

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

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Background

Lettuce drop, caused by *Sclerotinia minor*, can result in considerable yield losses worldwide (Adams, 1986). In Australia, yield losses ranging from 20 to 70% have been reported by growers (Porter *pers. comm.*). Control of this disease has relied on the application of a limited number of fungicides, predominantly Sumisclex, however over recent years the fungicide has been reported to be losing effectiveness. Soil sterilisation with metham sodium has been used on a limited basis and long crop rotations have reduced, but not consistently eliminated disease. There is also concern about the sustainability of fungicides and the associated problems with their continued use, including; i) damage to the environment, ii) food safety iii) development of resistance to the fungicides by *Sclerotinia*, and iv) enhanced degradation that may occur after repeated use.

Many reports have shown that experimental formulations of mycoparasitic fungi, such as *Coniothyrium minitans* and *Trichoderma* spp., have effectively controlled *Sclerotinia* diseases in controlled experiments but given unreliable results in the field (Turner and Tribe, 1976, Budge and Whipps, 1991). More recently commercial formulations of biological controls have been developed which have consistently given good control of *Sclerotinia* in New Zealand and Germany (Stewart, 2001). Development of biological controls is particularly important for sustainable control of *Sclerotinia*, because unlike fungicides, they can directly eradicate sclerotia from soils.

The objective of this cooperative Trans-Tasman research program was to compare the effectiveness of commercial formulations of biological controls available worldwide against a range of other nutrient, organic and chemical treatments for their efficacy of control of *Sclerotinia* diseases of lettuce and other horticultural crops. Within the project, a number of control strategies will be evaluated that seek to minimise the application of fungicides currently used for *Sclerotinia* control. These strategies include the use of an integrated control program that uses biocontrols to reduce/eradicate *S. minor* in the soil followed up by a reduced fungicide program for effective disease control. The results from the last two years of glasshouse experiments and field trials in Victoria and Tasmania are presented.

1. Biological versus chemical control

In field trials in Victoria, seven biocontrol agents applied to the soil using various methods of application were compared to the fungicide Sumisclex for their efficacy against *Sclerotinia minor*. In one field trial where disease incidence at harvest was low (<6%), some of the seven biocontrol products showed some potential for control of *Sclerotinia* on lettuce. In another trial where the disease incidence at harvest was very high (65%), Sumisclex sprays strategically applied in either 1000 or 2500 L water/ha were the best treatment for control of *Sclerotinia* (Fig. 1).

In Victoria, of the seven commercial formulations of biocontrol agents applied as transplant and soil treatments, only one (*Trichoderma* sp.) reduced lettuce drop incidence, compared to the untreated control but this level of disease control was not as good as with Sumisclex (Fig. 1). In a field trial in Tasmania, the biocontrol agent *Coniothyrium minitans*, applied as a pre-plant soil treatment six months before planting, significantly reduced *Sclerotinia* lettuce drop when compared to the untreated control. In another field experiment conducted in Bacchus Marsh Victoria, *S. minor* sclerotial germination was lowered significantly by *C. minitans* compared with the untreated control (Peta Easton *pers. comm.*) (Table 1).

Fig. 1. Effect of biological control agents and Sumisclex treatments on *Sclerotinia* lettuce drop incidence (%) at harvest in Victoria 2001.

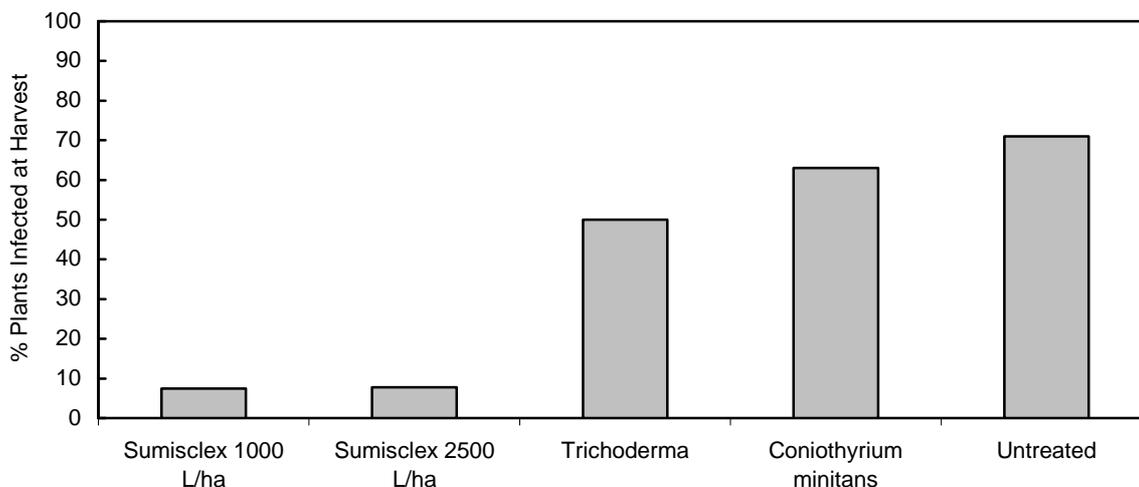


Table 1. The effect of *C. minitans* biocontrol on the viability of *S. minor* sclerotial germination.

| Treatment | Germination |
|--|-------------|
| Untreated control | 54.15% |
| <i>C. minitans</i> , soil | 15.85% |
| <i>C. minitans</i> , transplant + soil | 33.35% |

2. Resistance of *Sclerotinia* to the fungicide Sumisclex.

Ten *Sclerotinia minor* isolates collected in Victoria were tested *in vitro* for their sensitivity to Sumisclex (procymidone). The results showed that mycelial growth in all ten isolates tested was inhibited by lower doses of procymidone (<0.1 µg/ml) than those recommended for field use. This result suggests that recent reported losses of effectiveness of procymidone in lettuce crops in Victoria may be due to poor application by growers or enhanced soil breakdown of the fungicide rather than fungicide resistance.



Photo: *S. minor* mycelial growth and sclerotial production on PDA media, amended with procymidone (Sumisclex), 1-week after incubation at room temperature.

3. Post-planting foliar sprays

In field trials in Tasmania, two foliar sprays of Sumisclex, either applied alone or in combination with different chemicals, were applied to lettuce plants using 250L/ha water. The 1st spray was applied after planting and the 2nd spray applied 2 weeks later. The results showed that plants treated with mixtures of Sumisclex and calcium sulphate or Agri-Fos (phosphorus acid) had lower disease incidence compared to plants treated with Sumisclex alone (Table 2).

These results showed that calcium sulphate and Agri-Fos appeared to improve *Sclerotinia* lettuce drop control when applied with Sumisclex. These two products have no direct activities against *S. minor*, but they could be providing some control effect by increasing the plant's natural defence system and hence reducing plant susceptibility to fungal infection. This aspect of the research requires further investigation.

Table 2. Effect of Sumisclex sprays applied alone or in combination with different chemicals on the % of plants infected with Lettuce drop.

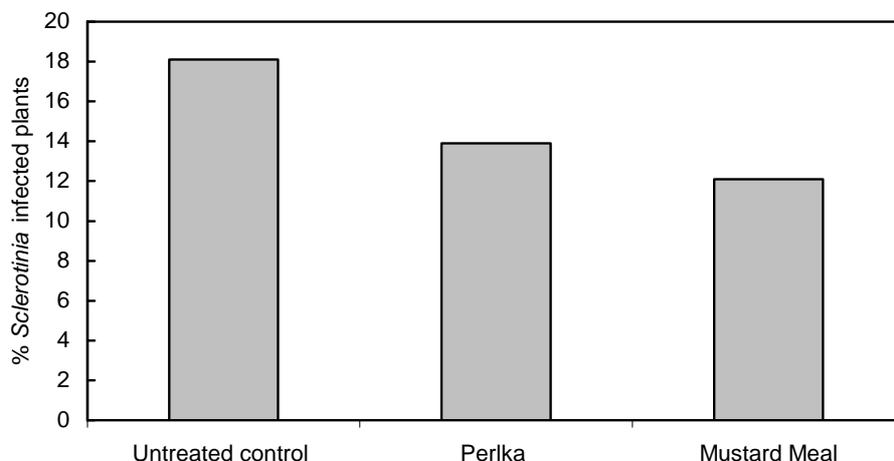
| Treatment | % Plants with disease |
|------------------------------|-----------------------|
| Sumisclex + Calcium sulphate | 1.8 a |
| Sumisclex + Bion | 2.0 a |
| Sumisclex + Agri-Fos | 2.4 a |
| Sumisclex + Natural Kelp | 3.2 ab |
| Sumisclex | 3.5 abc |
| Agri-Fos | 6.5 c |
| Bion | 6.7 c |
| Untreated control | 13.1 d |

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

4. Soil amendments

In a field trial in Tasmania, lettuces planted in soil treated with cold pressed mustard meal and calcium cyanamide (Perlka™) had marginally lower levels of *Sclerotinia* disease (Fig. 2). Laboratory tests showed that these two materials killed sclerotia of *S. minor* in soil.

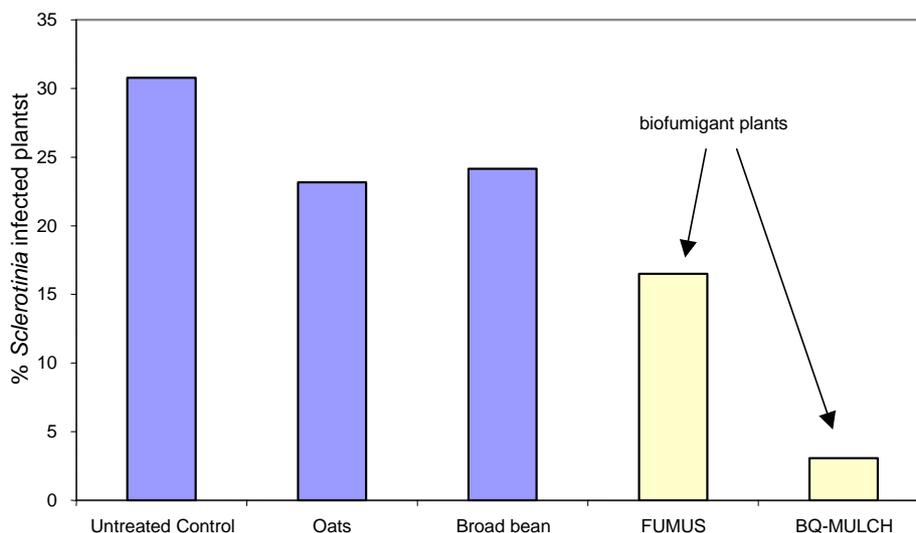
Fig. 2. Effect of pre-plant soil treatments on the incidence of *Sclerotinia* lettuce drop on iceberg lettuce.



5. Biofumigant crops

In trials in Tasmania, the biofumigant crops, BQ-MULCH and FUMUS, significantly reduced the percentage of plants with *Sclerotinia* disease (Fig. 3). BQ-MULCH appeared to be more effective in reducing the percentage of diseased plants compared to FUMUS. These two green manure crops were sown in winter, mulched and rotary hoed into the ground in October and November, and the lettuce crop planted in summer.

Fig. 3 Effects of green manure crops on the incidence of *Sclerotinia* on cos lettuce



6. Crop susceptibility to *Sclerotinia*

A better understanding of crop susceptibility to *Sclerotinia* will enable growers to determine the suitability of crops for use in rotations as part of *Sclerotinia* disease management. In a pot trial in Tasmania, seedlings of broad bean, beet, onion, spinach, tatsoi and mizuna, were relatively tolerant to *Sclerotinia*. The brassica plants, canola (BQ-Mulch), mustard (FUMUS) and rocket, were susceptible to *Sclerotinia* wilt at the early seedling stage.

In glasshouse and field experiments, the lower leaves of lettuce plants were found to be an important mode of infection for *S. minor*. Plastic film acting as barrier to minimise contact between lettuce leaves and soil reduced disease incidence from 80% to 30%, while in the greenhouse 50% to 60% of plants were infected after the lettuce leaves were held onto soil inoculated with sclerotia of *S. minor* (Easton *pers. comm*).

Summary

Field trials in Victoria have shown that disease control levels obtained with the biological control agents *Trichoderma* and *Coniothyrium minitans* were not as good as those obtained in New Zealand. This could be due to either poor survival of the biocontrol agents in the new commercial formulations being developed, or lack of survival of the biocontrols in the warmer soil conditions experienced at the time of application in Australian soils. The lack of survival could also be due to the lower level of organic matter found in Victorian soils where disease occurs.

To address these issues, a series of laboratory and field studies have been conducted in Victoria and at Lincoln University in New Zealand to ensure that biological control agents are adequately formulated and applied into grower's fields when conditions are optimal. These biological studies include the evaluation of i) the optimum conditions for biocontrol growth, ii) population densities required to maintain biocontrol effect, and iii) new formulations and application regimes for effective biocontrol effect. In addition, glasshouse and field studies are currently evaluating the use of organic soil amendments that either inhibit/kill sclerotia or change soil conditions so that microbial control is enhanced.

Next steps

- Development of a biological control strategy for *Sclerotinia minor* is still of high priority as there are no new chemicals besides procymidone (Sumisclex) in Australia that are sufficiently effective for control.
- More trials are required, however, before wide spread adoption of biocontrols can be accepted by industry. Therefore, efforts for the remainder of the project will be directed towards improving formulation technology, product delivery and field effectiveness of biological controls.
- In total 6 new field trials are being conducted in Victoria and Tasmania during the 2001-2002 season to evaluate cultural, chemical, biocontrol products and biofumigant crops (mustard, canola, and broccoli) for control of *Sclerotinia* disease of lettuce. In the trials in Victoria, new biocontrol formulations developed in NZ will be compared to commercial biocontrol products from Australia, Germany and USA.
- Field trials have also been established in Werribee Victoria to determine *Sclerotinia* population thresholds in soil to optimise the number and timing of Sumisclex sprays for *S. minor*.
- The new trials will also focus on evaluating soil amendments (organic and chemical) that either inhibit/kill sclerotia in the soil or change soil conditions so microbial control is enhanced. Addition of organic soil amendments with biocontrol agents will also be evaluated to enhance the activity of promising biocontrol agents.
- There will also be wider evaluation and adoption of the improved method to apply Sumisclex this season because of the larger scale of field trials that are being conducted throughout Victoria and Tasmania.
- In addition, integration of a range of potential new treatments, including chemicals, biofumigant crops and crops tolerant to *Sclerotinia*, will be evaluated in field trials in order to broaden the options available for improved management of *Sclerotinia* diseases.

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