biocontrol of *S. minor* on lettuce. In these two mixes, high colonisation of seedling transplants by T. hamatum 6Sr4 was probably prevented by the high population density of indigenous total fungi (including Penicillium species) colonising the Kiwi peat mix. The microorganisms (non-pathogenic) that had naturally colonised the components of commercial mixes are predominantly non-pathogenic and therefore have no risk for seedling production. The treatment (sterilisation) of these mixes could reduce the levels of indigenous microorganisms and allow biocontrol agents to colonise the mix at high levels. However, this will increase the costs of commercial mixes and the cost of seedling transplant production.

The effect of biocontrol treatments on seedling growth is summarised in Table 5.13. The mean fresh weight of lettuce seedlings grown in the two Kiwi peat commercial mixes (untreated and treated with the two biocontrol treatments) were significantly higher than the mean fresh weight of seedlings grown in the composted pine bark mix treatments. The mean dry weight of seedlings grown in the light Kiwi peat mix (plus Vermicullite/Perlite), either untreated or treated with the two biocontrol treatments, and seedlings grown in the untreated high Kiwi peat and composted pine bark mix treated with *T. hamatum* 6Sr4, were significantly higher than the mean dry weight of seedlings grown in all other treatments. The sizes of seedlings grown in all Kiwi peat mix treatments were significantly bigger than the sizes of seedlings grown in all composted pine bark mix treatments. The composted pine bark mix retained less water than the Kiwi peat mixes and could have been prepared with not fully matured pine bark. These two characteristics of this mix probably affected the growth of lettuce seedlings. The roots of seedlings grown in the high Kiwi peat (plus sand) mix treatments were significantly shorter than the roots of seedlings grown in the other treatments. This was probably caused by the more acidic pH in the high Kiwi peat mix.

Effect of biocontrol treatments on plant growth and disease control in pots

The mean population density (cfu g^{-1} mix) of biocontrol agents in seedling transplants and seedling growth measurements prior to potting up, and disease incidence and plant weights measured in pots infested with field sclerotia and blocks of agar with mycelium are summarised in Tables 5.14 and 5.15.

In untreated mixes, the mean count (cfu) of total culturable bacteria was significantly lower in the composted pine mix used in experiment 1 (HortiPine™ mix 9.0×10^6 cfu/g^{-1}) than in the composted pine mix used in experiment 2 (Debco Plugger Starter™ 222 mix 2.2×10^8cfu/g^{-1}). After two applications of Companion™ (*B. subtilis* GB03), the cfu counts of total culturable bacteria in the seedling transplants grown in the HortiPine™ significantly increased from 9.0×10^6 in untreated mix to 4.0×10^7 cfu/g^{-1} in treated mix. There were no significant increases in cfu counts of total culturable bacteria in seedling transplants grown in the Debco™ mix treated with Companion™. The results with the Debco mix indicated that the high levels of indigenous bacteria colonising this mix (10^8 cfu/g^{-1}) affected the ability of *B. subtilis* BG03 to colonise the region of roots of the seedling transplant at high levels, despite the high concentration of *B. subtilis* GB03 applied to the seedling transplants (1L/800L water - Companion™ 5.5×10^{10} cfu/mL).

The mean count (cfu) of the different *Trichoderma* species in seedling transplants (grown in two composted pine bark mixes) treated with the different biocontrol treatments, applied twice before potting up, ranged from 4.6×10^2 to 6.7×10^5 in the two pot experiments. There were no significant differences in *Trichoderma* levels among all biocontrol treatments in the two experiments. Although not significant, seedling transplants treated with Trich-A-Soil™ (*T. harziamun and T. viride*) had consistently the highest cfu counts in both experiments. The high level of colonisation of seedling transplants by *T. harziamun and T. viride* was probably due to the high concentration of spores in the formulation of this biocontrol product.

In experiment 1, there was no significant difference in the sizes of butterhead seedlings among all treatments. The fresh and dry weights of seedlings grown in the HortiPine™ untreated mix were generally heavier than the weights

of seedlings in all other treatments. The size of roots of seedlings treated with two applications of Trichoderma (Trich-A-Soil™ and Trichoshield™) and the *B. subtilis* GB03 treatments were significantly larger than the size of roots in all other treatments.

In experiment 2, crisphead seedlings grown in the Debco™ mix, treated with two applications of Companion™ (*B. subtilis* GB03), had significantly bigger sizes than seedlings in all other treatments. Fresh and dry weights of seedlings treated with Companion™ (*B. subtilis* GB03) were also significantly heavier than the weights of seedlings in all other biocontrol treatments, except for the seedlings in the untreated mix (fresh weight only). In contrast, the roots of seedlings treated with all *Trichoderma* treatments were significantly larger than roots of seedlings in untreated and Companion™ (*B. subtilis* GB03) treated mixes. There were no significant differences in root sizes between *Trichoderma* treatments.

In the two pot experiments, all lettuce plants from untreated and biocontrol treated seedling transplants were infected and killed by *S. minor* during the first two weeks after transplanting into pots. All seedlings transplants treated with the fungicide treatment (seedling drench - procymidone 2L/ha in 1000L water) survived until harvest approximately 5 weeks after transplanting into pots. The population densities of biocontrol agents, measured in the seedling transplants prior to potting up, were not optimal for effective biocontrol of *S. minor* infection under conditions of high disease pressure (sclerotia plus mycelium in pots) in both experiments. The level of disease pressure used in the two pot experiments is very unlike to occur in soils of commercial lettuce farms. Nevertheless, the results obtained suggest that high levels of biocontrol agents ($>10^5$ cfu g^{-1}) may be needed in the seedling transplants when planted in the field in order to provide some protection against *S. minor* infection, similar to that provided by procymidone. After lettuce seedlings are planted in the field, biocontrol agents in the seedling transplants have to colonise the region of roots and base of plants in order to protect the crown of plants and leaves touching the ground against *S. minor* infection. Additional applications of biocontrols may be needed in the field to increase the population density of biocontrol in the rhizosphere and base of plants to provide long season protection against *S. minor* infection. Alternatively, a late season application of fungicide (compatible with biocontrol products) could be integrated with early season applications of biocontrols to provide the long season protection needed at different disease pressure sites.

The third pot experiment was conducted using butterhead seedlings and soil with sclerotia. The mean counts (cfu) of biocontrol agents in seedling transplants and seedling growth measurements before potting up and cfu counts of biocontrol agents in region of roots of potted plants and disease incidence and plant weights taken 4 weeks after potting up are summarised in Tables 5.16.

The population density of total culturable bacteria in the untreated composted pine bark mix (HortiPine™) used for growing seedling transplants was very high (3.0×10^8 cfu/g^{-1}) and diverse (e.g. *Bacillus* spp, *Pseudomonas* spp., etc). Consequently, there was no significant increase in the levels of total culturable bacteria in the seedling transplants treated with two applications of Companion™ (*B. subtilis* GB03). Four weeks after seedlings were potted up, the levels of total culturable bacteria in the region of roots of plants in the untreated and Companion™ treatments were lower than the levels measured in the seedling transplants before potting up. The cfu counts of total bacteria in the region of roots of untreated plants in pots was significantly higher than levels measured in the rhizosphere of plants treated with *B. subtilis* GB03.

The population densities of the different *Trichoderma* species, measured in the seedling transplants treated with two applications of the four *Trichoderma* biocontrol treatments prior to potting up, ranged from 3.4×10^2 to 2.9×10^5 cfu/g^{-1}mix. Seedling transplants treated with Tric-A-Soil™ (*T. harziamun and T. viride*) had significantly higher Trichoderma cfu/g^{-1} mix counts than seedling transplants treated with all other biocontrol treatments. Four weeks after potting up, Trichoderma levels in region of roots of potted plants were generally similar to levels measured in the seedling transplants before potting up. In pots, plants treated with Trich-A-Soil™ (*T. harziamun and T. viride*) and Trichodry™ and Trichoflow™ (*T. hamatum* 6Sr4) had significantly higher *Trichoderma* counts (range 5.1×10^4 – 5.1×10^5) than plants treated with the other treatments containing *Trichoderma* species. Butterhead seedlings grown in untreated HortiPine™ mix were bigger and heavier than seedlings grown in mixes treated with all biocontrol treatments. There were no significant differences in root sizes between seedlings grown in untreated mix and seedlings grown in *Trichoderma* treated mix.

Disease levels were too low in this experiment to determine the effect of biocontrol treatments on suppression of *S. minor* infection on lettuce. The low levels of disease were probably the result of low viability of field and laboratory sclerotia in the soil. Although not significant, the fungicide treatment appeared to provide the best disease control.

However, this treatment was phytotoxic to lettuce plants resulting in a significant reduction in plant fresh weight when compared to the untreated control. Lettuce plants treated with biocontrol treatments, except Trichoshield™, were significantly heavier than plants in the untreated control 5 weeks after potting up. Companion™ (*B. subtilis* GB03) treated plants had the heaviest fresh weights.

Effect of four commercial mixes on biocontrol colonisation of seedling transplants in a nursery experiment

The population density of microorganisms that naturally colonised the four commercial seedling growing mixes and biocontrol agents in seedling transplants are summarised in table 5.17 and 5.18.

The population density of total fungi, which consisted mainly of *Penicillium* species and other wood/peat saprophytic fungi, was highest in mixes containing peat (eg Kiwi peat and Canadian peat) and lowest in a composted pine bark mix without peat. There were no significant differences in the levels of total fungi among the four untreated mixes. In untreated mixes, *Trichoderma* species (eg. *T. harzianum)* were detected only in the Canadian peat mix (1.6×10^4 cfu/g^{-1} mix). The population density of *T. hamatum* 6Sr4 in seedling transplants, grown for seven weeks in four commercial mixes treated with two application of Trichodry™ and Trichoflow™ (*T. hamatum* 6Sr4), ranged from 4.7×10^4 to 9.3×10^5 cfu/g^{-1} mix. *T. hamatum* 6Sr4 cfu counts were significantly higher in seedling transplants grown in the Canadian peat (TH-2™), composted pine bark plus Kiwi peat (HortiPine™) and Kiwi peat plus Vermicullite/Perlite mix (Velisha Bros™) than in seedling transplants grown in the composted pine bark mix (Debco™). In another experiment, however, levels of *T. hamatum* 6Sr4 cfu were very high (10^5-10^6 cfu/g^{-1} mix) in cauliflower seedling transplants grown in the composted pine bark mix Debco™ (data not shown).

The population density of total culturable bacteria in the four untreated mixes ranged from 3.1×10^7 to 2.2×10^7 cfu/g^{-1} mix. There were no significant differences in population density levels between the untreated mixes. Seven weeks after two applications of *B. subtilis* GB03 (Companion™) were made to seedling transplants, population density of total culturable bacteria, including *B. subtilis* GB03, was significantly higher in seedling transplants grown in Kiwi peat (Velisha Bros™) and composted pine bark (Debco™) mixes than in seedling transplants grown in the two corresponding untreated mixes. When considering cfu counts of *B. subtilis* GB03 alone, transplants in the same two mixes also had significantly higher levels of *B. subtilis* GB03 counts than seedling transplants in the two respective untreated mixes.

The effect of biocontrol treatments on seedling growth is summarised in Table 5.19 and 5.20. When comparing butterhead seedlings, there were no significant differences in seedling sizes and weights between treatments (Table 5.19). Crisphead seedlings grown in the Kiwi peat mix (Velisha bros™) had the largest sizes and heaviest fresh and dry weights. The size and fresh weight of seedlings grown in Kiwi peat mix (Velisha bros™), treated with two applications of Companion™ (*B. subtilis* GB03), were significantly larger than the sizes and weights of seedlings grown in untreated mix and in the other 2 mixes for all treatments. There were no significant differences in seedling sizes and fresh or dry weights between untreated and biocontrol treatments when comparing treatments within each mix type. Seedlings grown in the composted pine bark mix (Debco™) had the smallest sizes and lowest fresh and dry weights.

The effect of biocontrol treatments, applied twice to seedling transplants (crisphead) grown in three mixes in the nursery, on disease incidence and fresh weight of lettuce plants in the field are summarised in Table 5.21. Although not significant (P=0.05), the mean disease incidence in plots planted with lettuce transplants grown in the composted pine bark (Debco™) and treated with *T. hamatum* 6Sr4 (4.5×10^5 cfu/g^{-1}) was lower than the mean disease incidence in all other treatments. Disease incidence levels were generally lower in plots planted with seedling transplants grown in the composted pine bark (Debco™) and Canadian peat (TH-2™) mixes than in plots planted with transplants grown in a Kiwi peat mix (Velisha Bros™). There were no differences in fresh weights between the treatments.

Table 5.10 Description of biological agents antagonistic to *S. minor* and other antagonistic soil microorganisms formulated in commercial products which were evaluated for their potential to improve plant growth and protect lettuce plants against infection caused by *S. minor*.

Product	**Biological agent(s)**	**Origin/ Manufacturer**	**Target pathogen(s)**	**Target crop**	**Application**	**Mode of action**
Trichodry™	*Trichoderma hamatum 6Sr4*	Lincoln University NZ Agrimm Technologies NZ	*Sclerotinia minor*	Lettuce	Seedling mix	Competition, Mycoparasite Some antibiosis
Trichoflow™	*Trichoderma hamatum 6Sr4*	Lincoln University NZ Agrimm Technologies NZ	*Sclerotinia minor*	Lettuce	Seedling drench	Competition, Mycoparasite Some antibiosis
Trich-A-Soil™	*Trichoderma viride* *Trichoderma harzianum*	Organic Crop Protectants Pty Ltd Australia	Pathogenic soil fungi	Turf, Horticulture	Soil drench	Competition Growth promoter
TRI-D25™	*Trichoderma koningii* *Trichoderma harzianum*	JH Biotech, Inc, USA	Sclerotinia spp Botrytis spp	Horticulture	Soil drench	Competition Growth promoter
Companion™	*Bacillus subtilis* GB03	Growth Products USA	*Rhizoctonia* spp *Fusarium* spp	Horticulture	Plant drench Irrigation	Growth promoter Antibiosis
Nutri-life Trichoshield™	*Trichoderma harzianum* *Trichoderma lignorum* *Trichoderma virens* Bacterial soil microbes	Nutri-Tech Solutions Pty	Pathogenic soil fungi	Horticulture	Soil drench	Soil inoculant Competition

Table 5.12 Levels of native microbial communities and two biological control agents (*Trichoderma hamatum* 6Sr4 and *Bacillus subtilis* GB03) in three commercial lettuce seedling growing mixes.

Seedling mix	Before biocontrol application				5-weeks after biocontrol application	
	Moisture (% w/w)	pH	Total Fungi[A] (cfu/g)	Total Bacteria[B] (cfu/g)	*Trichoderma*[D] (cfu/g)	Total Bacteria[E] (cfu/g)
Composted pine bark and Kiwi peat	56	5.1	5.87 ($1.1x10^6$)[C]	7.91 ($4.0x10^7$)	6.12 ($1.3x10^6$)	7.53 ($8.2x10^7$)
Kiwi peat and Vermicullite/Perlite	51	5.7	6.31 ($2.1x10^6$)	6.99 ($6.9x10^6$)	4.51 ($3.4x10^4$)	6.11 ($1.0x10^7$)
Kiwi peat and sand	60	4.5	6.55 ($3.6x10^6$)	6.63 ($2.1x10^6$)	4.68 ($5.5x10^4$)	5.91 ($7.9x10^6$)
			0.04	0.01	<0.001	0.05
F-test						
lsd (P=0.05)[F]	ns	ns	0.47	0.67	0.26	1.38

[A] Total fungi cultured in Malt extract agar with antibiotic (MEA).
[B] Total culturable aerobic bacteria cultured in Tryptone soy agar (TSA).
[C] Population density (cfu/g) for each replication was transformed to $\log_{10}$ to obtain a constant variance. Natural values shown in parenthesis. LSD values calculated based on transformed data.
[D] *Trichoderma hamatum* strain 6Sr4 cultured in *Trichoderma* semi-selective medium (Stewart Lincoln University).
[E] Total culturable aerobic bacteria includes original bacteria in mix plus *Bacillus subtilis* strain GB03. A comparison between untreated and treated mix types showed no significant differences in total bacteria levels, except for the light Kiwi peat plus Vermicullite mix.
[F] Differences in cfu/g larger than the lsd (within columns) are significantly different ($P = 0.05$).

Table 5.13 Lettuce seedling growth (crisphead) after 5 weeks in three commercial seedling growing mixes with and without applications of two biological control agents (*Trichoderma hamatum* 6Sr4 and *Bacillus subtilis* GB03).

Seedling mix	Treatment[A]	Fresh weight (g)	Dry weight (g)	Shoot length (cm)	Root length (cm)
Composted pine bark and Kiwi peat	untreated	0.50[B]	0.03	8.03	6.28
	B. subtilis	0.29	0.02	6.54	5.38
	T. hamatum	0.59	0.04	8.22	6.86
Kiwi peat and vermicullite/Perlite	untreated	0.75	0.05	10.9	7.04
	B. subtilis	0.68	0.05	9.54	5.59
	T. hamatum	0.84	0.06	10.1	6.83
Kiwi peat and sand	untreated	0.62	0.04	10.2	4.52
	B. subtilis	0.53	0.03	10.5	4.74
	T. hamatum	0.68	0.03	11.0	4.66
		0.002	<0.001	<0.001	0.002
F-test					
lsd (P≤0.05)		0.23	0.02	1.7	1.5

[A] Biological treatments were applied as a drench to seedling trays. The first application was made after sowing and the second two weeks later.
[B] Seedlings were harvested 5 weeks after sowing.

Table 5.14 Effect of biocontrol treatments on seedling growth, incidence of lettuce drop and plant weight of butterhead lettuce plants (cv 'Nadine' in HortiPine™ mix) in pots – experiment 1.

Treatment	Seedlings before transplanting into pots[A]						Pots	
	Bacteria (cfu/g)	*Trichoderma* (cfu/g)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	% of Plants infected[B]	Plant weight (grs)
Untreated	$9.0x10^6$	0.0	7.31	8.05	3.97	0.56	**100**	-
Fungicide (procymidone)™	-	-	-	-	-	-	**0**	99
Trichodry (*T. hamatum* 6S4r)™	-	$6.6x10^3$	7.52	7.83	3.30	0.27	**100**	-
TRI-D25™	-	$1.6x10^3$	7.52	7.75	3.35	0.46	**100**	-
Trich-A-Soil™	-	$3.3x10^5$	7.17	8.50	3.14	0.38	**100**	-
Trichoshield™	-	$2.8x10^5$	7.67	9.23	3.58	0.38	**100**	-
Companion (*B. subtilis* GB03)™	$4.0x10^7$	-	7.63	8.73	3.73	0.46	**100**	-
F-test	0.001		0.644	0.047	0.027	<0.001		
lsd (P=0.05)[C]	$2.3x10^7$	ns	0.67	1.04	0.52	0.10		

[A] Assessments of biocontrol population density in seedling plugs seedling growth was conducted prior to transplanting into pots. Total culturable aerobic bacteria cultured in Tryptone soy agar (TSA). *Trichoderma* cultured in *Trichoderma* selective media.
[B] Lettuce plants were infected and killed by *S. minor* during the 2 weeks after transplanting into pots. Fungicide treated plants were harvested 30 days after transplanting into pots.
[C] Differences in cfu/g larger than the lsd (within columns) are significantly different ($P = 0.05$).

Table 5.15 Effect of biocontrol treatments on seedling growth, incidence of lettuce drop and plant weight of crisphead lettuce plants (cv 'Green harvest' in Debco™ mix) in pots – experiment 2.

Treatment	Seedlings before transplanting into pots[A]						Pots	
	Bacteria (cfu/g)	*Trichoderma* (cfu/g)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	% of Plants infected[B]	Plant weight (grs)
Untreated	$2.2x10^8$	0.0	7.03	7.28	1.56	0.10	**100**	-
Fungicide (procymidone)™	-	-	-	-	-	-	**0**	76
Trichodry (*T. hamatum* 6S4r)™	-	$4.6x10^2$	5.94	8.00	0.86	0.09	**100**	-
TRI-D25™	-	$2.9x10^3$	6.09	8.99	1.02	0.11	**100**	-
Trich-A-Soil™	-	$6.7x10^5$	6.24	8.76	1.08	0.08	**100**	-
Trichoshield™	-	$8.5x10^3$	6.49	8.25	1.12	0.11	**100**	-
Companion(*B. subtilis*GB03)™	$3.2x10^8$	-	7.96	7.50	1.77	0.21	**100**	-
F-test			<0.001	0.014	<0.001	<0.001		
lsd (P=0.05)[C]	ns	ns	0.88	1.06	0.28	0.04		

[A] Assessments of biocontrol population density in seedling plugs seedling growth was conducted prior to transplanting into pots. Total culturable aerobic bacteria cultured in Tryptone soy agar (TSA). *Trichoderma* cultured in *Trichoderma* selective media.
[B] Lettuce plants were infected and killed by *S. minor* during the 2 weeks after transplanting into pots. Fungicide treated plants were harvested 30 days after transplanting into pots.
[C] Differences in cfu/g larger than the lsd (within columns) are significantly different ($P = 0.05$).

Table 5.16 Effect of biocontrol treatments on seedling growth, incidence of lettuce drop and plant weight of butterhead lettuce plants (cv 'Nadine' in HortiPine™ mix) in pots.

Treatment	Seedlings before transplanting into pots[A]						Pots			
	Bacteria (cfu/g)	Trichoderma (cfu/g)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	Bacteria (cfu/g)	Trichoderma (cfu/g)	% of plants infected	Plant weight (grs)[C]
Untreated	$3.0x10^8$	1.98 ($3.4x10^2$)[B]	13.13	7.06	9.36	0.75	8.32 ($2.1x10^8$)[B]	1.58 ($7.4x10^2$)[B]	**10**	78.1
Fungicide (procymidone)™	-	-	-	-	-	-	-	-	**0**	41.7
Trichodry (*T. hamatum*)™	-	3.53 ($3.7x10^3$)	12.09	6.48	7.68	0.61	-	4.63 ($5.1x10^4$)	**10**	99.5
TRI-D25™	-	1.97 ($3.4x10^2$)	11.46	7.13	7.16	0.69	-	0.60 ($6.2x10^1$)	**20**	97.0
Trich-A-Soil™	-	5.44 ($2.9x10^5$)	11.79	7.59	8.03	0.64	-	5.70 ($5.1x10^5$)	**20**	93.0
Trichoshield™	-	3.61 ($5.3x10^3$)	12.90	6.81	8.51	0.64	-	4.01 ($1.0x10^4$)	**10**	81.8
Companion (*B. subtilis*)™	$2.1x10^8$	-	11.93	6.94	7.43	0.55	8.01 ($3.4x10^7$)	-	**10**	110.5
F-test	ns	<0.001	<0.001	<0.092	0.002	0.023	0.017	<0.001	ns	0.003
lsd (P=0.05)[D]		1.22	0.81	0.73	1.07	0.11	0.20	1.363		26.34

[A] Assessments of biocontrol population density in seedling plugs and seedling growth were conducted prior to transplanting into pots and biocontrol population density was tested again 3 weeks after transplanting into pots. Total culturable aerobic bacteria cultured in Tryptone soy agar (TSA). *Trichoderma* cultured in *Trichoderma* selective media.

[B] Population density (cfu/g) for each replication was transformed to $\log_{10}$ before analysis to obtain a constant variance. Natural values shown in parenthesis. LSD values calculated based on transformed data.

[C] Lettuce plants were harvested 30 days after transplanting into pots.

[D] Differences in cfu/g larger than the lsd (within columns) are significantly different ($P = 0.05$).

Table 5.17 Population density of total fungi in untreated commercial mixes and the biocontrol *Trichoderma hamatum* 6Sr4 applied twice to seedling transplants in a nursery experiment.

Treatment	pH (n=2)	Fungi[A] (cfu/g)	*Trichoderma*[C] (cfu/g)
Kiwi peat/Vermicullite/Perlite™	5.20	5.21 ($1.6x10^5$)[B]	0.00
Kiwi peat/Vermicullite/Perlite+*T. hamatum* 6Sr4™	5.22	5.98 ($2.4x10^5$)	5.94 ($8.9x10^4$)
Composted pine bark™	6.38	5.67 ($2.2x10^4$)	0.00
Composted pine bark+*T. hamatum* 6Sr4™	6.38	4.76 ($2.4x10^4$)	4.36 ($4.7x10^4$)
Canadian peat™	5.67	5.03 ($8.1x10^4$)	4.21 ($1.6x10^4$)
Canadian peat+*T. hamatum* 6Sr4™	5.68	4.70 ($2.7x10^4$)	5.48 ($5.5x10^5$)
Composted pine bark/Kiwi peat™	5.02	5.36 ($5.4x10^4$)	0.00
Composted pine bark/Kiwi peat+*T. hamatum* 6Sr4™	4.99	5.35 ($2.8x10^4$)	5.97 ($9.3x10^5$)
F-test		0.225	<0.001
lsd (P=0.05)[D]		ns	0.90

[A] Total fungi cultured in Malt extract agar.
[B] Population density (cfu/g) counts were transformed to $\log_{10}$ before analysis to obtain a constant variance. Natural values shown in parenthesis. LSD values calculated based on transformed data.
[C] *T. hamatum* strain 6Sr4 cultured in *Trichoderma* selective media. *T. hamatum* 6Sr4 (Trichodry™ and Trichoflow) was applied twice to seedling transplants in the nursery. Seedling transplants were assessed for levels of *T. hamatum* 6Sr4 (cfu/g) before planting in the field.
[D] Differences in cfu/g larger than the lsd (within columns) are significantly different ($P = 0.05$).

Table 5.18 Population density of total culturable bacteria in commercial mixes and the biocontrol *Bacillus subtilis* GB03 applied twice to seedling transplants in a nursery experiment.

Treatment	pH	Bacteria/*B. s*[A] (cfu/g)	*B. subtilis*[C] (cfu/g)
Kiwi peat/Vermicullite/Perlite™	5.20	7.51 ($3.2x10^7$)[B]	6.61 ($4.1x10^6$)
Kiwi peat/Vermicullite/Perlite+*B.subtilis* GB03™	5.22	8.89 ($7.9x10^8$)	8.58 ($3.9x10^8$)
Composted pine bark™	6.38	8.31 ($2.2x10^7$)	7.96 ($9.8x10^7$)
Composted pine bark+*B. subtilis* GB03™	6.38	7.45 ($2.8x10^8$)	6.85 ($7.4x10^6$)
Canadian peat™	5.67	7.54 ($3.5x10^7$)	7.30 ($2.0x10^7$)
Canadian peat +*B. subtilis* GB03™	5.68	7.51 ($3.4x10^7$)	6.93 ($1.1x10^7$)
Composted pine bark/Kiwi peat™	5.02	7.16 ($3.1x10^7$)	6.65 ($4.5x10^6$)
Composted pine bark/Kiwi peat+*B. subtilis* GB03™	4.99	7.48 ($1.9x10^7$)	6.26 ($2.1x10^6$)
F-test		<0.001	<0.001
lsd (P=0.05)[D]		0.47	0.44

[A] Total culturable aerobic bacteria and *B. subtilis* GB03 cultured in Tryptone soy agar.
[B] Population density (cfu/g) for each replication was transformed to $\log_{10}$ before analysis to obtain a constant variance. Natural values shown in parenthesis. LSD values calculated based on transformed data.
[C] *Bacillus subtilis* GB03 (Companion™) was applied twice to seedling transplants in the nursery. Seedling transplants were assessed for levels of total bacteria (cfu) before planting in the field.
[D] Differences in cfu/g larger than the lsd (within columns) are significantly different ($P = 0.05$).

Table 5.19 Effect of biocontrol treatments on growth of butterhead lettuce seedlings in a nursery experiment (Werribee) in Victoria, July-August 2003.

Treatment[A]	Fresh weight (g)	Dry weight (g)	Shoot length (cm)
Pine bark/Kiwi peat untreated	11.07	0.567	12.40
Pine bark/Kiwi peat+*B. subtilis* GB03™	10.50	0.467	13.33
Pine bark/Kiwi peat+*T. hamatum* 6Sr4™	10.70	0.533	13.50
F-test	0.87	0.28	0.07
lsd (P=0.05)	ns	ns	1.0

[A] *Trichoderma hamatum* 6Sr4 (Trichodry™ and Trichoflow) and *Bacillus subtilis* GB03 (Companion™) were applied twice to seedling transplants in the nursery and seedlings measured before sowing.

Table 5.20 Effect of two biocontrol treatments applied to three commercial mixes on growth of crisphead lettuce seedlings in a nursery experiment (Werribee) in Victoria, July-August 2003.

Seedling mix	Treatment[A]	Fresh weight (g)	Dry weight (g)	Shoot length (cm)
Composted pine bark™	untreated	3.66	0.400	8.33
	B. subtilis GB03	3.50	0.366	8.16
	T. hamatum 6Sr4	2.83	0.333	7.50
Kiwi peat/Vermicullite/Perlite™	untreated	5.70	0.600	9.66
	B. subtilis GB03	6.67	0.633	10.39
	T. hamatum 6Sr4	5.16	0.566	9.38
Canadian peat™	untreated	4.56	0.500	8.94
	*B. subtilis*GB03	4.56	0.500	8.83
	T. hamatum 6Sr4	4.46	0.550	8.83
F-test		<0.001	<0.001	<0.001
lsd (P=0.05)		0.56	0.067	0.51

[A] *Trichoderma hamatum* 6Sr4 (Trichodry™ and Trichoflow) and *Bacillus subtilis* GB03 (Companion™) were applied twice to seedling transplants in the nursery and seedlings measured 7 weeks after sowing.

Table 5.21 Effect of biocontrol treatments applied to three commercial mixes on lettuce drop incidence and fresh weights of crisphead lettuce in a field trial, spring 2003, Werribee Victoria.

Mix type	Treatment[A]	*Trichoderma* (cfu/g)[B]	*B. subtilis* (cfu/g)[B]	% plants infected[C] (n=2)	Plot FW (kg)[D]
Kiwi peat™	Untreated	0	4.1x106	11.68 (19.89)	2.15
	Trichoderma mix, transplant	8.9×10^4	-	11.21 (19.42)	1.56
	B. subtilis mix, transplant	0	3.9x108	11.68 (19.98)	2.01
Mean (n=6)				11.52	1.91
Canadian peat™	Untreated	1.6×10^4	2.0x107	7.99 (16.25)	1.76
	Trichoderma mix, transplant	5.5×10^5	-	10.19 (18.54)	1.98
	B. subtilis mix, transplant	0	1.1x107	5.12 (13.06)	1.89
Mean (n=6)				7.76	1.87
C. pine bark™	Untreated	0	9.8x107	6.54 (14.82)	1.53
	Trichoderma mix, transplant	9.3×10^5	-	3.79 (11.16)	1.43
	B. subtilis mix, transplant	0	7.4x106	8.33 (16.68)	1.25
Mean (n=6)				6.22	1.40
F-test				0.056	0.342
lsd (0.05)[E]				5.850	0.852

[A] Crisphead lettuce seedlings were grown and treated with biocontrols in an open plastic glasshouse as part of a replicated nursery experiment.
[B] Population density of biocontrols (cfu/g) in seedling transplants was measured as part of a nursery experiment (Tables 5.17 and 5.18).
[C] Disease incidence data was transformed to angular values before analysis to obtain a constant variance. Transformed values shown in parenthesis. LSD values calculated based on transformed data.
[D] Ten plants harvested from each replicate plot.
[E] Differences in disease incidence (transformed data) larger than the lsd (within columns) are significantly different ($P = 0.05$).

5.4 THE EFFECT OF BIOCONTROL AND CHEMICAL TREATMENTS ON THE INCIDENCE OF SCLEROTINIA LETTUCE DROP

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5.4.1 Summary

Eight field trials were conducted in Victoria over seven lettuce growing seasons at sites with different disease pressures to evaluate the effects of biocontrol and fungicide treatments on control of Sclerotinia lettuce drop (SLD).

Results from seven field trials showed that consistent good control of lettuce drop (80-95% disease reductions) was obtained with well-timed and strategic (plant-targeted) applications of procymidone (Sumisclex™). Two to three early season applications of procymidone (2L/ha in 1000L water/ha), applied the first after planting and thereafter approximately every 2-weeks, provided excellent control of SLD in low (<10% disease) and mod/high (10 and 70% disease) disease pressure sites. Better disease control was obtained when procymidone was applied with a high volume of water (2,500L/ha), which facilitated the penetration of the fungicide into the soil. The statistically significant and consistent reduction in disease incidence with procymidone treatments in the seven trials indicated that the reported decline in procymidone effectiveness could be due to inadequate timing and application of fungicide sprays by growers. Results from two of the seven field trials under moderate disease pressure (11-13% disease incidence) also showed that two strategic applications of the new fungicide BAS 510 (boscalid, BASF NuFarm) provided excellent SLD control, which was comparable to that provided by procymidone. BAS 510 is a new fungicide with good activity against Sclerotinia, making it suitable for use as an alternative or in alternation with procymidone.

All biocontrol treatments evaluated did not provide the consistent and effective SLD control provided by the fungicide treatments. A few biocontrol treatments showed some potential for disease control (eg. 50-76% disease reduction - *C. minitans* A69, *T. hamatum* 6Sr4, *B. subtilis* GB03) at 3 low disease pressure sites planted with butterhead lettuce. In trials 7 and 8, an integrated approach using biocontrol treatments (eg. *T. hamatum 6Sr4* and *B. subtilis* GB03) with a single early-season application of fungicide did not show potential for enhanced disease control in butterhead and iceberg lettuce. In trial 7, however, a combination of nursery and field treatments with *T. hamatum* 6Sr4 and *B. subtilis* GB03 showed some potential for disease control (70-83% disease reductions) in butterhead lettuce, which had a less dense canopy and more upright foliage and therefore was less susceptible to *S. minor* infection in late season. In trial 8, two application of fungicide were required to provide acceptable levels of disease control (71-86% disease reductions) on iceberg lettuce, which grows a more dense canopy with leaves in direct contact with soil in late season. This type of plant architecture makes iceberg plants more susceptible to Sclerotinia infection in late season. Under these conditions, *T. hamatum* 6Sr4 in combination with one early-season fungicide spray gave 56% disease reduction when compared to an untreated control. The low efficacy of biocontrol treatments was most likely the result of low levels of biocontrol agent colonisation in the region of roots of seedling transplants and field plants (soil, crown and base of plants). These low levels were not effective in preventing early season and especially late season (spread of disease from infected plants) infection by *S. minor*.

There was a positive correlation between the concentration of sclerotia of *S. minor* in soil and disease incidence in untreated plots in Victorian sites. Although the correlation was significant, regression analysis showed that only around 27% of the variation (R^2=27.7, P=0.015) in disease incidence could be explained by sclerotial concentration in the soil. The analysis indicated that counts of sclerotia (wet-sieving method) alone were not good predictors of disease pressure in Victorian sites. In addition to sclerotial counts, the effect of environment, history of disease and crop susceptibility on disease development must also be considered when developing recommendations for the management SLD in a given site.

5.4.2 Introduction

Sclerotinia pathogens cause serious economic losses in a number of important vegetable crops including lettuce, bean, carrot and cabbage. Sclerotinia control relies on the use of fungicides. In recent years, the inconsistency of chemical control and increasing public concerns over the use of chemicals have highlighted the need to find other control measures which can provide more effective sustainable control of Sclerotinia diseases. Many antagonistic fungal species have been shown to have potential biocontrol activity against sclerotial pathogens. Fungi belonging

the genera *Trichoderma* and *Coniothyrium* have been reported to act as sclerotial mycoparasites of Sclerotinia pathogens and therefore have the potential for biocontrol of Sclerotinia diseases in vegetable crops (Budge and Whipps 1991, Jones and Stewart 2000, Whipps and Budge 1990).

In addition to mycoparasitism, isolates of *Trichoderma* have also been reported to provide disease protection/suppression based on competition for nutrients and space and antibiosis in the region of roots. In Europe, the sclerotial mycoparasite *C. minitans* (Contans™) has been shown to reduce populations of *Sclerotinia minor* and *Sclerotinia sclerotiorum* in soil and this reduced Sclerotinia disease on lettuce plants grown in glasshouse and open fields (50-80% reduction disease incidence) (Luth 2001). Research field trials in New Zealand showed that the incorporation of the biocontrols *T. hamatum* 6Sr4 or *C. minitans* A69 into lettuce seedling transplants prior to field transplanting, provided good control (60-80% disease control) of Sclerotinia lettuce drop (Stewart and Rabeendran 2000). There are other biocontrol products available overseas and in Australasia for use in the nursery and in field crops to improve the growth of plants and the management of soilborne diseases.

Biocontrol formulations of mycoparasites of sclerotia (e.g. *C. minitans* Contans™) are recommended for field application several weeks prior to planting to allow time for the mycoparasite to effectively parasitise and kill sclerotia in the soil. Biocontrol formulations are also recommended for application shortly before or after planting to allow time for the biocontrol to colonise the region of roots and base of plants and thus protect the plant against Sclerotinia infection. In field trials in New Zealand, the incorporation of the biocontrols *C. minitans* A69 and *T. hamatum* 6Sr4 into the transplant mix (10^6 spores/g potting mix) or into soil at the time of planting (soil amendment - inoculum mixed into soil 10^6 propagules/g) were two effective methods of delivering the biocontrol agents into the region of roots of lettuce plants (Ridgway *et al*. 2001, Stewart and Rabeendran 2000, Stewart *et al.* 2001). The use of these two methods of inoculum incorporation resulted in good biocontrol of Sclerotinia lettuce drop (60-85%), under medium disease pressure (30-60% disease) and under conditions in which biocontrol colony numbers were maintained in the root region of plants at high levels (10^5-10^6 cfu/g) (Stewart and Rabeendran 2000, Stewart *et al*. 2001,). The use of both methods combined also gave a high level of control (77%) of Sclerotinia lettuce drop, which was significantly better than the standard fungicide treatment. The soil incorporation method (*C. minitans* A69) also gave excellent control (75%) of Sclerotinia disease in a direct seeded bean trial (Stewart *et al.* 2001).

Aims

A series of field trials were conduced in Victoria to evaluate the effects of biocontrol and chemical treatments on control of Sclerotinia lettuce drop disease. The trials aimed to evaluate the biocontrol agents *T. hamatum* 6Sr4 and *C. minitans* A69, and other promising biocontrol agents developed in Australasia and overseas, for their ability to kill sclerotia and prevent disease and therefore as an alternative treatment to fungicide for Sclerotinia control. The trials also aimed to evaluate the effects of different application methods, timing of application and number of applications for both biocontrol and fungicide treatments on disease control, and evaluate the use biocontrols in combination with fungicide. In addition, population densities of sclerotia were monitored in field trials with the aim of developing a method to predict crop loss and recommendations for application of biocontrol and chemical treatments.

5.4.3 MATERIALS AND METHODS

Trial locations and S. minor populations in soil

There were 8 field trials conducted over seven lettuce growing seasons during 2000-2003 in Victoria. All trials were conducted in commercial lettuce farms located in Bacchus Marsh and Werribee, Victoria. Details of trials are described in tables 5.22-5.23 (appendix).

In trials 1-3 after harvesting the crops, the concentration of sclerotia of *S. minor* in soil of each replicate plot was determined using the wet-sieving method. This was done to determine the effect of biocontrol treatments on sclerotial numbers in soil. In trials 4-8, the concentration of sclerotia in soil was determined prior to planting the crop. Several soil samples were collected at random from the entire field and included samples from untreated plots. Each soil sample consisted of four to five soil sub-samples collected from each replicate plot or sampling area (13 m^3 plots). Soil samples were collected using a soil core sampler or a scoop to depths of 10 and 15 cm. Soil sub-samples were pooled and 200 g used for sclerotial counts. Viability of sclerotia was determined in PDA medium containing antibiotic. Results were expressed as the number of sclerotia per kg (dry weight) soil.

The mean concentration of sclerotia in field soil (untreated plots) at the different field sites was correlated to disease incidence. These two variables were correlated to investigate the relationship between sclerotial concentration in soil and disease incidence in susceptible lettuce varieties. The sclerotial and disease data were collected in the 8 field trials conducted in Victoria. Sclerotial and disease incidence data collected from another 4 field trials, reported in this final report and 2 grower trials (Werribee) were also included in the correlation analysis. Soil temperature and pH were measured at each trial location. Growers applied their regular commercial pesticide spray program for control of insect and foliar diseases (e.g. *Botrytis* and Anthracnose). Irrigation (overhead sprinklers) was applied after biocontrol and fungicide applications, to drench the biological products and fungicides into the soil.

Trial product list

The biocontrol and chemical products, their active ingredients, suppliers, application details and product rates used in the field trials are listed in Tables 5.24-5.28 (appendix). Biocontrol treatments included biocontrol products formulated with fungal biocontrol agents antagonistic to *S. minor* (e.i. *T. hamatum 6Sr4* and *C. minitans* A69) and fungal and bacterial biocontrol agents antagonistic to a range of soil borne pathogens (Table 5.29).

The biocontrol products evaluated were: three commercial biocontrol products containing single isolates of *Trichoderma* species, *C. minitans* or *B. subtilis*; three laboratory formulations containing single isolates of *C. minitans* and one of *T. longipile*; and four commercial biocontrol products containing a mixture of *Trichoderma* species and bacteria. Except for *C. minitans* isolates A69, A, B and *T. longipile* isolate D, all other biocontrol products evaluated were commercial products. Products were obtained from manufacturers and store at 4-5°C until used. Procymidone (Sumisclex™) is the registered fungicide for control of Sclerotinia lettuce drop. BAS 510 is a new fungicide developed by BASF and belongs to the new chemical group Boscalid (benzaniline). Calcium cyanamide (Perlka™) is a slow release nitrogenous calcium fertiliser (SKW Trostberg, AG).

Biological treatments

Biocontrol products were used as recommended by manufacturers. *C. minitans* isolate Contans™ was reported to be a good mycoparasite of sclerotia of S. minor. *T. hamatum* isolate 6Sr4 and *C. minitans* isolate A69 were reported to be able to parasite sclerotia and mycelium of S. minor and grow well in the soil region of lettuce plants and therefore provide protection against *S. minor* infection. Therefore T. hamatum 6Sr4 and *C. minitans* A69 were used as the standard biocontrol treatments in all trials. The main mode of action for all other microorganisms in commercial biocontrol products was reported to be competition, and some antibiosis. These products were recommended for use in glasshouses and field crops to improve the growth of plants and the management of soil borne diseases. All other microorganisms were isolated from sclerotia of *S. minor* in Victoria and it was assumed these isolates would act as mycoparasites of sclerotia.
Inoculum (spores) of *T. hamatum* 6Sr4 were prepared in a dry formulation medium suitable for blending with seedling growing mixes (Trichodry™ – 10^6 spores/g of medium) and suitable for drenching of seedling trays/soil (Trichoflow™ – 10^8 cfu g^{-1}). Inoculum for all the other biocontrol products, except *B. subtilis*, were prepared in dry formulation media suitable for drenching of seedling trays/soil and for application in irrigation water (Tables 5.24-5.28). *B. subtilis* was formulated in a liquid formulation also suitable for drenching seedling trays/soil and for application in the irrigation water.

Application of biocontrol treatments

Tables 5.24-5.28 describe the methods and timings of biocontrol applications in all trials. Seedling growing mixes (no fungicide added) and lettuce varieties used in the trials and planting dates are described in Tables 5.22 and 5.23.

Biocontrol treatments were applied using a variety of methods and application timings. Biocontrol treatments were incorporated into the seedling growing mix at sowing (only *T. hamatum* 6Sr4, *C. minitans* A69), applied as a drench to seeded or seedling plug before field transplanting (transplant treatments), applied into the soil at transplanting (soil drench) or as a drench onto plants/soil and as a combination of both seedling transplant and drench treatments. In trials 1-3, biocontrol treatments (soil treatment) were also incorporated into bands (12.5 cm wide, 20 cm deep) along the transplant rows at transplanting. Trichodry™ (*T. hamatum* 6Sr4) was blended with mix at the rate of 2 kg of product per cubic metre of mix before filling the seeding trays. Seedling trays were filled with untreated mix or mix treated with Trichodry™ and seeded with pelleted lettuce seed. Trays with untreated mix were treated (drench) with solutions of the other biocontrol treatments. Before application, biocontrol products were mixed in distilled water and allowed to stand for 1-2 hrs to stimulate spore growth. Trays were drenched with the different solutions until saturation point. Control mixtures were made with the same mixes but were not blended or drenched with biocontrol

treatments. Treated and untreated trays were randomized on benches (RCB) in nurseries. Seedlings for trials 1-5 and 7 were grown in a commercial nursery (Boomaroo) in Lara, Victoria. Seedlings for trials 6 and 8 were grown in a commercial nursery (Velisha Bros) in Werribee, Victoria.

Lettuce seedlings were grown in nurseries for approximately 6-8 weeks depending on the time of the year. Before seedling release from the nurseries, seedling transplants were treated with the first or additional applications of biocontrol treatments 24-48 hrs before field transplanting. Biocontrol treatments were applied as a drench to seedling transplants to saturation point. In trials 1 and 2, seedling transplants were planted manually into plots and in trials 3-8 seedling transplants were planted using a 2 or 4-row seedling planter ensuring that planting tubes were cleaned between treatments. In trials 4-6, biocontrol treatments were also applied to individual plants in the field using a 'cup method' (plant-targeted applications or drench). In trials 7 and 8, biocontrol treatments were applied to lettuce rows using a 'banded application method' that delivered the biocontrol solution onto lettuce plants. Applications were made with a 5 L hand sprayer using 200 L water/ha and a spreader (Nu-Film™ – 17, Miller). Applications in the field were made 2 and 4 weeks after seedling transplanting. The whole trial areas were irrigated following the applications in order to drench the biological product onto the soil surface and the base of plants.

Application of chemical treatments

All biocontrol treatments were compared to the standard fungicide treatment procymidone (Sumisclex® 500SC – 2 L/ha). The fungicide treatments were applied using different water dosages per hectare and different methods and timings of application. In trials 1-4, fungicide applications were applied using the cup method which delivered the appropriate amount of product/ha (1000 L water/ha) to each plant. In trials 5, the cup method was compared to the banded application method that allowed the spraying of individual lettuce rows. Banded sprays were applied using a knapsack sprayer fitted with a 40 cm boom (2 nozzles) and calibrated to deliver 1000 L of water/ha. In trials 6-8, all applications were made using the banded method. The first spray was applied after transplanting and after that subsequent applications were made approximately every 2 weeks. The whole trial area was irrigated following the spray applications in order to drench the product onto the soil surface and the base of plants. In trials 7 and 8, fungicide treatments were also included to evaluate three rates of the new fungicide BAS 510 (Boscalid 0.8, 1.0 and 1.6 kg/ha) for control of lettuce drop. Fungicide sprays were applied using the banded application method (1000L water/ha). In trials 1-3, calcium cyanamide (Perlka®), a slow release nitrogen calcium fertiliser, was evaluated for its potential to kill or inhibit germination of sclerotia and prevent *S. minor* infection on lettuce. Calcium cyanamide was spread onto the total surface of treatment plots at transplanting at the rate of 300-500 kg/ha.

Populations of biocontrol

The concentration of different biological control agents in biocontrol products was determined *in vitro* prior to application using 10-fold serial dilutions and a variety of selective media (trials 4-8). *Trichoderma* species were cultured in PDA medium plus antibiotic and Triton x100 (Agrimm Technologies recipe) and *Bacteria* in TSA medium. The population density (cfu/g-^{1}) of biocontrol agents was measured in the region of roots of seedling transplants (mix/root) before seedlings were planted in the field (Trials 4-8).

The population density of biocontrol agents was also measured in the root/soil region of lettuce plants in the field 2-3 weeks after seedlings were planted in the field. *Trichoderma* species were cultured in *Trichoderma* selective medium (Lincoln University recipe) and *Bacteria* in TSA medium. For *in vitro* testing, each replicate sample consisted of four sub-samples taken from seedling trays (seedling transplants) or replicate plot. Each sub-sample included a seedling transplant (mix and seedling roots) or a sample of roots and soil (field sample) collected using a 10mm diameter steel core borer. Colonisation comparisons were made with untreated mix control. Media and culturing procedures are described in detail in section 5.3.

Measurements and experimental design

Plant survival was recorded 1 week after field transplanting and thereafter the number of infected plants recorded every week. Before commercial harvest, the percentage of infected plants in each replicate plot was calculated from the total number of plants assessed. At harvest, 10-12 lettuce heads were harvested to determine fresh weight yields from each plot. The experimental designs (RBD) used in the 8 trials are summarised in Tables 5.22-5.23 (appendix). All biological treatments, applied using variety of methods, were compared to the standard chemical treatment (procymidone) to identify promising biocontrol treatments for control of *S. minor*. The effect of different application methods and interactions between treatments were not examined in details in this report. Statistical analysis was conducted on disease and fresh weight data using analysis of variance (ANOVA). Data were transformed when

required to obtain a constant variance before analysis. Where ANOVA indicated a significant treatment effect, a lsd test (P=0.05) was used to compare the treatment means.

5.4.4 RESULTS AND DISCUSSION

Relationship between concentration of sclerotia in soil and disease incidence

Figure 5.3 shows the relationship between the mean number of sclerotia in soil at the different field sites (including other trials in this project) and the level of disease incidence observed in untreated plots. When all disease incidence data were included in the correlation analysis, the correlation was not significant. However, when the mean for the highest disease incidence observed in trial 3 (wettest autumn 2001 in the four year project) was removed from the analysis, disease incidence was positively correlated (r= 0.35; P=0.05) with the concentration of sclerotia in the soil. This relationship indicates that an increase in inoculum concentration will increase the amount of infection in lettuces (Dillard and Grogan 1985). Although the correlation was significant, regression analysis showed that only around 0.27% of the variation (R^2=27.7, P=0.015) in disease incidence could be explained by sclerotial concentration in the soil.
The regression line fitted to the data sets for the two variables examined was compared to a regression line fitted to data sets (n=100, R^2=0.063) collected from mini-plot experiments conducted in Victoria over two seasons in 2001 by Peta Easton (B.Sc. Hons) (Figure 5.3). The slopes of the two regression lines were not significantly different. However, the slope of a regression line fitted to data sets collected in the Tasmanian trials was significantly different to the slopes of the two regression lines for the Victorian trials. The three regression lines indicated that increases in inoculum concentrations would result in increases in the amount of infected plants. However, the slope of the Tasmanian regression line indicated that increases in inoculum concentrations would result in higher number of infected plants under Tasmanian conditions than under Victorian conditions.

The low R^2 coefficients of determination of the regression lines for Victoria would suggest that in addition to sclerotia in soil, environmental (dry and wet cycles) and soil factors (soil temperatures in autumn, winter and spring) would greatly influence the development of lettuce drop under Victorian conditions. In contrast, the R^2 of the regression line for Tasmania would suggest that increases in inoculum most likely would result in increases in disease because environmental conditions are more consistent (cool and wet weather) from season to season (lettuce production occurs mainly during spring/summer). The cooler and wetter conditions in Tasmania and the presence of two pathogens (*S. minor and S. sclerotiorium*) and sometimes two mechanisms of infection (mycelium and ascospores) can result in more favourable conditions for the development of lettuce drop. Temperature and soil moisture content can influence the growth, germination and infection process of *S. minor* (Abawi and Grogan 1979). Other factors also influence the development of lettuce drop. Variation in the numbers of sclerotia within and between plots was high in Victoria (range 0-160 sclerotia/kg soil). Viability of sclerotia within and between plots in Victoria was also variable (data not shown).

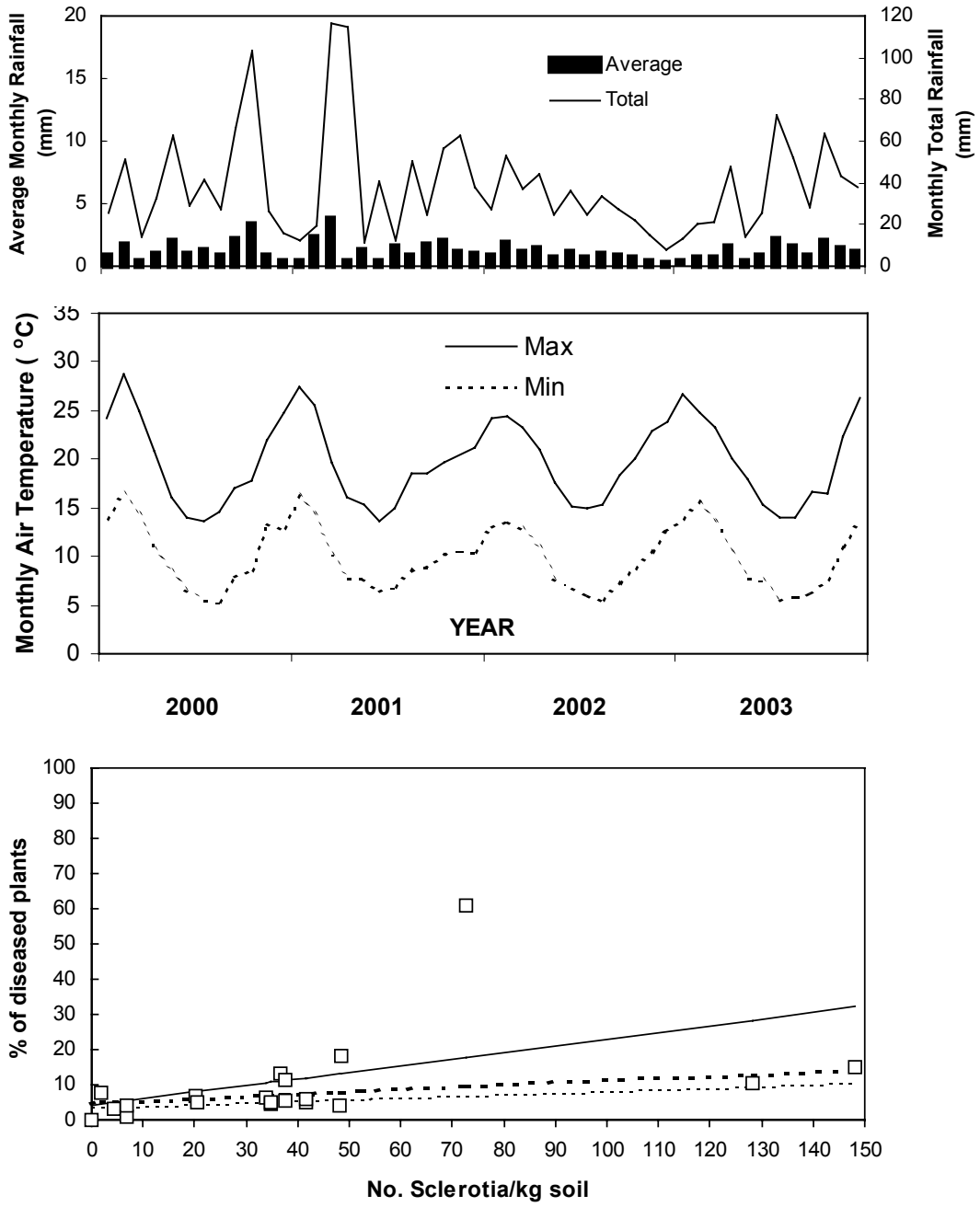

Figure 5.3 Weather records and scatter graph of the mean number of sclerotia of *S. minor* in the soil at the different Victorian trial sites plotted against percentage of plants infected observed in untreated plots during 2000-2003. The symbols are the means of concentrations of sclerotia in untreated plots and the trend lines represent three regression lines fitted to different data sets as follow: heavy dashed line is regression line ($y = 0.0633x + 4.75$, $R^2 = 0.30$) fitted to data sets (1 data points with highest disease not included in regression analysis) collected in Victorian trials; light dashed line is regression line ($y = 0.0486x + 3.42$, $R^2 = 0.063$) fitted to data sets (100 data points) collected from a mini-plot study in Victoria 2001 (Peta Easton B.Sc. Hons Thesis); and solid line is regression line ($y = 0.944 + 4.1287$, R^2 0.47) fitted to data sets collected in Tasmanian trials (expressed as no. sclerotia/kg soil).

Field trials

Eight field trials were conducted over seven lettuce growing seasons in Victoria to evaluate the effect of biocontrol and chemical treatments on control of lettuce drop. Soil temperatures during the autumn trials ranged from 20 to 10ºC, in winter from 5 to 11ºC and in spring from 10 to 25ºC, and soil pH (clay loamy soils) ranged from 6.5 to 7.6. In Victoria, the incidence of lettuce drop is commonly highest in the spring and autumn (pers. comm. F. Ruffo and S. Velisha).

Field trials 1-3

The effects of biocontrol and chemical treatments on the incidence of lettuce drop in trials 1-3 are summarised in Tables 3.30-3.32 and Figures 5.4 and 5.5. Despite the high levels of sclerotia measured in the soil (range 18-48/kg dry soil), disease levels in untreated plots were low (<7%) in trials 1 and 2 conducted during winter and spring 2000, respectively. In trials 1 and 2, three plant-targeted (cup method) applications of procymidone (2L/ha), applied the first after planting and thereafter approximately every two weeks, using either 1000 or 2500 L water/ha, significantly reduced the percentage of plants infected (84-96% disease reduction) compared to the untreated controls (6.55% and 3.94% disease incidences). The greatest disease control was obtained when procymidone was applied with a high volume of water (2,500L/ha), which facilitated the penetration of the fungicide into the soil. Under conditions of low disease pressure, a few biocontrol treatments provided reductions in disease incidence (50-76% disease reductions) worth mentioning. In trial 1, the mean disease incidences of two biocontrol treatments (*C. minitans* 69 and Nutri-life™), were significantly lower (P=0.05) than the mean disease incidence of the substrate base control. In trial 2 although not significant, the mean disease incidences in plots treated with *T. hamatum* strain 6Sr4 (transplant treatment) and *C. minitans* A69 (soil application) were lower than the mean disease incidence of untreated plots. In all these cases, the levels of disease control provided by these biocontrol treatments were not comparable to that provided by the fungicide treatment.

There was no difference in sclerotial levels (and viability – data not shown) between treatments, indicating that none of the biocontrol treatments reduced the population levels of *S. minor* sclerotia in the soil. There was also no difference in fresh weights of mature lettuce plants between treatments.

Disease levels were higher in the 2 lettuce crops in trial 3 than in lettuce crops in trials 1 and 2. In trial 3, the first lettuce crop was grow in autumn 2001 during a wet growing season in soil with high levels of sclerotia of *S. minor* (Table 5.32, Figure 5.3). Under these conditions in the autumn crop, three plant-targeted (cup method) applications of procymidone (2L/ha), applied the first after planting and thereafter approximately every two weeks, using either 1000 or 2500 L water/ha, significantly reduced the percentage of plants infected (90% disease reduction) compared to the untreated control (70.49%). None of the other chemical and biocontrol treatments provided commercially acceptable levels of disease control. Although not significant, lettuce plants grown in plots treated with procymidone had higher fresh weights than plants grown in all other treatments. A second crop was planted in the same field/plots in spring 2001 but this time the grower applied a fungicide program consisting of 2 procymidone sprays (2L/ha, 2000L water/ha) applied with a Silvan sprayer fitted with a spray boom. Although not significant, the greatest disease control was obtained when procymidone sprays were applied to plots previously treated with procymidone applications (cup method) in the previous autumn crop. The population density of sclerotia in plots treated with applications of procymidone in the two crops was significantly lower than the population density of sclerotia in all other treatments at harvest. This result indicated that the good disease control provided by the fungicide treatment prevented the build up of inoculum in soil.

Table 5.30 – Effects of biocontrol and chemical treatments on disease incidence and fresh weights of Green Mignonette lettuce in a field trial at Bacchus Marsh Vic, during winter 2000.

Treatment	*Application*	*Disease incidence (%)*	Disease reduction (% of control	Fresh weight (kg)	No. Sclerotia (kg/dry soil)[C]
C. minitans (B-Vic)	Transplant	4.76[A]	27.3	4.08[B]	44.2
	Soil drench	6.94	-	4.11	29.1
	Transplant+Soil	3.77	42.4	4.13	33.0
C. minitans (A69-NZ)	Transplant	3.57	45.5	3.96	43.0
	Soil drench	8.13	24.1	4.48	34.9
	Transplant+Soil	3.17	51.6	4.16	38.4
T. longipile (D-Vic*)*	Transplant	3.77	42.4	3.86	33.7
	Soil drench	3.77	42.4	3.34	24.2
C. minitans (Contans™)	Soil drench	3.37	48.6	3.52	38.4
Nutri-life 4/20	Soil drench	2.98	54.5	3.45	36.0
Sumisclex™ (procymidone)	Day 0,14,28 2L/1000L	**0.46**	**92.9**	3.60	22.0
Sumisclex	Day 0,14,28 2L/2500L	**0.23**	**96.5**	3.99	27.9
Perlka™	Soil application	6.35	3.1	4.17	46.5
Substrate base	Soil application	4.86	25.8	4.23	34.9
Untreated	-	6.55	-	4.53	33.7
lsd (P = 0.05)[D]		1.55		ns	ns

[A] Percentage of plants infected relative to the number of plants established after transplanting in each treatment.
[B] Fresh weight of 10 lettuce plants per replicate plot.
[C] Number of sclerotia measured from each plot after the trial was harvested.
[D] Differences in disease incidence larger than lsd (within columns) are significantly different (P = 0.05)

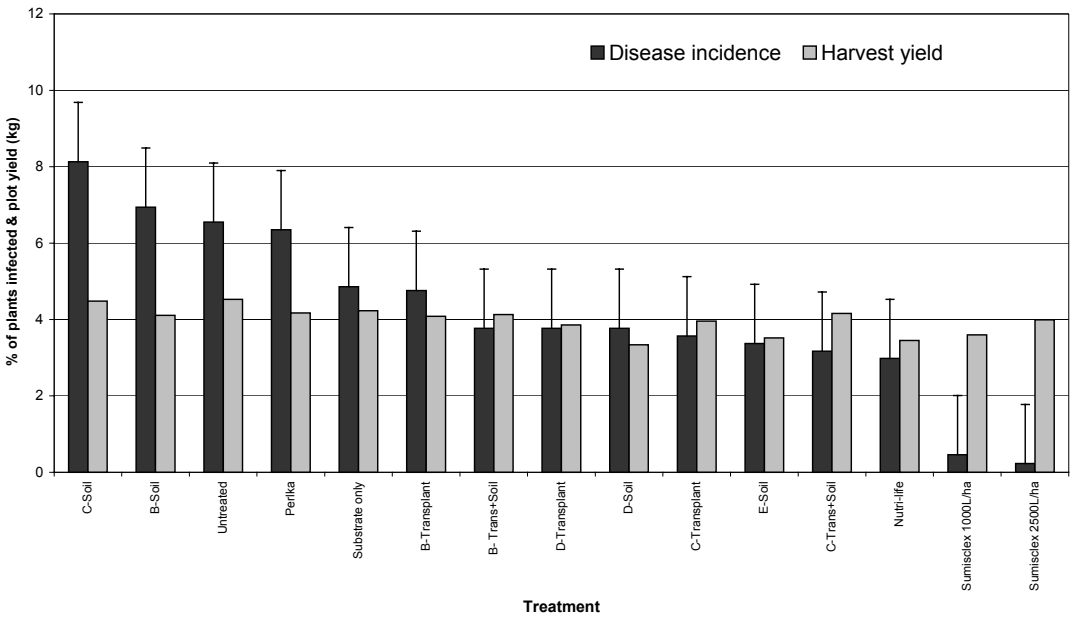

Figure 5.4 - Effects of biocontrol and chemical treatments on disease incidence and fresh weights of Green Mignonette at Bacchus Marsh, winter-spring 2000. (Bars above the histograms represent the lsd at P=0.05)

Table 5.31 – Effects of biocontrol and chemical treatments on disease incidence and fresh weights of Green Mignonette lettuce in a field trial at Bacchus Marsh Vic, during spring 2000.

Treatment	Application	Disease incidence (%)	Fresh weight (kg)	No. Sclerotia (kg dry soil)[C]
C. minitans (B -Vic)	Transplant	3.70[A]	6.34[B]	21.5
C. minitans (B -Vic)	Soil application	7.41	5.64	30.0
C. minitans (B -Vic)	Transplant+Soil	4.17	6.44	30.0
C. minitans (B -Vic)	Spot spray	5.79	6.34	44.3
C. minitans (B -Vic)	Pre-planting soil	2.55	5.92	40.0
C. minitans (A69)	Transplant	3.01	5.23	38.6
C. minitans (A69)	Soil application	**1.62 (58.9)**	6.00	35.6
C. minitans (A69)	Transplant+Soil	3.70	5.69	40.0
C. minitans (Contans™)	Soil application	2.78	6.88	44.3
Nutri-life 4/20™	Soil application	3.01	5.24	34.3
T. hamatum (6Sr4)	Transplant	**0.93 (76.4)**	6.90	40.0
T. hamatum (6Sr4)	Soil application	3.24	6.28	38.6
T. hamatum (6Sr4)	Transplant+Soil	4.63	7.12	42.9
Tri-D25™	Soil application	2.78	5.70	41.5
Trich-A-Soil™	Soil application	3.47	5.92	38.6
Sumisclex™ (procymidone)	Day 0,16,30 2L/1000L/ha	**0.60 (84.8)**	5.48	20.0
Sumisclex	Day 0,16,30 2L/2500L/ha	**0.23 (94.2)**	6.07	18.6
Substrate control	Soil application	5.09	5.82	41.5
Untreated (removal)	Infected plants	6.02	6.27	41.5
Untreated control	-	3.94	5.77	48.1
F-test		0.004	0.424	0.985
lsd (p=0.05)[D]		3.39	1.44	20.1

[A] Percentage of diseased plants relative to the number of plants established after transplanting in each treatment. In parenthesis best disease reduction (% of control).
[B] Fresh weight of 10 lettuce plants/plot.
[C] Number of sclerotia measured from each plot after trial was harvested.
[D] Differences in disease incidence larger than lsd (within columns) are significantly different (P = 0.05)

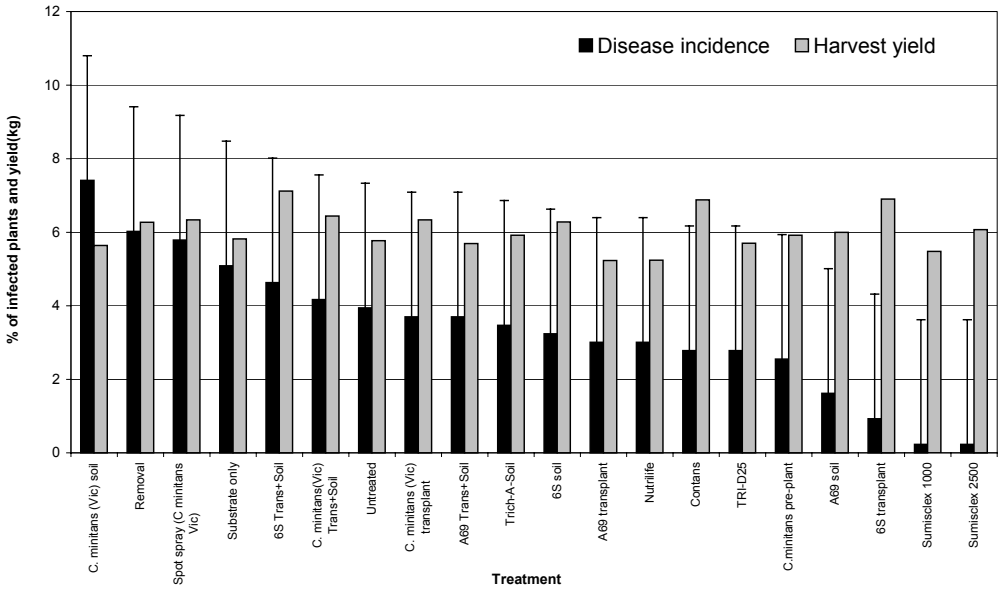

Figure 5.5 - Effects of biocontrol and chemical treatments on disease incidence and fresh weights of green Mignonette lettuce at Bacchus Marsh, winter-spring 2000. (Bars above the histograms represent the lsd at P=0.05).

Trials 4-6

The effects of biocontrol and chemical treatments on the incidence of lettuce drop in trials 4, 5 and 6 are summarised in Table 5.33 and Figures 5.6 and 5.7. As in trials 1 and 2, despite the levels of sclerotia measured in the soil (range 10-20 sclerotia/kg dry soil), disease incidence levels in untreated plots were low (<8%) in all 3 trials conducted during autumn/winter (trial 4) and spring 2002 (trials 5 and 6). The generally low disease incidence was the result of dry conditions during 2002.

The population density of *Trichoderma* species, *C. minitans* and *B. subtilis*, detected in the region of roots (soil/root samples) of field plants treated with nursery (seedling transplant) and field application of the different biocontrols, was below $10^4/g^{-1}$ soil in all 3 trials (data not shown). The Petri dish cultures (selective media) showed that other soil/mix microorganisms, mainly filamentous fungi and various soil bacteria, were detected at levels higher than those of biocontrol agents in the region of roots of field plants. These results suggested that the population densities of biocontrol agents in seedling transplant before planting in the field were not adequate (optimal 10^5-10^6 cfu/g^{-1}) for effective biocontrol of *S. minor* on lettuce. Additional applications of biocontrols treatments in the field apparently did not increase the biocontrol levels.

In trial 4, two plant-targeted (cup method) applications of procymidone (2L/ha), applied the first after planting and thereafter approximately every two weeks using 1000 water/ha, significantly reduced the percentage of plants infected from 6.67 to 1.67% (75% disease reduction) compared to an untreated control. There were no significant differences in disease control levels between 2 and 3 or 4 applications of procymidone. The greatest disease control (91% reduction) was obtained with 3 or 4 applications of procymidone applied at approximately 2 weeks intervals after transplanting. A single application of procymidone after field transplanting also significantly reduced disease incidence (62% reduction) when compared to the untreated control under conditions of low disease pressure.

Although population density of biocontrol agents in rhizosphere of plants were apparently low in this trial, six biocontrol treatments gave significant disease control levels (50-71% disease reduction), which were comparable to disease control levels provided by the less effective fungicide treatments. The greatest disease control (71% disease reduction) was obtained when *T. hamatum* 6Sr4 was applied as dual treatment (seedling transplant and plant/soil drench). The mean disease incidences of the other 8 biocontrol treatments were also significantly lower (P=0.05) than the mean disease incidence of untreated plots. Although these 8 treatments showed significant disease control, the levels of disease control were not comparable to those provided by fungicide treatments. There were no significant differences in fresh weights of mature lettuce plants between treatments.

In trial 5, all procymidone treatments (2L/ha 1000L water/ha) were effective in reducing disease incidence (50-71% disease reduction). Plants treated with 1, 2 or 3 applications of procymidone (cup method), applied the first after transplanting and thereafter approximately every 2 weeks, had significantly lower disease incidence (≤3.47%) than untreated plants (7.78%). Disease control obtained with 3 applications of procymidone, applied using the banded application method (spray knapsack), was similar to that obtained with either 1, 2 or 3 applications of procymidone applied using the cup method. None of biocontrol treatments reduced disease incidence when compared to the untreated control. There were no significant differences in fresh weight of mature plants between treatments.

In trial 6, disease incidence was very low and therefore there were no significant differences in disease incidence and fresh weight between treatments.

Table 5.29 - Description of biocontrol agents antagonistic to *S. minor* and other microorganisms in commercial biological products evaluated in field trials for control of lettuce drop in Victoria.

Product	Biological agent(s)	Origin/ Manufacturer	Target pathogen(s)	Target crop	Application	Mode of action
Trichoflow™	*Trichoderma hamatum* ***6Sr4***	Lincoln University NZ Agrimm Technologies NZ	*Sclerotinia minor*	Lettuce	Seedling mix Soil drench	Competition Mycoparasite
Trich-A-Soil™	*Trichoderma viride* *Trichoderma harzianum*	Organic Crop Protectants Pty Ltd Australia	Pathogenic fungi	Turf Horticulture	Soil drench	Competition Growth promoter
TRI-D25™	*Trichoderma koningii* *Trichoderma harzianum*	JH Biotech, Inc, USA	*Sclerotinia* spp *Botrytis* spp	Horticulture	Soil drench	Competition Growth promoter
Contans™	*Coniothyrium minitans*	Prophyta, Germany	*Sclerotinia* spp	Field crops/vegetables	Soil drench	Mycoparasite
Isolate A69	*Coniothyrium minitans* A69	Lincoln University Agrimm Technologies	*Sclerotinia minor*	Lettuce	Seedling mix Soil drench	Competition Mycoparasite
Companion™	*Bacillus subtilis* GB03	Growth Products USA	*Rhizoctonia* spp *Fusarium* spp	Horticulture	Plant drench Irrigation	Growth promoter Antibiosis
Nutri-life Trichoshield™	*Trichoderma harzianum* *Trichoderma lignorum* Bacterial soil microbes	Nutri-Tech Solutions Pty	Pathogenic fungi	Horticulture	Soil drench	Soil inoculant Competition
Nutri-life 4/20™	*Trichoderma harzianum* *Trichoderma lignorum* Bacterial soil microbes	Nutri-Tech Solutions Pty	Pathogenic fungi	Horticulture	Soil drench	Soil inoculant
Isolate A	*Coniothyrium minitans*	Victorian isolate	*Sclerotinia minor*	Lettuce	Soil drench	unknown
Isolate D	*Trichoderma longipile*	Victorian isolate	*Sclerotinia minor*	Lettuce	Soil drench	unknown
Isolate B	*Coniothyrium minitans*	Victorian isolate	*Sclerotinia minor*	Lettuce	Soil drench	unknown

Table 5.32 – Effects of biocontrol and chemical treatments on disease incidence and fresh weight of Green Butter lettuce in a field trial conducted over two growing seasons at Bacchus Marsh Vic, 2001.

Treatment	Application	% of plants infected[A]		No. Sclerotia (kg dry soil)[C]	
		Autumn crop	Spring crop	Autumn crop	Spring crop
C. minitans (B Vic)	Transplant	53.82 (3.38)[B]	9.60	79.0	133.0
	Soil application	64.41 (3.45)	13.65	88.3	158.6
	Transplant + Soil application	62.67 (3.72)	18.27	49.4	125.4
C. minitans (A69)	Transplant	59.40 (4.24)	12.70	50.4	158.7
	Soil application	61.98 (3.73)	12.20	53.5	147.2
	Transplant+Soil application	63.19 (3.76)	15.05	87.2	133.9
T. hamatum (6Sr4)	Transplant	69.94 (3.64)	20.08	43.0	141.5
	Soil application	65.97 (3.73)	16.04	46.5	132.0
	Transplant+Soil application	65.10 (3.82)	16.25	72.0	119.7
C. minitans (Contans)	Transplant	66.82 (3.77)	15.44	78.4	127.3
	Soil	66.37 (3.79)	13.76	52.3	116.8
	Transplant+Soil application	64.41 (3.71)	14.10	44.0	116.8
Nutri-life 4/20™	Soil drench	51.89 (3.90)	16.00	42.1	139.6
TRI-D25™	Soil drench	58.51 (3.42)	11.95	74.1	121.6
Trich-A-Soil™	Soil drench	49.51 (4.31)	17.90	62.3	99.7
Sumisclex 1000L/ha	Spray 0, 20, 42	**7.81 (4.38)**	**7.97**	79.3	**81.7**
Sumisclex 2500L/ha	Spray 0, 20, 42	**7.47 (4.93)**	**6.25**	44.5	**64.6**
Perlka™	Soil application	57.64 (3.20)	13.06	40.7	127.3
Substrate base	Soil application	61.11 (4.29)	14.83	72.6	157.7
Untreated		70.49 (3.70)	10.60	67.4	128.2
F-test		<0.001	0.655	0.526	0.001
lsd (P=0.05)[D]		11.45	11.32	38.77	41.66

[A] The autumn-winter crop was harvested on 19/6/01 and the winter-spring crop harvested on 22/10/01. Percentage of diseased plants relative to the number of plants established after transplanting in each treatment.
[B] Values in parenthesis are the means of fresh weight of lettuce plants (n=10) (F-test 0.293). Yields were not assessed in the second crop.
[C] Sclerotial levels were assessed after harvesting each of the two lettuce crops in the same field.
[D] Differences in disease incidence larger than lsd (within columns) are significantly different (P = 0.05)

Table 5.33 – Effects of biocontrol and fungicide treatments on disease incidence and yield of green butter (trials 4 and 5) and iceberg (trial 6) lettuce in three trials conducted in Victoria, during autumn (trial 4) and spring 2002 (trials 5 and 6).

Treatment	Application method	Trial 4 (B. Marsh -Butterhead)		Trial 5 (B. Marsh - Butterhead)		Trial 6 (Werribee - Crisphead)	
		% plants infected	Fresh weight (kg)	% plants infected	Fresh weight (kg)	% plants infected	Fresh weight (kg)
Untreated	-	6.67[A]	6.60[B]	7.78[A]	6.22[B]	4.03[A]	14.64[B]
Sumisclex™ (procymidone)	Spray day 0, 14, 28, 42	**0.56 (91.6)**	6.84	-	-	-	-
Sumisclex	Spray day 0, 14, 28	**0.56 (91.6)**	6.25	**3.12**	5.77	-	-
Sumisclex (banded spray)	Spray day 0, 14, 28	-	-	**2.22**	6.60	2.50	13.16
Sumisclex	Spray day 0, 14	**1.67 (74.9)**	6.27	**3.06**	6.49	1.85	14.06
Sumisclex	Spray day 0	2.50	7.10	**3.47**	6.65	-	-
T. hamatum (6Sr4™)	Potting mix, transplant, plant/soil drench	2.79	6.48	9.31	5.39	2.18	14.06
T. hamatum (6Sr4™)	Potting mix, transplant	4.18	6.69	-	-	-	-
T. hamatum (6Sr4™)	Transplant, plant/soil drench	**1.94 (70.9)**	5.86	-	-	-	-
T. hamatum (6Sr4™)	Transplant	2.80	5.92	-	-	-	-
C. minitans (A69™)	Potting mix, transplant, plant/soil drench	2.78	6.65	7.65	5.00	-	-
C. minitans (A69™)	Potting mix, transplant	5.00	7.40	-	-	-	-
C. mintans (Contans™)	Transplant, plant/soil drench	2.53	6.64	-	-	-	-
C. mintans (Contans™)	Transplant	3.38	6.67	-	-	-	-
T. koningii/harziamum (TRI-D25™)	Transplant, plant/soil drench	3.08	6.83	9.46	5.74	-	-
T. koningii/harziamum (TRI-D25™)	Transplant	4.45	6.19	-	-	-	-
T. viride/harzianum (Trich-A-Soil™)	Transplant, plant/soil drench	3.89	6.59	9.31	6.22	-	-
T. viride/harzianum (Trich-A-Soil™)	Transplant	4.48	6.06	-	-	-	-
B. subtilis (Companion™)	Transplant, plant/soil drench	3.62	5.86	10.99	5.97	2.19	14.22
B. subtilis (Companion™)	Transplant	4.72	6.05	-	-	2.20	14.52
F-test		<0.001	0.123	<0.001	0.112	0.386	0.099
lsd (P=0.05)[C]		1.46	0.821	3.58	1.16	1.83	1.29

Only some of the treatments evaluated in trial 4 were selected for further evaluation in trials 5 and 6.

[A] Percentage of diseased plants relative to the number of plants established after transplanting in each treatment. In parentheses the treatments with greatest disease reduction (disease reduction as % of control).

[B] Means of fresh weight of lettuce plants (n=12).

[C] Differences in disease incidence larger than lsd (within columns) are significantly different (P = 0.05).

Table 5.35b – Effect of biocontrol and fungicide treatments on disease incidence and fresh weights of Butterhead (trial 7) and Iceberg (trial 8) lettuce in 2 field trials conducted during spring 2003 at Werribee Vic.

Treatment[A]	Trial 7 (butterhead)			Trial 8 (iceberg)		
	% plants infected (week 5)	% plants infected (week 10)	Disease reduction (% of control)	% plants infected (week 5)	% plants infected (week 10)	Disease reduction (% of control)
Untreated control	3.2[B]	11.5	-	4.7	13.2	-
Untreated control (water only)	1.9	5.8	**49.6**[C]	3.3	13.1	-
Sumisclex (procymidone) 1 x (2 L/ha)	0.7	4.8	**64.3**	0.0	9.9	**25.0**[C]
Sumisclex (Procymidone) 2 x (2 L/ha)	0.0	5.3	**82.6**	0.0	1.8	**86.4**
BAS 510 1 x (1.0 kg/ha)	2.1	4.7	**59.1**	0.9	8.4	**36.4**
BAS 510 2 x (0.8 kg/ha)	0.7	1.9	**78.3**	1.9	7.9	**40.2**
BAS 510 2 x (1.0 kg/ha)	0.6	2.5	**84.4**	0.9	6.9	**47.7**
BAS 510 2 x (1.6 kg/ha)	0.0	1.4	**87.8**	1.8	3.8	**71.2**
T. hamatum 6Sr4 mix, transplant	1.5	9.4	**18.3**	0.9	10.3	**21.9**
T. hamatum 6Sr4 mix, transplant, drench 1x	1.3	3.4	**70.4**	2.3	8.2	**37.9**
T. hamatum 6Sr4 mix, transplant, drench 2x	0.0	3.4	**70.4**	0.5	12.2	**7.6**
T. hamatum 6Sr4 mix, transplant, drench 1x+1xSumisclex	0.7	4.3	**62.6**	1.9	5.8	**56.1**
B. subtilis mix, transplant[A]	0.6	4.8	**58.3**	1.4	8.0	**8.3**
B. subtilis mix, transplant, drench 1x	0.0	2.0	**81.7**	0.5	7.1	**46.2**
B. subtilis mix, transplant, drench 2x	2.1	1.9	**83.5**	1.4	14.3	**37.9**
B. subtilis mix, transplant, drench 1x+1xSumisclex	0.7	3.2	**72.2**	2.8	7.7	**41.7**
F-test	0.582	0.035		0.400	0.010	
lsd (P=0.05)[D]	1.566	5.35		1.966	6.192	

[A] Treatments of *T. hamatum* 6Sr4 or *B. subtilis* GB03 were applied to the transplant mix and to seedling plugs before transplanting in the field. The seedling transplants (mix and roots) were tested for levels of *Trichoderma* (cfu/g) and total culturable bacteria (cfu/g) prior to field transplanting.

[B] Percentage of diseased plants relative to the number of plants established after transplanting in each treatment.

[C] Disease reduction expressed as % of control for final assessment (week 10).

[D] Differences in disease incidence larger than lsd (within columns) are significantly different (P = 0.05).

Ten lettuce plants were harvested per plot at week 10. Lettuce plants were too small for the market due to inadequate irrigation (water shortage at Werribee). Therefore there were no significant differences in fresh weights between treatments.

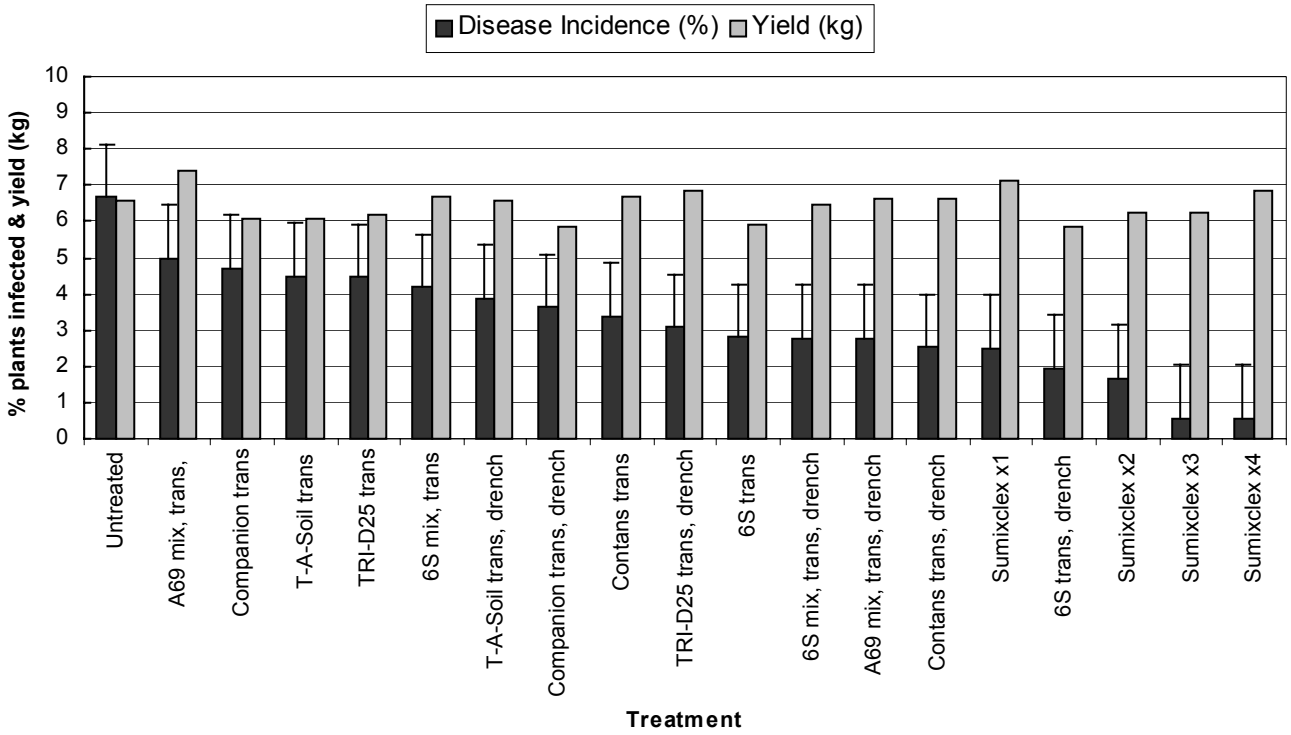

Figure 5.6 Trial 4 - Effects of biocontrol and chemical treatments on disease incidence and fresh weights of butter lettuce at Bacchus Marsh, autumn 2002. (Bars above the histograms represent the lsd at P=0.05)

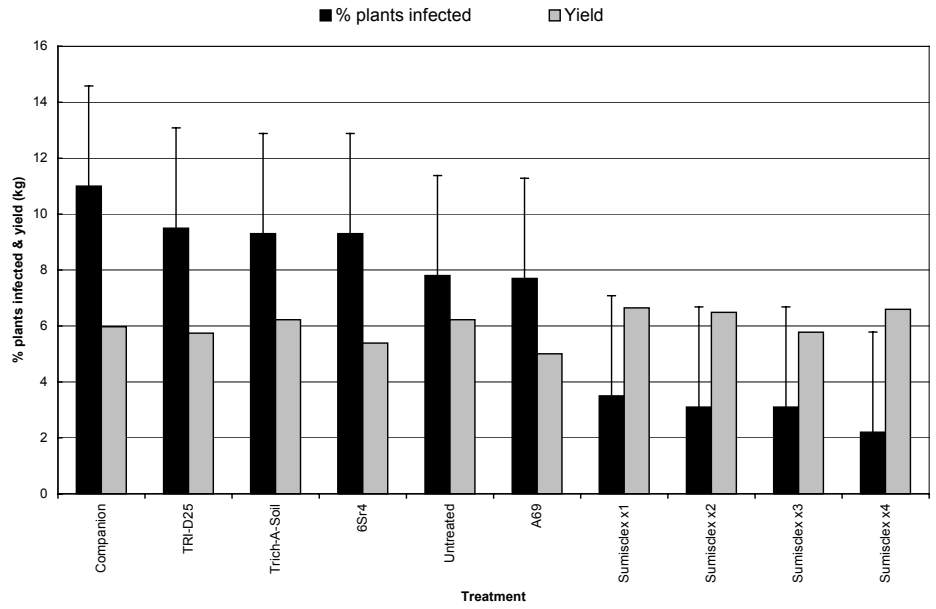

Figure 5.7 Trial 5 Effects of biocontrol and fungicide treatments on disease incidence and fresh weights of butter lettuce at Bacchus Marsh, spring 2002. (Bars above the histograms represent the lsd at P=0.05)

Trials 7 and 8

The effects of biocontrol and chemical treatments on the incidence of lettuce drop in trials 7 and 8 are summarised in Tables 5.34-5.35a and Figure 5.9. The two trials were conducted during spring/summer 2003. Levels of sclerotia of *S. minor* in the soil ranged from 27 to 49 sclerotia/kg of dry soil.

The population density of *T. hamatum* 6Sr4 in seedling transplants produced in a Kiwi peat mix (Velisha Bro™ - trial 8) averaged 3.4 x 10^4 cfu/g-^{1} mix when field transplanted. The population density of *T. hamatum* 6Sr4 in seedling transplants produced in a composted pine bark mix (HortiPine™ - trial 7) averaged 1.3 x 10^6 cfu/g-^{1} mix (Table 5.34). *Trichoderma* spp was not detected in untreated seedling transplants. The population density of total culturable bacteria in seedling transplants produced in the Kiwi peat mix averaged 6.9 x 10^6 cfu/g-^{1} mix and in the same mix treated with *B. subtilis* GB30 averaged 1.1 x 10^7 cfu/g-^{1} mix when field transplanted.The population density of total culturable bacteria in seedling transplants produced in the composted pine bark mix averaged 4.0 x 10^7 cfu/g-^{1} mix and in the same mix treated with *B. subtilis* GB30 averaged 7.5 x 10^8 cfu/g-^{1} mix.

Five weeks after transplanting in the field, disease incidence was very low in both trials and therefore there were no significant differences in disease incidences between treatments. After this period of warm and dry weather in Werribee, there was a period of 2 weeks of cool and wet weather that was conducive for Sclerotinia infection. After this wet period, disease spread from infected plants to healthy adjacent plants under wet conditions.

At harvest in trial 7 (butterhead plants), all fungicide treatments with procymidone and BAS 510 significantly reduced the percentage of plants infected (59-88% disease reduction) compared to the untreated control (11.5% disease incidence). Two sprays of procymidone (2L/ha 1000L water/ha), applied using the banded application method, were more effective than one application in reducing disease incidence (83% disease reduction). Increased rates of BAS 510 were also more effective against *S. minor* infection. Two applications of *T. hamatum* 6Sr4 to the seedling transplant before field transplanting did not reduce disease incidence when compared to the untreated control. However, 2 applications of *T. hamatum* 6Sr4 to the seedling transplant in combination with 1 or 2 soil/plant drenches of *T. hamatum* 6Sr4 to field plants at week 2 significantly reduced the percentage of plants infected by 70% when compared to the untreated control. All treatments with *B. subtilis* significantly reduced the percentage of infected plants (50-83% reduction) also compared to the untreated control. Plants of the butterhead cultivar used were less susceptible to *S. minor* infection in late season due to their less dense canopy and unright foliage, which reduced condictions for Sclerotinia infection. Under these conditions, biocontrol treatments offered some potential for early season disease control. There were no significant differences in fresh weights of mature butterhead lettuce plants between treatments. There was no significant interaction between the main treatments and sub-treatments (single or dual applications) only the effect of main fungicide or biocontrol treatments (Table 5.35a).

In trial 8 (iceberg plants), two sprays of either procymidone or BAS 510 (1.6 kg product/ha), applied using the banded application method, significantly reduced the percentage of infected plants (70-86% disease reduction) compared to the untreated control (13.2% disease incidence). There was no significant interaction between the main treatments and sub-treatments (single or dual applications) only the main effect of fungicide treatment (Table 5.35a). The greatest disease control provided by biocontrol treatments was obtained when seedling transplants and field plants were treated with *T. hamatum* 6Sr4 (3 applications) and 1 spray of procymidone (4 weeks after transplanting). These combined treatments significantly reduced the percentage of infected plants (56% disease reduction) compared to the untreated control. There were no differences in fresh weights of mature plants between treatments.

Two applications of procymidone or BAS 510, applied before the onset of the 2-week period of cool/wet weather, provided good disease control in the two trials. BAS 510 is a new fungicide, with good activity against Sclerotinia, making it suitable for use as an alternative to procymidone. Higher rates of BAS 510 tended to be more effective in reducing disease incidence in both trials. The availability of two effective fungicide products, for use in rotation to control Sclerotinia, is critical for preventing the loss of product effectiveness through overuse of a single product.

Biological treatments appeared to be less effective in providing protection against late season *S. minor* infection in crisphead (iceberg type) lettuce. One reason for this was probably the effect of plant architecture of crispheaf plants on disease development. As plants mature, leaves of crisphead lettuce grow more horizontally and are in direct contact with the soil surface. In contrast, leaves of butterhead lettuce have a more upright growth habit and plants have a less dense canopy therefore reducing the conditions favourable for infection.

Another reason could have been the low density of biocontrol agents in crisphead seedling transplants. Additional field applications of biocontrol treatments did not enhance disease control, suggesting that even if biocontrol levels

were increased by additional field applications of biocontrols, plant acquitecture was probably the main factor affecting the capacity of biocontrol agents to control disease under leaves and base of crisphead plants. The use of biological treatments in combination with fungicide treatments did not enhance disease control in the two trials. This poor response was probably due to the too early application timing (4 weeks after planting) of the fungicide spray. A later application timing at 6 or 8 weeks after transplanting may have been a more appropriate combination with early application of biological agents considering the importance plant growth habit of iceberg lettuce cultivars have on *S. minor* infection.

Table 5.34 Mean colony forming units of *Trichoderma hamatum* (6Sr4) and *B. subtilis* (GB03) in seedling transplants before transplanted in field trials 7 and 8.

Treatment	*Trichoderma*[A] (cfu/g)	Total culturable bacteria[B] (cfu/g)
Trial 8		
Kiwi peat/Vermicullite/Perlite mix	0	$6.9x10^6$
Kiwi peat/Vermicullite/Perlite+biocontrol	$3.4x10^4$	$1.1x10^7$
Trial 7		
Composted pine bark/Kiwi peat mix	0	$4.0x10^7$
Composted pine bark/Kiwi peat+biocontrol	$1.3x10^6$	$7.5x10^8$

[A] *T. hamatum* strain 6Sr4 cultured in *Trichoderma* selective medium. Treatments of *T. hamatum* 6Sr4 were applied to the transplant mix and to seedling plugs before transplanting in the field. The seedling plugs were tested for levels of *Trichoderma* (cfu/g) prior to field transplanting.
[B] Total culturable bacteria cultured in TSA medium. Treatments of *B. subtilis* GB03 were applied to the transplant mix and to seedling plugs before transplanting in the field. The seedling plugs were tested for levels of total culturable bacteria (cfu/g) prior to field transplanting.

Table 5.35a – Significance of main effects – includes the effect of adding a second fungicide spray or a single fungicide spray (dual) to biocontrol treatments on disease incidence in two field trials at Werribee Victoria, spring 2003.

	Application strategy		
Butterhead trial (trial 7)	**Single**	**Dual**	**Mean[A]**
Untreated	11.5	11.5	11.5a
Fungicide (BAS 510)	4.7	2.5	4.0b
T. hamatum 6Sr4	3.4	4.3	3.9b
B. subtilis GB03	1.9	3.2	2.6b
Mean	5.3	3.4	
[A] Significance of main treatment effects F-test 0.05 lsd (P=0.05) = 4.526. There were no significant interactions.			
Iceberg trial (trial 8)			
	Single	**Dual**	**Mean[B]**
Untreated	13.2	13.2	13.2a
Fungicide (Sumisclex)	9.9	1.8	5.9b
T. hamatum 6Sr4	12.3	5.8	9.0a
B. subtilis GB03	14.3	7.7	11.0a
Mean	10.4	7.3	
[B] Significant of main treatment effects F-test 0.032 lsd (P=0.05) = 4.762. There were no significant interactions.			

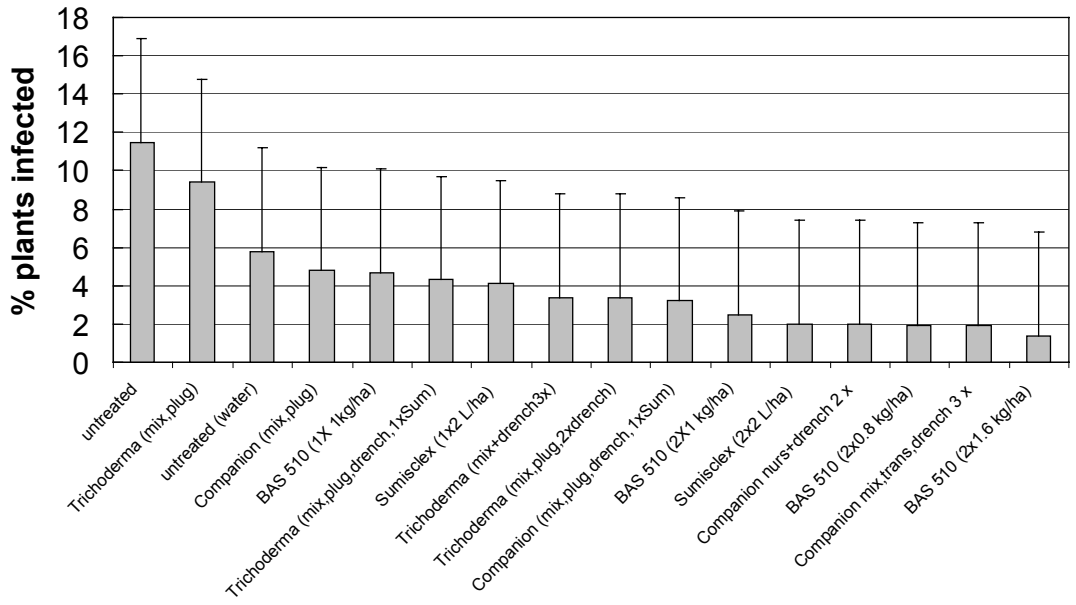

Figure 5.8– Effect of biocontrol and fungicide treatments on disease incidence of butter lettuce at Werribee, spring 2003. (Bars above the histograms represent the lsd at P=0.05)

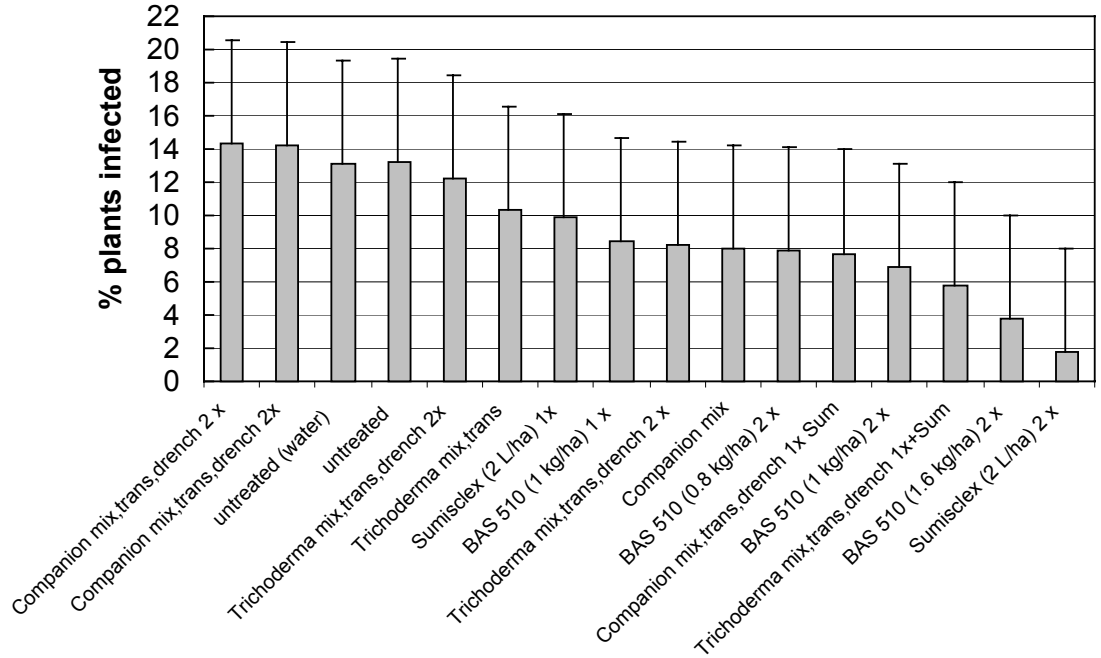

Figure 5.9 – Effect of biocontrol and fungicide treatments on disease incidence of iceberg lettuce at Werribee, spring 2003. (Bars above the histograms represent the lsd at P=0.05)

5.5 SENSITIVITY OF ISOLATES OF *S. MINOR* TO PROCYMIDONE AND EFFECT OF EXISTING AND NEW FUNGICIDES ON CONTROL OF SCLEROTINIA LETTUCE DROP.

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5.5.1 Summary

Petri dish assays were conducted to determine the sensitivity of eight isolates of *S. minor* to the fungicide procymidone. In the first 2 weeks of incubation, mycelial growth of *S. minor* was inhibited in media amended with 1 µg/ml of procymidone (lowest dosage used). After three weeks of incubation, some mycelial growth was measured on most isolate cultures amended with different dosages of procymidone. The mycelial growth in amended media was the result of fungicide breakdown in artificial media. The results indicated that all isolates of *S. minor* evaluated have not developed resistance to procymidone.

Two field trials were conducted to evaluate the effectiveness of fungicide treatments for controlling Sclerotinia lettuce drop (SLD) and Grey mold (*Botrytis* spp) on butterhead and Red Mignonette lettuce. Grey mold levels were too low in both trials to determine the full effect of fungicides on control of this disease. SLD levels were higher in butterhead lettuce than in Red Mignonette, indicating that the variety of butterhead used was very susceptible to *S. minor* infection. In the butterhead trial, results showed that three strategic (plant-targeted) and well-timed applications of procymidone (500g a.i./ha in 1000L water/ha - Sumisclex™) were very effective in controlling SLD on butterhead (80% disease reduction). The new fungicide BAS 510 (500g a.i./ha in 1000L water/ha - boscalid) was also very effective in controlling SLD (90% disease reduction). Three sprays of Bavistin™ reduced SLD by 65%. One spray of Sumisclex™, applied one day after transplanting followed by one spray of Bavistin™ and Rovral™, applied at 14 day-intervals, respectively, gave as good SLD control (80% disease control) as three sprays of Sumisclex™. This result indicated the potential for using BAS 510 and Bavistin™ or Rovral™ in alternation with Sumisclex™ for effective control of SLD.

5.5.2 Introduction

Procymidone (sold as Sumisclex™ or Fortress™) is the standard and predominant fungicide used for the control of SLD in south-eastern Australia. In recent years, lettuce growers have reported inconsistent control of SLD with procymidone. Possible reasons for inconsistent fungicide control could include 1) strains of *S. minor* have become resistant to procymidone, 2) fungicide applications are not being timed and applied properly and 3) continue use of fungicide has increased the density of microbial populations in soil that rapidly degrade the fungicide. Resistance to dicarboximide fungicides (e.g. iprodione, vinclozolin, and procymidone) has been reported in field isolates of *S. minor* from peanut in Virginia, USA (Detweiler *et al*. 1983). In New Zealand, resistance to dicarboximates was not found in field isolates of *S. minor* and *S. cepivorum* tested *in vitro* from a district with reported fungicide loss of effectiveness (A. Stewart, unpublished). However, it has been shown that *S. minor* has the capacity to develop resistance to dicarboximates *in vitro* (Hubbard *et al*. 1997). The evaluation of fungicides from different chemical groups is required to identify new fungicide treatments efficacious against *S. minor* infection for use in alternation with procymidone to prevent the loss of this product effectiveness with overuse.

Aims

Two Petri plate assays were conducted to determine the sensitivity of isolates of *S. minor* to procymidone. Two field trials were conducted to evaluate the efficacies of existing and new fungicides for the control of SLD and Gray mold (*Botrytis* spp) on lettuce.

5.5.3 Materials and methods

Sensitivity of S. minor isolates to procymidone

Eight *S. minor* isolates collected from commercial lettuce farms in Victoria were tested in Petri plate assays for their sensitivity to procymidone. The isolates, site of collection and history of procymidone use in the site of collection are listed in Table 5.36 (appendix).

Single sclerotial isolates of *S. minor* (sclerotia) were obtained from lettuce heads showing symptoms of lettuce drop and from soil samples collected from commercial lettuce farms in Werribee and Bacchus Marsh, Victoria. The locations were selected based on the history of procymidone use for disease management. Sclerotia of *S. minor* were isolated from soil using the wet-sieving method and 200 grams of soil washed through a 2 mm and 500 micron-screens. Sclerotial isolates were surfaced sterilised in a 1:1 v/v solution containing sodium hypochlorite (5%) and ethanol (90%) for 3 min and later rinsed in sterile distilled water for 3 min, and dried in a laminar flow cabinet on sterile filter paper. Single sclerotial isolates were then placed onto potato-dextrose agar (PDA) medium containing acromycin antibiotic. Eight single sclerotial cultures that produced strong and uncontaminated mycelial growth were selected for the experiments. A 8-mm diameter agar disk, containing mycelium, was cut from PDA cultures for each isolate and plated out on Petri dishes with PDA medium containing antibiotic. From these cultures, a 8-mm diameter agar disk, containing actively growing vegetative mycelium, was cut from the PDA culture of each isolate and plated out on PDA medium amended with 0, 1, 5, 10, 50 and 100µg/ml of a commercial formulation of procymidone (Sumisclex™ 500 a.i./L). All plates were incubated at room temperature (15-21ºC) and colony diameters measured weekly for 3 weeks.

The experiment was conducted twice. Colony diameter data were transformed to arsine values before analysis. Analysis of variance (ANOVA) was used to test the effects of experiment, procymidone concentration and interactions on colony growth. The appropriateness of an ANOVA for the data was checked by visual inspection of residual plots. An F-test was used to determine whether observations of the two tests could be pooled. Means of treatments for the two tests combined were compared using LSD test (P = 0.05). Analysis was conducted using Genstat (Genstat 5 Committee, 1993).

The effect fungicide treatments on control of lettuce drop

The fungicides, active ingredient, chemical group and rates of product used in the two field trials are listed in the Table 5.37 (appendix). The trial details are listed in Table 5.38 (appendix). Except for BAS 510 (boscalid™ – benzaniline), all other chemical products evaluated were commercial products.

The trial was set up in a commercial lettuce field at Bacchus Marsh, Vic. The soil was a clay loam with pH 6.8-7.0. Density of *S. minor* was determined from soil samples collected before planting using the wet-sieving method. Sclerotial levels ranged from 12 to 38 sclerotia per kilogram of dry soil. The lettuce cultivars used in the two trials were Red Mignonette (Trial 1 - cv 'Ember') and Green Butter (Trial 2 - cv 'Jerka'). There were 3 replicate plots (144 plants/plot) per treatment in each trial.

All sprays were applied using a knapsack sprayer fitted with a 40 cm boom (2 nozzles) calibrated to deliver 1000 of water L/ha. The appropriate amount of product/ha was delivered onto lettuce rows using the banded application method. The first spray was applied after transplanting, and the second and third sprays applied at 14 and 28 days after transplanting, respectively. After spraying, the trials were irrigated to drench the product into the soil and base of plants. Fertilisation, weed control and irrigation (overhead sprinklers) were conducted according to standard practices.

Plants were assessed for symptoms of lettuce drop and Botrytis every week after transplanting and a plant survival count conducted 1 week after transplanting. The percentage of infected plants in each replicate plot was calculated from the total number of plants assessed. The trial designs were RBD. Statistical analysis was performed on disease incidence and fresh weight data using ANOVA. If significant differences were observed a lsd test was used to compare the treatment means. Fresh weights were measured on 12 plants per plot.

5.5.4 Results and Discussion

Sensitivity of S. minor isolates to procymidone

The effect of different concentrations of procymidone on *S. minor* mycelial growth is presented in Table 5.39 and Figure 5.10. Little mycelial growth was observed in the amended plates in the first 2 weeks of the experiments. Therefore only colony diameter (mm) for the final assessment at week 3 is presented.

In untreated PDA medium, vegetative mycelium covered the 85-mm Petri dishes within 4 days and produced sclerotia within 2 weeks. On PDA amended with 1 µg/ml of procymidone (the lowest concentration used) all eight

isolates showed no growth at 10 days. However, after a period of 2 weeks of incubation on media amended, all cultures began growing at varying rates. In these cultures, colony morphology was irregular in appearance and colour, and sometimes individual sectors of the colony grew rather the whole colony. At week 3, 3 of the 9 isolates showed up to 50.7 mm of growth at 1 mμ/ml of procymidone. One isolate grew at least 0.8 mm at 10 mμ/ml and another one 21.3 mm at 100 mμ/ml. None of the isolates produced sclerotia in amended media. The limited and irregular growth of isolates in media amended after 10 days was probably the result of procymidone (commercial formulation) breakdown in artificial media.

Analysis of variance showed that the results from the two experiments were consistent (F-test) and therefore the results from both experiments were combined. Variation in colony diameter was explained by the concentration of procymidone, isolate type and time of assessment (analysis not shown, Figure 5.10). The interactions between isolate (limited colony growth) and fungicide concentration was significant indicating that there was a differential response of *S. minor* isolates to procymidone concentration.

Table 5.39 Mean colony diameter (mm) of *S. minor* isolates incubated in PAD medium amended with different concentrations of procymidone.

		Concentration (mμ/ml)[A]						
Isolate	**Source**	**0**	**1**	**5**	**10**	**50**	**100**	**Sclerotia[C]**
3AA	Soil	85[B]	39.2±3.7	3.7±1.5	0.8±0.6	0	0	No
4FR	Butterhead	85	22.8±5.7	0	0	0	0	No
2AA	Soil	85	22.8±5.7	0	0	0	0	No
5SV	Soil	85	36.3±7.1	0	0	0	0	No
1AA	Crisphead	85	46.5±9.3	1.0±1.0	0	0	0	No
7SV	Soil	85	40.0±1.5	0	0	0	0	No
6SV	Crisphead	85	50.7±19.8	0	0	0	0	No
8FR	Soil	85	30.2±10.0	13.3±3.5	37.5±1.5	21.8±3.8	21.3±3.8	No

[A] Mycelial growth was recorded weekly for 3 weeks. Very little growth was recorded in amended plates in the first 2 weeks thus values presented are colony diameter (mm) for final assessment (3 weeks).

[B] Means of two tests were combined based on F-test. (± = sem). Each test had three replicate plates for each fungicide dosage treatment. Data was transformed to arsine values to obtain a constant variance with dose, time of assessment and isolate as factors. There were significant (F-test <0.001) differences within isolates (lsd = 2.1), doses (lsd = 4.8) and time of assessment. There were also significant interactions (F-test <0.001) between dose x isolate (lsd = 6.5), dose x time of assessment, isolate x time of assessment, and isolate x dose x time of assessment.

[C] Production of sclerotia in plates amended with procymidone. On unamended PDA medium, all isolates reached the edge of Petri plates within 4-5 days and produced sclerotia in 2 weeks.

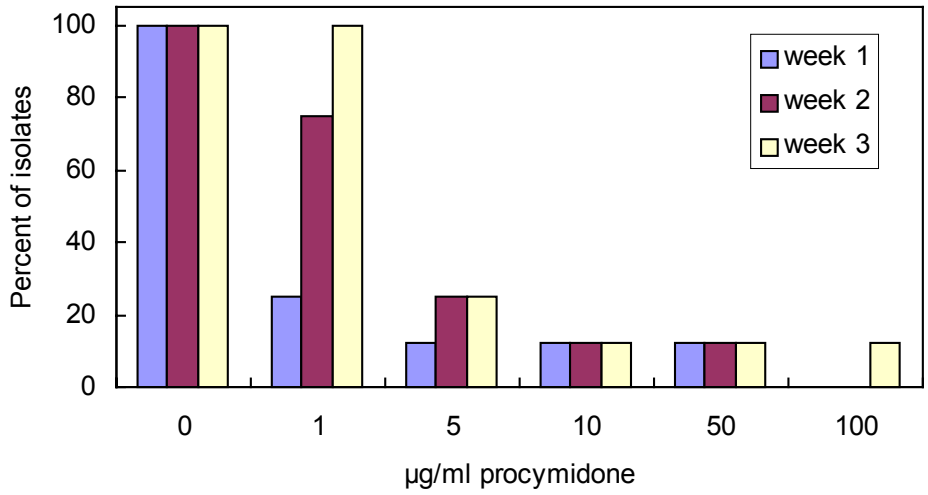

Figure 5.10 Percentage of 8 isolates of *S. minor* showing at least 2 mm of growth on PDA (potato-dextroxe agar) amended with different concentrations of procymidone. Growth was measure weekly for 3 weeks.

The effect of fungicide treatments on control of Sclerotinia and Botrytis

Sclerotinia lettuce drop incidence was higher in the trial planted with butterhead lettuce than in the trial planted with Red Mignonette lettuce. The percentage of plants infected by *S. minor* in the butterhead trial ranged from 1.6 to 18.1% and in the Red Mignonette trial ranged from 1.4 to 4.8% at harvest approximately 12 weeks after planting. The percentage of plants infected by *Botrytis* spp was below 2.5% in the two trials.

There were significant differences in the percentage of plants infected by *S. minor* between treatments at harvest in both trials (P = 0.05). In the butterhead trial, three sprays, applied the first after transplanting and thereafter every 2 weeks, using 500g a.i./ha and 1000L/water/ha, in five out of seven treatments significantly reduced the percentage of plants infected compared to the untreated control. Three of these five treatments (Sumisclex™, Bavistin™, BAS 510™) and the treatment with three fungicides used in alternation (Sumisclex, Bavistin, Rovral) caused the greatest levels of disease control (80-91% disease reductions). BAS 510™ provided the best disease control (90% disease reduction). In the Red Mignonette trial, three Sumisclex™ sprays caused the greatest level of disease control (60% disease reduction). Although Gray mold (*Botrytis* spp) levels were low in the butterhead trial, there were significant differences in the percentage of plants infected by *Botrytis* spp between treatments at harvest (P = 0.05). Three spray applications with either BAS 510™ or Scala™ caused the greatest level of disease control. There were no significant differences in disease incidence between treatments in the trial with Red Mignonette. The effects of fungicide treatments on the combined disease incidences of both Sclerotinia lettuce drop and Grey mold and fresh weights in the two trials are shown in Tables 5.41 and Figure 5.11. When disease incidence data for both diseases were combined, there were also significant differences in the percentage of plants infected by both *S. minor* and *Botrytis spp* between treatments in both trials (P = 0.05). The levels of disease reduction and treatments rankings were similar to those estimated for lettuce drop control. There were no significant differences in the fresh weights of plants between treatments in the two trials.

Strategic (plant-targeted) and well-timed applications of Sumisclex™ (500g a.i./ha or 1L/ha in 1000L water/ha) were very effective in the control of Sclerotinia lettuce drop and Grey mold on butterhead and Red Mignonette lettuce. Bavistin™ and BAS 510™, also evaluated at a rate of 500 g a.i./ha in 1000L water/ha, were also very effective in controlling both diseases when compared to Sumisclex™. Other fungicide treatments evaluated in the two trials were less effective on disease control. Results also showed that one spray of Sumisclex™ applied after transplanting, followed by one spray of Bavistin™ and Rovral™ applied at 14 day intervals, respectively, gave as good disease control as three sprays of Sumisclex™. These results showed the potential for using Bavistin™ and Rovral or BAS 510 in alternation with Sumisclex™ for effective and consistent disease control, thereby reducing the number of procymidone (Sumisclex™) applications required per crop/season.

Table 5.40 Effect of fungicide treatments on the incidence of Sclerotinia lettuce drop and Gray mold (*Botrytis* spp) in two trials with different lettuce cultivars during spring 2002, Bacchus Marsh Vic.

	Trial 1 Green Butterhead		Trial 2 Red Mignonette	
	Sclerotinia	Botrytis	Sclerotinia	Botrytis
Treatment	**% plants infected**	**% of plants infected**	**% plants infected**	**% of plants infected**
	18.1	2.55	3.47	0.69
Untreated				
Sumisclex	3.49	0.69	1.39	0
Bavistin	6.08	0.46	2.78	0.23
Rovral	12.1	0.93	4.86	0.69
Teldor	9.96	0.69	3.24	0.46
BAS 510	1.62	0.23	2.31	0
Scala	13.2	0	2.78	0.46
Sumisclex, Bavistin, Rovral	3.71	0.46	2.78	0.46
F-test	<0.001	0.009	0.042	0.705
lsd (P = 0.05)	5.5	2.2	1.83	ns

Table 5.41 Effects of fungicide treatments on the incidence of *Sclerotinia* lettuce drop and Grey mold and fresh weights in two trials with two lettuce cultivars during spring 2002, Bacchus Marsh Vic.

	Trial 1 Green Butterhead			Trial 2 Red Mignonette		
Treatment	**% plants infected**	**Fresh weight (kg)**	**Disease reduction (% of control)**	**% plants infected**	**Fresh weight (kg)**	**Disease reduction (% of control)**
Untreated	20.6	6.38[A]	-	4.29	4.65	-
Sumisclex	4.17	6.27	**79.8**	1.40	3.95	**67.4**
Bavistin	6.55	6.45	**68.2**	3.02	3.51	**29.6**
Rovral	13.1	7.28	**36.4**	5.58	4.33	**-**
Teldor	10.6	5.20	**48.5**	3.71	3.94	**13.5**
BAS 510	1.85	7.84	**91.0**	2.31	4.29	**46.2**
Scala	13.2	6.56	**35.9**	3.25	3.81	**24.2**
Alternation*	3.94	7.42	**80.8**	3.25	4.11	**24.2**
F-test	<0.001	0.713		0.01	0.889	
lsd (P = 0.05)	5.6	ns		1.9	ns	

[A] Fresh weights of 12 lettuce plants harvested from each plot.
*Sumisclex, Bavistin, Rovral

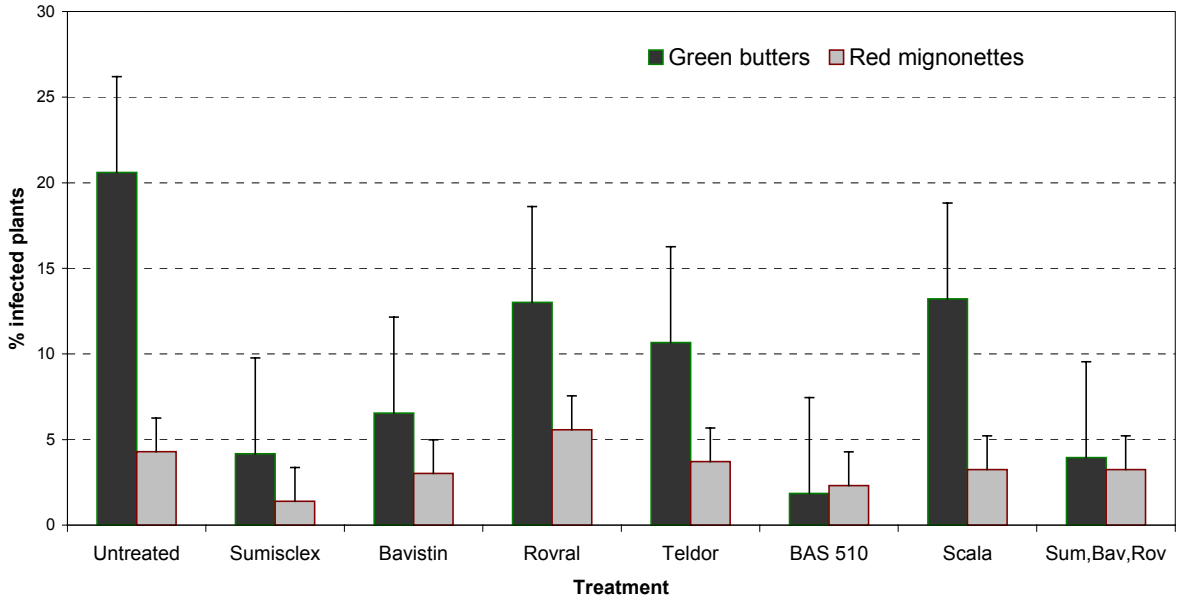

Figure 5.11 - Effects of fungicide treatments on the combined incidences of Sclerotinia lettuce drop and Botrytis (grey mold) in two trials with Green Butterhead and Red Mignonette lettuce cultivars during spring 2002, Bacchus Marsh Vic. (Bars above the histograms represent the lsd at P =0.05)

5.6 The effect of soil amendment and biocontrol treatments on reduction of *S. minor* inoculum and control of Sclerotinia lettuce drop

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5.6.1 Abstract

Three field trials were conducted to evaluate the potential of different chemical and organic soil amendments and biocontrol treatments for reducing the number of sclerotia of *S. minor* in soil and Sclerotinia lettuce drop.

Despite the high levels of sclerotia in the soil, disease levels were low in the two trials that evaluated pre-planting treatments due to dry weather in autumn and spring 2002. As a consequence, there were no significant differences in disease levels between treatments. The trials showed that two of the nitrogenous amendment treatments evaluated (Urea™ and Perlka™) were toxic to lettuce plants when applied at high rates (500 kg/ha) 7-14 days prior to planting a lettuce crop. The trials also showed that fresh weights of plants from plots treated with certified green compost (raked or mulched) were significantly higher than fresh weights of plants in untreated plots and those treated with nitrogenous treatments. Bacterial pathogens (e.g. *E. coli* and coliforms) were not detected on leaves of plants in plots treated with certified green compost but were detected at low levels in irrigation river water.

In the third trial (soil treatments applied 3 months before transplanting), cold pressed mustard meal, urea and blood and bone meal fertiliser were found to be effective in reducing the viability of sclerotia of *S. minor* in soil when used at high rates/ha. These three materials contain or can convert into toxic (HNO^2-, $NH4^+$, ITCs – mustard meal) compounds, which behave like soil fumigants. Blood and bone meal fertiliser, used at 20 t/ha, gave the highest reduction in sclerotial viability. Unfortunately, disease levels at harvest were also too low in this trial to determine the full effect of nitrogenous and biofumigation treatments on disease control. More research is required with soil amendments to optimise their potential for reducing sclerotial inoculum and controlling of Sclerotinia diseases.

5.6.2 Introduction

Soil treatments such as solarisation and chemical fumigation can be effective in reducing inoculum levels and control diseases caused by *S. minor* and *S. sclerotiorum* on lettuce (Porter and Merriman 1985). However, soil fumigation is considered too expensive and solarisation is impractical because it can only be used for 2-3 months each year. Many studies have shown that chemical and organic soil amendments such as nitrogenous fertilisers, animal manures and plant residues (biofumigation), and biocontrol agents (eg. *Coniothyrium minitans*) have the potential to reduce populations of soilborne pathogens, including *Verticillium dahliae*, *Pythium ultimum*, *Sclerotium rolfsii*, *S. minor* and *Fusarium oxysporum* (Gamliel and Stapleton 1993, Lazarovits *et al.* 1999, Luth 2001, Nico et al. 2003). The research overseas has shown the potential of soil amendments for reducing inoculum in soil and therefore disease pressure. A reduction in disease presssure could result in reduced use and expenses of fungicides for Sclerotinia control, ensuring that fungicides are only used when required.

Aim

Three field trials were conducted to evaluate the effect of different chemical and organic soil amendments and biocontrol treatments on reduction of sclerotia of *S. minor* in soil and control of Sclerotinia lettuce drop. The treatments were evaluated for their capacity to kill sclerotia in soil and suppress infection when applied as pre-planting soil applications.

5.6.3 Materials and Methods

Details of trials are described in table 5.42 (appendix). The soil amendment materials evaluated were selected based on their availability and total content of nitrogen. The materials (fertilisers) evaluated were urea (49% total nitrogen), calcium cyanamide (19% total nitrogen), blood and bone meal (7.8% total nitrogen) and fish emulsion (8% total nitrogen). These nitrogenous materials were compared to the organic amendments chicken manure (<3% total nitrogen), certified green compost (1.6%) and cold pressed defatted mustard meal (biofumigation). The biocontrol products Contans™ (*C. minitans*) and Trich-A-Soil™ (*T. harziamum and T. koningii*) were also evaluated in the trials for their capacity to kill sclerotia in soil and suppress disease.

Effect of pre-planting treatments on disease incidence (trials 1 and 2)

The materials and treatments evaluated in trials 1 and 2 are listed in Tables 5.43 and 5.44 (appendix). The two trials were set up in fields used for commercial lettuce production at Bacchus Marsh, Victoria. Before applying the soil treatments, the levels of sclerotia of *S. minor* in soil were determined using the wet-sieving method. Several soil samples were collected at random from the entire field, including all untreated plots, using a soil core sampler to depths of 10 and 15 cm. Four soil sub-samples were collected from each plot area (9m x 1.5m). The soil sub-samples were mixed and a 200g sub-sample was used for sclerotial counts. Viability of sclerotia was determined in PDA medium containing antibiotic. The materials were broadcasted onto the soil surface, and then mixed well with a rake into the top-soil to a depth of 5-10 cm. The treatments were applied 7 and 14 days before lettuce transplanting in trials 1 and 2, respectively. The trial designs were randomised complete blocks with four replicates. Biocontrol treatments (Trich-A-Soil™) were applied to seedling transplants (drench) to saturation point 24 hrs prior to transplanting. All fungicide sprays (procymidone) were applied using a knapsack sprayer fitted with a 0.4 m boom (2 nozzles) calibrated to deliver 1000 L of water/ha. The appropriate amount of product/ha was delivered onto lettuce rows using a banded application method. The first spray was applied after transplanting and the second spray 14 days later. The trial site was managed as a commercial lettuce crop.

Effect of soil treatments on inoculum reduction (trial 3)

The materials and treatments evaluated are listed in Table 5.45 (appendix). The trial was set up in a field used for commercial lettuce production at Bacchus Marsh, Victoria. The nitrogenous and organic materials were broadcasted onto the soil surface, and then mixed well with a rake into the top-soil to a depth of 5-10 cm. The biocontrol treatment Contans™ (*C. minitans*), a mycoparasite of *S. minor*, was applied to soil as a drench (2 kg/ha in 2000L water/ha) and later raked into soil. The treatments were applied three months before lettuce planting. The trial design was a randomised complete block with four replicates. Soil pH was measured at monthly intervals after treatment application to determine the effect of nitrogenous products on soil pH. Four soil sub-samples were taken at 5 and 10 cm depths from each plot, mixed well and a sub-sample of this (50 g) used to measure soil pH. Two weeks before planting, the levels of sclerotia of *S. minor* were determined using the wet-sieving method. Four soil sub-samples were collected from each replicate plot, mixed well and from this composite sample 200 g of soil sub-sample used for sclerotial counts. Soil samples were taken with a soil core sampler to depths of 10 and 15 cm. Viability of sclerotia was determined in PDA medium containing antibiotic. Fungicide sprays were applied as described previously. The trial site was managed as a commercial lettuce crop.

Population density of microorganisms

In trial 2, plants in plots treated with certified organic green compost were tested for the presence of soil/water human pathogenic bacteria. Serial dilutions and selective medium (Petrifilm 3M) were used to determine population densities of *E. coli*, faecal coliforms and total culturable bacteria. Lettuce plants were randomly selected in each plot and 4 leaves removed from outside and inside of each plant. Leaves were shredded and stomached for 2 min in peptone agar. The population density of bacteria on leaves of untreated plants was compared to the population density of bacteria on leaves of plants treated with compost. River and irrigation water was also tested. Samples were diluted (10-fold dilutions) and plated onto Petrifilm total aerobic bacteria count plates (3M) and Petrifilm *E. coli* plates (3M) and incubated at 37ºC for 48 hrs. The population density of total culturable aerobic bacteria was determined by counting the red bacterial colonies (cfu/g) on the surface of the Petrifilm. *E. coli* was identified by counting the blue colonies with an adjacent gas bubble. Faecal coliforms were identified by counting red colonies with an adjacent gas bubble.

In trial 3, 10-fold serial dilutions and selective medium were used to determine the population density of total filamentous fungi (MEA - malt agar medium) and total culturable bacteria (TSA) in soil treated with the different soil amendments. Soil samples were collected 5 and 60 days after application of treatments. Four soil sub-samples were collected from each plot (n=4) at a depth of 5 and 10 cm, mixed well and 10 g of the pooled soil sample used for serial dilutions.

Trial management and disease assessment

Growers applied their regular commercial pesticide spray program for control of insect and foliar diseases (Botrytis and Anthracnose). Irrigation (overhead sprinklers) was applied after application of soil treatments and Sumisclex sprays. Plant survival was recorded 1 week after transplanting and thereafter disease incidence recorded every week. Before commercial harvest, the percentage of infected plants in each replicate plot was calculated from the total

number of plants assessed. At harvest, fresh weights were measured on 12 plants per plot. Statistical analysis was conducted on disease and fresh weights data using ANOVA. If significant differences were observed a lsd test was used to compare the treatment means.

5.6.4 Results and discussion

The effect of pre-planting soil treatments on disease incidence (trials 1 and 2)

In both trials, *S. minor* levels in soil ranged from 10 to 80 sclerotia/kg dry soil (means 36 and 38). Despite the high levels of sclerotia measured in soil, disease levels were low in both trials conducted during two relatively dry growing seasons in autumn and spring 2002. As a consequence, there were no significant differences in disease levels between treatments (Table 5.46). In trial 2, although not significant, the fungicide (Sumisclex™) treatment had the lowest disease incidence at harvest. In trial 1, the urea treatment, applied 7 days prior to lettuce transplanting, had the lowest percentage of infected plants compared to the untreated control. The urea treatment, however, affected lettuce growth (stunted plants) in both trials when applied 7 and 14 days before planting. As a result, plant fresh weights were significantly lower than those measured in fungicide and soil amendment treatments (trial 1) or untreated and soil amendment treatments (trial 2). Similarly in both trials, plants in plots treated with Perlka™ were affected (stunted and small) by this treatment early in the season. However, after this period, plants recovered and fresh weights at harvest were no different to those measured in untreated and other treatments.

In both trials, plants in the plots treated with certified green compost (raked and mulch) had the highest plant weights at harvest (Table 5.46). In trial 1, fresh weights of plants treated with certified green compost (mulch) were significantly higher than fresh weights of plants in the untreated plots. In trial 2, fresh weights of plants treated with mulch and biocontrol treatments were also significantly higher than the fresh weights of plants in untreated and nitrogenous treatments. Certified green compost contains low levels of nitrogen (<1.6% of total nitrogen), which is in a form of nitrogen readily available for plant uptake. This compost also contains a range of other micronutrients and high levels (>1.3 x 10^7) of total culturable bacteria (data not shown) that apparently contributed to improved plant growth. Unfortunately, the batches of compost used in the two trials also contained small pieces of plastic and broken glass which rendered the compost unsuitable for commercial lettuce production.

The levels of bacteria measured in leaves collected from plots untreated and treated with certified green compost are summarised in Table 5.47. *E. Coli* was not detected in lettuce leaves but it was detected at very low levels in irrigation and river water. Levels of faecal coliform bacteria detected in all samples were well below the allowable detectable limits. The coliforms detected were most likely not indicators of faecal contamination but natural soil coliforms. Although not significant, populations density (cfu/g counts) of total culturable bacteria was higher in irrigation water samples than on lettuce leaves (unwashed).

Table 5.46 Effects of pre-planting applications of high nitrogen fertilisers, organic soil amendments and biocontrol treatments on disease incidence and fresh weight of plants in two field trials at Bacchus Marsh, 2002.

	Trial 1 (autumn/winter)			Trial 2 (winter/spring)	
Treatment	**% of plants infected**	**FW (kg)**	**Treatment**	**% of plants infected**	**FW (kg)**
Untreated	4.7	5.99	Untreated	5.4	5.9
Compost™ + Trich-A-Soil™	3.5	7.42	Compost™ + Trich-A-Soil™	5.1	6.7
Trich-A-Soil ™	2.4	6.73	Compost™ (raked)	4.3	5.9
Urea™	1.4	4.83	Urea™	4.7	5.2
Fish emulsion™	3.3	6.54	$CaSo^4$ Mycrogyp™	3.3	6.6
Compost™ (Mulch)	2.4	7.47	Compost™ (Mulch)	3.3	7.4
Pulverised mustard meal™	2.0	6.57	Pulverised mustard meal™	3.5	6.1
Perlka™	2.1	6.17	Perlka™	3.1	6.2
Sumisclex™ 500F	2.6	6.36	Sumisclex™ 500F	1.4	5.4
F-test	0.079	0.004		0.121	<0.001
lsd (P = 0.05)	2.07	1.25		2.66	0.74

Table 5.47 Population density of culturable bacteria detected on lettuce leaves collected from plots untreated and treated with certified green compost.

Treatment	*E. coli* (cfu 100ml or 100g)	Faecal coliforms (cfu/g)	Total aerobic bacteria (cfu/g)
Leaves from untreated plots	0	<7.7	2.6×10^5
Leaves from plots treated with compost	0	<38.5	5.6×10^5
Lettuce leaves grower's crop	0	1.0×10^4	1.0 to 10^7
Dam & river water	10^2/100 mls	1.0×10^3	1.0×10^6
Irrigation water	10^1/100 mls	1.0×10^4	1.0×10^7

The effect of soil treatments on S. minor inoculum (trial 3)

There were no significant differences in the number of sclerotia/kg of dry soil between treatments (Table 3.48, Figure 5.12). However, there were significant differences in the viability of sclerotia between treatments. The application of nitrogenous treatments to soil, applied 3 months before soil sampling, significantly reduced the proportion of viable sclerotia that germinated on PDA medium compared to the untreated control. Blood and bone meal fertiliser, used at 20 t/ha, gave the highest reduction in sclerotial viability.

Measurements of soil pH indicated that nitrogenous amendment treatments (e.g. urea and blood and bone meal) affected the ability of sclerotia of *S. minor* to survive in soil due to the accumulation and persistence of toxic levels of nitrogen forms (e.g. ammonium, nitrate) in soil for several weeks (Figure 5.13). Based on an ANOVA analysis, there was a significant effect of high-nitrogen amendments on soil pH over time as determined by lsd for each time of assessments and interaction of means (P = 0.05). Blood and bone meal and urea significantly increased soil pH from 7 to 8 during the first 15 days after application compared to the untreated control. The soil pH in plots treated with blood and bone fertiliser remained above 8 (possibly due to NH4+) for three months until it declined and was not significantly different from that in the untreated soil prior to planting. Soil pH in plots treated with urea decreased from 8 to 6.8 after 2 weeks and this treatment was significantly different to soil pH in untreated plots. Thereafter the soil pH in plots treated with urea and poultry manure were significantly lower (more acidic) than the soil pH in untreated soil for the two months prior to planting (possibly due to accumulation of nitrate or nitrous acid). Soil pH in plots treated with nitrogenous materials returned to levels comparable to pH in untreated soil three months after treatment application and after soil was rotary hoed two months after treatment application.

Analysis of microbial communities in soil showed that nitrogenous and poultry manure amendment treatments significantly reduced (P=0.05) the population density of filamentous total fungi from 3.6 x 10^4 to ≤8.8 x 10^3 cfu g-^{1} of dry soil at 5 days after application compared to an untreated control (Table 5.49). In contrast, nitrogenous and poultry manure treatments significantly increased the population density of total culturable bacteria from 3.2 x 10^7 to ≥6.6 x 10^7 cfu g-^{1} dry soil at 5 days after application compared to an untreated control. Population densities of both total fungi and bacteria in plots treated with urea and poultry manure returned to levels comparable to those in untreated soil at 60 days after treatment application. However, population density of total fungi in plots treated with blood and bone meal fertiliser was significantly lower than all other treatments at 60 days after treatment application. In contrast, the levels of total culturable bacteria in plots treated with blood and bone meal fertiliser was significantly higher than all other treatments at 60 days after application. The experimental rates of nitrogenous materials evaluated were probably too high for field use. More research is required to determine the mode of action and mechanisms used by nitrogenous products to kill sclerotia of *S. minor* in the soil so that only optimum rates of product/ha are used with minimum enviromental damage.

The application of pulverised mustard meal (biofumigation) and poultry manure treatments into soil, 3 months before soil sampling, also significantly reduced the viability of sclerotia when compared to the untreated control. Mustard meal, which was defatted and cold pressed meal, was probably effective reducing *S. minor* viability because it contains concentrated levels of ITCs which were toxic to sclerotia (R. Harding, SARDI, pers. comm.). Poultry manure was probably effective in reducing *S. minor* viability due to its nitrogen content and high mcrobial carrying capacity. The reduction levels on sclerotial viability observed with mustard meal and poultry manure suggest that very high levels of mustard meal (ITCs) and poultry manure would be required per hectare for effective eradication of sclerotia of *S. minor* in soil. *Coniothyrium minitans* (Contans™), a mycoparasite of *S. minor*, used at 2 kg/ha did not reduce the number or viability of *S. minor* sclerotia compared to the untreated control. In a pot trial in Tasmania, however, application of the same isolate of *C. minitans* into soil at a higher rates (10 kg/ha) and 6 months prior to planting, resulted in a significant reduction in the percentage of plants infected by *S. minor* compared to untreated soil (section 6.4).

Although disease levels were low at harvest, there were significant differences in the percentage of infected plants between treatments. Two sprays of Sumisclex (standard grower practice), applied to plots treated with poultry manure, and the biocontrol treatment provided the greatest reduction in disease incidence. The application of blood and bone meal fertiliser and pulverised mustard meal to soil also reduced the percentage of plants infected at harvest compared to the untreated control. There were no significant differences in plant fresh weights between treatments indicating that high-nitrogen and other amendment treatments, applied 3 months prior to planting, had no negative effects on lettuce growth.

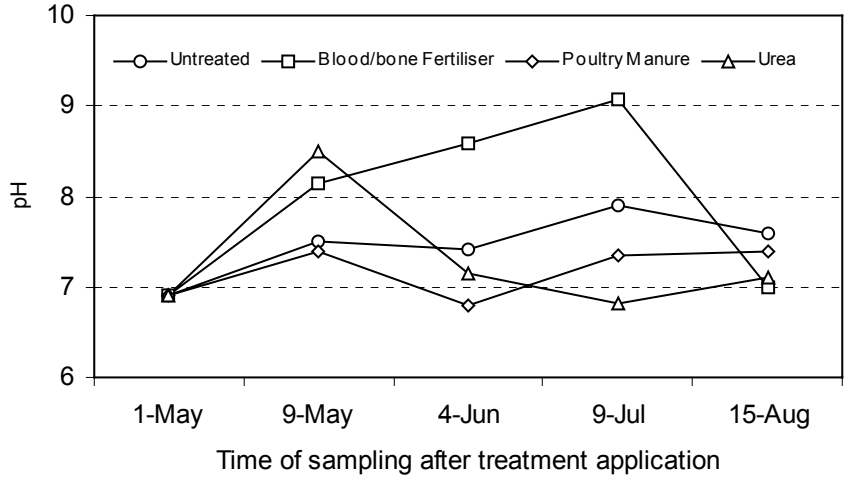

Figure 5.13 Effects of application of nitrogen fertilisers and chicken manure amendment treatments to soil 3 months before (fallow) a lettuce crop on changes in soil pH over time. Each data point is the mean of 4 replicate plots. ANOVA analysis significant at P = 0.05 for comparison of means within each assessment date (lsd 1 May = 0; 9 May = 0.23; 4 June = 0.30; 9 July = 0.45; 15 Aug = 0.42) and for interactions (treatment x assessment date).

Table 5.48 Effect of applications of nitrogenous fertilisers, mustard meal and chicken manure amendments and biocontrol (mycoparasite of *S. minor*) treatments into soil 3 months before a lettuce crop on density and viability of *S. minor* sclerotia, disease incidence and fresh weight in a field trial at Bacchus Marsh, winter-spring 2002.

Treatment	Density of Sclerotia (no./kg dry soil)	Proportion of viable sclerotia	% of infected plants	FW(kg)
Untreated	25	0.90	5.22	7.45
Blood & Bone meal™	30	0.36	2.26	8.47
Poultry manure & Sumisclex™	21	0.53	1.74	8.93
Urea™	35	0.53	3.83	7.69
Pulverised mustard meal™	25	0.47	2.78	8.39
C. minitans (Contans™)	34	0.64	1.56	7.78
F-test	0.398	0.023	0.011	0.557
lsd (P = 0.05)	14.9	0.30	1.99	1.89

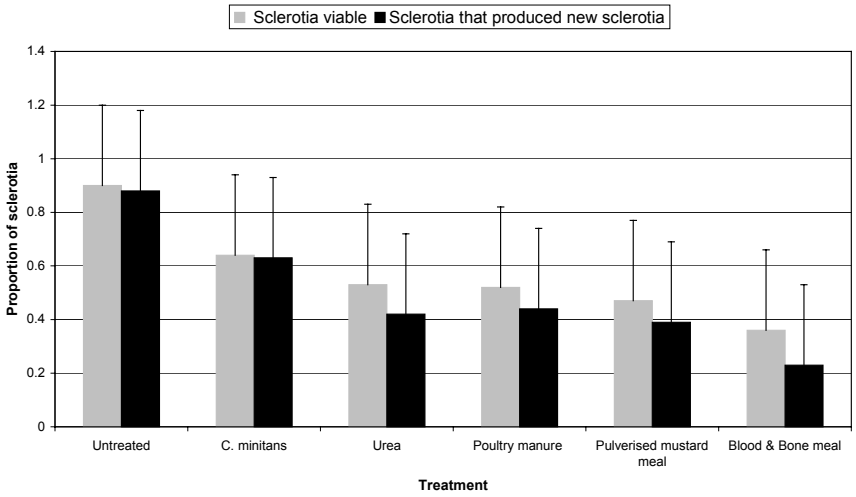

Figure 5.13 Effects of applications of nitrogenous fertilisers, mustard meal and chicken manure amendments and biocontrol (mycoparasite of *S. minor*) treatments into soil 3 months before a lettuce crop on the proportion of *S. minor* sclerotia viable in a field trial at Bacchus Marsh, winter-spring 2002. (Bars above the histograms represent lsd at P=0.05)

Table 5.49 Effects of nitrogenous soil amendments on changes in microbial levels over time in soil in a field trial at Bacchus Marsh, winter-spring 2002.

Treatment	Total fungi 5 days (cfu g^{-1})	Total fungi 60 days (cfu/g^{-1})	Total bacteria 5 days (cfu/g^{-1})	Total bacteria 60 days (cfu/g^{-1})
Untreated	$3.6x10^{4A}$	$3.5x10^{5}$	$3.2x10^{7}$	$7.3x10^{7}$
Blood & Bone meal™	$3.9x10^{3}$	$5.7x10^{4}$	$7.7x10^{7}$	$5.5x10^{8}$
Poultry manure	$8.8x10^{3}$	$4.9x10^{5}$	$8.1x10^{7}$	$3.7x10^{7}$
Urea™	$6.6x10^{3}$	$3.9x10^{5}$	$6.6x10^{7}$	$6.9x10^{7}$
F-test	<0.001	0.005	<0.001	<0.001
lsd (P= 0.05)	$8.1x10^{3}$	$1.9x10^{5}$	$2.7x10^{7}$	$1.13x10^{8}$

[A] Means (n=4) are cfu/g of soil samples collected 5 and 60 days after application of treatments. Trial area was left fallow for 3 months after application of treatments. Comparisons of means between assessment dates for each functional microbial group and interactions were significant at P = 0.05.

TECHNICAL REPORT PART B – Evaluation of biocontrol, chemical and cultural strategies for Sclerotinia control in Tasmania

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Serve-Ag Research

6.1 Summary (Tasmanian Trials)

Trial studies were conducted in Tasmania as part of the HAL project VG00048, "Developments of biological controls for *Sclerotinia* diseases of horticultural crops in Australia". The studies examined and identified a range of new options that could be used in an integrated *Sclerotinia* disease management strategy, incorporing biological, cultural, and chemical methods. The underlying strategies were aimed at detecting, eradicating and reducing pathogen levels in the soil, suppressing the pathogen, reducing plant susceptibility, and preventing fungal invasion. Almost all trials in Tasmania, were conducted within commercial lettuce crops, which are highly susceptible to *Sclerotinia* and have a relatively short growing period, enabling consecutive trials in the same growing season. The research conducted in Tasmania can be divided into six main topics, and the key findings are summarized below.

Fungicide control
Field trials conducted in Tasmania showed that the fungicide procymidone (sold as Sumisclex and Fortress) gave consistent and effective control of *Sclerotinia* disease caused by *S. minor* and *S. sclerotiorum,* under high disease pressure. Procymidone is usually the fungicide of choice and is frequently the only fungicide used for *Sclerotinia* disease control in many crops in Tasmania.

Boscalid (BAS510-01F), a new class of fungicide, was shown to be highly effective against *S. sclerotiorum* and *S. minor* on bean, lettuce and pyrethrum crops in Tasmania. Under conditions that are highly favourable to *Sclerotinia* disease, the consistency and efficacy of Boscalid were similar to procymidone. This makes it a suitable alternative to procymidone for *Sclerotinia* control in a range of horticultural crops. Procymidone is a dicarboximide fungicide, while Boscalid belongs to benzanilide chemical group.

Effective fungicide application methods are essential for good disease control, with appropriate spray volume and timing in different types of horticultural crops.

Reducing plant susceptibility
In trials conducted in Tasmania, two products, Agri-Fos (phosphorus acid) and MicroGyp (natural gypsum or calcium sulphate), consistently improved disease control when applied in combination with Sumisclex. Sumisclex plus Agri-Fos or MicroGyp were shown to further reduce the percentage of diseased plants by 1% to 5%, when compared to Sumisclex alone.

Unlike Sumisclex, Agri-Fos and MicroGyp have no direct activities against *Sclerotinia*, but may have reduced infection indirectly by increasing the plant's natural defence system and reducing plant susceptibility to fungal infection. These two materials were not as effective as Sumisclex when applied on their own.

Although the additional level of improvement in disease control by Sumisclex plus Agri-Fos or MicroGyp are considered to be relatively small, ranging from 1% to 5% compared to Sumisclex alone, the low cost of the latter products could still make it cost effective.

Agri-Fos and MicroGyp are fertilizers, which do not leave any chemical residues on treated plants. These findings indicate the importance of plant health in combating plant diseases. Plants have their own mechanisms for preventing fungal invasion, and an unhealthy crop with nutrient deficiencies is usually more susceptible to disease than a healthy crop.

Biocontrol agents
Contans, based on *C. minitans* biocontrol fungus, was identified as the most promising potential biocontrol agent for *S. sclerotiorum* and *S. minor* control in three initial biocontrol trials conducted in Tasmania. Under low disease pressure, Contans could provide early disease control when applied as a pre-plant soil treatment or post-plant spray applications.

With other biocontrol products that were evaluated in Tasmania, they were less effective and there were inconsistencies in their levels of disease control under different conditions, indicating that further improvements in their performance are required before commercial use can be recommended.

With many biocontrol agents, including Contans, their efficacy for prolonged *Sclerotinia* control appeared to be limited. They appeared to be less effective under high disease pressure, and against actively growing *Sclerotinia* pathogens under favourable wet conditions, at the late crop stage. In commercial crops, *Sclerotinia* infection rarely occurred early in the crop stage, and prolonged disease control is vital as it spreads rapidly under ideal moist conditions in a dense crop canopy during the late crop stage. Therefore, under high disease pressure, fungicide applications following early biocontrol agent treatments are still recommended.

Brassica green manures

This study indicated that brassica green manure plants that produce high plant biomass and high concentrations of biofumigants may offer advantages over non-brassica green manure plants for *Sclerotinia* disease control. BQ-Mulch, which produces high levels of isothiocyanates (ITCs) in roots, was found to be more effective for *Sclerotinia* control than Fumus, which produces high levels of ITCs in its foliage. The effectiveness of BQ-Mulch may be related to the high level of the non-volatile, but more persistent and toxic, 2-phenylethyl-ITC produced in its root tissues.

The fumigating activities, either by ITCs or other toxic bioactive compounds produced by the green manures, are likely to diminish rapidly after their release or formation in soil, and their effects for disease suppression are expected to be relatively short term. As a result, poor control of *Sclerotinia* may occur late in the crop's development, on lettuce planted after the brassica green manure crops. Therefore, under conditions that are ideal for the *Sclerotinia* disease, fungicide control methods should also be used, in conjunction with brassica green manures, for disease management.

This study showed that the high plant biomass and deep tap root systems of brassica green manures, helped reduce soil crusting, improved infiltration, increased organic matter and reduced sub-soil compaction. These overall soil improvement effects are likely to increase soil fertility and improve soil structural properties, thereby contributing to improved crop health and disease control.

Soil amendments with mustard meal, PERLKA and urea

A preliminary laboratory test conducted in this study showed that cold pressed mustard meal, urea and PERLKA were highly effective in killing sclerotia of *S. minor* at very high rates (10 g/kg soil or equivalent to 16 tonnes/ha). These three materials contain, or can convert into, toxic compounds, which behave as soil fumigants.

However, when applied at much lower rates, i.e. 500 and 1000 kg/ha, in field studies, they did not eradicate the *S. minor* pathogen. The results indicate that unless very high rates are used, the defatted mustard meal, urea and PERLKA are unlikely to be effective for long-term management over the fallow period, but may be useful for suppressing disease in the short-term. They are unlikely to replace post-plant fungicide applications, but could be considered for use as an additional tool for an integrated disease management practice.

Detection

Sclerotinia may cause seedling damping-off, and the susceptibility of seedlings appeared to be closely associated with plant architecture. Plant architecture could therefore be a useful guide when selecting plant varieties for use in crop rotations, particularly in ground that has high *Sclerotinia* pressure.

There was a positive correlation between the sclerotia levels and disease incidences on lettuces in the field. This indicated that the wet sieving method for sclerotia population may be useful for detecting high levels of sclerotes, and hence for identifying high disease pressure sites.

In Tasmania, apart from mycelial growth from sclerotes, *S. minor* was noted to produce apothecia on sclerotes. Infection by *S. minor,* however, is still predominantly due to mycelial infection at the base of plants. In contrast, widespread *Sclerotinia* disease due to *S. sclerotiorum* in Tasmania is frequently associated with infection by ascospores of the fungus. Effective disease control methods in different crops would be determined by the species of *Sclerotinia* pathogen and its mode of infection, as well as crop growth and susceptible periods. Selection of paddocks that enable good air flow and rapid drying of crops, in order to minimize risk to *Sclerotinia* disease, are critical. Effective disease control can be difficult to achieve in the presence of field conditions that are highly conducive to the pathogen.

6.2 Introduction

In Tasmania, *Sclerotinia* diseases caused by *S. sclerotiorum* and *S. minor* have become widespread in the north-west and southern regions, where intensive horticultural cropping occurs. They affect many crops, such as beans, brassicas, carrots, lettuces, peas, potatoes and pyrethrum. *Sclerotinia* produces sclerotia that can survive in soil over a long period of time. As a result, with intensive cropping, *Sclerotinia* inoculum in the soil could increase to such a high level that fungicide spray programs alone may not give adequate control of the disease in highly susceptible horticultural crops.

Among the horticultural crops, lettuce is considered to be the most susceptible crop to *Sclerotinia* infection, which results in lettuce drop. In north-west and southern Tasmania, many lettuce crops cannot be grown without the regular use of procymidone fungicide spray program. In some areas, where lettuces are sown every year, high losses ranging from 10% to 20% can still occur despite a regular fungicide spray program. Other crops in Tasmania, such as beans and carrots, can also sustain heavy losses due to *Sclerotinia* diseases. *Sclerotinia* disease on beans is caused by *S. sclerotiorum*, primarily through infected flowers by ascospores. Carrot crops infected by *S. sclerotiorum* in the field are rejected for export to overseas markets due to increased risk of post-harvest storage rot.

The perennial crop, pyrethrum, is susceptible to both species of *Sclerotinia*, resulting in wilting of young plants as well as direct flower infection of matured plants. Increasing frequency of *S. sclerotiorum* infection on peas, potatoes, and poppies have also been observed in the field. However, the impact of the disease on crop yield is unknown in these crops. Late infections of these crops have been noted to cause dramatic increases in sclerotia populations in soil, which can then pose a threat to subsequent crops in the rotations. Apart from obvious infections on maturing and matured plants, *Sclerotinia* pathogens, particularly *S. minor* can also cause seedling damping-off of most vegetable and field crops. The seedling damping-off caused by the *Sclerotinia* pathogens resembles that caused by other common soil pathogens such as *Fusarium, Pythium*, and *Rhizoctonia*, and is therefore often mistakenly attributed to the latter.

In many vegetables and field crops, *Sclerotinia* control programs consist of a regular fungicide application at the onset of *Sclerotinia* disease in each growing season. Although *Sclerotinia* diseases on the susceptible crops can usually be controlled effectively with a regular fungicide spray program, these fungicide sprays are an additional cost to crop production. There are also occasions where frequent rainfall and dense crop canopy help create an ideal environment for the disease. Adverse weather conditions can prevent spray applications, and hence affecting the timing of fungicide sprays, and therefore reducing the effectiveness of disease control. Poor control of *Sclerotinia* diseases will lead to losses in yield and quality, as well as market share, in an industry that values consistency, quality and timely delivery of produce. The over reliance on fungicides for disease control could also result in their reduced efficacies. Fungicide resistance and enhanced degradation of benomyl, iprodione and vinclozolin have been reported following frequent use (Subbarao 1998).

Procymidone (sold as Sumisclex and Fortress) is often used for *Sclerotinia* control in Australia. Since 1990s, it is widely used for *Sclerotinia* control in Tasmania, and therefore, it is considered to be a relatively new fungicide. Although no enhanced degradation or resistance has been found in this project or other studies (Pung & O'Brien 2000), we must still be cautious and vigilant in its use to ensure its availability for long-term use. High disease pressure, poor fungicide application methods, multiple fungicide sprays, and lack of other suitable fungicides for use in alternation with procymidone, could result in a loss of efficacy.

This project takes a new approach to examining and developing a long term *Sclerotinia* disease management strategy. The aims of the studies conducted in Tasmania were to evaluate and develop both short and long-term management strategies that are suitable for *Sclerotinia* control in horticultural crops. Long-term strategies that were investigated included the evaluation of promising biological methods, such as commercial biocontrol agents, green manures and break crops. The short-term strategies examined were pre-plant and post-plant chemical control methods to determine if there are other products that could be used in combination or in alternation with the current fungicide program.

As lettuce is the most susceptible crop to *Sclerotinia* wilt, almost all trials in Tasmania were conducted with lettuce as the benchmark crop. Although *Sclerotinia sclerotiorum* can also cause *Sclerotinia* wilt, *S. minor* is the most common cause of lettuce wilt. Disease management for *S. sclerotiorum* is similar to that used for *S. minor*. Therefore, suitable disease management method identified in these studies could also be used to manage susceptible crops and sites that are known to have high levels of *S. sclerotiorum*.

The areas of study examined in the Tasmanian trials were:

- The effectiveness of bacteria and fungal biocontrol agents, used in pre- and post-plant treatments, for use in wilt disease management.
- The effectiveness of alternative chemical soil treatments, used in pre-plant treatments, for use in wilt disease management.
- Methods for improving *Sclerotinia* control on crops in the field after planting, with the use of new fungicides and fungicide mixtures containing materials that enhance plant defence systems.
- The effectiveness of a range of green manure crops, including brassica green manures, for use in high *Sclerotinia* pressure areas, for long-term disease management.

6.3 Preliminary evaluation of potential alternative biocontrol and chemical products for *Sclerotinia* wilt control

Abstract

Three preliminary trials were conducted in Tasmania at the beginning of the project, to evaluate the efficacies of potential biological control agents, as well as new chemical products, for *Sclerotinia* control on lettuces. Sumisclex was found to be highly effective in the control of *Sclerotinia* disease caused by *S. minor* and *S. sclerotiorum*. Other new fungicide products evaluated in three trials, Amistar and Maxim, were less effective compared to Sumisclex. The trials conducted also consistently indicated that two sprays of Bion applied at 14 day intervals following Sumisclex at planting, gave a similar level of *Sclerotinia* disease control as three sprays of Sumisclex. Bion belongs to a new category of plant protection products called plant activators, which work by stimulating or inducing the development of systemic acquired resistance in plants. On its own, Bion has no direct effect on the *Sclerotinia* pathogens, and gave poor disease control. The significance of these findings was the potential of harnessing and enhancing the plant's own natural defense mechanism to improve *Sclerotinia* disease control. Among the biocontrol products evaluated, Contans, based on *C. minitans* biocontrol fungus, was identified as the most promising potential biocontrol agent for *S. sclerotiorum* and *S. minor* control in the three trials. Inconsistencies in the levels of disease control with the different biocontrol treatments in the three trials, indicates that further improvements in their performance are required before commercial use can be recommended.

Aims

Three preliminary trials were conducted at the beginning of the project in Tasmania. The biocontrol and chemical products, and their active ingredients, used in the trials are listed in Table 6.1. Trial details in appendix. Trials 1.1 and 1.2 were field trials conducted to evaluate the efficacies of potential biological control agents, as well as new chemical products, for *Sclerotinia* control on lettuces (Table 6.2). Trial 1.3 was conducted after the two field trials, to further evaluate the potential of Bion and Contans, applied alone or in combination with Sumisclex (Table 6.3).

Except for BCA002 and BCA003, all of the new products examined were commercial products. Treatments for biological control agents included different application methods for the products BCA001 and BCA002. Product rates and application details for the treatment application are in Table 6.4 for Trials 1.1 and 1.2, and in Table 6.5 for Trial 1.3.

Materials and Methods

Table 6.1: Product list, Trials 1.1 – 1.3

Product	Active ingredient (a.i.)
Amistar	Azoxystrobin
Contans	*Coniothyrum minitans* (Contans™)
BCA002	*Coniothyrum minitans* (NZ isolate A69)
BCA003	*Trichoderma* spp. (NZ isolate A6R)
BCA004	*Trichoderma koningii* + *Trichoderma harzianum* (TRI-D25™)
BCA005	*Trichoderma* spp. + beneficial fungi/bacteria (Nutri-life 4/20™)
Bion	Acibenzolar-S-methyl
Maxim	50% fludioxinol
Neem	Emulsified cold-pressed neem oil (Nutri-Life)
Elexa	Chitosan
Sumisclex	50% procymidone

Table 6.2: Treatment list for Trials 1.1 and 1.2

No.	Treatment	Product application method	
		Before or at planting	Post-planting
1	Sumisclex (3 sprays)	Spray at planting	2 more sprays at 14 day intervals
2	Amistar (3 sprays)	Spray at planting	2 more sprays at 14 day intervals
3	Sumisclex / Bion / Bion	Sumisclex spray at planting	2 sprays of Bion at 14 day intervals
4	Sumisclex / Elexa / Elexa	Sumisclex spray at planting	2 sprays of Elexa at 14 day intervals
5	Neem (3 sprays)	Spray at planting	2 more sprays at 14 day intervals
6	Contans transplant plug	Spray drench of transplant plug	None
7	Contans (2 sprays at 4 kg/ha)	Spray at planting	2nd spray at 4 weeks after planting
8	BCA002 (transplant plug)	Product mixed into transplant plug	None
9	BCA002 (750 kg/ha soil application at planting)	In-soil application just before planting	None
10	BCA003 (transplant plug)	Product mixed into transplant plug	None
11	Contans (1 spray at 8 kg/ha at planting)	Spray at planting	None
12	BCA004 (2 sprays)	Spray at planting	2nd spray at 4 weeks after planting
13	BCA005 (2 sprays)	Spray at planting	2nd spray at 4 weeks after planting
14	Untreated control	None	None

Table 6.3: Treatment list for Trial 1.3

No.	Treatment	Product application method	
		Before or at planting	Post-plant
1	Sumisclex (3 sprays)	Spray at 1 day after planting	2 more sprays at 7 day intervals
2	Maxim (3 sprays)		
3	Bion (3 sprays)		
5	Sumisclex / Bion / Bion	Sumisclex spray applied 1 day after planting	2 sprays of Bion at 7 day intervals
6	Contans (pre-plant application)	Pre-plant soil application	None
7	Contans (transplant plug)	Transplant drench	
8	Contans (3 sprays)	Spray at 1 day after planting	2 sprays of Bion at 7 day intervals
9	Contans (pre-plant application) / Bion (3 sprays)	Pre-plant soil application of Contans & Bion spray 1 day after planting	
10	Contans (transplant plug) / Bion (3 sprays)	Transplant drench with Contans & Bion spray at 1 day after planting	
11	Contans (transplant plug) / Bion / Bion / Sumisclex	Transplant drench & Bion spray 1 day after planting	2nd Bion spray at 7 days after planting, and Sumisclex spray at 14 days after planting
12	Untreated control	None	None

Table 6.4: Application details for Treatments 1-13 in Trials 1.1 and 1.2

Treatment	Product	Product rate & Treatment method
1, 3, 4	Sumisclex*	2 L/ha - spray applications
2	Amistar*	500 g/ha - spray applications
3	Bion*	200 g/ha - spray applications
4	Elexa*	100 L/ha - spray applications
5	Neem*	2 L/ha - spray applications
6	Contans (transplant plug)	Applied into peat moss mix in seedling trays before sowing. Product was mixed with water (1 g product/1 L water), and then applied as a drench onto the peat moss medium in trays at 4 L per tray until saturation. Product rate used was 1.14 g/L peat moss.
7	Contans (spray) *	4 kg/ha - spray applications
8, 10	BCA002 & BCA003 (transplant plug)	Applied into seedling medium prior to sowing, by mixing the product with peat moss in a plastic bag. Product rate used was 1.0 g/kg peat moss.
9	BCA002 (soil application)	Applied 450 g per plot, by broadcasting onto soil surface, then raking into soil to 10 cm depth (i.e. 3.6 kg per trial for 8 replicate plots, or 750 kg/ha)
11	Contans (spray)*	8 kg/ha - spray applications
12	BCA004*	1 kg/ha - spray applications
13	BCA005*	Prepared concentrated brew prepared as per instructions on label; then diluted to 1:1 ratio with water and applied as spray application at 900 L mix/ha.

* Following all spray application treatments at planting and post-plant, the trial area was irrigated with 20 mm water in order to drench the sprayed product into ground & base of plants.

In Trial 1.3, *Sclerotinia* colonized barley grains were used to inoculate the potting soil mix. *S. minor* inoculum was produced on autoclaved barley grains (inoculated and incubated for 3 weeks before use). The appropriate pot was first filled with soil mix, and then covered to the top with 10 cm deep *Sclerotinia* inoculated soil mix. The inoculated soil mix in each pot contained 30 g of colonized barley grains, and the pathogen consisted of both mycelium and sclerotia.

Table 6.5: Application details for Trial 1.3

Treatment No.	Product	Product rate & Treatment method
1, 5, 11	Sumisclex (spray)	2 L/ha - spray applications
2	Maxim (spray)	2 L/ha - spray applications
3, 5, 9, 10, 11	Bion (spray)	0.2 kg/ha - spray applications
8	Contans (spray)	4 kg/ha - spray applications
6, 9	Contans (pre-plant application)	4 kg/ha - spray application
7, 10, 11	Contans (transplant plug)	Product applied as a drench onto plants in seedling trays at 14 days before transplanting. Application rate was 8 g product/1 L water and 2.5 ml per cell/seedling, which was equivalent to 2×10^7 spores per seedling. Serial dilution test of BCA001 transplant drench indicates a population of 2.25×10^7 cfu applied per seedling in each cell.

The details of product applications in Trials 1.1 and 1.2 were as described in Table 6.4. All post-plant spray applications were applied with 900 L/ha of water at 250 kPa, using an air-pressurized knapsack precision sprayer fitted with a 1.5 m boom. The whole trial area was irrigated following the post-plant spray applications in order to drench the product onto the soil surface and the base of plants.

A hand-pressurized sprayer was used for all spray applications in Trial 1.3. The appropriate amount of product was delivered in 25 ml water per pot. Following all spray application treatments, before or after transplanting, the whole trial area was irrigated using an overhead sprinkler with 10 mm water, in order to drench the sprayed product onto the soil surface and the base of plants. Soils in the pots were kept constantly moist with overhead sprinkler irrigation every day.

Assessment & Analysis

Plants were assessed for *Sclerotinia* infection at 43 and 49 days after planting (DAP) in Trial 1.1, at 49 and 59 DAP in Trial 1.2, and at 15 and 29 DAP in Trial 1.3. The percentage of wilted plants in each replicate plot was then tabulated from the total number of plants assessed. Statistical analysis was performed on arcsine transformed data values all the trials, and pair-wise comparisons, using the least significant difference (LSD) procedure, were applied to the mean values.

Results

Trial 1.1

Wilted plants in Trial 1.1 were caused by *S. sclerotiorum*. The *Sclerotinia* disease levels in the trial area were considered to be very low, ranging from 0.5 to 3% at 49 DAP, one day prior to commercial harvest (Table 6.6). As a result of the low disease levels, there were no significant differences in the percentage of wilted plants between treatments ($p = 0.1$ at 43 DAP, and $p = 0.15$ at 49 DAP). In this trial, diseased plants occurred in random clusters, with many infected plants found in the north-west corner of the trial area.

Although not significant, Sumisclex sprays (Treatment 1), and the consecutive sprays of Sumisclex, Bion and Bion (Treatment 2), appeared to give the greatest disease control (Table 6.6). The biocontrol treatments that appeared to show potential included Contans applied in one application at planting, at 8 kg/ha (Treatment 11), or applied in two sprays at 4 kg/ha at planting and four weeks later (Treatment 7), and BCA003 applied into the transplant plug (Treatment 10).

Table 6.6: Treatments effects on lettuce wilt caused by *S. sclerotiorum* in Trial 1.1

No.	Treatment	Average no. of plants per plot at 43 DAP	% Wilted plants at 43 DAP*	% Wilted plants at 49 DAP*
1	Sumisclex (3 sprays)	51	0.5	0.5
3	Sumisclex /Bion /Bion	52	0.3	0.5
11	Contans (1 spray at 8 kg/ha)	49	0.8	1.0
2	Amistar (3 sprays)	52	0.2	1.2
7	Contans (2 sprays at 4 kg/ha)	51	1.0	1.2
10	BCA003 (transplant plug)	50	1.0	1.3
5	Neem (3 sprays)	50	1.3	1.6
8	BCA002 (transplant plug)	50	1.6	1.6
9	BCA002 (soil application)	49	1.0	1.6
12	BCA004 (2 sprays)	51	0.5	1.7
6	Contans (transplant plug)	49	1.6	2.1
4	Sumisclex /Elexa /Elexa	51	1.5	2.5
13	BCA005 (2 sprays)	48	2.1	2.9
14	**Untreated control**	50	2.0	3.0

DAP = days after planting
Treatments were ranked in an ascending order, based on mean values at 49 DAP.
* Mean values were not significantly different in the analysis of variance ($p > 0.05$).

Trial 1.2

Unlike Trial 1.1, *S. minor* was the cause of wilted plants in Trial 1.2. The *Sclerotinia* disease levels in the trial area were low at 49 DAP, but increased substantially 10 days later. Diseased plants were randomly distributed throughout the trial area, and ranged from 3.0% to 11.3% at 59 DAP, one day before commercial harvest (Table 6.7).

There were significant differences in the percentage of wilted plants between treatments at both assessments ($p = 0.04$ at 49 DAP, and $p = 0.03$ at 59 DAP) (Table 6.7). At 49 DAP, no significant disease control was noted by any of the biocontrol or chemical treatments when compared to the untreated control. However, a significantly higher percentage of diseased plant was recorded with Neem (Treatment 5). Therefore, although neem has been reported to have anti-microbial activity, it did not appear to have any adverse effects on *S. minor* and may even encourage its growth by suppressing other soil microbes.

At 59 days after planting, only plants treated with Sumisclex / Bion / Bion (Treatment 3) significantly reduced the percentage of wilted plants, when compared to the untreated control (Table 6.7). In addition to Treatment 3, Treatments 1 and 7, also appeared to reduce the disease. All other treatment methods, including the new fungicide Amistar and the new product Elexa, did not cause a significant reduction in the percentage of wilted plants at 59 days after planting. This suggests that the effectiveness of the biocontrol treatments may be hampered by poor residual effect over a long period of time.

All seedlings used in this trial were drenched with Sumisclex (100 ml/100L) by mistake by farm staff, on arrival from the plant nursery. The seedlings were transplanted to the trial area 4 days after the fungicide application. Therefore, the efficacies of the biocontrol agents, especially the transplant plug treatments, may have been adversely affected by this Sumisclex application. Nevertheless, the two biocontrol treatments with Contans, applied into transplant plugs (Treatment 6), and as 2 sprays at 4 kg/ha (Treatment 7) showed potential in this trial.

Table 6.7: Treatment effects on % lettuce wilt caused by *S. minor* in Trial 1.2

No.	Treatment	Average plants per plot	% Wilted plants at 49 DAP *	% Wilted plants at 59 DAP *
3	Sumisclex/ Bion/ Bion	49	0.2 a	3.0 a
1	Sumisclex (3 sprays)	49	0.5 a	4.3 ab
7	Contans (2 sprays at 4 kg/ha)	49	0.5 a	4.7 abc
6	Contans (transplant plug)	48	1.6 ab	6.3 abcd
8	BCA002 (transplant plug)	49	1.3 a	6.9 abcde
4	Sumisclex/ Elexa/ Elexa	49	1.5 a	7.5 abcde
2	Amistar (3 sprays)	49	0.3 a	8.0 bcde
9	BCA002 (soil application at 4 kg/ha)	50	1.0 a	8.0 bcde
13	BCA005 (2 sprays)	49	1.0 a	8.1 bcde
14	**Untreated Control**	50	1.0 a	8.7 bcde
5	Neem (3 sprays)	50	3.0 b	9.4 cde
10	BCA003 (transplant plug)	48	0.5 a	9.4 cde
11	Contans (1 spray only at 8 kg/ha)	50	0.7 a	10.1 de
12	BCA004 (2 sprays)	49	1.5 a	11.3 e

DAP = days after planting

Treatments are ranked in an ascending order, based on mean values at 59 DAP.

* Within the same column, means followed by the same letter are not significantly different at the 5% level according to LSD Test.

Trial 1.3

Very high incidence of infection on lettuces by *S. minor* was recorded in Trial 1.3, due to soil inoculation with high levels of *S. minor* inoculum, as well as wet soil condition with frequent irrigations. The percentage of wilted plants ranged from 6 - 45% at 15 days after planting, and 25 - 88% at 29 days after planting (Table 6.8).

There were significant differences in the percentage of wilted plants between treatments at both assessments ($p = 0.008$ at 15 DAP, and $p < 0.001$ at 29 DAP). At 15 DAP, only two treatments (Treatments 1 and 5) significantly reduced diseased plants compared to the untreated control. The Sumisclex / Bion / Bion treatment appeared to caused the greatest level of disease control (Table 6.8). Bion applied on its own in three sprays (Treatment 3) have no effect in preventing *S. minor* infection.

At 29 DAP, nine out of ten treatments significantly reduced the percentage of wilted plants compared to the untreated control (Table 6.8). Three consecutive sprays of Sumisclex gave the greatest level of disease control at 29 DAP, followed by one early or one late Sumisclex application in conjunction with Bion in Treatments 5 and 11. The disease spread very rapidly from infected plants to healthy adjacent plants under wet conditions. Therefore, the results indicate that an early Sumisclex spray, applied at one day after planting, was more effective than the late application.

Although the biocontrol product Contans and the new fungicide Maxim showed significant disease control at 29 days after planting, the percentage of infected plants was still very high ranging from 54 - 74% (Table 6.8). This indicates that pre-plant inoculation at 14 days before planting with the biocontrol product did not prevent plants from becoming infected after they were transplanted. Contans applied at 7 DAP also failed to control the *S. minor* infection.

It should be noted that the *S. minor* inoculum was based on fresh culture that had just produced black sclerotia, and its mycelium was primed for active growth under wet conducive soil condition. Seedlings in the pot were also planted close together. Therefore, infected plants were noted at 7 days after planting. These findings suggest that the biocontrol is unlikely to be effective against an actively growing *S. minor* under conducive conditions, and under high disease pressure.

Table 6.8: Treatment effects on % lettuce wilt caused by *S. minor* in Trial 1.3

No.	Treatment	% Infected 15 DAP	% Infected 29 DAP
1	Sumisclex (3 sprays)	13 ab	25 a
5	Sumisclex / Bion / Bion	6 a	32 a
11	Contans (transplant plug) / Bion / Bion / Sumisclex	35 c	54 b
10	Contans (transplant plug) / Bion (3 sprays)	33 c	60 bc
7	Contans (transplant plug)	31 c	60 bc
9	Contans (pre-plant application) / Bion (3 sprays)	31 bc	65 bc
8	Contans (3 sprays)	35 c	67 bcd
2	Maxim (3 sprays)	39 c	72 cd
6	Contans (pre-plant application)	39 c	74 cd
3	Bion (3 sprays)	45 c	80 de
12	**Untreated**	**39 c**	88 e

Discussion

Sumisclex was found to be highly effective in the control of *Sclerotinia* disease caused by *S. minor* and S. *sclerotiorum*. Other new fungicide products evaluated in three trials, Amistar and Maxim, were less effective compared to Sumisclex.

Results also consistently indicated that two sprays of Bion applied at 14 day intervals following Sumisclex at planting, gave as good *Sclerotinia* disease control as three sprays of Sumisclex. These findings show the potential for using Bion in alternation with Sumisclex, thereby reducing the number of Sumisclex applications for consistent disease control. A greater significance of these findings was the potential of harnessing and enhancing the plant's own natural defense mechanism to improve *Sclerotinia* disease control.

Bion belongs to a new category of plant protection products called plant activators, which work by stimulating or inducing the development of systemic acquired resistance in plants. This activity gives rise to natural protection mechanisms against invading pathogens. In contrast, Sumisclex only has a direct effect in preventing infection by *Sclerotinia* pathogens. On its own, Bion has no direct effect on the *Sclerotinia* pathogens, and gave poor disease control. This indicates that these types of products are likely to be effective only when applied in alternation or in combination with Sumisclex against aggressive pathogens like *Sclerotinia* spp.

Another new product, Elexa, based on chitosan, has a similar mode of activity to Bion, whereby it can also activate plant's own defense mechanisms against invaders. However, in the two field trials conducted, when applied with Sumisclex in a similar manner to Bion, it had little or no effect in reducing disease plants. This suggests that different types of products may activate different plant mechanisms, and exhibit different levels of efficacy and suitability for *Sclerotinia* control. Therefore, further trials with alternative products to Bion were conducted, and are discussed in Sections 5 and 6.

Contans, based on *C. minitans* biocontrol fungus, was identified as the most promising potential biocontrol agent for *S. sclerotiorum* and *S. minor* control in the three trials. Contans, produced by Prophyta Ltd, Germany, is a commercial product that is now registered for *Sclerotinia* control in Europe and the United States of America. Its efficacy for prolonged *Sclerotinia* control, however, may be limited close to harvest by the spread of the biocontrol agent as well as the speed at which it could contain actively growing *Sclerotinia* pathogens under favourable wet top-soil conditions. *C. minitans* is a relatively slow growing fungus compared to *Sclerotinia* pathogens. In commercial crops, *Sclerotinia* infection rarely occurred early in the crop stage due to rapid drying of exposed top-soil by wind and sunlight. However, as plants mature, their leaves spread out, covering the gaps between plants, and creating ideal moist conditions beneath the plants for *Sclerotinia* infection as well as its spread to adjacent plants.

Other biocontrol agents tested in the preliminary trials were less effective against *Sclerotinia*. These include another *C. minitans* based product (BCA002). Some of the poor control may be associated with poor product formulation, and hence poor survival of the biocontrol agents, before or after application. The method of biocontrol application could also be critical. With Contans, drench application onto seedlings plugs prior to planting and post-plant spray applications were identified as effective methods.

In commercial crops, consistent and effective disease control is vital. The Sumisclex (3 sprays) or Sumisclex / Bion / Bion treatments in all three trials met these two important criteria. Unfortunately, with the biocontrol treatments, there were inconsistencies in the levels of disease control with different products, and different application methods in the three trials. Therefore, in order to meet the criteria for commercial use, further improvements in the performance of the products under different field conditions must be made.

6.4 The efficacy of biocontrol agents applied in soil treatments prior to lettuce planting for *Sclerotinia* wilt control

Abstract

Two field trials were conducted to further evaluate the effectiveness of bacterial and fungal biocontrol agents, used in pre-plant treatments, for use in wilt disease management. At a high disease pressure site, Contans, applied at the high rate of 10 kg/ha six months prior to lettuce planting, was shown to be effective in reducing *Sclerotinia* wilt due to *S. minor*. Companion, applied on its own to lettuce transplants, had no effects in *Sclerotinia* disease control. Companion is a commercial product based on *Bacillus subtilis*, and is sold in Australia as a plant root growth promoter. Species of *Bacillus* and *Lysobacter*, inoculated and applied with composted fish medium, appeared to perform better than liquid cultures of the bacteria. This demonstrates the potential of using a suitable organic medium as a delivery system for bacterial biocontrol agents. However, a 40% reduction in diseased plants by the best biocontrol treatments, with the bacteria alone, still caused a loss of 22% and 25% plants due to *Sclerotinia* wilt, when compared to untreated control. These losses are still considered to be too high in a commercial lettuce crop.

Aims

In 2001/02, two field trials were conducted to evaluate bacterial and fungal biocontrol treatments for use in wilt disease management.

Materials & Methods

In 2001/02, two trials were conducted on the same farm at Cambridge, in southern Tasmania, to evaluate biocontrol treatments for Sclerotinia control on lettuces. The two sites were known to have high levels of *Sclerotinia* disease in previous lettuce crops. Trial details in appendix.

Table 6.9: Treatment list for Trial 2.1

No.	Treatment	Active ingredient	Application Method
1	Untreated Control	N/a	N/a
2	5 kg/ha Contans	*C. minitans*	Sprayed with 620 L/ha water, then hoed and mixed into top-soil (10 cm deep).
3	10 kg/ha Contans	*C. minitans*	
4	560 kg/ha BCA002	*C. minitans* (NZ isolate)	Broadcast the dry granular product evenly onto the soil surface and then hoed and mixed into top-soil (10 cm deep).

In Trial 2.1, *C. minitans* (Contans and BCA002), a fungal parasite of *Sclerotinia*, was applied onto fallow ground on 5th July 2001, approximately 6.5 months prior to lettuce planting, to evaluate the use of biocontrol agents for long-term disease control (Table 6.9). A suspension of Contans at the appropriate concentration was applied with a boom sprayer, and then mixed into top-soil with a hoe to 10 cm deep. BCA002 was broadcast and mixed into the soil with a hoe. The trial area was left as fallow ground until the lettuce crop was planted. A hard crust formed over the soil surface for almost all of the time it was fallow.

In Trial 2.2, bacterial biocontrol agents were applied one week immediately prior to lettuce planting, to determine their effectiveness for short-term disease control (Table 6.10). The lettuce crop was planted on 21st March 2002. Companion™ is a commercial bioproduct based on *Bacillus subtilis*, which is used to improve soil and root growth. The other bacterial biocontrol agents, BCA10 & BCA11 were isolated from pyrethrum, and BCA12 was isolated from lettuce (isolated by Y. Ramona, PhD student, University of Tasmania).

Disease assessments were conducted, when plants were close to full maturity, just before commercial harvest. The number of plants with *Sclerotinia* wilts and other disorders that affect marketability of the lettuces were counted in each plot (1 bed wide x 6 m^2 in Trial 2.1, and x 1 m^2 in Trial 2.2). The percentages of plants with disease or disorders were then tabulated from the total number of plants in the area assessed. Lettuces planted at the same time as Trial 2.2, in an adjacent ground on the same beds , but treated as in regular commercial practice (2 L/ha Sumisclex at planting, and then at two weekly intervals), were also assessed for the percentage of diseased plants. The disease incidence of the latter was not included in the data analysis for Trial 2.2, but is presented in Table 6.12 as a demonstration of the level of disease control from the commercial fungicide spray program.

Statistical analysis was performed on data values of both trials, and pair-wise comparisons, using the least significant difference (LSD) procedure, were applied to the mean values.

Table 6.12: Treatment list for Trial 2.2

No.	Pre-plant treatment	Product Rate*	Active ingredient	Application Method
1	Untreated control	N/a	N/a	N/a
2	BCA10 in pulp compost	1 L/plot or 4,733 kg/ha	*Bacillus polymyxa* UT1	Broadcast evenly onto the soil surface and then hoed and raked it into soil (5 cm deep). Applied one week before lettuce planting.
3	BCA11 in fish compost	2 L/plot or 12,133 kg/ha	*Bacillus* sp. SAG3	
4	BCA12 in fish compost	2 L/plot or 12,133 kg/ha	*Lysobacter antibiotics* BG	
5	BCA10 (liquid culture)	2 L suspension (10^7 cfu/ml)	*Bacillus polymyxa* UT1	Drench application, by applying the suspension with a watering can onto lettuce transplants in seedling trays. Applied just before transplanting.
6	BCA11 (liquid culture)	2 L suspension (10^7 cfu/ml)	*Bacillus* sp. SAG3	
7	BCA12 (liquid culture)	2 L suspension (10^7 cfu/ml)	*Lysobacter antibiotics* BG	
8	Companion	10.5 ml/10L (7.1 x 10^4 cfu/ml)	*Bacillus subtilis*	Drench application just before transplanting as above.
9	Companion + Sumisclex	10.5 ml/10L + 50 ml/10L	*Bacillus subtilis* + procymidone	
10	Fish compost only	2 L/plot or 12,133 kg/ha	N/a	Broadcast evenly onto the soil surface and then hoed and raked into soil (5 cm deep). Applied one week before lettuce planting.

* Equivalent weight of 2 L fish waste compost = 1.456 kg; 1 L wood fiber waste compost = 0.568 kg

Results

Trial 2.1

In Trial 2.1, only Contans, applied at the high rate of 10 kg/ha, approximately six months before lettuce planting, significantly reduced *Sclerotinia* wilt due to *S. minor*, when compared to the untreated control (Table 6.11). Both Contans and BCA002 were based on the biocontrol fungus *C. minitans*. Contans, supplied by Prophyta Ltd, Germany, is a commercial product that is now registered for *Sclerotinia* control in Europe and the United States of America. The BCA002 was still under development and the formulation used in this study was bulky and had poor viability. This may explain the relatively poor level of disease control, even when applied at a very high application rate.

Table 6.11: The effects of fungal biocontrol agents based on *C. minitans,* for *Sclerotinia* wilt disease on Red Oak lettuce in Trial 2.1.

No.	Treatment	No. of sclerotes / 200g soil		% Plants with *Sclerotinia* wilt
		Initial count (5/07/01)	Final count (28/12/01)	
1	**Untreated Control**	**22**	**20**	**23 b**
2	5 kg/ha BCA001	14	22	17 ab
3	10 kg/ha BCA001	17	18	10 a
4	560 kg/ha BCA002	11	19	18 ab

Within each column, means followed by the same letters are not significantly different at the 5% level according to LSD Test.

The sclerotia levels between plots were variable, and at the final sclerotia assessment, the biocontrol treatments appeared to have little or no effect on viable sclerotia levels in the soil. This indicated that the reduced *Sclerotinia* incidence by 10 kg/ha Contans might not be due to reduced sclerotia inoculum level, but possibly to other modes of biocontrol activity by the fungal parasite.

Trial 2.2

Companion, applied on its own to lettuce transplants, caused little or no reduction in *Sclerotinia* infected plants (Table 6.12). In contrast, the Companion + Sumisclex drench treatment significantly reduced the level of *Sclerotinia* disease compared to all other treatments. This treatment also appeared to have a much lower disease level compared to plants from an adjacent area, which were treated with the commercial standard fungicide applications.

Table 6.12: The effects of bacterial biocontrol agents for *Sclerotinia* wilt disease on Red Oak lettuce in Trial 2.2

No.	Treatment	Total no. plants/plot	% Plants with *Sclerotinia* wilt	
			week 5	week 8
1	**Untreated control**	**12**	**25 b**	**40 b**
2	BCA10 in pulp compost	12	17 b	30 b
3	BCA11 in fish compost	12	12 b	22 b
4	BCA12 in fish compost	12	12 b	25 b
5	BCA10 (liquid culture)	12	20 b	34 b
6	BCA11 (liquid culture)	12	22 b	32 b
7	BCA12 (liquid culture)	12	23 b	30 b
8	Companion	12	17 b	32 b
9	Companion + Sumisclex	12	2 a	7 a
10	Fish compost only	12	23 b	40 b
	Commercial standard	**118**	**11**	**18**

Within each column, means followed by the same letters are not significantly different at the 5% level according to LSD Test.
* *Sclerotinia* infection of plants from outside the trial area, but adjacent to it, assessed as a comparison. Plants were treated with the commercial standard fungicide application (ie. 2 L/ha Sumisclex after planting, and then at two weekly intervals).

In Trial 2.2, when applied at much lower rates in an infected field site, Treatments 2-7, containing BCA10, BCA 11 & BCA12, appeared have lower *Sclerotinia* infected plants, but the differences were not significantly different to that of the untreated control (Table 6.12). The disease levels in these treatments were still considered to be too high and unacceptable in commercial practice. However, in terms of disease reduction, the best two treatments, Treatments 3 and 4, applied in fish compost, gave an average of 40% reduction in the percentage of diseased plants when compared to the untreated control.

Discussion

At a high disease pressure site, Contans, applied at the high rate of 10 kg/ha, six months prior to lettuce planting, was shown to be effective in reducing *Sclerotinia* wilt due to *S. minor*. Pre-plant application of Contans, several months or weeks prior to planting of a crop susceptible to *Sclerotinia* disease, is the preferred application method recommended by the manufacturer. The time lag between application and crop planting is believed to be necessary to allow the biocontrol fungus sufficient time to germinate from spores, spread and come into contact with the pathogen's sclerotia in soil, parasitise the sclerotia and eventually kill it. However, in this study, there was little or no difference between the initial and final sclerotia levels, following pre-plant Contans applications. This indicates that the Contans pre-plant treatments have little or no effect on viable sclerotia levels in the soil, although there might be other modes of biocontrol activity by the fungal parasite.

Companion, applied on its own to lettuce transplants, had no effects on *Sclerotinia* disease control. Companion + Sumisclex drench treatment, however, appeared to reduce the level of *Sclerotinia* disease. Unfortunately, as there was no Sumisclex only treatment in the trial, the possible synergistic or additive effects by Companion + Sumisclex

could not be confirmed. Further field trials are recommended to compare Companion + Sumisclex against Sumisclex alone, to ensure that the result can be repeated with replicated plots and under different soil types. Companion is a commercial product based on *Bacillus subtilis*, and is sold in Australia as plant root growth promoter.

The experimental biocontrol agents *Bacillus* spp. and *Lysobacter* spp., when applied at a very high rate of 60 tonnes/ha, have been shown to be highly effective in pot trials under glasshouse conditions, with up to 40% reduction in *Sclerotinia* infection (Y. Ramona, pers. comm.). The high level of disease control obtained in the preliminary glasshouse study, with extremely high rates of biocontrol-inoculated composts, is impractical on a commercial farm, and further developmental work is clearly required in improving the delivery of the biocontrol agents.

In this study, species of *Bacillus* and *Lysobacter*, inoculated and applied with composted fish waste medium, appeared to perform better than liquid cultures of the bacteria. This demonstrates the potential for recycling and making use of the abundant organic waste material for large scale biocontrol production and delivery (Ramona & Line 2002). In this system, a relatively low level of the bacterial or fungal biocontrol agent could be inoculated onto a suitable organic medium, such as composted fish waste or pulp mill waste. The bacterial biocontrol agent can then multiply and establish itself onto the organic medium, which may improve its survival on application in the field environment, and be prime for disease control.

Note, however, in a high disease pressure site, a 40% reduction in diseased plants by the best treatments with the bacteria, compared to the untreated control, still caused a loss of 22% and 25% plants due to *Sclerotinia* wilt. These losses are considered to be too high in commercial lettuce crops. It is possible however, that these biocontrol agents may be suitable for use on sites that have relatively low disease pressure, or under conditions that are less favourable to the pathogens.

6.5 The effects of green manure crops on *Sclerotinia* disease incidence on subsequent lettuce crops

Abstract

This study indicates that brassica green manure plants that produce high concentrations of biofumigants offer advantages over non-brassica green manure plants for *Sclerotinia* disease control. Fodder rapes, which produce high levels of isothiocyanates (ITCs) in their roots, were more effective for *Sclerotinia* control than mustards, which produce high levels of ITCs in their foliage. Brassica green manures did not eliminate *S. minor* inoculum in soil, and short-term disease suppression is believed to be the mode of action against *Sclerotinia*. In this study, high plant biomass and deep tap root systems, which reduce soil crusting, improved infiltration, increased soil fertility, increased organic matter and reduced sub-soil compaction. These overall soil improvements might also contribute to improved crop health and disease control.

Aims

Four trials were conducted to determine the feasibility of incorporating green manure crops in between vegetable crops for long-term disease management.

Materials & Methods

Trials 3.1 and 3.2 were conducted in 2001/02 (Table 6.13) to determine the effects of brassica and non-brassica green manures in comparison to fallow ground on *Sclerotinia* disease on subsequent lettuce crops. Trials 3.3 and 3.4 were conducted in 2002/03 (Table 6.13) to determine the suitability of different brassica green manures and a legume-oat seed mixture for use *Sclerotinia* disease management. Trial details in appendix.

Trials 3.1 & 3.4 were conducted at Forth and Cuprona, both located in north-west Tasmania, in Red Ferrosol soils, which have a relatively good structure, with friable soil that drained well when wet. Trials 3.2 & 3.3 were conducted in the same farm at Cambridge, located in south-east Tasmania, in Brown Dermosol soil, which has poor structure, formed a hard surface crust when dry, and drained slowly when wet.

Table 6.13: Treatment list for the pre-plant green manure trials

Green manure treatment		
Trial 3.2	**Trial 3.1**	**Trials 3.3 and 3.4**
Untreated Control (fallow)	Untreated Control (fallow)	10 kg/ha BQ-Mulch
18 plants/m2 Broccoli	120 kg/ha Oats	10 kg/ha BQ-Graze
250 kg/ha Broad bean	250 kg/ha Broad bean	10 kg/ha Fumus
16 kg/ha Fumus (mustard)	16 kg/ha Fumus (mustard)	10 kg/ha Nemfix
16 kg/ha BQ-Mulch (rape)	16 kg/ha BQ-Mulch (rape)	5 kg/ha BQ-Mulch + 5 kg/ha Fumus
-	-	5 kg/ha BQ-Mulch + 50 kg/ha Biomax

BQ-Mulch, BQ-Graze, Fumus and Nemfix were the brassica green manure varieties examined. These varieties were selected in plant breeding programs for high isothiocyanates (ITCs) production. The first two are fodder rapes and the latter two are mustards. Broccoli, oats and broad beans were also examined in Trials 3.1 and 3.2. Biomax, a green manure cover crop consisting of a blend of oats, beans, peas and vetch was used in Trials 3.3 and 3.4.

Green manure incorporation

When the mustard and rape crops reached full flowering stage in October and November, the green manure crops were chopped up and incorporated into soil with a rotary hoe to a depth of 25 cm. Following green manure crops, and ground preparation, iceberg lettuce was planted in December 2001 in Trial 3.1, and cos lettuce was planted in January 2002 in Trial 3.2, and in February 2003 in Trial 3.3, and January 2003 in Trial 3.4. Sumisclex fungicide (2 L/ha) was applied to the whole trial area. In Trial 3.1, the fungicide was applied at two and four weeks after planting. In Trial 3.2, the fungicide was applied only once after lettuce planting. No fungicide was applied in Trial

3.3. In Trial 3.4, 2 L/ha Sumisclex + 3 L/ha Agri-Fos 600 was also applied once after lettuce planting on to the whole trial area.

In Trial 3.1, broccoli transplants were re-sown in 23/08/01 after initial transplants were eaten by sheep, while in Trial 3.2 oats and broad beans had to be re-sown on 10/09/01 to replace seedlings eaten by birds. The brassica green manure crops were unpalatable to the animals, and hence, there were no loss of plants.

Plant analysis

At one week before incorporation, shoot and root samples of BQ-Mulch and Fumus were collected to analyse the type and levels of ITCs. About one week before rotary hoeing the biofumigant crops, shoot and root samples of BQ-Mulch and Fumus were collected and stored in a freezer. The frozen samples were later sent to the CSIRO laboratory in Perth, Western Australia to analyse the type and levels of isothiocyanates (ITCs), biocidal compounds or plant biofumigants. Sub-samples of the plant varieties were taken from each plot as they were sown, and the sub-samples from the different replicate plots in each trial were then bulked together as one large sample of roots or shoots.

In Trials 3.3 and 3.4, plant biomass was also measured on plant materials taken from two half-metre quadrants in each plot, and after drying in an oven.

Soil compaction measurements

Soil compaction was also assessed in Trials 3.3 and 3.4, by measuring soil penetration resistance with a mechanical cone soil penetrometer (3 readings from each plot), on soil at field capacity. The soil was at field capacity during the measurements. The average of soil penetration resistance at 0 to 210 mm and 215 to 410 mm was tabulated for each plot.

Sclerotia analysis

S. minor sclerotia levels in 200 g of soil from each treatment plot were determined by a wet sieving method. Soil was assessed at the beginning of the trials, just prior to the green manure crops. The soil sample was taken from a bulked sample of twenty sub-samples of soil in each plot; these samples were collected with a hand trowel to a depth of 5 cm and at 0.5 m spacing. In Trial 3.2, soil samples were also taken between lettuce plant rows at the end of the trial, after the commercial harvesting of lettuces, to determine the final sclerotia levels in the soil. In Trial 3.3, only a soil sample from one replicate plot per treatment was assessed at the end of the trial. The final sclerote populations were not determined in Trials 3.1 and 3.4, as the initial sclerote numbers were very low and almost undetectable. The overall effects of the levels of sclerotes in field soils on disease incidence are discussed in Section 7.

Disease assessments

Disease assessments were conducted as plants were close to full maturity and just before commercial harvest. The number of plants with *Sclerotinia* wilts and other disorders that affect marketability of the lettuce were counted in each plot (in 5 m x 1.2 m for Trials 3.1 to 3.3, and in 8 m x 1.8 m for Trial 3.4). The percentages of plants with disease or disorders were then tabulated from the total number of plants that were assessed.

Analysis

Statistical analysis was conducted on data values of all trials using Statgraphics Plus 4.1, and pair-wise comparisons using the least significant difference (LSD) procedure were applied to the mean values.

Results

Trial 3.1 (Forth)

Trial 3.1 was located at Forth in an intermittently cropped paddock, and soils from the trial plots had negligible levels of sclerotia (ranging from 0 to 1 sclerotia in 200 g soil). This finding was consistent with the negligible levels of *Sclerotinia* wilt on lettuces in the trial. Tipburn and bacterial soft rot were the main cause of unmarketable lettuces in Trial 3.1 (Table 6.14). Only Fumus planted prior to the lettuce caused a significant increase in the percentage of marketable lettuces (88% in Fumus treatment compared to 72% in control - fallow treatment). BQ-Mulch, broccoli and broad beans had little or no effect on tipburn and bacterial rot or marketable yield compared to the control fallow treatment.

Table 6.14: The effects of green manure crops on *Sclerotinia* disease incidence on a subsequent iceberg lettuce crop in Trial 3.1 at Forth, Tasmania

Pre-plant treatment	No. sclerotes / 200 g soil Initial count*	Total ITCs (μmole/g)		% Marketable plants**	% Unmarketable plants**		
		Root	Shoot		% Tip burn & soft rot	% Other***	% *Sclerotinia* wilt
Control - Fallow	0.0	N/a	N/a	71.9 a	24.1 a	3.5 a	0.4 a
8 plants/m^2 Broccoli	0.3	N/a	N/a	78.2 a	18.8 a	3.0 a	0.0 a
240 kg/ha Broad bean	0.0	N/a	N/a	78.5 a	19.2 a	1.3 a	0.9 a
16 kg/ha Fumus	0.3	23.35	32.22	87.8 b	8.7 a	2.6 a	0.9 a
16 kg/ha BQ-Mulch	0.0	42.23	2.69	73.5 a	22.3 a	4.2 a	0.0 a

* Initial: soil sampled prior to green manure crops.
** Within each column, means followed by the same letters are not significantly different according to LSD test ($P = 0.05$).
*** Plants rejected due to physiological disorder such as poor shape, appearance and maturity.

Bacterial rot often occurred on the inner leaves of plants that also showed tipburn symptoms. Both symptoms have been associated with plant tissue breakdown as a result of calcium deficiency. In Red Ferrosol soil, Fumus was observed to have the highest level of plant biomass compared to other green manures. Soil nutrient analysis showed there were no obvious differences in the levels of calcium between treatments (Table 6.15). Therefore, there are likely to be multiple contributing factors involved in the improvement of calcium uptake in the Fumus treatment.

Table 6.15: The effects of green manure crops on nutrient levels of soils sampled at the end of the subsequent lettuce crop in Trial 3.1 at Forth, Tasmania

Pre-plant treatment	NO3 (mg/kg)	P (mg/kg)	K (mg/kg)	S (mg/kg)	Ca (meq/100g)
Control - Fallow	29	290	720	6.4	18.00
8 plants/m^2 Broccoli	34	310	740	9.5	17.75
240 kg/ha Broad bean	69	310	840	8.3	18.00
16 kg/ha Fumus	48	300	750	14.0	17.25
16 kg/ha BQ-Mulch	37	300	790	13.0	17.25

Trials 3.2 & 3.2 (Cambridge)

Trials 3.2 and 3.3 were conducted on the same farm, and *Sclerotinia* wilt caused by *S. minor* was the only major disease. The biofumigant crops, BQ-Mulch and Fumus, significantly reduced the percentage of plants with *Sclerotinia* wilt in Trial 3.2 (Table 6.16). BQ-Mulch appeared to be more effective in reducing the percentage of wilted plants compared to Fumus. Oat and broad bean crops had little or no effect on the disease. It should be noted, however, that plants from the re-sown broad bean and oat crops to replace lost plants due to birds, were still relatively immature, when they were rotary hoed.

Table 6.16: The effects of green manure crops on *Sclerotinia* disease incidence on a subsequent lettuce crop in Trial 3.2 at Cambridge, Tasmania

Pre-plant treatment	No. sclerotes / 200 g soil*		Total ITCs (μmole/g)		% *Sclerotinia* wilted plants**
	Initial	Final	Root	Shoot	
Control - Fallow	26 ± 3	10 ± 2	N/a	N/a	30.8 c
20 kg/ha Oats	16 ± 5	-	0	0	23.2 bc
240 kg/ha Broad bean	15 ± 2	11 ± 2	0	0	24.2 bc
16 kg/ha Fumus	16 ± 3	9 ± 2	5.92	6.14	16.5 ab
16 kg/ha BQ-Mulch	25 ± 6	7 ± 1	42.23	2.69	3.1 a

* Initial: soil sampled prior to green manure crops; Final: in soil sampled at the end of lettuce crop.
** Within each column, means followed by the same letters are not significantly different according to least significance difference test (P = 0.05).

In Trial 3.3, *Sclerotinia* disease appeared late in the lettuce crop, at close to harvest. Frequent rainfall, and high humidity at about two weeks prior to harvest, created ideal field conditions for the rapid spread of *S. minor* disease. High incidence of *Sclerotinia* wilt, ranging from 24% to 31% was recorded at crop maturity (Table 6.17). There was no significant difference in the disease incidence between the different brassicas, BQ-Graze, BQ-Mulch, Fumus and Nemfix (P = 0.917). Unfortunately, an early disease assessment that might have detected differences between the green manure pre-plant treatments, was not carried out. The high disease incidence at the end of the trial might also have been compounded by the absence of any fungicide application.

Table 6.17: The plant densities, plant biomass, total ITCs concentration and *Sclerotinia* disease as affected by the pre-plant green manures in Trial 3.3 at Cambridge, Tasmania

Pre-plant treatment	No. sclerotes /200g soil* Initial (1/07/02)	No. green manure plants (/m^2)	Plant biomass (kg/m^2)	Total ITCs (μmole/g)		% *Sclerotinia* wilted plants **
				Root	Shoot	
10 kg/ha BQ-Mulch	9 ± 2	186	1.28	15.76	5.51	27.3 a
10 kg/ha BQ-Graze	14 ± 3	238	1.50	21.40	10.88	26.0 a
10 kg/ha Fumus	12 ± 6	75	0.94	3.41	7.71	29.7 a
10 kg/ha Nemfix	13 ± 4	186	1.10	4.25	14.27	31.3 a
5 kg/ha BQ-Mulch + 50 kg/ha Biomax	10 ± 5	97	0.92	N/a	N/a	24.2 a
5 kg/ha BQ-Mulch + 5 kg/ha Fumus	13 ± 3	109	0.93	N/a	N/a	31.1 a

*Initial: soil sampled prior to green manure crops
** Within each column, means followed by the same letters are not significantly different according to least significance difference test (P = 0.05).

In Trial 3.3, the BQ-Mulch + Biomax treatment resulted in mainly BQ-Mulch and oat plants. Even though Biomax contained a mixture of pea, bean, vetch and oat seed mix for plant species diversity, only the oat plants survived and competed well with BQ-Mulch plants, producing massive fibrous root systems. The combination of strong and deep tap root systems of BQ-Mulch plants, and the massive fibrous roots of oat plants, resulted in the lowest sub-soil penetration resistance (Table 6.18).

In the final assessment for sclerotia levels in soil samples in Trial 3.2, even though the BQ-Mulch and Fumus plots tended to have lower sclerotia levels, the differences between these plots and other treatments were relatively small (Table 6.16). The sclerotes in Trial 3.3 were highly variable between plots, as well as within each plot (Table 6.17). As a result of the high variability, we believe that soil sampling for sclerotes is too time consuming and is unlikely to detect any differences between treatments in the trials.

Table 6.18: The effects of pre-plant green manure treatments on top-soil and sub-soil compaction in Trial 3.3 at Cambridge, Tasmania

Pre-plant treatment	Soil penetration resistance (kPa)	
	Top-soil (Average of 0-210 mm)	Sub-soil (Average of 225-420 mm)
10 kg/ha Fumus	467	1026
10 kg/ha Nemfix	436	896
10 kg/ha BQ-Graze	471	905
10 kg/ha BQ-Mulch	509	963
5 kg/ha BQ-Mulch + 50 kg/ha Biomax	508	829
Fallow ground	552	1092

Soil improvement was noted following BQ-Mulch in Trial 3.2, with reduced surface crusting and improved infiltration. In Trial 3.3, further evidence of soil structural improvement after brassica green manures was noted with reduced soil compaction. A linear regression analysis showed a positive correlation between the average penetration resistance of sub-soil of 225 to 420 mm depth at the 90% confidence level ($P = 0.081$, r = 0.753), where increased soil compaction increased *Sclerotinia* wilt disease (Figure 6.1). This indicated that sub-soil compaction might influence plant disease, possibly through poor infiltration and impeded root growth. However, there was no correlation between the two in the top-soil of 0 to 210 mm depth ($P = 0.859$) (Figure 6.1). The lack of relationship in the top-soil is not surprising, as soil was cultivated with a rotary hoe to a depth of 25 cm in preparation.

Figure 3.1: The relationship between top-soil and sub-soil compaction and disease incidence in Trial 3.3

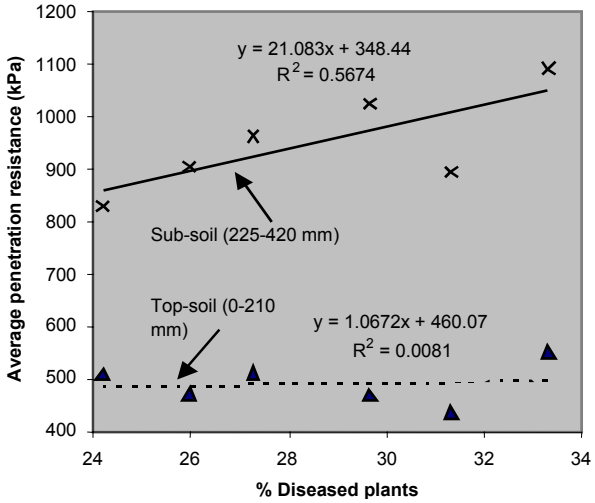


Figure 3.2: The relationship between total ITCs levels and disease incidence in Trial 3.3

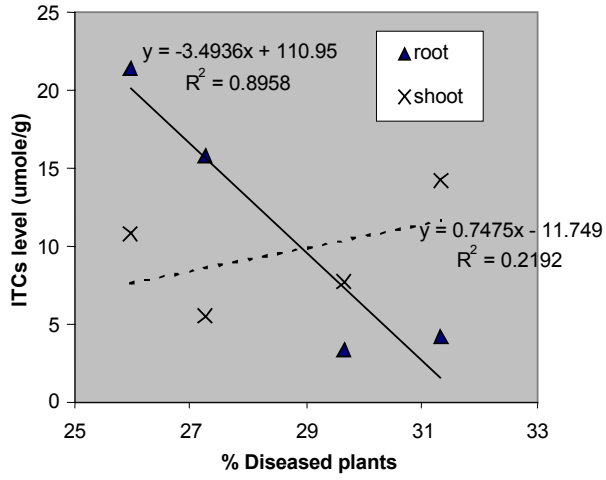
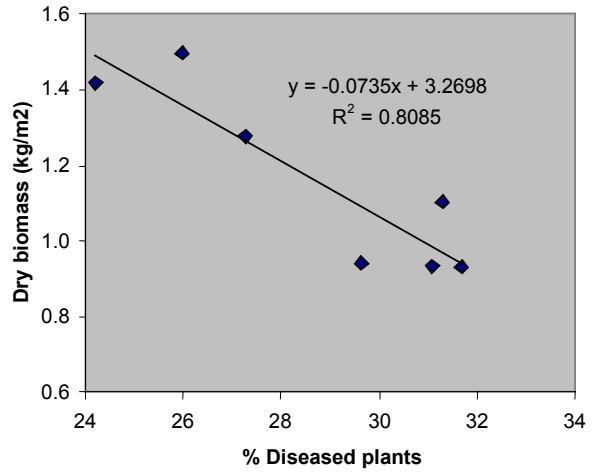


Figure 3.3: The relationship between plant biomass levels and disease incidence in Trial 3.3



With Trials 3.2 and 3.3, linear regression analysis showed that the ITCs levels in the green manure roots were negatively correlated to disease incidence ($P = 0.010$, r = -0.959 in Trial 3.2, and $P = 0.050$, r = -0.950 in Trial 3.3) (Figure 6.2). In contrast, there was no relationship between the ITCs levels in the shoots and the disease incidence ($P = 0.333$ in Trial 3.2, and $P = 0.539$ in Trial 3.3). This indicates that high levels of ITCs levels in the roots could be the main cause of disease reduction with the brassica green manure treatments.

In both trials, the fodder rape such as BQ-Graze and BQ-Mulch produced much higher levels of ITCs in the roots compared to the mustard, such as Fumus and Nemfix (Table 6.4). It is interesting that the highest plant biomasses recorded in Trial 3.4 were with the two fodder rapes BQ-Graze and BQ-Mulch, and that the plant biomass was negatively correlated to disease incidence (P = 0.006, r = -0.901) (Figure 6.3). However, this relationship could be coincidental with the suitability of fodder rapes in Brown Dermosol soil at this farm.

Trial 3.4 (Cuprona)

In Trial 3.4, there were relatively low levels of *Sclerotinia* diseased plants, ranging from 0.4% to 1.9% on matured plants just prior to harvest. The sclerote population in the trial was also very low and almost undetectable, ranging from 0 to 3 sclerotes per 200 g soil in treatment plots. *S. minor* was the cause of all *Sclerotinia* infections in Trial 3.4. As a result of the low disease incidence, there were no significant differences in the two disease assessments between treatments. However, it is interesting to note that lettuces planted in plots previously sown with BQ-Mulch, either on its own or in combination with Biomax or Fumus, tended to have lower disease than the other brassica green manures, BQ-Graze, Fumus and Nemfix. This trend was consistent with Trial 3.2, and could be associated with the higher ITCs produced in the roots of BQ-Mulch.

In contrast to Trial 3.3, higher plant biomass was evident with Fumus, and BQ-Mulch + Biomax. The high biomass in the latter treatment was due to massive oat plants. As in Trial 3.3, the BQ-Mulch + Biomax treatment in Trial 3.4 also resulted in mainly a mixture of BQ-Mulch and oat plants. In contrast, the few pea, bean or vetch plants that survived were stunted.

Table 6.19: The plant densities, plant biomass, total ITCs concentration and *Sclerotinia* disease as affected by the pre-plant green manures in Trial 3.4 at Cuprona, Tasmania.

Pre-plant treatment	No. sclerotes / 200g soil Initial*	No. green manure plants/m^2	Dry plant biomass (kg/m^2)	Total ITCs (µmole/g)		% *Sclerotinia* wilted plants**	
				Root	Shoot	(21/2/03)	(28/2/03)
10 kg/ha BQ-Mulch	0.8	186	0.85	21.85	13.37	0.0	0.4
10 kg/ha BQ-Graze	0.3	204	0.89	7.70	8.16	0.8	1.9
10 kg/ha Fumus	0.5	138	1.10	5.54	30.09	0.5	1.6
10 kg/ha Nemfix	0.3	179	0.89	4.54	17.94	0.9	1.6
5 kg/ha BQ-Mulch + 50 kg/ha Biomax	0.3	249	1.79	N/a	N/a	0.0	0.6
5 kg/ha BQ-Mulch + 5 kg/ha Fumus	0.0	189	1.00	N/a	N/a	0.0	0.8

* Initial: soil sampled prior to green manure crops.
** Means were not significantly different according analysis of variance (P = 0.05).

Discussion

Different brassica plants are known to produce different types and levels of biofumigant chemicals, collectively known as isothiocyanates (ITCs). ITCs are biocidal compounds, similar to metham sodium, are produced when glucosinolades produced in the plant tissues are hydrolysed (Fenwick et al 1983). The fodder rapes, BQ-Mulch and BQ-Graze, and two mustards, Fumus and Nemfix, are brassica green manures that are selectively bred for high ITCs production. These varieties, however, differ in the types and levels of ITCs produced in their roots and shoots. Plant pathogenic fungi have different sensitivity to the different types of ITCs (Sarwar et al 1998).

This study indicates that brassica green manure plants that produce high concentrations of biofumigants offer advantages over non-brassica green manure plants for disease control. Fodder rapes such as BQ-Mulch, which produce high levels of ITCs in their roots, are more effective for *Sclerotinia* control than mustards such as Fumus, which produce high levels of ITCs in their foliage. The different effects noted might also be related to the relatively high levels of 2-phenyl-ethyl ITC that are produced almost exclusively in root tissues (Tables 6.20 and 6.21). Different parts of the brassica plant contain different glucosinolates, producing isothiocyanates that have different properties (Stirling & Potter 1998). Leaf glucosinolates generally produce volatile ITCs such as 2-propenyl-ITC that

are lost rapidly, whereas root glucosinolates often produce non-volatile ITCs such as 2-phenylethyl-ITC that can also persist over longer periods in soil. Generally, aromatic ITCs such as benzyl ITC and 2-phenyethyl-ITC are less volatile, but more toxic, than aliphatic ITCs such as propenyl-ITC and butenyl-ITC (Sarwar et al 1998).

Brassica green manures did not eliminate *S. minor* inoculum in soil, and short-term disease suppression is believed to be the mode of action against *Sclerotinia*. As ITCs levels will diminish rapidly after incorporation into soil, their effects for disease suppression are expected to be relatively short term. Therefore, it is not surprising that the brassica green manures did not control *Sclerotinia* infection at later crop stages. Matthiessen & Kirkegaard (2002) showed that only a fraction of the glucosinolates in plant tissue converts into the toxic ITCs in soil following plant incorporation with a rotary hoe, and that improvements in maceration and incorporation techniques increased the levels of ITCs released into soil. It should be noted, however, that their studies largely concentrated on increasing ITCs from leaf tissues. The effect of different crop incorporation techniques on root ITCs release is unknown. It is also possible that non-volatile ITCs, which are released slowly or can persist in soil over long period of time, are better for soilborne disease and plant nematode control compared to volatile ITCs that degrade rapidly (Stirling & Potter 1998).

Therefore, while the concept of biofumigation with ITCs produced by brassica green manures for *Sclerotinia* control may be an attractive natural process, we must also be realistic in our expectations. As with any biological systems, the success of the biofumigation process will be subjected to variability in response to plant varieties, soil types, locations, environment, and crop management practices. Under conditions that are ideal for the *Sclerotinia* disease, fungicide control methods should also be used in conjunction with brassica green manures to ensure effective disease control. Ideally, early disease suppression resulting from brassica green manures may help eliminate one early fungicide application in the subsequent crop.

Other benefits from green manures that can produce longer lasting effects are often overlooked. In this study, high plant biomass and a deep tap root system reduced soil crusting, improved infiltration, increased organic matter and reduced sub-soil compaction. These soil improvements may contribute to disease management and crop health. Good plant growth, resulting in high plant biomass and the subsequent increase in organic matter, are important factors in reducing sub-soil compaction. All of the different brassica plant varieties performed equally well in reducing the sub-soil compaction. Poor drainage due to soil compaction will create a favourable wet soil environment for lettuce drop disease due to *Sclerotinia minor*. Improved crop health as a result of increased soil fertility and improved soil structural properties, may also help reduce plants' susceptibility to fungal infection.

Table 6.20: Plant analysis for types and levels of isothiocyanates (plant biofumigants) in Trials 3.1 and 3.2 in 2001/02.

			ITC concentrations (umole/g)								
Source - Trial	Variety	Tissue	2-Propenyl	3-Butenyl	4-Pentenyl	3-MeThiopropyl	Benzyl	4-MeThiobutyl	2-Phenylethyl	5-MeThiopentyl	Total ITC concentration
Trial 3.1-Forth	BQ-Mulch	Shoot	0.000	1.921	0.768	0.000	0.000	0.000	0.000	0.000	2.690
		Root	0.548	7.092	3.429	0.321	0.267	6.065	22.735	1.778	42.234
	Fumus	Shoot	24.720	5.772	0.000	0.000	0.000	0.000	1.730	0.000	32.222
		Root	6.536	1.396	0.000	0.507	0.000	0.000	14.913	0.000	23.351
Trial 3.2 - Cambridge	BQ-Mulch	Shoot	0.000	2.149	0.545	0.000	0.000	0.000	0.000	0.000	2.694
		Root	0.000	4.240	2.056	0.000	0.000	1.815	7.955	0.546	16.611
	Fumus	Shoot	3.687	2.076	0.000	0.000	0.000	0.000	0.384	0.000	6.148
		Root	0.596	0.504	0.000	0.000	0.000	0.000	4.824	0.000	5.923

Table 6.21: Plant analysis for types and levels of isothiocyanates (plant biofumigants) in Trials 3.3 and 3.4 in 2002/03

			ITC concentrations (umole/g)								
Source - Trial	**Variety**	**Tissue**	**2-Propenyl**	**sec-Butyl**	**3-Butenyl**	**4-Pentenyl**	**3MeThiopropyl**	**4-MeThiobutyl**	**2-Phenylethyl**	**5-MeThiopentyl**	**Total ITC concentration**
Trial 3.3 - Cambridge	BQ-Mulch	Root	0.000	0.000	6.244	2.141	0.286	1.315	5.139	0.640	15.764
		Shoot	0.000	0.000	3.999	1.374	0.000	0.000	0.140	0.000	5.513
	BQ-Graze	Root	0.321	0.000	9.862	3.491	0.231	1.693	5.125	0.674	21.398
		Shoot	0.219	0.000	7.745	2.680	0.000	0.000	0.231	0.000	10.875
	Fumus	Root	1.301	0.000	0.357	0.000	0.000	0.000	1.756	0.000	3.414
		Shoot	6.330	0.000	0.966	0.000	0.000	0.000	0.417	0.000	7.713
	Nemfix	Root	2.671	0.000	0.000	0.000	0.000	0.000	1.581	0.000	4.251
		Shoot	13.460	0.000	0.000	0.000	0.000	0.000	0.814	0.000	14.273
Trial 3.4 - Cuprona	BQ-Mulch	Root	0.000	0.000	5.716	2.538	0.333	2.185	10.446	0.630	21.847
		Shoot	0.382	0.000	9.905	2.735	0.000	0.000	0.347	0.000	13.370
	BQ-Graze	Root	0.000	0.000	1.821	0.468	0.000	0.712	4.694	0.000	7.696
		Shoot	0.000	0.000	6.331	1.506	0.000	0.000	0.319	0.000	8.156
	Fumus	Root	1.627	0.000	0.418	0.000	0.000	0.000	3.498	0.000	5.543
		Shoot	22.195	0.236	6.792	0.000	0.000	0.000	0.865	0.000	30.088
	Nemfix	Root	1.890	0.000	0.000	0.000	0.000	0.000	2.649	0.000	4.539
		Shoot	16.813	0.000	0.000	0.000	0.000	0.000	1.129	0.000	17.942
	Mix – BQ-Mulch	Root	0.000	0.000	2.479	1.261	0.000	0.539	8.480	0.156	12.915
		Shoot	0.000	0.000	3.973	0.715	0.000	0.000	0.611	0.000	5.299
	Mix – Fumus	Root	1.977	0.000	0.442	0.000	0.000	0.000	2.847	0.000	5.266
		Shoot	25.781	0.263	2.551	0.000	0.000	0.000	0.749	0.000	29.344

6.6 The effects of pre-plant soil treatments with urea, calcium cyanamide & mustard meal on *Sclerotinia* disease control

Abstract

In the laboratory test, 20 biocontrol agents and soil amendment materials were tested for their potential to kill sclerotia of *S. minor* in soil. Cold pressed mustard meal, urea and calcium cyanamide (PERLKA) were found to be highly effective in killing sclerotia of *S. minor* at very high rates (10 g/kg soil or equivalent to 16 tonnes/ha). These three materials contain or can convert into toxic compounds, which behave as soil fumigants. However, when applied at much lower rates of 500 and 1000 kg/ha in two field trials, they did not appear to eradicate the pathogen. The mustard meal and PERLKA suppressed disease at the early lettuce crop stage, when applied one day before planting. Applications of the mustard meal at six weeks before planting, had no effect in *Sclerotinia* disease control. Urea applied at 500 kg/ha, tended to result in slightly lower *Sclerotinia* disease on lettuces planted six weeks after the soil treatment. Therefore, unless very high rates are used, the mustard meal, urea and PERLKA are unlikely to be effective for use in long-term disease management, but may be useful for suppressing disease in the short-term. They are unlikely to replace post-plant fungicide applications, but could be considered for use as an additional tool for an integrated disease management practice.

Aims

A laboratory study was initially conducted to evaluate 20 different materials, including biocontrol agents and soil amendment materials, for their potential to kill sclerotia of *S. minor* in soil (Table 6.22). Consequently, the three materials that showed the greatest potential in reducing sclerotia viability in the laboratory study were examined further in two field trials in pre-plant soil treatment applications at one day and 48 days before planting for *Sclerotinia* control on lettuces (Tables 6.23 and 6.24). Trial details in appendix.

Materials & Methods

Laboratory study

The *S. minor* sclerotia used in this study were produced on sterile barley grains and were air-dried before use. The appropriate material was mixed into a sandy loam soil (3 kg), and placed in three plastic trays (1 kg soil in each tray). The tray size was 16.5 cm x 11 cm x 5 cm. Fifty black sclerotia or barley grains containing sclerotia were mixed with approximately 20 g of the treated soil and wrapped in a porous weed fibre mat, which was then buried with the rest of the treated soil in a plastic tray. There were three replicates for each treatment. Untreated field soil was used in the control.

The trays were kept in an incubator at 15^{o}C, and the soil moisture in the tray was adjusted to field capacity once a week. At 12 weeks after incubation, the bag containing buried sclerotia was removed from each tray to determine the number of viable sclerotia in the treated soil. At 22 weeks after incubation, the bag containing colonised barley grains was removed, and the barley grains were recovered, and the number of barley containing viable sclerotia was determined, and expressed as an average of sclerotia or barley grains in the three bags.

Table 6.22: Treatment list for the laboratory study

No.	Soil treatment	Application rate in 3 kg soil
1	Defatted mustard seed meal A	30 g
2	Fresh mustard seed meal B	30 g
3	Soy meal	30 g
4	Chopped broccoli roots	30 g
5	Ground Dynamic Lifter	30 g
6	Contans	0.4 g in 100 ml of water
7	BCA002	30 g
8	PERLKA	30 g
9	Serenade	0.4g mixed in 100 ml of water

10	Nutri-Life	Brew as per label instruction, then add 100 ml solution
11	Neem	0.2 g in 100 ml water
12	Sumisclex	0.2 g in 100 ml of water
13	Lime	0.2 g in 100 ml of water
14	Unpasteurised milk	30 g
15	Nubark	30 ml in 70 ml of water (100 ml)
16	Pine bark	30 g
17	Eucalyptus bark	30 g
18	Fresh eucalyptus leaf pieces	30 g
19	Chopped grass	30 g
20	Ground urea	30 g
21	Untreated Control	N/a

Note: 10 g/kg soil is equivalent to about 16,000 tonnes/ha.

Field trials

In the two field trials, the materials were broadcast onto the soil surface, and then mixed into top-soil to a depth of 5 cm with a rake. The treatments were applied at one day before lettuce planting in Trial 4.1, and 48 days before lettuce planting in Trial 4.2. Trial 4.1 was conducted on Red Ferrosol soil at Cuprona, and Trial 4.2 was conducted on Brown Dermosol soil at Cambridge. Both trial sites are known to had high levels of *Sclerotinia* disease in previous lettuce crops, and the trial design was randomised complete blocks with six replicates.

As both trials were set up within commercial crops, and plants were to be harvested for sales, the growers at the two sites also applied their regular commercial fungicide spray program for *Sclerotinia* and other disease control. Irrigation was applied in Trial 4.2 after fungicide applications, to drench the fungicides into the soil. Fortress and Sumisclex were applied for *Sclerotinia* control, while the other fungicides were applied for the control of other lettuce diseases such as *Botrytis* and anthracnose.

Table 6.23: Treatment list for Trial 4.1

No.	Pre-plant treatment			Post-plant treatment
	Treatment	Product rate/ha	Application schedule	
1	Untreated control	N/a	N/a	Fortress was applied as a drench treatment (50 ml/10L) to all seedlings, followed by foliar sprays of 2 L/ha Fortress + 100 g/ha Benlate at 2 weeks after planting, and 2L/ha Fortress + 2 L/ha Rovral at 4 week after planting.
2	Mustard meal	500 kg	One day before planting	
3	PERLKA	500 kg		

Note: Fortress has the same rate of active ingredient, procymidone, as Sumisclex

Three and two disease assessments were conducted in Trials 4.1 and 4.2, respectively, as plants were close to full maturity and just before commercial harvest. The number of plants with *Sclerotinia* wilts and other disorders that affect marketability of the lettuce were counted in each plot (3 m x 1.8 m in Trial 4.1 and 3 m x 1.2 m in Trial 4.2). The percentages of plants with disease or disorders were then tabulated from the total number of plants in the area assessed. The number of weeds in Trial 4.1 was also counted in each plot, because of claims that PERLKA could also help control weed seeds in treated soil.

Table 6.24: Treatment list for Trial 4.2

No.	Pre-plant treatment			Post-plant treatment
	Treatment	Product rate/ha	Application schedule	
1	Untreated control	N/a	N/a	2 L/ha Sumisclex was applied as foliar sprays, just after planting, and then 2 L Sumisclex + 2.2 kg/ha Dithane + 1.8 L/ha Bravo sprayed at 2 week intervals.
2	Urea	500 kg	Applied 48 days before lettuce planting	
3	Mustard meal	500 kg		
4	Mustard meal	1000 kg		

Results

Laboratory study

In the laboratory study, mustard meal A, urea and PERLKA were the three materials that consistently reduced sclerotia viability in soil and in the colonised barley grains (Table 6.25). These three materials have soil fumigating effects.

In the laboratory study, the biocontrol agents based on *C. minitans*, Contans and BCA002, appeared to have little or no effect in reducing the number of viable sclerotia in soil or in the colonised barley grains (Table 6.25). *C. minitans* is a natural fungal parasite of *Sclerotinia*, which is often isolated directly from colonised sclerotia in the field, and is believed to reduce the viability of sclerotia. It is possible that the conditions used in the study were unfavourable to the growth of *C. minitans*. It is interesting to note that in Sections 2 and 3, Trials 2.1 and 3.2 with pre-plant biocontrol treatments using Contans and BCA002, also showed little or no sclerotia reduction in the treated soils.

Table 6.25: The effects of different materials in soil treatments on sclerotia viability in a laboratory study

		No. viable sclerotia *	
No.	**Soil treatment**	**In soil after 12 weeks****	**In colonised barley grains after 22 weeks *****
1	Mustard meal A (cold pressed defatted meal)	**0**	**0**
2	Mustard meal B (ground seed meal)	38	52
3	Soy meal	42	53
4	Chopped Broccoli roots	49	51
5	Ground Dynamic lifter	49	53
6	Contans	49	43
7	BCA002	46	51
8	PERLKA	**37**	**12**
9	Serenade	51	69
10	Nutri-Life	53	48
11	Neem	50	53
12	Sumisclex	54	65
13	Lime	50	72
14	Unpasteurised Milk	50	57
15	Nubark	52	52
16	Pine bark	47	52
17	Eucalyptus bark	48	63
18	Fresh eucalyptus leaf pieces	76	53
19	Chopped grass	53	45
20	Ground urea	**5**	**0**
21	**Untreated Control**	**56**	**55**

* Average of samples from three replicates.

Field trials

In field Trial 4.1, where PERLKA and mustard meal were applied one day prior to lettuce planting, there was a reduction in *Sclerotinia* infected plants in the treated soil compared to the untreated control (Table 6.26). At 49 days after planting, there was a significantly lower percentage of *Sclerotinia* infected plants in mustard meal and PERLKA treated plots. PERLKA and mustard meal A had no obvious effects on weed numbers (Table 6.26).

Table 6.26: The effects of pre-plant soil treatments, applied one day before iceberg lettuce planting, on *Sclerotinia* disease in Trial 4.1

No.	*Soil treatment*	Product Rate/ha	No. weeds/3m^2		% *Sclerotinia* infected plants	
			38 DAP	36 DAP	49 DAP	58 DAP
1	Untreated control	N/a	14.4 a	1.4 a	6.5 b	18.1 a
2	Mustard meal A	500 kg	16.8 a	0.4 a	2.0 a	12.1 a
3	PERLKA	500 kg	18.6 a	0.0 a	2.2 a	13.9 a

Within each column, means followed by the same letters are not significantly different at the 5% level according to LSD Test.
DAP = days after planting

In Trial 4.2, where the soil treatments were applied seven weeks before lettuce planting, there was no significant difference in the percentage of infected plants between treatments at 43 (P = 0.188) and 55 (P = 0.132) days after planting (Table 6.27). Although not significant, plants in urea treated soils tended to have a lower disease incidence at 43 and 55 days after planting (Table 6.27).

No obvious difference was observed in plant growth between the treatments in Trials 4.1 and 4.2, indicating that organic matter and nitrogen due to the mustard meal, PERLKA and urea have no adverse effects on the lettuce plants.

Table 6.27: The effects of pre-plant soil treatments, applied seven weeks before cos lettuce planting, on *Sclerotinia* disease in Trial 4.2

No.	*Soil treatment*	Product Rate/ha	% *Sclerotinia* infected plants	
			43 DAP	55 DAP
1	**Untreated control**	**N/a**	**11.4 a**	**18.0 a**
2	Urea	500 kg	9.1 a	14.7 a
3	Mustard meal A	500 kg	15.9 a	20.9 a
4	Mustard meal A	1000 kg	15.0 a	22.1 a

Within each column, means followed by the same letters are not significantly different at the 5% level according to LSD Test.
DAP = days after planting

Discussion

In the laboratory study, mustard meal A, urea and PERLKA were highly effective in killing sclerotes of *S. minor* at very high rates (10 g/kg soil or equivalent to 16 tonnes/ha). All of these materials have soil fumiganting activities. In the test, sclerotes in the treated soil crumbled easily, indicating complete degradation of the affected sclerotes. It is possible that the active ingredients of the materials may have weakened the sclerotes, thereby enhance their degradation by other soil microbes.

Mustard meal A, which was defatted and cold pressed mustard meal, was effective against sclerotes. In contrast, mustard meal B, which was unprocessed, ground mustard seeds, had no effect on sclerotes. Mustard meal after the extraction of oil contains concentrated levels of ITCs (R. Harding, SARDI, pers. comm.). Mustard meal B was freshly ground seed obtained from a health food store. Tests have shown that fresh ground seed meal has very low level of ITCs (R. Harding, SARDI, per. comm.). This indicates that a very high level of ITCs is essential for eradication of sclerotes. Laboratory studies have shown that ITCs in mustard meal suppress mycelial growth of many fungal pathogens (Harding 2001).

The active ingredient of PERLKA is calcium cyanamide, which converts into calcium dihydroxide and hydrogen cyanamide in soil (Cornforth 1971, Klasse 2000). Hydrogen cyanamide decomposes in soils within several days into urea, which in turn forms ammonium and nitrate nitrogen. Hydrogen cyanamide is phytotoxic and has fungicidal properties that can inhibit soil fungi including *Sclerotinia* (Mattusch 1984). High levels of ammonium nitrogen in the soil are also believed to be toxic to soil microbes. Therefore, PERLKA is both a fertilizer and soil fumigant. Similarly, urea can convert to nitrous acid in acidic soil, or into ammonia in alkaline soil, and if present at very high concentrations, both by-products can act as soil fumigants. PERLKA was also found to have adverse effects *S. sclerotiorum,* reducing the viability of its sclerotes, as well as affecting their carpogenic germination (Huang & Sun 1991).

When applied at much lower rates (urea and PERLKA at 500 kg/ha, and mustard meal A at 500 and 1000 kg/ha) in field trials, these materials did not appear to eradicate the sclerotes of *S. minor*. Instead, mustard meal A and PERLKA, applied one day before planting, seem to suppress disease on lettuces only at the early crop stage. In the Tasmanian trial, no toxic effect was noted when these materials were applied one day before lettuce planting. In contrast, toxic effects were noted in trials conducted in Victoria (O. Villalta, pers. comm.). The lack of toxic effects may be associated to the good buffering capacity in the Red Ferrosol soil in the Tasmanian trial. These findings indicate that we must be cautious when using these materials close to planting. Accumulation of high levels of nitrogen in soil and plant tissue is also undesirable. Moreover, the toxic biocidal activities of the materials may also have adverse effects on antagonistic microbes that can help suppress soilborne plant pathogens, as well as beneficial soil microbes and invertebrates such as fungi and free-living nematodes that help recycle organic matter and nutrients, and maintain soil quality.

The defatted mustard meal, applied seven weeks before planting, had no effect on *Sclerotinia* disease control. This indicated that the rates of mustard meal, applied at 500 and 1000 kg/ha, were not high enough to kill the sclerotes, and may only be effective in suppressing the pathogen over a short period of time. Therefore, when lettuces were planted seven weeks after treatment, there was no reduction in the percentage of diseased plants. Any ITCs released by the mustard meal into soil are likely to dissipate or degrade into non-toxic by-products within several days of application. Urea, applied at 500 kg/ha, tended to result in lower *Sclerotinia* infection of lettuces planted seven weeks later. The level of disease reduction by the urea was not consistent, however, and disease suppression, rather than eradication of sclerotes, is a more likely cause. It is also possible that the top-soil treatments to 10 cm deep, with mustard meal and urea at seven weeks before lettuce planting, only affected sclerotes to 10 cm deep, and that soil preparation prior to lettuce planting might have brought sclerotes from soil below the 10 cm depth.

This study indicates that unless very high rates are used, the defatted mustard meal, urea and PERLKA are unlikely to be effective for use in long-term management over the fallow period, but may be useful for suppressing disease in the short-term. They are unlikely to replace post-plant fungicide applications, but could be considered for use as an additional tool for an integrated disease management practice.

6.7 The effects of procymidone mixture with other products in spray applications for improving disease control

Abstracts

Trials in this section were conducted to further evaluate the potential of combining Sumisclex with other products to improve *Sclerotinia* disease control. Agri-Fos and MicroGyp are low cost products, which when applied in combination with Sumisclex, consistently resulted in lower *Sclerotinia* disease incidence compared to Sumisclex alone in the two trials. Although Agri-Fos, applied alone, was also effective in reducing *Sclerotinia* disease incidence, it was not as effective as Sumisclex. Bion, on its own, increased the disease at Cambridge, but reduced the disease at Cuprona. The performance of Bion with Sumisclex was also not consistent in the two trials. Agri-Fos, Bion and MicroGyp have no direct activities against *S. minor*, but they may help reduced infection indirectly by increasing the plant's natural defence system, therefore reducing plant susceptibility to fungal infection. The seaweed extract, Natural Kelp, applied with Sumisclex showed no improvement in disease control compared to Sumisclex alone. Contans, applied with MicroGyp or Agri-Fos,also reduced the disease incidence, but to a lesser degree compared to the same products, when applied with Sumisclex.

Aims

Following promising results in preliminary trials (Section 6.3), two field trials were conducted to evaluate methods for improving *Sclerotinia* control on crops in the field after planting, using materials that can enhance natural plant defence systems, alone or with Sumisclex. These include Agri-Fos (phosphorous acid), Bion and MicroGyp (natural gypsum or calcium sulphate), and Natural Kelp (seaweed extract). The efficacy of Contans, applied with Agri-Fos or MicroGyp, was also evaluated for possible improvements in disease control.

Materials & Methods

Trials 5.1 and 5.2 were conducted within commercial crops at Cambridge and Cuprona, respectively. At planting, the farm's commercial standard fungicide treatment was applied in both trials. Sumisclex or Fortress (50 ml in 10 L water) was applied as a drench treatment onto all seedlings just before planting. Fortress and Sumisclex have the same rate and type of active ingredient, procymidone. The brand of fungicide used at each farm was the preferred fungicide used by the grower. Trial details in appendix.

The post-plant treatments are described in Tables 6.28 and 6.29. Sprays were applied using a knapsack precision sprayer fitted with 1.5 m boom at 300 kPa and 250 L/ha water. Except for the untreated control, two sprays were applied in all treatments. The first spray was applied two weeks after planting, and the second spray was applied four weeks after planting.

Table 6.28: Treatment list for Trial 5.1

No.	Treatment	Product Rate/ha	Application Schedule
1	Untreated control	N/a	N/a
2	Sumisclex	2 L	2 sprays applied with 250 L/ha water & irrigated Timing: 1st spray at 2 weeks after planting, 2nd spray at 4 weeks after planting
3	Agri-Fos	5 L	
4	Bion	200 g	
5	Agri-Fos + Contans	5 L + 4 kg	
6	Contans + MicroGyp	4 kg + 2.5 kg	
7	Sumisclex + Agri-Fos	2 L + 5 L	
8	Sumisclex + Bion	2 L + 200 g	
9	Sumisclex + MicroGyp	2 L + 2.5 kg	
10	Sumisclex + Natural Kelp	2 L + 10 L	

Trial 5.1 was conducted on cos lettuce at Cambridge and Trial 5.2 was conducted on iceberg lettuce at Cuprona. Both trial sites are known to have high levels of *Sclerotinia* disease in previous lettuce crops, and the trial designs were randomised complete blocks, with six and five replicates in Trial 5.1 and Trial 5.2, respectively.

Disease assessments were conducted at 36 days after planting in Trial 5.1, and at 42 and 55 days after planting in Trial 5.2. The number of plants with *Sclerotinia* wilts and other disorders that affect marketability of the lettuces were recorded in a 3 m x 1.2 m area in Trial 5.1, and 4 m x 1.8 m in Trial 5.2, in each plot. The percentages of plants with disease or disorders were then tabulated from the total number of plants in the area assessed. The average number of plants assessed in each plot was 58 in Trial 5.1, and 54 in Trial 5.2.

Table 6.29 Treatment list for Trial 5.2

No.	Treatment	Product Rate/ha	Application Schedule
1	Untreated control	N/a	N/a
2	Sumisclex	2 L	2 sprays applied with 260 L/ha water & irrigated at 2 week intervals
3	Agri-Fos	5 L	
4	Bion	200 g	
5	Contans + MicroGyp	4 kg + 2.5 kg	
6	Sumisclex + MicroGyp	2 L + 2.5 kg	
7	Sumisclex + Agri-Fos	2 L + 5 L	
8	Sumisclex + Bion	2 L + 200 g	

Results

Trial 5.1

Sclerotinia wilt due to *S. minor* was the only disease present in Trial 5.1. All foliar spray treatments significantly reduced *Sclerotinia* disease incidence compared to untreated control (Table 6.30). Treatments with Sumisclex (Treatments 2, 7, 8, 9 and 10) tended to be more effective than those without the fungicide. Sumisclex mixtures with MicroGyp, Bion or Agri-Fos (Treatments 9, 8 and 7, respectively) tended to be more effective than Sumisclex alone. Kelpak, applied with Sumisclex, had little or no effect on the level of disease control.

Treatments with other products, alone or in combinations, were not as effective as those with Sumisclex alone or in combination with MicroGyp, Bion and Agri-Fos. Bion and Agri-Fos, applied on their own in Treatments 4 and 3, also reduced the disease incidence compared to untreated control. There was no additional advantage in combining Agri-Fos with Contans. The level of disease control by Agri-Fos + Contans was similar to the control by Agri-Fos alone. The level of disease control noted with Contans + MicroGyp may have been due to MicroGyp alone.

Table 6.30: The effects of foliar spray treatments on *Sclerotinia* wilt incidence on cos lettuce at 36 days after planting at Cambridge in Trial 5.1

No.	Treatment	% Plants with *Sclerotinia* wilt *
9	Sumisclex + MicroGyp	1.8 a
8	Sumisclex + Bion	2.0 a
7	Sumisclex + Agri-Fos	2.4 a
10	Sumisclex + Natural Kelp	3.2 ab
2	Sumisclex	3.5 abc
6	Contans + MicroGyp	4.6 bc
3	Agri-Fos	6.5 c
5	Agri-Fos + Contans	6.6 c
4	Bion	6.7 c
1	Untreated control	13.1 d

* Means followed by the same letters are not significantly different at the 5% level according to LSD Test.
* Mean values are ranked in an ascending order.

Trial 5.2

In Trial 5.2, *Sclerotinia* wilt due to *S. minor* was also the main disease in the trial area (Table 6.31). *Botrytis* was also present, but at low and variable levels between replicate plots, ranging from 0 to 1.8% infected plants at 55 days after planting. Therefore, it was not possible to evaluate the treatment effects on the *Botrytis* disease.

At 42 days after planting, there was no significant difference in the percentage of *Sclerotinia* infected plants between all treatments ($P > 0.05$) (Table 6.31). However, there was a trend of slightly lower *Sclerotinia* disease incidence on plants treated with Sumisclex plus Agri-Fos or MicroGyp (Treatments 7 & 6, respectively) compared to Sumisclex alone (Table 6.31). At 55 days after planting, only Sumisclex + Agri-Fos significantly reduced the *Sclerotinia* wilt incidence compared to the untreated control. Plants treated with Bion only, had siginificantly higher *Sclerotinia* incidence compared to all other treatments.

Table 6.31: The effects of foliar spray treatments on *Sclerotinia* wilt incidence on iceberg lettuce plants at Cuprona in Trial 5.2.

No.	Treatment	% *Sclerotinia* infected plants*		% *Botrytis* infected plants
		42 DAP**	55 DAP	55 DAP
7	Sumisclex + Agri-Fos	1.1 a	4.8 a	0.0
6	Sumisclex + MicroGyp	1.5 a	9.0 ab	0.0
2	Sumisclex	2.2 a	9.8 ab	1.8
8	Sumisclex + Bion	3.6 a	10.3 ab	1.1
3	Agri-Fos	3.6 a	12.5 bc	0.4
5	Contans + MicroGyp	3.0 a	12.8 bc	0.7
1	Untreated control	3.7 a	13.2 bc	0.0
4	Bion	5.4 a	17.1 d	0.0

* Within each column, means followed by the same letters are not significantly different at the 5% level according to LSD Test.
* Mean values are ranked in an ascending order.
** DAP = days after planting

Discussion

In the preliminary study with Trials 1.1 and 1.2 (Section 1), one Sumisclex spray at planting followed by two sprays of Bion applied at 14 day intervals gave as good *Sclerotinia* disease control as three sprays of Sumisclex. The significance of the findings of this study, is that relatively low cost products such as Agri-Fos and MicroGyp, when applied with Sumisclex, tended to consistently result in lower *Sclerotinia* disease incidence compared to Sumisclex alone in two trials. The improvement in disease control by these products was also repeated in several field trials conducted for *S. minor* control in pyrethrum crops (Pung & Cross 2003). In addition, phosphorous acid and gypsum are also fertilizers, which do not leave any chemical residues on treated plants. Bion, however, is an agricultural chemical and its availability is dependent on its registration for use. The performance of Bion with Sumisclex also tended to be less consistent. Kelpak, applied with Sumisclex, had little or no effect on the level of disease control. The active ingredients of Kelpak are the natural phyto-hormones, auxin and cytokinin, and it has been promoted as a stimulant for plant growth, resulting in healthier plants that are more resistant to disease.

Agri-Fos and MicroGyp are believed to have no direct activities against *S. minor*, but they may help to reduce infection indirectly by increasing the plant's natural defence system, and therefore reducing plant susceptibility to fungal infection. Phosphorous acid (Agri-Fos) is an alternative compound that is also known to activate the plant's defence mechanism, while calcium in natural gypsum (MicroGyp) is vital for cell wall integrity. Oxalic acid, which is produced by *Sclerotinia* and is essential for its pathogenicity, has a high affinity for calcium ions (Cessna et al 2000). The leaching of the stabilizing calcium ions from the host plant cell walls by oxalic acid is believed to weaken plant barriers to fungal invasion (Dutton & Evans 1996). The application of calcium sulphate may have inactivated the oxalic acid by binding, and hence immobilizing, and preventing its harmful effects on plant cell barriers.

6.8 The efficacy of a new fungicide, Boscalid, and procymidone mixtures for improving disease control

Abstract

The new fungicide Boscalid (BAS510-01F) was highly effective against *S. sclerotiorum* and *S. minor* on bean and lettuce crops, respectively. Different rates of the product evaluated indicate that 800 g/ha of Boscalid may be adequate for *Sclerotinia* control. The level of disease control by Boscalid was similar to that of procymidone (Sumisclex), and it is, therefore, a suitable alternative fungicide for *Sclerotinia* control. As with previous trials in this project (Sections 1 and 5), this study also showed that further improvement in *Sclerotinia* disease control could be obtained by combining Sumisclex with materials that can improve plant health and activate plant's natural defense mechanisms. Sumisclex, applied in combination with MicroGyp, Agri-Fos and potash, tended to result in lower disease incidence than Sumisclex alone, in both trials. The improvement in disease control was more evident in the trial on beans than lettuces, due to its impact in reducing disease severity of infected bean plants. This study, therefore, confirms the additional benefits of combining low cost fertilizer products like Agri-Fos and MicroGyp with Sumisclex. Although the level of improvement in disease control by Sumisclex plus Agri-Fos or MicroGyp are considered to be relatively small, ranging from 1% to 5% of further reduction in disease plants compared to Sumisclex alone, the low cost of the latter products could still make it cost effective. Assuming that there are an estimated 16 plants/m2 or 160,000 plants/ha, a 1% improvement in disease control will result in an additional 1,600 plants/ha for sale with a total cost of $800, based on a wholesale price of $0.50 per plant. The current costs of 2 L/ha Sumisclex application is $130 per hectare, 3 L/ha Agri-Fos 600 is $17.60, and 2.5 kg/ha MicroGyp is $1.50.

Aims

Two trials were conducted to evaluate the efficacies of different rates of a new fungicide product Boscalid (BAS 510-01F) for *S. minor* and *S. sclerotiorum* control. The trials would also further examine Sumisclex and its combination with MicroGyp, Agri-Fos and potash for *Sclerotinia* control.

Materials & Methods

Trial 6.1 was conducted within a commercial green bean crop in 2002/03 (Table 6.32). Trial 6.2 was conducted within a commercial cos lettuce crop in 2003 (Table 6.33). Trial details in appendix.

Initially, in Trial 6.1, the BAS 510-01F product rates of 250, 500 and 1000 g/ha were examined. However, following the outcomes of this trial, the rates of BAS510-01F used in Trial 6.2 on lettuce were revised to 800, 1000 and 1200 g/ha.

Table 6.32: Treatment list for Trial 6.1 on a green bean crop

No.	Treatment	Application Schedule
1	Untreated control	N/a
2	250 g/ha BAS 510-01F	Three sprays applied at 41, 51 and 63 days after sowing : 1st spray applied at 5% flowering; 2nd and 3rd sprays at 7-10 day intervals
3	500 g/ha BAS 510-01F	
4	1000 g/ha BAS 510-01F	
5	1.5 L/ha Sumisclex	
6	1.5 L/ha Sumisclex + 2.5 kg/ha MicroGyp	
7	1.5 L/ha Sumisclex + 5.0 L/ha Agri-Fos 600	
8	1.5 L/ha Sumisclex + 2.5 kg/ha potash (potassium sulphate)	

Note that a new formulation of Agri-Fos (Agri-Fos 600) was used in these trials. In Trial 6.1, the Agri-Fos product rate was mistakenly applied at 5 L/ha based on the old formulation of Agri-Fos 400. In Trial 6.2, the correct rate of 3 L/ha Agri-Fos for the new formulation was used.

Table 6.33: Treatment list for Trial 6.2 on a cos lettuce crop

No.	Treatment	Application Schedule
1	Untreated control	N/a
2	800 g/ha BAS 510-01F	Two spray applications at 0 and 14 days after planting
3	1000 g/ha BAS 510-01F	
4	1200 g/ha BAS 510-01F	
5	2 L/ha Sumisclex	
6	2 L/ha Sumisclex + 2.5 kg/ha MicroGyp	
7	2 L/ha Sumisclex + 3.0 L/ha Agri-Fos 600	
8	2 L/ha Sumisclex + 2.5 kg/ha potash	

Disease assessments were conducted at 70 and 75 days after sowing in Trial 6.1, and at 45 and 51 days after planting in Trial 6.2.

In Trial 6.1, bean plants from a 5 m length of the middle row of each plot were assessed for disease incidence and severity. The disease incidence was tabulated as a percentage of the total of number of plants per plot infected by *Sclerotinia*. The average number of plants assessed in each plot was 80. The disease severity index was calculated from the disease severity ratings as the sum of the total number of diseased plants multiplied by their rating values, and divided by 3. The disease severity was assessed according to the following severity ratings:

1 = mild infection – infection of single stem, leaf or bean;
2 = moderate infection – infection of multiple stem branches;
3 = severe infection – infection affecting the whole plant.

In Trial 6.2, the total number of lettuce plants and number of *Sclerotinia* infected plants were counted within 4 m x 1.2 m in each plot. The average number of plants assessed in each plot was 79. The percentages of plants with disease were then tabulated.

All data was analysed using Statgraphics Plus, and if significantly different in the analysis of variance, the mean data was separated using the Least Significant Difference Test ($P = 0.05$). All data in both trials were log transformed before analysis.

Results

Trial 6.1

In Trial 6.1, *Sclerotinia* disease in the green bean crop was caused by *S. sclerotiorum*. In the trial, *Sclerotinia* disease became obvious late in the crop, when the crop canopy became denser and wet conditions persisted longer within the plant canopy.

Table 6.34: The effects of different fungicide treatments on bean disease incidence and severity caused by *S. sclerotiorum*

No.	Treatment	*Sclerotinia* infected plants*			
		70 DAS & 7 DAA**		75 DAS & 12 DAA	
		Severity index	% Disease incidence	Severity index	% Disease incidence
1	Untreated control	3.3 c	8.7 d	6.3 c	23.5 e
2	250 g/ha BAS 510-01F	2.7 bc	5.9 cd	6.0 c	18.9 de
3	500 g/ha BAS 510-01F	2.9 bc	7.1 cd	4.3 bc	12.7 cd
4	1000 g/ha BAS 510-01F	0.3 a	1.0 ab	0.9 a	3.2 ab
5	1.5 L/ha Sumisclex	1.1 ab	2.7 bc	1.7 ab	5.4 bc
6	1.5 L/ha Sumisclex + 2.5 kg/ha MicroGyp	0.6 a	1.3 ab	1.0 a	3.0 ab
7	1.5 L/ha Sumisclex + 5.0 L/ha Agri-Fos 600	0.1 a	0.4 a	0.6 a	2.2 a
8	1.5 L/ha Sumisclex + 2.5 kg/ha potash	0.5 a	1.3 ab	0.7 a	2.9 ab

* Within each column, means followed by the same letters are not significantly different at the 5% level according to LSD Test.
** DAS = days after sowing & DAA = days after last fungicide application

A rapid increase in disease incidence was noted on untreated control plants in the second disease assessment at 75 days after sowing (DAS) (Table 6.34). At 70 and 75 DAS, all the Sumisclex treatments applied alone or in combinations with Agri-Fos, MicroGyp and potash, were highly effective in reducing *Sclerotinia* incidence and severity (Table 6.34). Plants treated with Sumisclex + Agri-Fos had significantly lower disease incidence and severity compared to those treated with Sumisclex alone. Although not significant, the other combined treatments, Sumisclex with MicroGyp or potash, also showed similar improvements in disease control.

Increasing levels of disease control were noted with increasing rates of BAS510-01F (Table 6.34). At 70 DAS, the levels of disease on plants treated with 250 and 500 g/ha of BAS510-01F were similar to that of the untreated control plants. At 1000 g/ha, it was highly effective in reducing *Sclerotinia* incidence and severity (Table 6.34). BAS510-01F at 1000 g/ha also appeared to be more effective than Sumisclex at 2 L/ha.

Trial 6.2

In Trial 6.1, *S. minor* caused *Sclerotinia* disease in the lettuce crop. All fungicide treatments in the trial were highly effective in reducing *Sclerotinia* disease incidence, where the average percentage of infected plants was 8% in the fungicide treated plants compared to 33% on untreated plants in the final disease assessment at 51 days after planting. The high disease reduction at 20 and 26 days after the last fungicide spray applications also indicates good residual activity by Sumisclex and BAS510-01F. The levels of disease control by the three rates of BAS510-01F were similar.

Although not significantly different, there was a trend of lower disease incidence in the combined applications of Sumisclex with Agri-Fos or potash. These improvements in disease control were consistent with similar trends in disease reduction noted in Trial 6.1, as well as in other lettuce trials conducted earlier in 2001/02 (Trials 5.1 & 5.2).

Table 6.35: The effects of different fungicide treatments on lettuce disease incidence and severity caused by *S. minor*

		% *Sclerotinia* infected plants*	
No.	**Treatment**	**45 DAP & 20 DAA****	**51 DAP & 26 DAA**
1	Untreated control	23.8 b	33.3 b
2	800 g/ha BAS 510-01F	4.1 a	8.1 a
3	1000 g/ha BAS 510-01F	2.9 a	7.7 a
4	1200 g/ha BAS 510-01F	5.9 a	8.3 a
5	2 L/ha Sumisclex	5.5 a	9.5 a
6	2 L/ha Sumisclex + 2.5 kg/ha MicroGyp	6.2 a	8.6 a
7	2 L/ha Sumisclex + 3.0 L/ha Agri-Fos 600	4.2 a	7.6 a
8	2 L/ha Sumisclex + 2.5 kg/ha potash	4.6 a	7.5 a

* Within each column, means followed by the same letters are not significantly different at the 5% level according to LSD Test.
** DAS = days after sowing & DAA = days after last fungicide application

Discusssion

Sclerotinia was the only disease observed in the two trials. The disease in the bean crop was caused mainly by the lodging of flowers infected by ascospores of *S. sclerotiorum*. On the lettuce crop, the disease was caused by *S. minor* mycelium infection. The new fungicide BAS510-01F was found to be highly effective against *S. sclerotiorum* on beans at the high rate of 1000 g/ha, but was less effective at the two lower rates of 250 and 500 g/ha. In the subsequent trial on lettuces, the revised rates of BAS510-01F at 800, 1000 and 1200 g/ha, were shown to be equally effective against *S. minor*. This suggests that 800 g/ha of BAS510-01F may be adequate for *Sclerotinia* control, and that it can be effective against both species of *Sclerotinia*. At the appropriate rates, the efficacy of this fungicide for *Sclerotinia* control was similar to Sumisclex. Similar trials conducted in Victoria in this project showed that disease control by this product was repeatable in different locations and environments. Disease control by BAS510-01F was also observed in a recent trial on a pyrethrum crop against wilt disease caused by both *S. sclerotiorum* and *S. minor* in 2003. Therefore, BAS510-01F could be a suitable alternative to procymidone-based fungicides like Sumisclex and Fortress, to be used for *Sclerotinia* control in a range of crops. Furthermore, Sumisclex is a dicarboximide fungicide, while BAS510-01F is a benzanilide, a new fungicide chemical group.

As with previous trials in this project (Sections 1 and 5), this study also shows the potential of improving *Sclerotinia* disease control by combining materials that can improve plant health and activate plant's natural defense mechanisms. Sumisclex, applied in combination with MicroGyp, Agri-Fos and potash, tended to result in lower disease incidence than Sumisclex alone, in both trials. The improvement in disease control was more evident in the trial on beans than on lettuces. Interestingly, greater improvement in disease control was observed against *S. minor* by similar treatments, particularly by Sumisclex + MicroGyp, in pyrethrum crops (Pung & Cross 2003). Excellent improvement in white mould control on dry beans by calcium sulphate + benomyl, compared to benomyl alone, was also reported by Gross & Lamppa (2001). This might indicate that plants such as beans and pyrethrum, which are more tolerant to *Sclerotinia* infections than lettuces, respond better to MicroGyp and Agri-Fos. *Sclerotinia* infection on bean and pyrethrum plants may be localized and arrested in the presence of dry conditions, and the combined products might reduce its spread to other parts of the plants. In the bean trial, the reduction in disease severity was noted by the combined product treatments. In pyrethrum crops, Sumisclex + MicroGyp was found to have improved recovery of infected plants and reduce disease severity (Pung & Cross 2003). On lettuces, however, all infected plants completely wilted within two to three days after infection, and are therefore unmarketable.

Although the level of improvement in disease control by Sumisclex plus Agri-Fos or MicroGyp are considered to be relatively small, ranging from 1% to 5% of further reduction in disease plants compared to Sumisclex alone, the low cost of the latter products could still make it cost effective. Assuming that there are an estimated 16 plants/m2 or 160,000 plants/ha, a 1% improvement in disease control will result in an additional 1,600 plants/ha for sale with a total value of $800, based on a wholesale price of $0.50 per plant. The current cost of a 2 L/ha Sumisclex application is $130 per hectare, 3 L/ha Agri-Fos 600 is $17.60, and 2.5 kg/ha MicroGyp is $1.50.

6.9 Sclerotia levels and host susceptibility

Abstracts

Investigations were conducted to study seedling infections, host plant susceptibility, and soil inoculum levels. Pot trials showed that *S. minor* caused seedling damping-off, and the susceptibility of seedlings appeared to be closely associated with plant architecture. Seedlings of Rocket, BQ-Mulch and Fumus, which have lower leaves in contact with soil over a long period, were highly susceptible to infection. However, as these seedlings matured and became taller, they were no longer susceptible to *S. minor* infection. In contrast, young seedlings of onions, beets, and spinach, which have upright foliage, were less susceptible to damping-off. Plant architecture could, therefore, be a useful guide when selecting plant varieties for use in crop rotations, particularly in ground that has high *Sclerotinia* pressure. There was a positive correlation between the sclerotia levels and disease incidences on lettuces in the field. This indicated that the wet sieving method for sclerotia population may be useful for detecting high levels of sclerotes, and hence for identifying high disease pressure sites.

Materials & Methods

This section investigated the relationships between *S. minor* inoculum levels and plant disease incidence, and different host plants' susceptibility to *Sclerotinia* infection. The former also examined the levels of sclerotia in field soils and the resulting disease incidence of lettuce planted in those soils.

S. minor inoculum levels - pot trial

Pot Trial 1 was conducted to examine the effects of different levels of sclerotia and mycelium in colonised barley grains on *Sclerotinia* infection on lettuces. The trial design was complete randomised block, with four replicates for each inoculum level, and each replicate consisted of four pots (one plant per pot; pot size was 24.5 cm x 24 cm x 19 cm). Soil mix used in pots consisted of pasteurized potting mix with a slow-release fertilizer (prepared to Australian Approved Standard) and field sandy loam soil at 7:3 ratio. *S. minor* inoculum was produced on autoclaved barley grains (inoculated and incubated for 3 weeks before use). The appropriate container was first filled with soil mix, and then covered to the top 10 cm with the *Sclerotinia* inoculated soil mix. The levels of inoculum and type of inoculum used are given in Table 6.37. The fungus in the colonized barley grains consisted mainly of mycelium. Six-week-old iceberg lettuce transplants (cv. Oxley) were used for the trial. Soils in the containers were kept constantly moist every day using a sprinkler irrigator. Plants were assessed for *Sclerotinia* infection at weekly intervals until 42 days after transplanting. The average percentage of wilted plants in each inoculum level was then tabulated.

Host susceptibility

Two pot trials were conducted to examine plant host susceptibility to *S. minor* inoculum. Pot Trial 2 examined the susceptibility of brassica green manure crops (BQ-Mulch and Fumus) and rotation crops (broccoli, onion and broad beans) to *Sclerotinia* infection. Pot Trial 3 examined the susceptibility of other salad vegetables (beet, spinach, mizuna, rocket, and tatsoi) to *Sclerotinia* infections. These salad vegetables are grown in close rotations with lettuces. Pot soil was prepared as described above. The trial design for the two pot trials was complete randomized block, with four replicate pots for each plant variety. The number of seeds or transplants sown in each pot was determined by their relative sizes. In Pot Trial 2, 30 seeds of BQ-Mulch, Fumus and onion were sown, while 20 broad bean seeds and 10 broccoli transplants were sown in each pot. In Pot Trial 3, 30 seeds were sown in each pot for beet, spinach, mizuna, rocket, and tatsoi. The different plant varieties were sown in *S. minor* inoculated and uninoculated potting mix. *S. minor* inoculum consisted of dry soil infected with *S. minor* sclerotes, placed in top-soil (10 cm depth).

The number of surviving plants was assessed at 36 and 43 days after sowing or transplanting in Pot Trials 1 and 2, respectively. The percentage of reduction of surviving plants was then tabulated as follows in order to account for non-germinated seeds in the untreated control:

$$\% \text{ Reduction of plants} = 100 - 100 \times (\text{Treatment A}/\text{Treatment C})$$

Table 6.36: Treatment details of pot trials examining host plant susceptibility to *S. minor* infection

Pot trial 1	Pot trial 2	Soil treatment
Broccoli	Tatsoi	Inoculated with non-active *S. minor* sclerotia from previous lettuce pot trials
BQ-Mulch	Rocket	
Broad bean	Mizuna	
Fumus	Beet	
Onion	Spinach	
Broccoli	Tatsoi	Untreated soil – no pathogen
BQ-Mulch	Rocket	
Broad bean	Mizuna	
Fumus	Beet	
Onion	Spinach	

Field sclerotes levels

The initial sclerotes populations in the field soils at the different field sites and locations were examined for relationships between sclerotes populations and *Sclerotinia* disease incidence. *S. minor* was the causal pathogen for *Sclerotinia* disease on lettuces in all the trials. The sclerotes populations in field soils were determined in the green manure trials at Forth (Trial 3.1), Cambridge (Trials 3.2 & 3.3) and Cuprona (Trial 3.4) (Section 3). The initial soil sclerotes populations and *S. minor* disease incidence in Trial 2.1 at Cambridge was also included in the correlation analysis.

Soil was assessed for *S. minor* sclerotes in 200 g soil samples from each treatment plot at the beginning of the trials, by a wet sieving method. The soil sample was taken from a bulked sample of twenty sub-samples of soil in each plot; these samples were collected with a hand trowel to a depth of 5 cm and at a 0.5 m spacing. The average initial levels of sclerotes in the field soils were then plotted against the average *Sclerotinia* incidence on lettuces that were planted approximately six months later.

Results

Table 6.37: The effects of *S. minor* inoculum levels on *Sclerotinia* disease incidence on lettuces in a pot trial

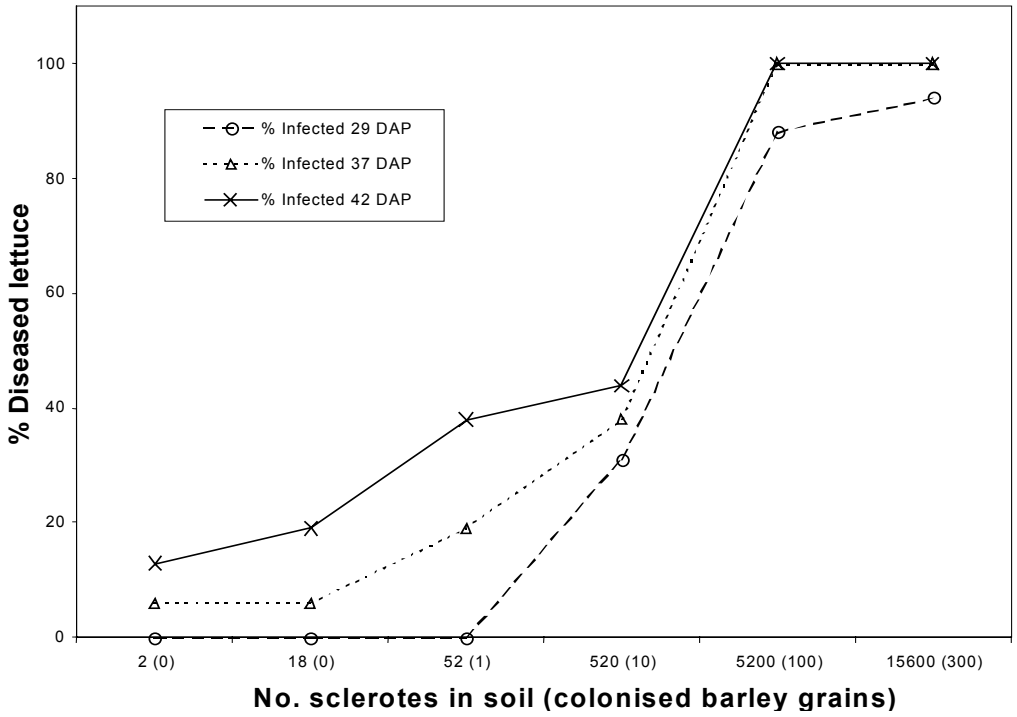
Treatment Code	No. of sclerotia	No. of colonised barley grain	% Lettuce plants infected by *S. minor*					
			7 DAP	15 DAP	23 DAP	29 DAP	37 DAP	42 DAP
A	2	0	0	0	0	0	6	13
B	18	0	0	0	0	0	6	19
C	52	1	0	0	0	6	19	38
D	520	10	6	25	31	31	38	44
E	5200	100	88	88	88	94	100	100
F	15600	300	63	94	94	94	100	100

S. minor inoculum levels - pot trial

The pot trial shows that increased sclerotes number and colonized barley grains increased the incidence of *Sclerotinia* infections on lettuce plants (Table 6.37, Figure 6.4). High levels of colonized grains (≥ 10) and sclerotes (≥ 520) caused rapid infections, with infected plants evident at 7 days after planting. In contrast, no infection was evident in soil inoculated with low levels of colonized grains (≤ 1) and sclerotes (≤ 52) until 29 days after planting. The fungal mycelium in the colonized grains was primed for active growth, and hence plant infection occurred rapidly. The increased in the number of colonized grains would have also increased their contact with the lettuce

plants. On the sclerotes however, eruptive germination must occur first, and the fungal mycelium must spread out to come in contact with the plants.

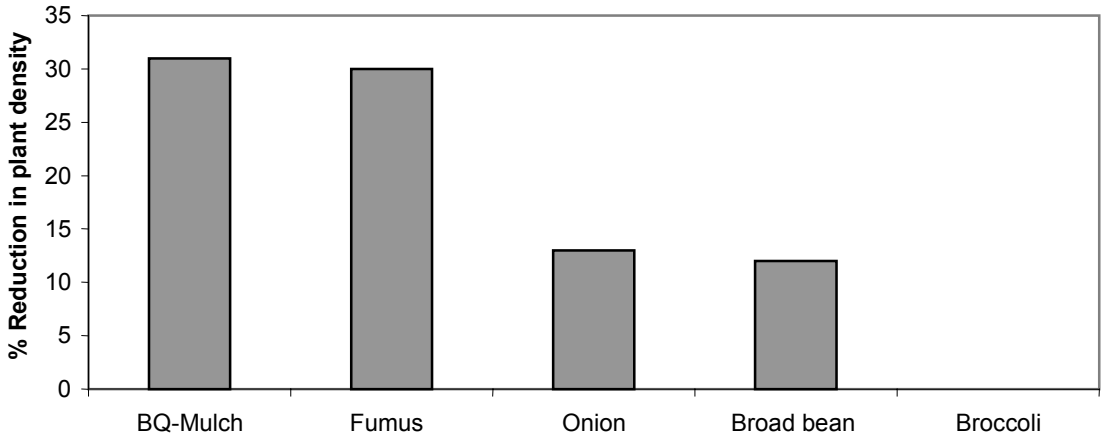
Figure 6.4: The relationship between *S. minor* inoculum and *Sclerotinia* disease incidence on lettuces in a pot trial



Host susceptibility – Pot Trials 2 & 3

In Pot Trial 2, the average plant densities for the uninoculated control were 20 broad bean, 10 broccoli, 26 onion, 27 BQ-Mulch and 22 Fumus plants per pot. In Pot Trial 3, the average plant densities were 30 beet, 26 spinach, 25 Mizuna, 26 Tatsoi and 24 Rocket plants per pot. Many of the plant varieties examined were susceptible to damping-off by *S. minor* (Figures 6.5 & 6.6).

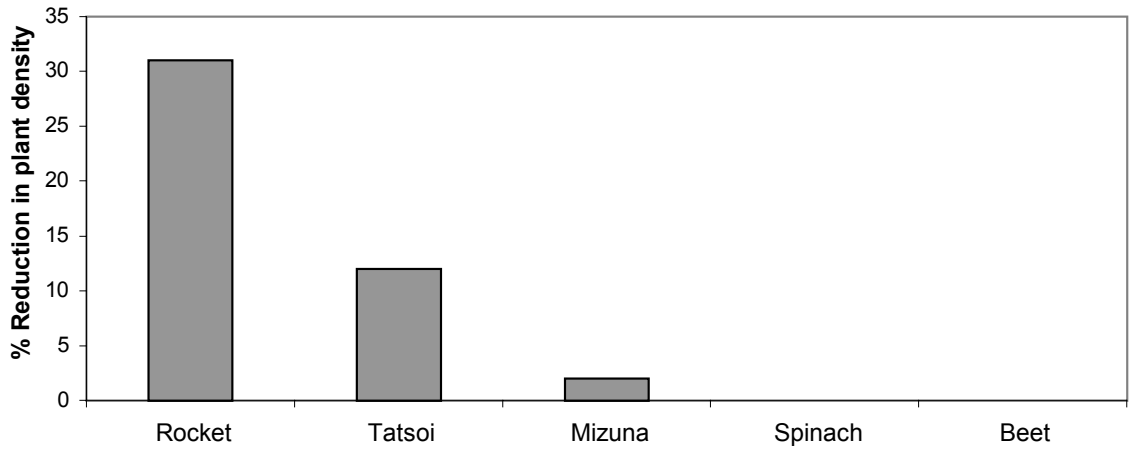
Figure 6.5: The percentage of reduction in plant density in soil inoculated with *S. minor* sclerotes at 36 days after sowing or planting in Pot Trial 2



It is interesting that the two brassica green manures specially selected for high biofumigant activities, BQ-Mulch and Fumus, are also susceptible to *S. minor* infection at the early seedling stage. The seedlings that survived the early infection became less susceptible to infection. These seedlings were most susceptible when their cotyledons were in contact with the soil surface, and therefore likely to come in contact with *S. minor* mycelium. As the seedlings mature, the separating distance between their leaves and soil reduces their susceptibility to infections.

Onion and broad bean seedling densities were less affected by *S. minor* infection, due to the upright foliage growth, where their cotyledons were not in contact with the infected soil. Similarly, broccoli transplants were not susceptible to *S. minor* infection, as there was no foliage contact with the infected soil.

Figure 6.6: The percentage of reduction in plant density in soil inoculated with *S. minor* sclerotes at 43 days after sowing in Pot Trial 3



In Pot Trial 3, among the other salad vegetables that are sown in rotation with lettuces, spinach and beet were less susceptible to *S. minor* infection. Rocket, however, was highly susceptible, followed by Tatsoi and Mizuna. Rocket, Tatsoi and Mizuna are brassica varieties. As in Pot Trial 2, seedling susceptibility could be associated to growth rate, and the length of contact between their cotyledons and infected soil.

Field sclerotes levels

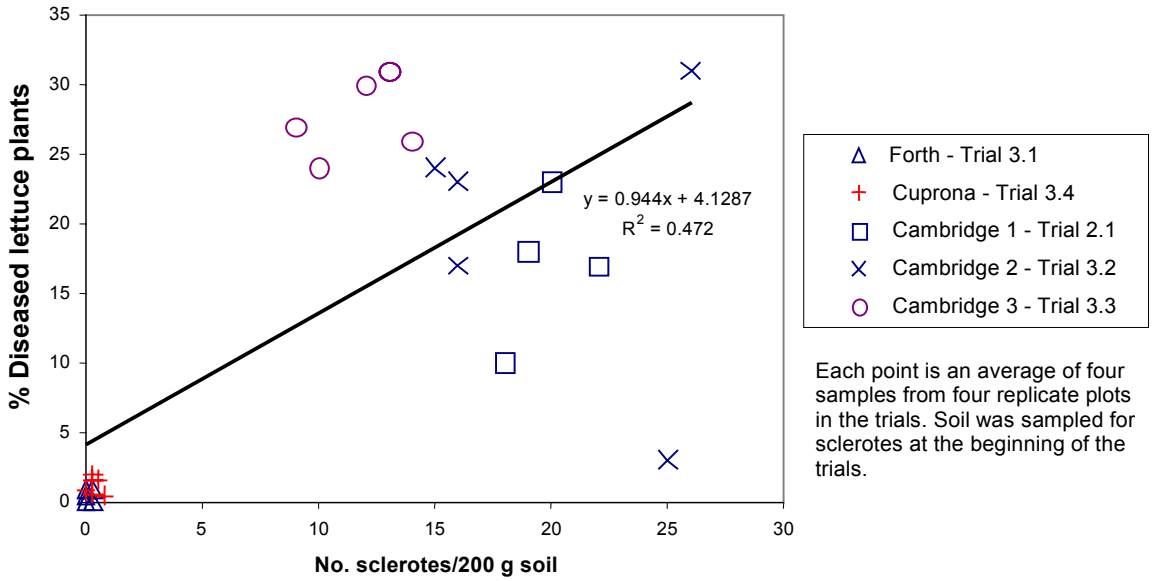
There was a relatively good linear and positive correlation between the sclerotia levels and disease incidence (r^2 = 0.472) (Figure 6.7). The sclerotia population appeared to be related to the locations and cropping history of the sites. The soil sclerotia levels determined in treatment plots at the beginning of the green manure trials showed relatively high levels of sclerotes in the three trial sites at Cambridge. With one exception, high disease incidences were always associated to the high sclerotia populations at the Cambridge trial sites (Figure 6.7). The one exception was as a result of disease suppression by BQ-Mulch crop residue. The sites at Cuprona and Forth have low levels of sclerotes, which were almost undetectable, and these sites also have very low *Sclerotinia* disease incidence (Figure 6.7). The sites at Cambridge have a long history of intensive lettuce productions, which might account for the high disease pressure. The site at Forth was not intensively cropped and has been under pasture prior to the green manure trial and lettuce crop. The Cuprona site has only been used recently for intensive vegetable production.

Apart from locations and cropping history, the different soil types may also contribute to the differences in the sclerotia population. The Brown Dermosol soil at Cambridge has a high clay content, low organic matter and poor structural properties that drained poorly when wet. In contrast, the Red Ferrosol soil at Cuprona and Forth have relatively high organic matter, and good structural properties that drain well and have good water holding capacity. Soil analysis showed 1.8%, 5.8% and 4.2% organic carbon in the soil samples at Cambridge, Cuprona and Forth.

Apart from mycelial growth from sclerotes, *S. minor* was also noted to produce apothecia on sclerotes in the lettuce crop in Trial 1.2 at Margate, where frequent wet conditions persists in a sheltered paddock. Subsequent crops in the same paddock later in the year suffered heavy losses in spite of multiple Sumisclex applications, due to widespread *Sclerotinia* infections. Although the paddock was relatively new to vegetable production, it is possible that the

widespread disease observed in the paddock later on was as a result of spread of *S. minor* through ascospores infections. Apothecia were not observed in other lettuce field trials, and infections were caused by mycelial infection at the bases of plants. In contrast, *Sclerotinia* disease due to *S. sclerotiorum* on beans was mainly associated with primary infection on flowers by ascospores.

Figure 6.7: The relationship between the levels of *S. minor* sclerotes in field soil at the different trial sites and *Sclerotinia* incidence on the subsequent lettuce crops



Discussion

It is interesting that the sclerotes inoculum in a pot trial in this study showed a time lag period before *Sclerotinia* infections on lettuce plants. This is similar to that observations in the field, where lettuce drop usually started to occur when plants reached the rosette stage, and then increased rapidly as the plants were close to harvest. The onset of the disease on lettuce crops in the field usually coincided with plant canopy closure of the space between plants, and with increasing contact of the lower leaves and soil surface.

The study on host plant susceptibility shows that *S. minor* could cause seedling damping-off. The seedling susceptibility appears to be closely associated with plant architecture, whereby seedlings that have lower leaves in contact with soil over a long period were highly susceptible to infection. In contrast, seedlings such as onions, beets, and spinach, which have upright foliage, were less susceptible. Susceptible seedling varieties included the brassica green manures, BQ-Mulch and Fumus, as well as the brassica vegetables, Rocket and Tatsoi. It should be noted, however, that as these seedlings mature and become taller, they become less susceptible to *S. minor* infection. Plant architecture could, therefore, be used as a guide when selecting plant varieties for use in crop rotations, particularly in ground that has high *Sclerotinia* pressure. Even within the same plant type, some cultivars may be less susceptible to infection due to differences in their growth habits. A good example of this is the lower susceptibility of cos lettuce compared to iceberg lettuce, to *Sclerotinia*.

There is a positive correlation between the sclerotia levels and disease incidence in field soils. This indicates that the wet sieving method for sclerotia population may be useful for detecting high levels of sclerotes, and hence for identifying high disease pressure sites. However, as a result of variability within each site, sclerotia count appears to be less useful for detecting differences between treatments in a trial. It should be noted that the threshold levels for critical sclerotia populations are likely to vary depending on crop susceptibility, the presence or absence of wet conditions during the cropping period, and soil types. It is possible that sclerotia survival and carry-over might be lower in soils with high organic matter, which also tended to have higher levels of microbes that are antagonistic to *Sclerotinia*. Meanwhile, a relatively low sclerotia level could still cause a rapid increase in disease incidence in a poorly drained soil and under wet conditions. Therefore, in determining threshold levels for sclerotia populations, local environments must also be taken into account.

Under frequent wet conditions in a sheltered paddock, *S. minor* was also noted to produce apothecia and ascospores that become airborne, hence enabling rapid spread of the pathogen. Effective disease control is difficult to achieve in

the presence of field conditions that are highly conducive to the pathogen. This indicates the importance of selecting paddocks based on their orientations and surrounding areas, that enable good air flow and rapid drying of crops, in order to minimize risk of *Sclerotinia* disease. However, infection by *S. minor* is predominantly due to mycelial infection at the base of susceptible plants. In contrast, widespread *Sclerotinia* disease due to *S. sclerotiorum* in Tasmania is frequently associated with infection by airborne ascospores. Effective disease control methods in different crops are expected to be determine by the species of *Sclerotinia* pathogen, its mode of infections, as well as crop growth and susceptible periods.

6.10 Discussion (Tasmanian trials)

Fungicide control

Since the mid 1990s, procymidone (Sumisclex) has set a new benchmark for *Sclerotinia* control in Australia. Field trials conducted in Tasmania in this project, showed that the fungicide, procymidone (sold as Sumisclex and Fortress) gave very consistent and effective control of *Sclerotinia* disease caused by *S. minor* and *S. sclerotiorum,* under high disease pressure. Most other registered fungicides, such as benomyl and iprodione, as well as many relatively new fungicides such as azoxystrobin, fluazinam, fludioxinol and tebuconozole, were found to be less effective than procymidone in the presence of high disease pressure, or conditions conducive to *Sclerotinia* pathogens in this study, as well as in a previous project (Pung & O'Brien 2000). As a result, procymidone is usually the fungicide of choice and is frequently the only fungicide used for *Sclerotinia* disease control in many crops. The sole reliance on procymidone for *Sclerotinia* control poses a risk of losing the fungicide through overuse. Although there is still no evidence of fungal resistance or enhanced degradation of procymidone, it is still good practice to use no more than two applications in a crop or to alternate its use with a fungicide from a different chemical group. Sumisclex is a dicarboximide fungicide, while BAS510-01F is a benzanilide, a new fungicide chemical group.

In Tasmania, Boscalid (BAS510-01F), a new class of fungicide, was shown to be highly effective against *S. sclerotiorum* and *S. minor* on bean, lettuce and pyrethrum crops. Different rates of the product evaluated indicated that 800 g/ha of Boscalid was adequate for *Sclerotinia* control. Under conditions that are highly favourable to *Sclerotinia* disease, the consistency and efficacy of Boscalid were similar to procymidone. Therefore, Boscalid is a suitable alternative to procymidone-based fungicides like Sumisclex and Fortress, for *Sclerotinia* control over a range of horticultural crops. Boscalid is currently being registered for use on grapes in Australia, and therefore, there is potential for extending its use for *Sclerotinia* control in horticultural crops.

Residual fungicidal effects by procymidone, when applied at the early crop stage before canopy closure, approximately 2 and 4 weeks after lettuce planting, appeared to be effective in controlling late infection. Fungicide residues in soil appeared to be critical in controlling the mycelial growth of *Sclerotinia* and in preventing lower leaf infections. Therefore, an effective fungicide application method is essential for good disease control. In Tasmania, procymidone spray application is often followed by irrigation in order to drench the fungicide into the plant base and top-soil. This method of drench application helps optimise disease control on lettuce crops. In other crops such as beans, spray timing was shown to be critical for effective control (Pung & O'Brien 2000).

Reducing plant susceptibility

Three preliminary trials conducted in Tasmania indicated that two sprays of Bion applied at 14 day intervals following procymidone at planting, gave as good *Sclerotinia* disease control as three sprays of procymidone (Sumisclex). Bion belongs to a new category of plant protection products called plant activators, which work by stimulating or inducing the development of systemic acquired resistance in plants. On its own, Bion has no direct effect on the *Sclerotinia* pathogens, and gave poor disease control. Unfortunately, the availability of Bion is uncertain and it is also expected to be a relatively expensive. However, these initial trial findings highlight the potential of harnessing and enhancing plants' own natural defense mechanisms to improve *Sclerotinia* disease control.

Further trials conducted in Tasmania revealed that two other products, Agri-Fos (phosphorus acid) and MicroGyp (natural gypsum or calcium sulphate), also showed similar improvement in disease control when applied in combination with Sumisclex. Sumisclex plus Agri-Fos or MicroGyp were shown to further reduce the percentage of diseased lettuce and bean plants by 1% to 5%, when compared to Sumisclex alone. The improvement in *Sclerotinia* disease control by these products was also repeated in field trials conducted for *S. minor* control in pyrethrum crops (Pung & Cross 2003). The use of Sumisclex mixtures with Agri-Fos or MicroGyp, provided two different modes of activity for *Sclerotinia* control. Sumisclex is highly effective in directly inhibiting the fungal pathogen. Agri-Fos and MicroGyp have no direct activities against *Sclerotinia*, but may reduced infection indirectly by increasing the plant's natural defence system and reducing plant susceptibility to fungal infection. Therefore, these two materials were not as effective as Sumisclex if it is applied on their own.

Although the level of improvement in disease control by Sumisclex plus Agri-Fos or MicroGyp are considered to be relatively small, ranging from 1% to 5% of further reduction in disease plants compared to Sumisclex alone, the low cost of the latter products could still make it cost effective. Assuming that there are an estimated 16 plants/m2 or 160,000 plants/ha, a 1% improvement in disease control will result in an additional 1,600 plants/ha for sale with a

total value of $800, based on a wholesale price of $0.50 per plant. The current cost of 2 L/ha Sumisclex application is $130 per hectare, 3 L/ha Agri-Fos 600 is $17.60, and 2.5 kg/ha MicroGyp is $1.50.

Biocontrol agents

Contans, based on *C. minitans* biocontrol fungus, was identified as the most promising potential biocontrol agent for *S. sclerotiorum* and *S. minor* control in three initial biocontrol trials conducted in Tasmania. It is a well formulated product that is easy to use and has a relatively good shelf-life. Under low disease pressure, Contans could provide early disease control when applied as a pre-plant soil treatment, drench application onto seedlings plugs prior to planting or post-plant spray applications. Its efficacy for prolonged *Sclerotinia* control however, appeared to be limited. It was less effective against actively growing *Sclerotinia* pathogens under favourable wet top-soil conditions, at the late crop stage. This might be related to the relatively slow growing *C. minitans* compared to the *Sclerotinia* pathogens. Contans had been registered for use to control *Sclerotinia* in Europe and United States of America. In Australia, unless there is severe restriction in the use of fungicides as in Europe and USA, its availability is uncertain due to competition with effective fungicides, its relatively high registration cost, and the small market size in Australia.

With other biocontrol products that are available in Australia (but not registered for disease control) and that were evaluated in this study, there were inconsistencies in the levels of disease control in different trials, indicating that further improvements in their performance are required before commercial use can be recommended.

Some of the biocontrol agents, including Contans, when applied at planting or after planting, have been noted to reduce *Sclerotinia* disease on lettuces at the early crop stage. Unfortunately, they were found to be less effective in late disease control. With commercial crops, early *Sclerotinia* infection rarely occurred early in the crop stage due to the upright seedling growth, and rapid drying of exposed top-soil by wind and sunlight. The delay in infection may also be associated with a lag period before the pathogen become active in soil and the onset of favourable conditions. Our pot trial study indicates that sclerotes in soil have a lag period of about 29 days before plants were infected. This lag period may be associated to stimuli required for eruptive germination of the sclerotes and mycelial growth. Moreover, as plants mature, their lower leaves spread out and come in contact with soil surface, covering the gaps between plants. This helps create ideal moist conditions beneath the plants for *Sclerotinia* infection as well as its rapid spread to adjacent plants.

Brassica green manures

This study indicates that brassica green manure plants that produce high concentrations of biofumigants offer advantages over non-brassica green manure plants for disease control. Fodder rapes such as BQ-Mulch, which produce high levels of ITCs in their roots, are more effective for *Sclerotinia* control than mustards such as Fumus, which produce high levels of ITCs in their foliage. These differences may be related to the different types of ITCs with different properties (Stirling & Potter 1998). Leaf glucosinolates generally produce volatile ITCs that are lost rapidly, whereas root glucosinolates often produce non-volatile but more toxic ITCs that can also persist over a longer period in soil (Sarwar et al 1998).

Apart from ITCs released by the brassica green manure, other modes of activity may also be involved. These activities could include other toxic bioactive compounds that are released or produced in soil. The fumigating activities, either by ITCs or other toxic bioactive compounds are likely to diminish rapidly after their release or formation in soil, and therefore their effects for disease suppression are expected to be relatively short term. As a result, at close to harvest, poor control of *Sclerotinia* may occur on lettuces planted after the brassica green manures. Therefore, under conditions that are ideal for the *Sclerotinia* disease, fungicide control methods should also be used in conjunction with brassica green manures for disease management.

While the concept of biofumigation with ITCs produced by brassica green manures for *Sclerotinia* control may be an attractive natural process, we must also be realistic in our expectations. As with any biological systems, the success of the biofumigation process will be subject to variability in response to plant varieties, soil types, locations, environment, and crop management practices. Under conditions that are ideal for the *Sclerotinia* disease, fungicide control methods should also be used in conjunction with brassica green manures to ensure effective disease control. Ideally, early disease suppression resulting from the brassica green manure may help reduce one or two early fungicide applications in the subsequent crop.

Other benefits from green manures that can produce longer lasting effects are often overlooked. In this study, their high plant biomass and deep tap root systems helped reduce soil crusting, improved infiltration, increased organic

matter and reduced sub-soil compaction. These overall soil improvement effects are likely to increase soil fertility and improve soil structural properties, thereby also contributing to crop health and disease management.

Soil amendments with mustard meal, PERLKA and urea

A preliminary laboratory test showed that cold pressed mustard meal, urea and PERLKA were highly effective in killing sclerotia of *S. minor* at very high rates (10 g/kg soil or equivalent to 16 tonnes/ha). The common theme among these three materials is that they contain, or can convert into, toxic compounds, which behave as soil fumigants. However, when applied at much lower rates (500 and 1000 kg/ha) in field studies, they did not eradicate the *S. minor* pathogen.

The mustard meal and PERLKA appeared to suppress disease at the early crop stage, when applied one day before planting. Urea applied as soil treatment at 500 kg/ha, six weeks before planting, did reduce *Sclerotinia* infection of the subsequent lettuce crop at the early crop stage. Like the brassica green manure, BQ-Mulch, the disease suppressive activities of these materials appear to be temporary, and higher disease incidence similar to the untreated control were recorded on lettuce plants, at close to harvest. Therefore, unless very high rates are used, the cold pressed mustard meal, urea and PERLKA are unlikely to be effective for use in long-term management over the fallow period, but may be useful for suppressing disease in the short-term. They are unlikely to replace post-plant fungicide applications, but could be considered for use as an additional tool for an integrated disease management practice. These materials must also be used with great caution as very high levels of these materials might also be toxic or adverse to crop health and beneficial soil microbes.

Detection

Sclerotinia may cause seedling damping-off, and the susceptibility of seedlings appeared to be closely associated with plant architecture. Seedlings of Rocket, BQ-Mulch and Fumus, which have lower leaves in contact with soil over a long period, were susceptible to infection. As these seedlings matured and became taller, they were no longer susceptible to *S. minor* infection.

In contrast, young seedlings of onions, beets, and spinach, which have upright foliage, were less susceptible to damping-off. Plant architecture could, therefore, be a useful guide when selecting plant varieties for use in crop rotations, particularly in ground that has high *Sclerotinia* pressure. There was a positive correlation between the sclerotia levels and disease incidences on lettuces in the field. This indicated that the wet sieving method for sclerotia population may be useful for detecting high levels of sclerotes, and hence for identifying high disease pressure sites. However, in determining the threshold levels for sclerotia populations, local environments must also be taken into account, as a rapid increase in disease incidence could occur with relatively low sclerotia populations in a poorly drained soil and under prolonged wet conditions.

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8 Appendix

Trial details (Victoria)

Table 5.22 Details of trials 1-4.

	TRIAL 1	TRIAL 2	TRIAL 3[A]	TRIAL 4
Year	2000	2000	2001	2002
Location	B. Marsh	B. Marsh	B. Marsh	B. Marsh
Soil Type	Clay loam	Clay loam	Clay loam	Clay loam
Lettuce variety	Green Mignonette 'Domino'	Green Mignonette 'Nadine'	Green butter 'Domino'	Green butter 'Extremo'
Trial Design	RCB	RCB	RCB	RCB
Replicates	7	8	8	5
Plot Size	9 m x 0.9 m	9 m x 0.9 m	9 m x 0.9 m	9 m x 1.5 m
Seedling mix	CPB	CPB	CPB	CPB
Planting date	10/05/00	24/08/00	08/02/01	24/04/02
Harvest date	29/07/00	12/11/00	19/06/01	01/07/02

A = In this trial, treatments were evaluated during two crops the first grown in autumn/winter and the second winter/spring 2001.
RCB = Randomised complete block
CPB = 85% composted pine bark, 15% Kiwi peat and macro and micronutrients (Boomaroo nursery - Hortipine™).
KPM = 60% Kiwi peat, 30% Vermicullite, 10% perlite and macro and micronutrients (Velisha Bros™, Werribee).

Table 5.23 Details of trials 5-8.

	TRIAL 5	TRIAL 6	TRIAL 7	TRIAL 8
Year	2002	2002	2003	2003
Location	B. Marsh	Werribee	Werribee	Werribee
Soil Type	Clay loam	Clay loam	Clay loam	Clay loam
Lettuce variety	Green butter 'Extremo'	Iceberg lettuce 'Green harvest'	Green butter 'Nadine'	Iceberg type 'Green harvest'
Trial Design	RCB	RCB	RCB (factorial)	RCB (factorial)
Replicates	5	6	4	4
Plot Size	9 m x 1.5 m	10.8 m x 1.3 m	5.0 m x 1.5 m	5.0 m x 1.5 m
Seedling mix	CPB	KPM	CPB	KPM
Planting date	29/08/02	21/08/02	5/09/03	5/09/03
Harvest date	30/11/02	15/11/02	15/12/03	15/12/03

RCB = Randomised complete block
CPB = 85% composted pine bark, 15% Kiwi peat and macro and micronutrients (Boomaroo nursery - Hortipine™).
KPM = 60% Kiwi peat, 30% Vermicullite, 10% perlite and macro and micronutrients (Velisha Bros™, Werribee).

Table 5.24 - Description of biocontrol and chemical treatments evaluated in trial 1 for control of lettuce drop at Bacchus Marsh Vic, winter 2000.

Treatment	Method of application and timing	Rate of application (kg/L/ha)
Untreated control	-	-
Substrate control	-	-
Sumisclex™ (procymidone)	Cup method day 0, 14, 28	2L/ha in 1000L water
Sumisclex	Cup method day 0, 14, 28	2L/ha in 2500L water
C. minitans (isolate B Vic)	Transplant, soil drench, transplant + soil drench	50L/ha (10^6 spores/g)
C. minitans (isolate A69 - NZ)	Transplant, soil drench, transplant + soil drench	50L/ha (10^6 spores/g)
C. minitans (Contans™ E Vic)	Soil drench	5kg/ha (10^6 spores/g)
T. longipile (isolate D Vic)	Transplant, soil drench	50L/ha (10^6 spores/g)
Nutri-life 4/20™	Soil drench	500g/L (10^6 spores/g)
Calcium cyanamide (Perlka™)	Soil application	300kg/ha

Table 5.25 - Description of biocontrol and chemical treatments evaluated in trial 2 for control of lettuce drop at Bacchus Marsh Vic, spring 2000.

Treatment	Method of application and timing	Rate of application (kg/L/ha)
Untreated control	-	-
Substrate control	Soil application	-
Untreated (removal)	Removal of infected plants (weekly)	-
Sumisclex™ (procymidone)	Cup method day 0, 16, 30	2L/ha in 1000L water
Sumisclex	Cup method day 0, 16, 30	2L/ha in 2500L water
C. minitans (isolate B Vic)	Spot-spray infected plants	100ml (10^6 spores/g)
C. minitans (isolate B Vic)	Transplant, soil drench, transplant + soil drench	50L/ha (10^6 spores/g)
C. minitans (isolate B Vic)	Pre-planting soil treatment	50L/ha (10^6 spores/g)
C. minitans (isolate A69 - NZ)	Transplant, soil drench, transplant + soil drench	50L/ha (10^8 spores/g)
C. minitans (Contans™ E Vic)	Soil drench	10kg/ha (10^6 spores/g)
T. hamatum (isolate 6Sr4 NZ)	Transplant, soil drench, transplant + soil drench	12kg/ha (10^6 spores/g)
T. Koningii/harzianum (TRI-D25™)	Soil drench	5.5kg/ha (10^6 spores/g)
T. viride/harzianum (Trich-A-Soil™)	Soil drench	5.5kg/ha (10^{10} spores/g)
Nutri-life 4/20™®	Soil drench	800g/L (10^6 spores/g)
Calcium cyanamide (Perlka™)	Soil application and rotary hoed	300kg/ha

Table 5.26 - Description of biocontrol and chemical treatments evaluated in trial 3 for control of lettuce drop at Bacchus Marsh Vic, autumn and winter-spring 2001.

Treatment	Method of application and timing	Rate of application (kg/L/ha)
Untreated control	-	-
Substrate control	Soil application	-
Sumisclex™ (procymidone)	Cup method day 0, 20, 42	2L/ha in 1000L water
Sumisclex	Cup method day 0, 20, 42	2L/ha in 2500L water
C. minitans (isolate B Vic)	Transplant, soil drench, transplant + soil drench	50L/ha ($2x10^7$/mL)
C. minitans (isolate A69 - NZ)	Transplant, soil drench, transplant + soil drench	50L/ha ($2x10^8$ spores/g)
C. minitans (Contans™ E)	Transplant, soil drench, transplant + soil drench	10kg/ha (10^6 spores/g)
T. hamatum (isolate 6Sr4 NZ)	Transplant, soil drench, transplant + soil drench	12kg/ha (10^6 spores/g)
T. Koningii/harzianum (TRI-D25™)	Soil drench	3kg/ha (10^6 spores/g)
T. viride/harzianum (Trich-A-Soil™)	Soil drench	3kg/ha (10^{10} spores/g)
Nutri-life 4/20™	Soil drench	1kg/ha (10^6 spores/g)
Calcium cyanamide (Perlka™)	Soil application and rotary hoed	500kg/ha

Table 5.27 - Descriptions of biocontrol and fungicide treatments evaluated in trials 4, 5 and 6 for control of lettuce drop at Bacchus Marsh and Werribee Vic, autumn and spring 2002.

Treatment	*Method of application and timing*	*Rate of application*[A] *(kg/L/ha)*
Untreated	-	-
Sumisclex™ (procymidone)	Cup method day 0, 14, 28, 42	2L/ha in 1000L water/ha
Sumisclex	Cup method day 0, 14, 28	2L/ha in 1000L water/ha
Sumisclex	Spray day 0, 14, 28	2L/ha in 1000L water/ha
Sumisclex	Cup method day 0, 14	2L/ha in 1000L water/ha
Sumisclex	Cup method day 0	2L/ha in 1000L water/ha
T. hamatum (6Sr4™)	Potting mix, transplant, drench	2kg/m^3 mix (10^6 spores/g)
T. hamatum (6Sr4™)	Potting mix, transplant	2kg/m^3 mix (10^6 spores/g)
T. hamatum (6Sr4™)	Transplant, soil drench	1 kg/100 L water drench (10^9 spores/g)
T. hamatum (6Sr4™)	Transplant	1 kg/100 L water drench (10^9 spores/g)
C. minitans (A69™)	Potting mix, transplant, drench	2kg/m^3 mix (10^6 spores/g)
C. minitans (A69™)	Potting mix, transplant	1 kg/100 L water drench (10^9 spores/g)
C. minitans (Contans™)	Transplant, soil drench	2kg/ha in 100 or 1000L water (10^6/g)
C. minitans (Contans™)	Transplant	2kg/ha in 100L water
T. koningii/harziamum (TRI-D25™)	Transplant, soil drench	1kg/ha in 100 or 1000L water ($5x10^7$/g)
T. koningii/harziamum (TRI-D25™)	Transplant	1kg/ha in 100L water
T. viride/harzianum (Trich-A-Soil™)	Transplant, soil drench	1.25kg/ha in 100 water ($2 x10^8$/g)
T. viride/harzianum (Trich-A-Soil™)	Transplant	1.25kg/ha in 100L water
B. subtilis (Companion™)	Transplant, soil drench	0.5L/400L water ha ($5.5 x 10^{10}$/g)
B. subtilis (Companion™)	Transplant	0.5L/400L water ha

Table 5.28- Descriptions of biocontrol and fungicide treatments evaluated in trials 7 and 8 for control of lettuce drop at Werribee Vic, spring 2003.

Treatment	Method of application and timing	Rate of application[A] (kg/L/ha)
Untreated	-	-
Sumisclex™ (procymidone)	Spray day 0	2.0 L/ha in 1000L water/ha
Sumisclex	Spray day 0, 30	2.0 L/ha in 1000L water/ha
BAS 510 (Boscalid™)	Spray day 0	1.0kg/ha in 1000L water/ha
BAS 510 (Boscalid™)	Spray day 0, 30	1.0kg/ha in 1000L water/ha
BAS 510 (Boscalid™)	Spray day 0, 30	0.8 kg/ha in 1000L water/ha
BAS 510 (Boscalid™)	Spray day 0, 30	1.6 kg/ha in 1000L water/ha
T. hamatum (6Sr4™)	Mix, transplant	2kg/m^3 mix; 1kg/100L water
T. hamatum (6Sr4™)	Mix, transplant, x1 soil/plant drench	2kg/m^3 mix; 1kg/100L water
T. hamatum (6Sr4™)	Mix, transplant, x2 soil/plant drench	2kg/m^3 mix; 1kg/100L water
T. hamatum (6Sr4™)	Mix, transplant, x2 soil/plant drench+x1Sumisclex	2kg/m^3 mix; 1kg/100L water
B. subtilis (Companion™)	Mix, transplant	0.5L/400L water ha
B. subtilis (Companion™)	Mix, transplant, x1 soil/plant drench	0.5L/400L water ha
B. subtilis (Companion™)	Mix, transplant, x2 soil/plant drench	0.5L/400L water ha
B. subtilis (Companion™)	Mix, transplant, x2 soil/plant drench+x1Sumisclex	0.5L/400L water ha

Table 5.36 Description of isolates of *S. minor* tested *in vitro* for their sensitivity to procymidone.

	Isolate	Host/Site	Source	History of procymidone usage
1	*S. minor* AA	Crisphead Werribee Vic	Plant	5 yrs
2	*S. minor* AA	Crisphead Werribee Vic	soil	5 yrs
3	*S. minor* AA	Crisphead Werribee Vic	soil	5 yrs
4	*S. minor* FR	Butterhead B. Marsh Vic	plant	7 yrs
5	*S. minor* SV	Crisphead Werribee Vic	plant	6 yrs
6	*S. minor* SV	Crisphead Werribee Vic	soil	6 yrs
7	*S. minor* SV	Crisphead Werribee Vic	soil	6 yrs
8	*S minor* FR	Butterhead B Marsh Vic	soil	7 yrs

Table 5.37 – Fungicide treatments used in two field trials for the control of lettuce drop and Botrytis of Green Butterhead and Red Mignonette lettuce in Bacchus Marsh Victoria spring 2002.

Treatment	Active ingredient	Chemical group	Rate a. i./ha	Rate product/ha
Sumisclex™ 500SC	procymidone	Dicarboximate	500g/ha	1L/ha
Bavistin™ 500FL	carbendazim	Benzimidazole	500g/ha	1L/ha
Rovral™ 500SC	iprodione	Dicarboximide	500g/ha	1L/ha
Teldor™ 500SC	fenhexamid	Hydroxyanilide	500g/ha	1L/ha
BAS 510™WG	boscalid	Anilines	500g/ha	1kg/ha
Scala™ 400 SC	pyrimethanil	Anilinopyrimidine	500g/ha	1.25L/ha
Sumisclex,Bavistin,Rovral	As above	As above	As above	As above

Table 5.38 Field trials details

Description	Activity
Type of trial	Field trial within commercial crop
Year	Spring 2002
Location	Bacchus Marsh
Soil Type	Clay loam
Lettuce variety	Trial 1 Red mignonette (cv 'Ember') Trial 2 Green butter type (cv 'Jerka')
Trial design	Complete randomised block
Replicates	3 of each cv/treatment
Plot size	1.5 m bed x 9 m (13.5 m2)
Plant spacing	30 cm, 144 plants/plot
Plant density	3 plant rows per 1.2 m bed
Sowing date at plant nursery	Transplants were sown in composted pine bark 80%/copra peat 20% mix plus nutrients at Boomaroo nursery. Seeds were sown with a precision air seeder.
Planting date	1 October 2002
Harvest date	15 December 2002

Table 5.42 Trials Summaries and products evaluated

	Trial 1	**Trial 2**	**Trial 3**
Year	2002	2002	2002
Location	B. Marsh	B. Marsh	B. Marsh
Soil Type	Clay loam	Clay loam	Clay loam
Lettuce variety	Green butter 'Domino'	Green butter 'Jerka'	Green butter 'Domino'
Trial Design	RCB	RCB	RCB
Replicates	4	4	4
Plot Size	9 m x 1.5 m	9 m x 1.5 m	9 m x 1.5 m
Soil treatment	17/04/02	15/08/02	02/05/02
Planting date	24/04/02	28/08/02	29/08/02
Harvest date	17/07/02	12/11/02	12/11/02

RCB = Randomised complete block

Table 5.43 Details of pre- and post-planting treatments in a field trial during autumn-winter, Bacchus Marsh 2002.

Treatment	*Application*	*Product rate (kg/L/ha)*
Untreated	-	-
Sumisclex™500F Fungicide	Sprays 0 and 14 days after planting	2L/ha
Certified green compost™	Mulch after transplanting	40t/ha
Compost ™ + Trich-A-Soil™ biocontrol	Raked into 10 cm soil/Seedling plug drench	40t/ha/1.25kg/ha
Trich-A-Soil™ Biocontrol	Seedling plug drench	1.25kg/ha/200L
Fish emulsion™ nutrient	Soil drench and raked into 10 cm soil depth	5L/ha
Calcium cyanamide Perlka™ fertiliser	Raked into 10 cm soil depth	500kg/ha
Pulverised mustard meal™ biofumigation	Raked into 10 cm soil depth	500kg/ha
Urea™ fertiliser	Raked into 10 cm soil depth	500kg/ha

Table 5.44 Details of pre- and post-planting treatments in a field trial during winter-spring, Bacchus Marsh 2002.

Treatment	**Application**	**Product rate (kg/L/ha)**
Untreated	-	-
Sumisclex™500F Fungicide	Sprays 0 and 14 days	2L/ha
Certified green compost™	Mulch, 14 days after transplanting	40t/ha
Certified green compost ™	Raked into 10 cm soil depth	40t/ha
Compost™ + Trich-A-Soil™ biocontrol	Raked into 10 cm soil, Seedling plug drench	40t/ha/1.25kg/ha
Calcium sulphate Mycrogyp™	Spray 0 and 14 days	2.5kg/ha
Calcium cyanamide Perlka™ fertiliser	Raked into 10 cm soil depth	500kg/ha
Pulverised mustard meal™ biofumigation	Raked into 10 cm soil depth	500kg/ha
Urea™ fertiliser	Raked into 10 cm soil depth	500kg/ha

Table 5.45 Details of pre-crop treatments in a field trial during winter-spring, Bacchus Marsh 2002.

Treatment	**Application**	**Product rate (kg/ha)**
Untreated	-	-
Sumisclex™500F Fungicide	Sprays 0 and 14 days after transplanting	2L/ha
Blood and bone meal fertiliser™	Raked into 10 cm soil depth	40t/ha
Chicken manure compost	Raked into 10 cm soil depth	20t/ha
Pulverised mustard meal™/biofumigation	Raked into 10 cm soil depth	500kg/ha
Urea™ fertiliser	Raked into 10 cm soil depth	500kg/ha
C. minitans (Contans™) biocontrol	Soil drench, raked into 10cm soil depth	2kg/ha

Trial Summaries (Tasmania)

	TRIAL 1.1	TRIAL 1.2	TRIAL 1.3
Type of trial	Field trial within commercial crop	Field trial within commercial crop	Pot trial
Year	2000	2000	2001
Location	Forth	Margate	Bellfield
Soil Type	Ferrosol	Sandy loam	70% Potting mix + 30% sandy loam
Lettuce variety	Iceberg lettuce (cv. Target)	Iceberg lettuce (cv. Target)	Iceberg lettuce (cv. Oxley)
Trial design	Complete randomised block	Complete randomised block	Complete randomised block
Replicates	8	8	4
Plot size	1.2 m bed x 5 m	1.2 m bed x 5 m	Each replicate consisted of 24 plants in two pots (60 cm x 40 cm x 20 cm, and 20 L soil mix). High planting density was used to create ideal conditions for *Sclerotinia* disease.
Plant spacing	30 cm	30 cm	7 cm
Plant density	3 plant rows per 1.2 m bed, 50 plants per plot	3 plant rows per 1.2 m bed, 50 plants per plot	24 plants per replicate
Sowing date at plant nursery	13/9/00	13/9/00	01/02/01
Planting date	25/10/00	31/10/00	13/03/01
Harvest date	13/12/00	29/12/00	N/a
Other details	In transplant plug treatments, the biocontrol products were applied one day before seeding onto the transplant medium (100% peat moss plus fertilizers).		*S. minor* colonized barley grains used as inoculum
Lettuce transplants	Transplants were sown in 100% peat moss on 13 September 2000at Hills Transplant Pty Ltd. Seeds were machine-sown with a precision air seeder. Each cell contained 18 ml of peat moss.		

Trial Summaries

	TRIAL 2.1	TRIAL 2.2
Type of trial	Field trial within commercial crop	Field trial within commercial crop
Year	2001/02	2002
Location	Cambridge	Cambridge
Soil type	Brown Dermosol	Brown Dermosol
Lettuce variety	Cos lettuce (Red Oak cv. Kendai)	Cos lettuce (Red Oak cv. Kendai)
Trial design	Randomised complete block	Randomised complete block
Replicates	4	6
Plot size	1.2 m x 15 m	1.2 m x 1 m
Biocontrol application date(s)	05/07/01	13/03/02 - Treatments 2 - 4 14/03/02 - Treatment 10 21/03/02 - Treatments 5 - 9
Planting date	17/01/02	21/03/02
Disease assessment date(s)	22/02/02	26/04/02 08/05/02
Harvest date	27/02/02	10/05/02
Post-plant fungicide + herbicide application	2 L/ha Sumisclex (fungicide) + 1.5 L/ha Kerb (herbicide) applied onto whole trial area just after lettuce planting, and drenched into soil with irrigation.	None

Trial Summaries

	TRIAL 3.1	TRIAL 3.2	TRIAL 3.3	TRIAL 3.4
Year	2001/02	2001/02	2002/03	2002/03
Type of trial	Field trial	Field trial	Field trial	Field trial
Location	Forth	Cambridge	Cambridge	Cuprona
Lettuce variety	Iceberg lettuce	Cos lettuce (Red Oak)	Cos lettuce (Red Oak)	Iceberg lettuce
Soil type	Red Ferrosol	Brown Dermosol	Brown Dermosol	Red Ferrosol
Trial design	RCB	RCB	RCB	RCB
Replicates	4	4	4	4
Plot size	1.2 m x 15 m	1.2 m x 15 m	1.2 m x 20 m	1.8 m x 8.5 m
Green manure sowing dates	19 - 20/07/01 23/08/01 - broccoli re-planted	05/07/01 10/09/01 - oats & broad beans re-sown	06/08/02	09/08/02
Incorporation of green manure dates	24/10/01 01/11/01 27/11/01	24/10/01 - Fumus only 15/11/01 12/12/01 16/01/02	31/10/02 12/12/02 04/02/03	12/11/02 05/12/02 16/01/03

Lettuce planting date(s)	05/12/01	17/01/02	05/02/03 (Replicates 1-3) 12/02/03 (Replicate 4)	16/01/03
Disease assessment date(s)	23/01/02 24/01/02	22/02/02 27/02/02	26/03/03	06/03/03
Other assessments	Plant samples for isothiocyanates analysis. Soil nutrient analysis.	Plant samples for isothiocyanates analysis.	Plant samples for isothiocyanates analysis and plant biomass. Measurements for soil penetration resistance. Soil nutrient analysis.	Plant samples for isothiocyanates analysis and plant biomass. Measurements for soil penetration resistance. Soil nutrient analysis.
Other comments	2 L/ha Sumisclex was applied two weeks after planting and then at 2 week intervals.	Only one 2 L/ha Sumisclex fungicide spray, applied after planting, and then drenched into soil with irrigation.	No fungicide applied.	2 L/ha Sumisclex + 3 L/ha Agri-Fos 600 applied after planting.

RCB = Randomised complete block

Trial Summaries

	LABORATORY STUDY	**TRIAL 4.1**	**TRIAL 4.2**
Type of trial	Laboratory study	Field trial	Field trial
Year	2001	2002	2002
Location	Devonport	Cuprona	Cambridge
Soil Type	Sandy loam	Red Ferrosol	Brown Dermosol
Lettuce variety	N/a	Iceberg lettuce	Cos lettuce (Red Oak)
Trial Design	RCB	RCB	RCB
Replicates	3	6	6
Plot Size	plastic tray	1.8 m x 4 m	1.2 m x 5 m
Soil treatment	06/06/01	21/01/02	25/01/02
Planting date	N/a	22/01/02	14/03/02
Harvest date	N/a	28/03/02	10/05/02
Disease assessment dates	12 weeks and 22 weeks after trial set up	27/02/02 12/03/02 21/03/02	26/04/02 08/05/02

RCB = Randomised complete block

Trial Summaries

	TRIAL 5.1	TRIAL 5.2
Year	2002	2002
Location	Cambridge	Cuprona
Soil type	Brown Dermosol	Red Ferrosol
Lettuce variety	Cos lettuce (Red Oak)	Iceberg lettuce
Trial design	Randomised complete block	Randomised complete block
Replicates	6	5
Plot size	1.2 m x 5 m	1.8 m x 5 m
Planting date	17/01/02	16/01/02
Fungicide application date	31/01/02 13/02/02	30/01/02 13/02/02
Disease assessment date	22/02/02	27/02/02 12/03/02
Commercial harvest date	27/02/02	18/03/02
Other comments	After all fungicide sprays at 300 kPa and 250 L/ha water, whole trial area irrigated for 10 mins to 6 mm water	After all fungicide sprays at 300 kPa and 260 L/ha water, whole trial area irrigated for 5 mins to 3.5 mm water

Trials Summary

	TRIAL 6.1	TRIAL 6.2
Year	2002/03	2003
Location	Merseylea, north-west Tasmania	Cambridge, southern Tasmania
Crop type	Green bean (cv. Rapier)	Cos lettuce (Red Oak cv. Kendai)
Soil type	Ferrosol	Brown Dermosol
Trial design	Randomised complete block	Randomised complete block
Replicates	6	6
Plot size	1.2 m x 6 m	1.2 m x 6 m
Planting date	13/12/02	25/02/03
Fungicide application dates	24/01/03 02/02/03 14/02/03	04/03/03 22/03/03
Disease assessment dates	21/02/03 26/02/03	11/04/03 17/04/03
Harvest date	25/02/03	18/04/03