

Development of Biological Controls for Sclerotinia Diseases of Horticultural Crops

Final Report VG00048

Horticulture Australia Project

(June 2004)

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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

Funding acknowledgments

The researchers acknowledge the financial support for this project from Horticulture Australia Limited (HAL), AUSVEG, the Federal Government and the Department of Primary Industries, Primary Industries Research Victoria.

Primary Industries Research Victoria

Knoxfield Centre



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Acknowledgments

There were many people and organisations that provided assistance to make this research possible. They include:

- Susan Pascoe, Barbara Czerniakowski, Rosa Crnov and Peta Easton (PIRVic, DPI Vic) for their invaluable assistance in conducting laboratory and field work and in compiling data. Nigel Crump for advice on soil amendments and Craig Murdoch for technology transfer.
- Field trials at Cambridge and Margate in southern Tasmania were conducted with the assistance of Dennis Patten and Lee Peterson, Serve-Ag staff based in Hobart. Other Serve-Ag staff who also assisted in this project were Peter Aird and Sarah Lamprey.
- Mark Shakelton at CSIRO Entomology, Perth, conducted plant analysis for isothiocyanates in brassica green manure tissues.
- The staff at the PIRVic, Department of Primary Industries, Knoxfield for their assistance in establishing and harvesting field trials.
- The lettuce growers in Victoria and Tasmania who graciously allowed trials on their farms and provided assistance in their establishment, maintenance and harvest.
- The commercial nurseries in Victoria and Tasmania which graciously produced seedlings for trials and allowed experiments on their glasshouses and provided assistance in their establishment and maintenance.
- Biometricians Graham Hepworth and Nam Nguyen for their input into trial design and data analysis.
- Agrochemical companies for providing samples of fungicides and other companies for supplying biocontrol products for laboratory, glasshouse and field work.
- The authors thank the members of the advisory group, Kon Koroneos, Stan Velisha, Frank Ruffo for their valuable contribution to this project.

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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 ($\leq 7\%$ 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, pesticides) 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 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) suppressive 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 occurring 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% disease 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 Sumiscler™) 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 improve 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 benzimidazole. 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.

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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.