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Improvement in lettuce quality by reduction in losses due to soil borne diseases

Dominie Wright
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VG99015

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Horticulture Australia

FINAL REPORT

HORTICULTURE AUSTRALIA PROJECT VG99015

IMPROVEMENT IN LETTUCE QUALITY BY REDUCTION IN LOSSES DUE TO SOIL-BORNE DISEASES

Ms Dominie Wright *et al.*

Department of Agriculture, Western Australia

Tasmanian Institute of Agricultural Research, Tasmania

Department of Agriculture
Government of Western Australia

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Horticulture Australia

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HORTICULTURE AUSTRALIA PROJECT VG99015

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This is the final report of project VG 99015 Improvement in lettuce quality by reduction in losses due to soil borne diseases. It covers research into the control of Sclerotinia lettuce drop, and lettuce big vein disease and integrated disease control.

January 2004

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Media summary

Lettuce (*Lactuca sativa* L.) production is an important component of the Australian fresh vegetable industry, having in 1999 a gross value of $89.1 M with export earnings of $6.7 M over the same year. Nationally, two of the main market issues for Australian lettuce producers are: static fresh lettuce exports to south-east Asia, and crop losses due to microbial diseases such as Sclerotinia lettuce drop (SLD) and Lettuce big vein (LBV).

SLD can be controlled to some extent, through the use of fungicides. The current project examined the use of fungicide alternatives for SLD control. Potential use of a biological control agent was investigated, and soil amendment strategies (eg lime, compost) were compared with the use of registered chemicals to control SLD during crop production. This work parallelled research in Tasmania. These alternatives to fungicides were not promising in Western Australia. However, the results from experiments conducted in Tasmania were quite different. The use of calcium hydroxide (with soil conditioning polymers) applied to the surface of the soil at a rate of 2.5 t/ha maintained the soil pH above 8.5 and resulted in a significant reduction (85%) in disease severity. Further experiments showed that an integrated approach using the calcium hydroxide treatment combined with strategies such as fungicides, rotation and drip irrigation offer an opportunity for enhanced and sustainable disease control of *Sclerotinia minor* in lettuce.

Lettuce big vein disease (LBVD) is a widespread virus disease problem for lettuce production in Australia. The viral disease complex vector is the soil-borne root-infecting fungus, *Olpidium brassicae*. Management of LBVD is difficult as the virus can persist in soils with the fungal vector for extended periods (up to 20 years) in absence of the host. Furthermore, symptom expression is environmentally driven. Nursery grown seedlings are commonly symptomless but infected, thus nursery producers unwittingly spread infections if careful hygiene practices are not maintained. In Western Australia, over 90% of the winter grown crops show symptoms and in a Perth seedling nursery LBVD incidence was considerable. In Perth, new land for lettuce production was rapidly becoming contaminated with LBVD by the introduction of infected transplants from nurseries. Surveys of Tasmanian nurseries showed little evidence of LBVD within seedling stocks. A possible link between nursery hygiene and sporadic occurrences of LBVD is discussed for the Tasmanian work.

In Tasmania other studies showed compost, oat cover crops, and fumigation with Metham sodium increases the incidence of LBV disease contrary to expectations. In Western Australia, an integrated disease management strategy was devised for: lettuce seedling nurseries, growers producing lettuce on virgin land, growers producing lettuce on old infested land and growers producing lettuce using hydroponics.

Technical Summary

Lettuce (*Lactuca sativa* L.) production is an important component of the Australian fresh vegetable industry, having a gross value in 1999 of $89.1 M, with export earnings of $6.7 M over the same year. Nationally, two main market issues for Australian lettuce producers are; static fresh lettuce exports to south-east Asia, and crop losses due to microbial diseases such as Sclerotinia lettuce drop (SLD) and Lettuce big vein (LBV).

Sclerotinia Lettuce Drop (SLD) is caused by either *Sclerotinia minor* or *Sclerotinia sclerotiorum*, although *S. minor* tends to predominate. *S. minor* has small (0.5-2.0 mm diameter) sclerotia that seldom produce apothecia, and thus field infection occurs predominantly by direct infection from germinating sclerotia. Studies examining the importance of sclerotia from differing soil depths toward disease have shown that those within the top few centimetres of soil are responsible for the vast majority of infections, which typically occur at the root crown inducing a collar rot symptom, leading to eventual collapse of the whole plant.

Lettuce big vein disease (LBVD) is caused by a complex of two viruses; lettuce big vein virus and Mirafiori lettuce virus (MiLV). Infection in seedlings impairs the formation of hearts. It diminishes yield and its symptoms impair lettuce quality and depress market value. Symptoms consist of vein banding, giving the typical 'big-vein' appearance accompanied by crinkling of leaves. Symptom development is favoured by cool growing conditions.

Controlling Sclerotinia lettuce drop

The lettuce industry relies on the regular use of fungicides to control the disease with varying degrees of success. The use of cultural control strategies such as crop rotation or the addition of soil amendments is not used within the industry. Reduced efficacy of fungicides and consumer concerns over pesticides used in food crops suggest integrated disease management reducing reliance on chemical control is advisable for sustainable production.

In Western Australia, the use of soil amendments failed to control SLD, and in some cases (such as the use of compost) increased the severity of SLD. The effectiveness of the fumigant Metham sodium in controlling SLD was examined. Results indicated that, in some situations Metham sodium was effective in reducing the disease pressure, and in other situations no reduction or control was achieved. The possibility of enhanced biodegradation of Metham sodium occurring was investigated. Soil testing indicated that on the commercial grower's property enhanced biodegradation was occurring and thus the effectiveness of the fumigant was reduced.

The use of different fungicide strategies to control SLD was investigated. These included; a) current growers standard best practice (fungicides applied weekly in rotation), b) reduced fungicide application (fungicides applied fortnightly), c) strategic application of fungicides (use of a systemic fungicide after infection observed), d) minimal fungicide use (not applied until infection was observed), e) drenching seedlings after planting, and f) the use of a single fungicide applied weekly. The results were variable and were very much dependent upon the site used and the disease pressure. The four field experiments were conducted on two sites, and showed that the current best practice used by growers provided the best control. However, there is the option of using a reduced fungicide strategy if disease pressure is not high and the soil fumigant if Metham sodium can be used.

In Tasmania, results were quite different. The use of soil surface applications of a highly alkaline material (finely powdered calcium hydroxide) to inhibit sclerotia germination of the lettuce drop pathogen and reduce infection at the collar region of lettuce plants was successful. A linear relationship was achieved between rate applied and level of disease control, with complete disease suppression at a rate of 10 t/ha. In field trials, a rate of 2.5 t/ha maintained soil surface pH above 8.5 for 1-3 weeks. Use of soil conditioning polymers applied to the calcium hydroxide layer prolonged pH elevation through reduction in losses of the alkaline material due to wind. At this rate, significant reductions in lettuce drop disease were shown (up to 85% disease reduction). Integration of calcium hydroxide treatment with a conventional procymidone based fungicide drench showed a significant synergistic effect, further improving disease control.

Other studies showed compost, oat cover crops, and fumigation with Metham sodium did not influence the disease severity of SLD, which was contrary to expectations. Compost amendments also exacerbated lettuce drop disease when used on a site with poor soil nutrition.

Controlling Lettuce big vein disease

A survey at a Perth seedling nursery found that LBVD occurred commonly. . New land for lettuce production was rapidly becoming contaminated with LBVD by the introduction of infected transplants from nurseries. A survey of Tasmanian nurseries only detected low levels of LBV in lettuce seedlings. It appears the majority of LBVD infections observed in Tasmanian production result from infections from contaminated soils.

Field experiments in Western Australia showed that, with early symptom expression, not only did more lettuces fail to produce hearts than with later symptom expression but also in those that produced them, heart formation was poorer. In contrast, symptoms were most severe with late infection. The magnitude of the yield and the quality losses measured warrant a re-appraisal of the importance of LBVD in Australia and elsewhere.

Other field experiments showed that the use of black plastic mulch combined with partial resistance to LBVD diminished incidence significantly. The mulch acted by reducing soil moisture and increasing soil temperature, one or both of which decreased activity of the viruliferous zoospores of the *O. brassicae* vector resulting in less virus transmission to lettuce roots. However, combining them was less effective when conditions were more conducive for spread. Thus, plastic mulch should always be integrated with other control measures (such as manipulating planting date, avoiding poorly drained land, decreasing irrigation inputs, and removing old crop debris). Considerable economic benefits are likely to arise from adopting integrated disease management tactics to control the LBVD.

In Tasmania, field experiments showed that compost treatments significantly increased LBVD levels. Furthermore, fumigation, oat green manure, and calcium hydroxide treatments also increased LBVD induced disease. As Tasmania did not have a diagnostic test for the LBVD disease causal agent, symptom expression was relied upon for scoring infection. It is unclear whether the resulting differences observed were due to enhanced infection events, or enhanced expression of disease symptoms with control treatments becoming infected but not expressing LBVD.

Technology transfer

Refereed Scientific Papers

1. Latham LJ and Jones RAC (2004). Deploying partially resistant genotypes and plastic mulch on the soil surface to suppress spread of lettuce big-vein disease in lettuce. Australian Journal of Agriculture Research (*in press*).

2. Latham LJ, Jones RAC and McKirdy SJ (2004). Lettuce big-vein disease: sources, patterns of spread and losses. Australian Journal of Agricultural Research (*in press*).

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12. Wilson CR. "Novel control of lettuce drop disease". American Phytopathology Society Annual Meeting, New Orleans, 2000 (Poster).

Extension Articles

1. Latham L, Jones R (2002) Growers, nurseries on lettuce alert. Countryman January pg 14

2. Latham L. (2001) Lettuce disease research in WA. *Leafy Crops Newsletter* Vol 1 No2. pp.6

3. Latham L., Jones R. (2001) Managing lettuce big vein virus. *Good Fruit and Vegetables* Vol. 12 No. 1, 40.

4. Annual Tasmanian vegetable industry ARAC presentations (oral) - each of these was also published as a 1 page abstract for ciculation to industry

   - 1999 - "Soil surface pH amendments as a potential means of reducing lettuce drop incidence" Devonport 1999.
   - 2000 - "Novel management of lettuce drop disease" Devonport 2000
   - 2001 - "Improvement in lettuce quality by reduction in losses due to soil-borne diseases" Devonport 2001
   - 2002 - "Improvement in lettuce quality by reduction in losses due to soil-borne diseases" Devonport 2002

5. Wood CE. (2001). Sclerotinia Research in WA. *Leafy Crops Newsletter* Vol 1. No 2 2001

6. Wood CE. (2001) Update on Sclerotinia research. *Leafy Crops Newsletter* Vol 1. No 4. 2001.

7. Wood CE. (2002). Reducing field losses due to Sclerotinia. *Leafy Crops Newsletter* July 2002.

8. Wright DG, Reid AF. (2002). Common diseases of Lettuce. Poster for Karragullen Field Day September 2002.

9. Wood CE, Reid AF. (2002). Controlling Sclerotinia in Lettuce. Poster for Karragullen Field Day September 2002.

Workshops / Field walks

1. Workshops for lettuce growers in WA were held on the 22nd September 2000 and 4th May 2002 at Wanneroo.
2. In August 2001, a field walk was held at the Medina Research Station for growers to discuss the results of this trial.

Financial Analysis of the project

Life of Project 1999/2000 Onwards

Funding Received	**Budget** *as per Project Variation dated 05/02/03	**Actual**	**Variance**
1999/2000	$75,310.00	$37,655.00	$37,655.00
2000/01	$39,953.00	$77,607.73	-$37,654.73
2001/02	$74,449.00	$39,953.00	$34,496.00
2002/03	$44,767.00	$79,263.00	-$34,496.00
2003/04 *as at 31/12/03	$7,000.00	$3,500.00	$3,500.00
Totals	**$241,479.00**	**$237,978.73**	**$3,500.27**

Expenditure Actuals	**Operating**	**Capital**	**Total**
1999/2000	$58,156.75	$4,200.00	$62,356.75
2000/01	$85,266.91	$0.00	$85,266.91
2001/02	$41,050.24	$0.00	$41,050.24
2002/03	$31,312.72	$0.00	$31,312.72
2003/04 *as at 31/12/03	$7,726.31	$0.00	$7,726.31
Totals	**$223,512.93**	**$4,200.00**	**$227,712.93**

Funds Surplus/(Deficit)	as at 31st December 2003	
Budget Revenue vs Actual Exps	(5.7% variation to total budget)	$13,766.07
Actual Revenue vs Actual Exps		$10,265.80

SECTION 1.0

SURVEY FOR BIOCONTROL AGENT

Ms Dominie Wright and Ms Christine Wood

Department of Agriculture, Western Australia

Summary

Six properties were selected for the survey, that had a history of continuous production of lettuce and/or Brassica cropping over a period of several years, and had a history of Sclerotinia lettuce drop. Some of the properties were located in the Wanneroo district (20-30 km north of Perth), which is the main area of winter lettuce and brassica production in the Perth metropolitan area. One property was located in the Manjimup horticultural district, approximately 400 km south of Perth. This is the major district for Brassica production in Western Australia.

Both soil samples and infected lettuce plants were collected to test for the presence of the mycoparasite *Sporidesmium sclerotivorum.*

The mycoparasite *S. sclerotivorum* was not detected in Western Australian soils and hence the biocontrol section of this project did not continue.

1.1 The potential for controlling SLD with the biocontrol agent *Sporidesmium sclerotivorum*

Introduction

Lettuce grown in Western Australia is generally grown on sandy soils in the metropolitan area of Wanneroo during the winter months. In the summer months, it is grown further south, in the Manjimup district, where the temperatures are cooler and the soil has a higher loam content.

Sclerotinia lettuce drop (SLD) is caused by two different pathogens in Western Australia. These are *Sclerotinia minor* and *Sclerotinia sclerotiorum.* The disease is worse during the cooler months of the year when the maximum daytime temperature is below 15 C.

The literature has shown that the mycoparasite *Sporidesmium sclerotivorum* can reduce inoculum levels and hence disease levels in a crop. Thus a survey was conducted to determine if the mycoparasite *S. sclerotivorum* was present in Western Australian soils and crops.

Methods

Site details

Six properties (A - F) were selected that had a history of continuous production of lettuce and/or Brassica cropping over a period of several years, and had a history of SLD (Table 1). Properties A, B, C, D and E were located in the Wanneroo district (20-30 km north of Perth), which is the main area of winter lettuce and brassica production in the Perth metropolitan area. Property F was located in the Manjimup horticultural district, approximately 400 km south of Perth. This is the major district for Brassica production in Western Australia.

Sampling methods

Two types of samples were collected for tests to check for the presence of *S. sclerotivorum*:

(1) Infected lettuce plants were sampled for ‘sclerote incubation test’; and

(2) Soil samples were collected for ‘soil bait test’.

SLD infected lettuce plants that contained sclerotia on lower leaf and/or crown tissues were collected from properties B, D and E.

Soil samples were collected from properties A, B, C, D and F. At each site, bulked soil samples (each approximately 1 kg) were collected from 2 separate beds with SLD infections. Each sample comprised of 5 sub-samples collected with a hand trowel from the first 10 cm of the soil profile and placed into a plastic zip-lock bag. The 2 bulked samples were then mixed to provide a single 2 kg soil sample per site.

Plant and soil samples were stored in a cool-room at the Department of Agriculture, Western Australia (DAWA) until ‘sclerote incubation tests’ and ‘soil bait tests’ were performed to check for presence of *S. sclerotivorum*.

Sclerote incubation test

Sclerotia were removed from lettuce plants using sterile tweezers. The pathogen causing SLD, either *S. minor* or *S. sclerotiorum*, was identified by observing the size range of sclerotia present on plant tissue. Sclerotia were placed on a piece of moistened filter paper in a petri dish and incubated in the dark, by wrapping in foil at 22°C for 14 days (Adams and Ayers, 1981). Sclerotia were then examined for the presence of characteristic *S. sclerotivorum* macroconidia (Uecker *et al*. 1978).

If fungal identification was ambiguous hyphae were scraped from sclerotia and plated onto SM-4, a media specific for the growth and sporulation of *S. sclerotivorum* (Ayers and Adams, 1983). Plates were incubated in the dark for 2 weeks at 22°C before further microscopic examination.

Soil bait test

Preparation of soil baits

S. sclerotiorum sclerotia were removed from the outer crown region of a crisphead lettuce collected from commercial property D. Some sclerotia were tested for the presence of inherent *S. sclerotivorum* by the 'sclerote incubation testing' method. The remaining sclerotia were plated to obtain pure 'single sclerote' isolates of *S. sclerotiorum*.

Sclerotia were surface sterilised in 5 per cent sodium hypochlorite (NaOHCl) solution for two minutes, rinsed three times with sterile distilled water and air-dried. Individual sclerotia were then plated onto potato dextrose agar amended with Aureomycin (40 mg/L Aureomycin hydrogen chloride (Apex Laboratories, NSW) (PDA+A) agar plate.

A clean single sclerote isolate was chosen to bulk up sclerotia for soil bait testing for *S. sclerotivorum*. Ten to twelve plates yielded approximately 2 g of mature *S. sclerotiorum* sclerotia.

Soil bait test

The baiting technique used was similar to that described by Adams and Ayers (1981). Soils were slightly air-dried by leaving zip-lock collection bags open on the bench for approximately 48 hours. Soils were sieved using a 2 mm sieve to remove rocks and large pieces of organic matter. The soil sample (100 g) was placed into a plastic pot and then a 'pouch' containing 2 g of sclerotia was then buried within the soil sample. The pouch consisted of two pieces of dressmakers 'tulle' which had been stapled together. Two pots were set up per sample. Soil was moistened to approximately 8 bars (with 100 ml distilled water). Pots were sealed with parafilm (to minimise evaporation), covered with foil (to create a dark environment) and incubated at 22°C for 28 days. Sclerotia 'pouches' were then removed from the soil and rinsed in sterile distilled water. Sclerotia were then tested for presence of *S. sclerotivorum* using the 'sclerote incubation test' method.

If fungal identification was ambiguous hyphae were scraped from sclerotia and plated onto SM-4, a media specific for the growth and sporulation of *S. sclerotivorum* (Ayers and Adams, 1983). Plates were incubated in the dark for 2 weeks at 22°C before further microscopic examination.

Results

Sclerote incubation test

S. sclerotivorum was not detected from any of the sclerotia collected from properties B, D, and E (Table 1).

Soil bait tests

The soil bait tests were run in conjunction with the sclerote incubation tests. No *S. sclerotivorum* was detected in any of the soil samples collected and tested (Table 1).

Discussion

The lack of detection of *S. sclerotivorum* by either the soil bait tests or the sclerote incubation test does not indicate that this mycoparasite does not exist within the state. The soil baiting technique used by Adams and Ayres (1981 and 1985) can successfully detect low levels (10 macroconidia per gram of soil) of the mycoparasite but the technique did not always detected *S. sclerotivorum* in fields with a history of *S. minor* or *S. sclerotiorum.* The soil samples chosen for this work were from areas that had a history of Sclerotinia diseases. Adams and Ayres (1985) have detected *S. sclerotivorum* in soils from Tasmania and Victoria and the soil types ranged from organic and mineral soils with a pH ranging from 5.5-8.5. The pH of the soil samples used in this experiment ranged from 5.0 to 7.0 suggesting this was not the limiting factor in detecting the mycoparasite. The other factor that Adams and Ayres (1985) observed from their survey work was that *S. sclerotivorum* has only been detected in temperate zones at latitudes grater than 35°N and 35°S. Western Australia does not fit into these requirements and this may explain the lack of *S. sclerotivorum* detection.

To improve the likelihood of detection, a greater number of samples should have been collected and tested. However, time and space constraints limited the opportunity to do further investigations.

In conclusion, the mycoparasite *S. sclerotivorum* was not detected in Western Australian soils and hence the biocontrol section of this project did not continue.

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Table 1. Site details of soil samples collected and screening tests used. SI = Sclerote incubation test, SB = Soil bait test

Property	Location	Soil type/pH	Crop type	Sample type	*Sclerotinia* sp. on plant material	Type of test screening test used	*S. sclerotivorum* detected
A	Wanneroo	Sand	Lettuce	Soil	N/A	SB	No
B	Wanneroo	Sand	Lettuce	Soil and plant	*S. minor*	SB/SI	No
C	Wanneroo	Sand	Lettuce	Soil	N/A	SB	No
D	Wanneroo	Sand	Lettuce	Plant	*S. sclerotiorum*	SI	No
E	Wanneroo	Sand	Lettuce	Plant	*S. sclerotiorum*	SI	No
F	Manjimup	Sandy loam	Brassicas	Soil	N/A	SB	No

SECTION 2.0

LABORATORY TESTING OF FUNGICIDES

Ms Dominie Wright and Ms Christine Wood

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Summary

Two experiments were conducted in the laboratory to examine the efficacy of fungicides in suppressing the growth of *S. minor* and *S. sclerotiorum.* Currently registered fungicides for lettuce were tested with non-registered fungicides that were registered for the control of *Sclerotinia* in other crops.

The mycelial growth and production of sclerotia of *S. minor* and *S. sclerotiorum* isolates were measured. Results indicated that the currently registered fungicides Benlate®, Rovral®, Sumisclex® and Marvel® provided adequate control of both *S. minor* and *S. sclerotiorum*. Benlate® is no longer registered for use and has been replaced with Marvel®. Bavistan® and Bayfidan® also gave adequate control of *S. minor* isolates in the plate tests.

The fungicides Amistar®, Chlorothalonil® and Mancozeb plus® failed to provide control of the *S. minor* and *S. sclerotiorum* isolates so no further work will be done with these fungicides.

2.1 First year screening of fungicides *in vitro* for potential use for controlling SLD in Western Australia

Introduction

Prior to this study, there was no information available on the ability of fungicides to suppress the growth of Western Australian isolates of either *S. minor* or *S. sclerotiorum* collected from lettuce crops.

In vitro fungicide screening tests were carried out to determine which fungicides should be investigated further in field trial studies. The technique used (fungicide amended agar) is a relatively cheap and a quick technique for gaining efficacy data for fungicides compared to field trials. Given funding and time limitations, it was decided to screen a range of currently registered and non-registered fungicides for suppression of fungal growth *in vitro*, to determine which fungicides should be involved in field trials.

Methods

Choosing fungicides for in vitro *efficacy tests*

Four fungicides, Benlate®, Rovral®, Sumisclex® and Amistar® were selected to test their potential in controlling *Sclerotinia minor* and *Sclerotinia sclerotiorum* isolates. These fungicides were short listed using InfoPest® (May 2000), and previous results from other researchers on the control of Sclerotinia diseases. The active ingredient for each chemical is listed in Appendix 1.

Three of these fungicides (Benlate®, Rovral®, Sumisclex®) were currently registered for the control of SLD in Western Australia and Amistar® was not registered.

Isolates tested

Two isolates of *S. minor* (WAC9869, WAC9870) and one isolate of *S. sclerotiorum* (DC22) were tested. The *S. minor* isolates were collected from a commercial lettuce property in Wanneroo and the *S. sclerotiorum* isolate was collected from another commercial lettuce property in Bullsbrook. Sclerotia were obtained from soil samples by wet sieving or removed from infected lettuce tissue. Single sclerotial isolates were obtained by placing sclerotia onto PDA plates and sub-culturing clean mycelial growth. All of these isolates have been lodged in the Department of Agriculture's Culture collection (WAC).

Preparation of fungicide amended agar

Potato dextrose agar (PDA), (containing 20 g agar (Davis), 15 g dextrose, 500 ml potato broth and 500 ml de-ionised water), was amended with each of the fungicides separately, or left unamended for a control. The agar was cooled to 55°C before adding a suspension of the fungicides to produce 0 ppm (control), 0.5 ppm, 1.0 ppm, 5 ppm, 10 ppm, 50 ppm and 100 ppm of active ingredient in the agar.

The amended PDA was then dispensed aseptically into Petri dishes, 15 ml per plate, using a peristaltic pump. Once solidified, plates were stored in a coolroom for no more than 2 days before use.

Each concentration of the fungicide amended media was replicated five times.

Preparation of S. minor *and* S. sclerotiorum *isolates*

Multiple sub-cultures of test isolates were made several days prior to inoculation of agar plates to provide sufficient mycelium for agar disks.

Inoculation and incubation of fungicide amended plates

Agar disks (5 mm) were taken from the growing edge of the test isolates using a sterilised cork borer and placed in the centre of PDA test plates. Inoculated plates were stored on a bench next to a window at room temperature for 5 days. Colony diameter (cm) was then measured along x- and y-axes on each test plate. These 2 values were then averaged to provide a single measurement of colony growth for each plate. Plates were incubated further, and then assessed for sclerotia production 14 days after they were inoculated.

Data analysis

The percentage growth and percentage sclerotia production data were analysed using Genstat® Release 4.1. Percentage data were arc-sin transformed prior to ANOVA. The effect of fungicide on growth and sclerotial production were compared using Fisher's least significance difference ($P<0.05$).

Results

Inhibition of growth

Figures 1, 2, and 3 showed that Sumisclex®, Benlate® and Rovral® all inhibited the growth of *Sclerotinia minor* and *S. sclerotiorum.* At a concentration of 5 ppm or greater there was no significant difference ($P<0.001$) between these fungicides. Amistar® showed no inhibition of growth of any of the isolates at any of the concentrations tested (Figures 1, 2 and 3).

Inhibition of sclerotial production

There was no significant difference ($P<0.001$) between Sumisclex®, Benlate® and Rovral®, in the inhibition of sclerotia production at a concentration of 5 ppm. However, these inhibited sclerotia production significantly more than Amistar® (Figures 4 and 5).

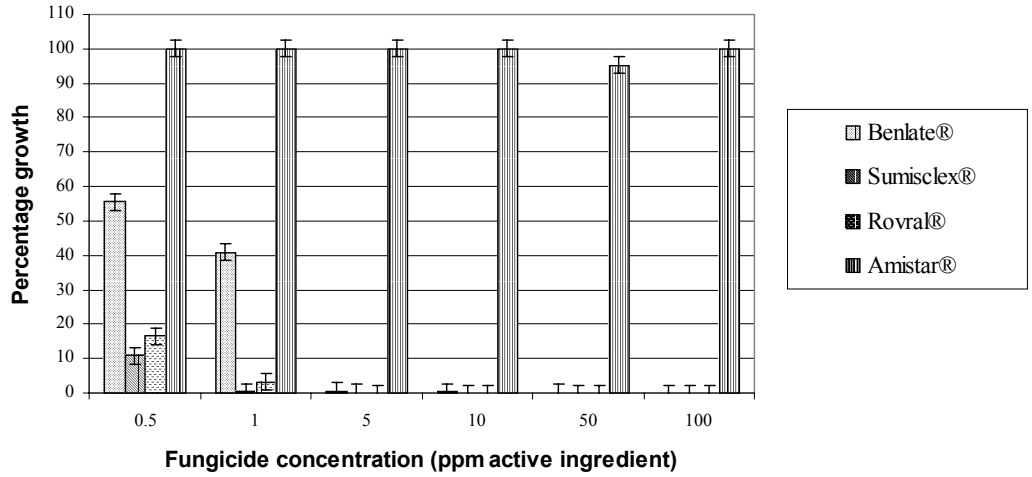

Figure 1. Percentage growth of S. minor isolate (WAC9869) for four different fungicides at varying concentrations. The percentage growth rate is relative to the control plates.

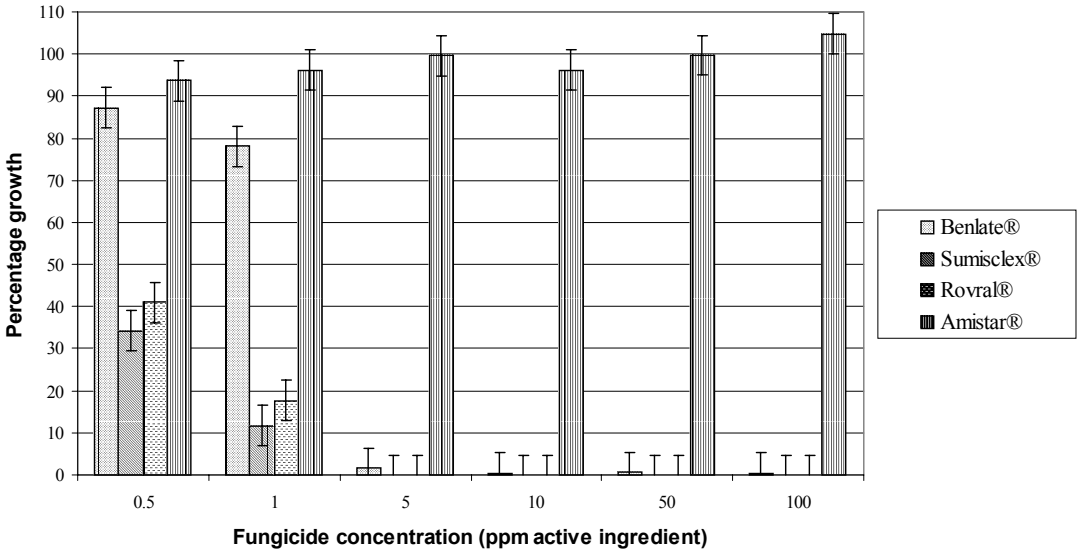

Figure 2. Percentage growth of S. minor (isolate WAC9870) for four different fungicides at varying concentrations. The percentage growth rate is relative to the control plates.

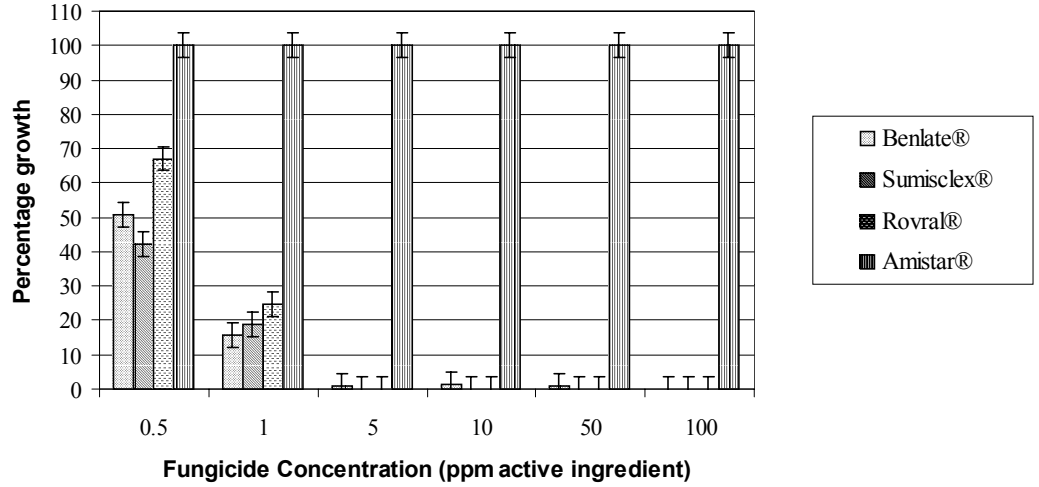

Figure 3. Percentage growth of S. sclerotiorum (isolate DC22) for four different fungicides at varying concentrations. The percentage growth rate is relative to the control plates.

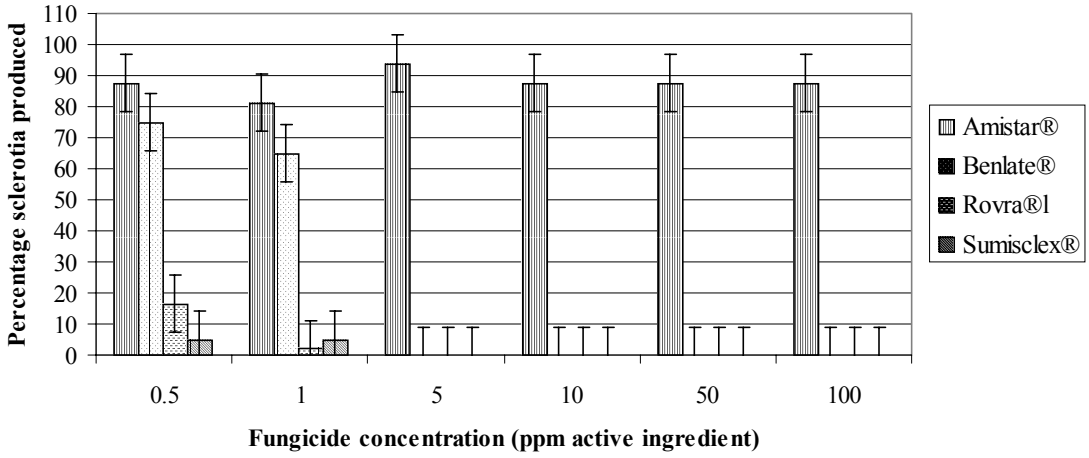

Figure 4. The percentage of sclerotia produced for S. minor isolate WAC 9870 for four different fungicides at varying concentrations. The percentage sclerotia produced is relative to the control plates.

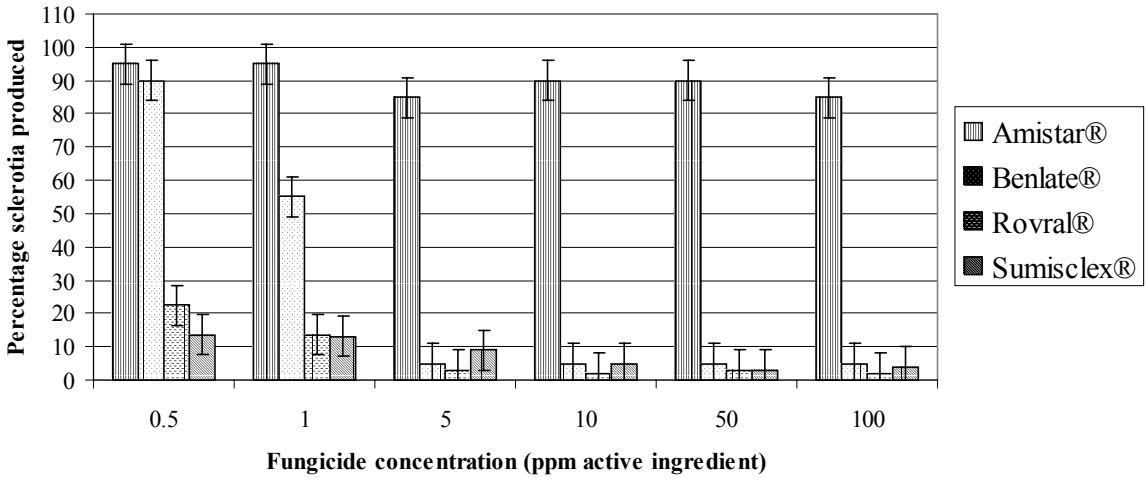

Figure 5. The percentage of sclerotia produced for S. minor isolate WAC 9869 for four different fungicides at varying concentrations. The percentage sclerotia produced is relative to the control plates.

Discussion

These plate tests indicate that the currently registered fungicides Benlate® (which has been removed from the market), Sumisclex®, and Rovral® inhibit the growth of *S. minor* and *S. sclerotiorum* at a concentration of 5 ppm or greater. Due to the removal of Benlate® from the market, further plate testing will be done to test possible replacement fungicides.

Further work with Amistar® for possible chemical control will not continue since this study showed poor control of the *S. minor* and *S. sclerotiorum* isolates.

The results also indicate that the production of sclerotia is related to the growth of mycelia of the fungus *S. minor.* Thus by controlling *S. minor* by using fungicides, the build up of inoculum for the following season should be reduced.

2.2 Second year of screening of fungicides *in vitro* for potential use for controlling SLD in Western Australia

Introduction

Prior to this study, there was no information available on the ability of fungicides to suppress the growth of Western Australian isolates of either *S. minor* or *S. sclerotiorum* collected from lettuce crops.

In vitro fungicide screening tests were carried out to determine a replacement fungicide for Benlate® as this fungicide was being removed from the market. Given funding and time limitations, it was decided to screen a range of new and non-registered fungicides for suppression of fungal growth in vitro, to determine which fungicides should be involved in field trials.

Methods

Choosing fungicides for in vitro efficacy tests

Six fungicides, Benlate®, Bavistin®, Marvel®, Bayfidan®, Chlorothalonil® and Mancozeb plus® were selected to test their potential in controlling *Sclerotinia minor* isolates. These fungicides were short listed using InfoPest® (December 2001), and previous results from other researchers on the control of Sclerotinia diseases. The active ingredient for each of these chemicals is listed in Appendix A.

Two of these fungicides (Benlate® and Marvel®) were currently registered for the control of SLD in Western Australia and the remaining four were not registered.

Isolates tested

Two isolates of *S. minor* (WAC9869 and WAC10172) were tested. The WAC9869 isolate was collected from a commercial lettuce property in Wanneroo and the WAC10172 isolate was collected from another commercial lettuce property in Carabooda. Sclerotia were obtained from soil samples by wet sieving or removed from infected lettuce tissue. Single sclerotial isolates were obtained by placing sclerotia onto PDA plates and sub-culturing clean mycelial growth. All of these isolates have been lodged in the Department of Agriculture's Culture collection (WAC).

Preparation of fungicide amended agar

Potato dextrose agar (PDA), (Oxoid), was amended with each of the fungicides separately, or left unamended for a control. The agar was cooled to 55°C before adding a suspension of the fungicides to produce 0 ppm (control), 0.5 ppm, 1.0 ppm, 5 ppm, 10 ppm, 50 ppm and 100 ppm of active ingredient in the agar.

The amended PDA was then dispensed aseptically into Petri dishes, 15 ml per plate, using a peristaltic pump. Once solidified, plates were stored in a coolroom for no more than 2 days before use.

Each concentration of the fungicide amended media was replicated five times.

Preparation of S. minor and S. sclerotiorum isolates

Multiple sub-cultures of test isolates were made several days prior to inoculation of agar plates to provide sufficient mycelium for agar disks.

Inoculation and incubation of fungicide amended plates

Agar disks (5 mm) were taken from the growing edge of the test isolates using a sterilised cork borer and placed in the centre of PDA test plates. Inoculated plates were stored in a regulated growth room at 22°C under blacklight and fluorescent light (12 hour photoperiod) for 2 days. Colony diameter (cm) was then measured along x- and y-axes on each test plate. These 2 values were then averaged to provide a single measurement of colony growth for each plate. Plates were incubated further, and then assessed for sclerotia production 14 days after they were inoculated.

Data analysis

The percentage growth and percentage sclerotia production data were analysed using Genstat® Release 4.1. Percentage data were arc-sin transformed prior to ANOVA. The effect of fungicide on growth and sclerotial production were compared using Fisher's least significance difference ($P<0.05$).

Results

Inhibition of growth

Figures 6 and 7 show that Bavistan®, Bayfidan®, Benlate® and Marvel® all inhibited the growth of the two *S. minor* isolates at a concentration of 5 ppm or greater. At 5 ppm there was no significant difference ($P<0.05$) between these fungicides. The fungicides Chlorothalonil® and Mancozeb® plus showed no control of either isolate at any of the concentrations tested (Figures 6 and 7).

Inhibition of sclerote production

Figures 8 and 9 show that Bavistan®, Benlate® and Marvel® all inhibited the production of sclerotia of the two *S. minor* isolates at a concentration of 5 ppm or greater. At 5 ppm there was no significant difference ($P<0.001$) between these fungicides. The fungicides Chlorothalonil® and Mancozeb® plus showed no control of either isolate at any of the concentrations tested (Figures 8 and 9).

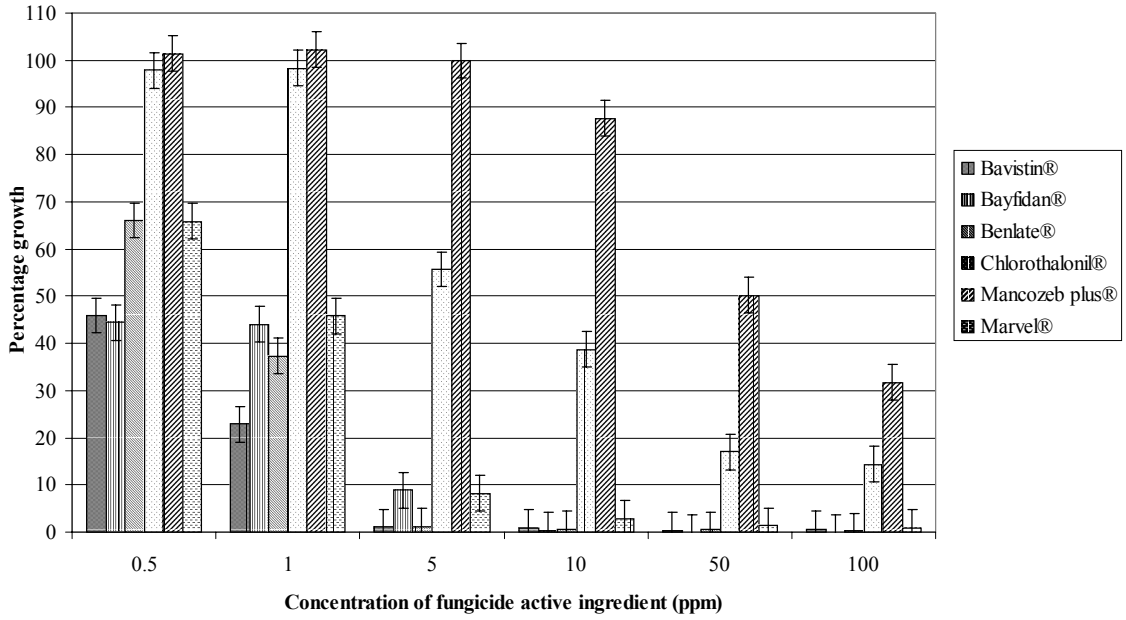

Figure 6. Percentage growth of S. minor isolate (WAC 9869) for 6 different fungicides at varying concentrations. The percentage growth is relative to the control plates.

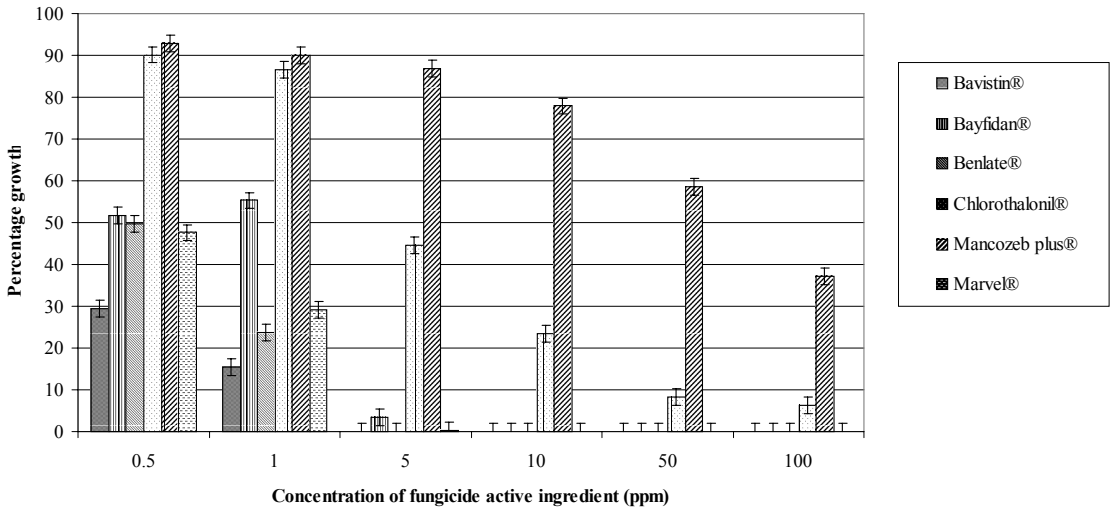

Figure 7. Percentage growth of S. minor isolate (WAC 10172) for 6 different fungicides at varying concentrations. The percentage growth is relative to the control plates.

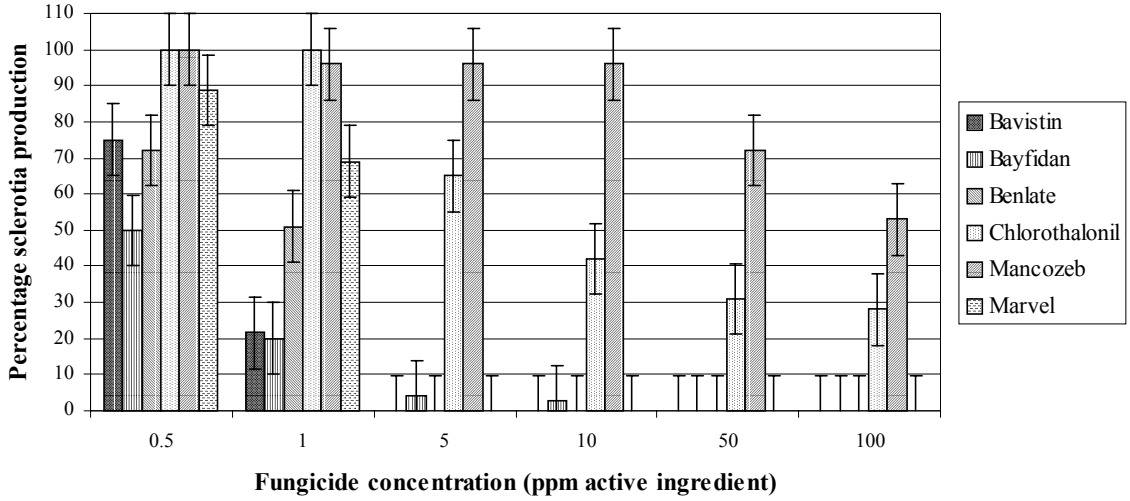

Figure 8. Percentage sclerote production of S. minor isolate (WAC 9869) for six different fungicides at varying concentrations. The percentage growth is relative to the control plates.

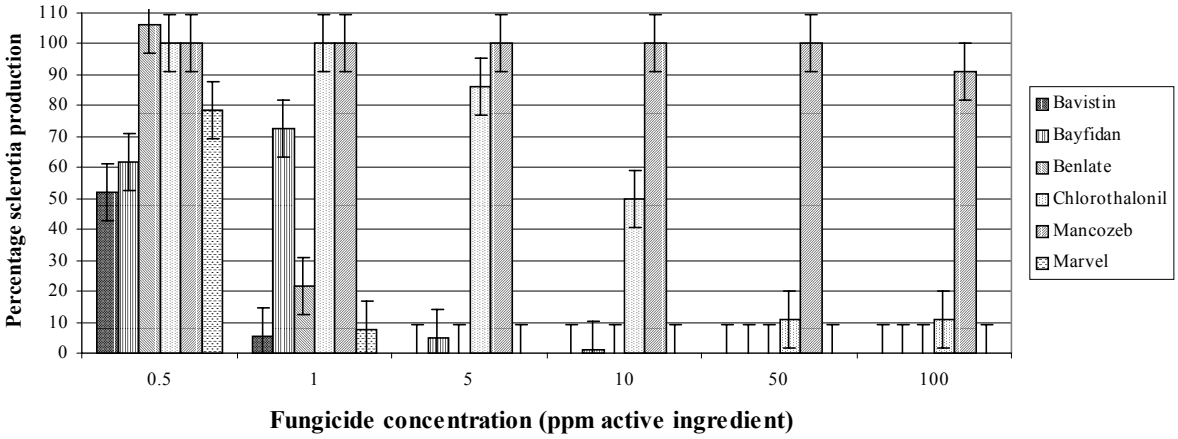

Figure 9. Percentage sclerote production of S. minor isolate (WAC 10172) for six different fungicides at varying concentrations. The percentage growth is relative to the control plates.

Discussion

These plate tests indicate that the currently registered fungicide Marvel® inhibits the growth of *S. minor* at a concentration of 5 ppm or greater. The results were not significantly different to those in this study using Benlate®. This confirms that the use of Marvel® as the replacement fungicide for Benlate® as being adequate.

Bavistan® and Bayfidan® also gave adequate control of *S. minor* isolates in the plate tests. As Chlorothalonil® and Mancozeb plus® failed to control any growth of *S. minor* no further work will be done with these fungicides.

The results show that the production of sclerotia is directly related to mycelial growth of the fungus *S. minor.* Thus by controlling *S. minor* by using fungicides, the build up of inoculum for the following season should be reduced. Further testing of these fungicides needs to be done to determine if they inhibit the germination of sclerotia. SLD occurs when seasonal conditions are favourable for the germination of sclerotia to infect plants.

References

1. Infopest® (May 2000). The complete reference of Australian registered agricultural and veterinary (Agvet)) chemicals and their uses. Queensland, Department of Primary Industries.

2. Infopest® (December 2001). The complete reference of Australian registered agricultural and veterinary (Agvet)) chemicals and their uses. Queensland Department of Primary Industries.

3. Genstat® (1998). Release 4.1. Lawes Agricultural trust (Rothamsted Experimental Station)

SECTION 3.0

FIELD EXPERIMENTS CONDUCTED IN WESTERN AUSTRALIA TO DETERMINE CONTROL STRATEGIES FOR SCLEROTINIA LETTUCE DROP (SLD)

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Summary

Four field experiments were done over three years to determine possible control strategies for SLD. Two of the experiments were conducted at the Department of Agriculture, Western Australia research station at Medina. A site had been picked where SLD had not been detected and this was inoculated with the pathogen *Sclerotinia minor*. The other two experiments were conducted on a commercial lettuce production property in the Wanneroo district. This site had been cropping lettuce for 16 years and had a history of SLD.

The effectiveness of the fumigant Metham sodium in controlling SLD was examined. Results indicated that in some situations Metham sodium was effective in reducing the disease pressure and in other situations no reduction or control was achieved. The possibility of enhanced biodegradation occurring was investigated. Soil testing indicated that on the commercial grower's property enhanced biodegradation was occurring and thus the effectiveness of the fumigant was reduced (Section 3.3). Results from the Research Station, Medina, showed that enhanced biodegradation was not occurring (Section 3.2), thus the use of the soil fumigant Metham sodium was effective in reducing the amount of SLD present in the soil.

The use of different fungicide strategies to control SLD was investigated. These included; a) current growers standard best practice (fungicides applied weekly in rotation), b) reduced fungicide application (fungicides applied fortnightly), c) strategic application of fungicides (use of a systemic fungicide after infection observed), d) minimal fungicide use (not applied until infection was observed), e) drenching seedlings after planting, and f) the use of a single fungicide applied weekly. The results were very mixed and were very much dependent upon the site used and the disease pressure. Most of the strategies tested provided some control except for the drenching of lettuce seedlings (Section 3.2) where phyto-toxicity occurred. The effectiveness of the use of fungicides was increased when used in conjunction with the soil fumigant Metham sodium (Section 3.1). However, there was very little significant difference between the different fungicide strategies used in all of the experiments conducted (Sections 3.1, 3.2 and 3.4).

The use of cultural control strategies such as soil amendments (lime and compost), and the addition of higher fertiliser rates was also investigated. The incorporation of compost into the lettuce beds (Section 3.1) increased disease incidence significantly ($P < 0.05$) and the application of lime (Section 3.1) was not significantly different ($P < 0.05$) to the control plots. The higher fertiliser rates (Section 3.4) was not significantly different ($P < 0.001$) to

the control plots. The addition of the product Microgyp® to the fungicide spray (Section 3.4) was not significantly different ($P < 0.001$) to the use of the fungicide spray on its own.

In conclusion, the experiments showed that current standard best practice strategy is effective in controlling SLD, however there is the option of using a reduced fungicide strategy if disease pressure is not high and the soil fumigant Metham sodium can be used.

3.1. Assessment of reduced fungicide applications and soil amendment strategies for adequate control of SLD in winter lettuce crops in Western Australia

Introduction

Lettuce production in Western Australia is in the winter months (May to October). However, during the summer lettuce can be grown in southern areas where daily temperatures are cooler and the soil has a higher loam content. In Perth, most lettuce production occurs in the Wanneroo area on sand soil type (Karrakatta sand).

The pathogen of Sclerotinia Lettuce Drop (SLD) can either be *Sclerotinia minor* or *Sclerotinia sclerotiorum*, although *S. minor* tends to predominate. The lettuce industry relies on the regular use of fungicides to control the disease with varying degrees of success. The use of cultural control strategies such as crop rotation, the addition of soil amendments, is not used within the industry. This experiment replicates some of the work being conducted in the Eastern States on the use of cultural control strategies including the reduction of fungicide application for the control of SLD.

Materials and Methods

An experiment was planted at Medina Research Station in July 2000, using the lettuce cv. Oxley, as 4-week-old transplants. Lettuce seedlings were grown in the glasshouse at DAWA, South Perth. The trial was cropped according to local industry practices, which include harvesting approximately 11 to 12 weeks after transplanting.

Pre trial preparation

Lettuce seedlings

To minimise the chances of *Olpidium* (a fungal vector of Lettuce Big Vein Virus) being introduced into the experiment, pelleted lettuce seeds (cv. Oxley) were surface sterilised before sowing. Outer pellets were removed by crushing with a knife and rubbing pellet residue off in water. Seeds were surface sterilised by soaking in 1.25 per cent sodium hypochlorite (NaOHCl) for 15 minutes. They were then rinsed thoroughly in sterile distilled water and air dried. Seeds were sown into sterilised potting mix in seedling trays. Seedlings were grown in the glasshouse at South Perth until they were 4 weeks old. Plants were then hardened off by placing them outside the glasshouse for another two weeks before being transported to the Medina Research Station.

Sclerotinia minor inoculum

An isolate of *S. minor* (WAC 9869), was grown on PDA for five days in the dark at 22°C. This was then used to inoculate sterilised barley seed in conical flasks. Flasks were sealed and placed under diurnal light (12/hrs) for six weeks at 15°C. Flasks were shaken by hand every two days to redistribute the inoculum evenly. The inoculum was then placed into sealed paper bags for drying before application in the field.

Application of *S. minor* to experiment

The inoculum was placed into buckets and mixed by hand. This was then sprinkled over the plots by hand at a rate of 21 g/m^2. The inoculum was mixed into the topsoil using a rake. The plots were left for one week before the Metham sodium treatments were applied. Lettuce seedlings were planted into the plots one week after the Metham sodium treatments had been applied.

Site details

The experiment was established at the Medina Research Station, 30 km south of Perth. The soil type was Karrakatta sand. The Medina site had no history of Sclerotinia Lettuce Drop (SLD) prior to this experiment.

Trial designs and treatments

The experiment was a split-plot design with 12 treatments (2 main treatments by 6 sub-treatments) replicated four times. Each plot was 1.2 m x 5.6 m with 42 lettuce plants in three rows. Between all treatments were lettuce buffers (1.2 m x 1.6 m, with 12 lettuce plants). Row spacing in all plots, including buffers, was 40 cm both within and between the rows.

Overhead irrigation was applied daily during early to mid-morning throughout the trial. The herbicide Kerb® was applied at standard rates as required, and prior to transplant. Superphosphate and trace elements were applied prior to transplant and additional fertilisers were applied as required.

Main treatments

1. Plus Metham sodium.
2. Minus Metham sodium.

Sub-treatments

The scheduling of all fungicide applications and their relevant rates are shown in Table 1. The active ingredient and current registration for each fungicide is listed in Appendix A.

T1 Full fungicide - Benlate®, Sumisclex® and Rovral® were applied in rotation weekly from transplant until crop maturity. Seedlings were drenched with Rovral® just prior to transplant.

T2 Minimal fungicide - Benlate®and Sumisclex® were applied in rotation weekly from 5 weeks after transplant until crop maturity. Seedlings were drenched with Rovral® just prior to transplant.

T3 Strategic applications - Sumisclex® 1 mL/L was applied at either 1000 L/ha or 1200 L/ha to all replicate plots whenever a 5 per cent disease threshold (i.e. 2 plants per plot) was observed in any single replicate plot. A total of five Sumisclex® applications were made between transplant and crop maturity with two additional sprays of Benlate® and Rovral®.

T4 Compost - Compost was incorporated into plots at 200 m^3/ha pre-plant.

T5 Hydrated lime - Hydrated lime was applied to plots at 10 g/m^2 after transplant.

T6 Untreated control.

Table 1. Trial 1 treatment schedule showing dates of all fungicide, compost and hydrated lime applications and application rates. Exceptions to general application dates are shown in parentheses in the treatment columns

Application (days after transplant)	T1 Full fungicide	T2 Minimal fungicide	T3 Strategic applications	T4 Compost	T5 Hydrated lime	T6 Untreated control
0	Rovral® 1 mL/L (drench)	Rovral® 1 mL/L (drench)		200 m^3/ha (incorporated during bed formation)		
5					10 g/m^2	
14	Benlate® 1 g/L at 800 L/ha					
21	Sumisclex® 1 mL/L at 800 L/ha					
28	Benlate® 1 g/L at 800 L/ha		Sumisclex® 1 mL/L at 1000 L/ha			
35	Rovral® 1 mL/L at 1000 L/ha	Benlate® 1 g/L at 1000 L/ha	Sumisclex® 1 mL/L at 1000 L/ha			
42	Benlate® 1 g/L at 1000 L/ha	Sumisclex® 1 mL/L at 1000 L/ha	Benlate® 1 g/L at 1000 L/ha			
49	Sumislex® 1 mL/L at 1000 L/ha	Benlate® 1 g/L at 1000 L/ha	Sumislex® 1 mL/L at 1000 L/ha			
51			Rovral® 1 mL/L at 1000 L/ha			
56	Benlate® 1 g/L at 1000 L/ha	Sumisclex® 1 mL/L at 1000 L/ha	Sumisclex® 1 mL/L at 1200 L/ha			
63	Sumislex® 1 mL/L at 1000 L/ha	Benlate® 1 g/L at 1000 L/ha	Sumislex® 1 mL/L at 1200 L/ha			

Measurements and data analysis

Disease incidence

Plots were scored weekly for SLD incidence by determining the cumulative number of plant deaths per plot from SLD. SLD infections were determined by the drooped appearance of outer leaves, combined with the subsequent collapse of the head in older plants, and/or the presence of white mycelium and/or black sclerotia underneath outer leaves near the crown region. The position of infected plants within plots was recorded.

Soil pH

Soil samples for pH analysis were collected from the hydrated lime plots (treatment 5) after the first application of the treatment, and then every week for a month. The initial soil sampling included collecting soil from the root zone using a pogo stick as well as collecting soil from the topsoil. All other soil collections were from the topsoil only. To do this, five subsamples were collected using a 10 cm^2 quadrat placed on the soil surface at intervals along a W-pattern within each plot. Subsamples were bulked together from each plot and mixed thoroughly prior to determination of soil pH. The pH of soil samples was determined using $CaCl_2$ according to the methods described by Piper (1950) and Loveday (1974).

Soil sclerote counts

Soil samples for sclerote counts were collected 2 weeks after harvest from each control plot that either had Metham sodium applied or not (sub treatment 6). Ten subsamples were collected using a 10 cm^2 quadrat placed on the soil surface at intervals along a W-pattern within each plot. Subsamples were bulked together from each plot and mixed thoroughly prior to determination of soil sclerote density. The number of sclerotia per 100 cc of soil was determined by the wet-sieving method of Adams (1979).

Data analysis

Disease incidence data were analysed using Genstat 4.1. Percentage data were arc-sin transformed prior to ANOVA. Data were analysed as a split-plot design. Treatment means were compared using Fisher's least significance difference ($P < 0.05$).

Results

Disease incidence

First infection of SLD was seen in the plots 27 days after transplant. The percentage of SLD infection within the plots ranged form 24 to 100 per cent infection at harvest, with an average of 74 per cent (raw value) infection.

Figure 1, shows that the number of plants infected with SLD increased with time and that the rate of increase is greater in the minus Metham sodium plots. There was a significant ($P < 0.001$) difference between the plus and minus Metham sodium treatments. The mean percentage of plants dead at harvest for all plots fumigated with Metham sodium was 51.23 per cent compared to 97.12 per cent in the non-fumigated plots (Table 2).

At harvest there was a significant interaction between Metham sodium application and the sub-treatments ($P < 0.005$), indicating that treatment means needed to be compared separately for plots plus or minus Metham sodium. There was no significant difference between the sub-treatment plots within the minus Metham sodium treated plots (Table 2).

Within the Metham sodium treated plots there was a significant difference in disease incidences between the sub-treatments (Table 2). At harvest, there was a significantly

lower disease incidence for sub-treatments 1 (weekly fungicide applications) and 3 (strategic applications) compared with all other sub-treatments (Table 2). Sub-treatments (1 and 3) were not significantly different from each other. The disease incidence levels in the minimal fungicide application and the hydrated lime treatments (sub-treatments 2 and 5, respectively) were not significantly different ($P < 0.05$) to levels within the control plots (sub-treatment 6). The addition of compost (sub-treatment 4) increased the number of plant deaths significantly higher than any other sub-treatment.

Table 2 Mean percentage lettuce plants dead from SLD per plot at harvest*. Statistical analysis is based on angular transformed data (de-transformed means are shown in parentheses). Sub-treatment means are shown for plots with and without Metham sodium fumigation. Each value is derived from the average of four replicate plots

Sub-treatment	Plus Metham Sodium*	Minus Metham Sodium*
1 (Full fungicide)	33.99 (31.25) *a***	82.36 (98.23) *a*
3 (Strategic applications)	35.12 (33.10) *a*	75.22 (93.49) *a*
2 (Minimal fungicide)	44.85 (49.74) *b*	81.12 (97.62) *a*
6 (Untreated control)	48.82 (56.65) *b*	82.41 (98.26) *a*
5 (Hydrated lime)	51.29 (60.89) *b*	78.86 (96.27) *a*
4 (Compost)	60.50 (75.75) *c*	83.91 (98.87) *a*
Average	45.76 (51.23)	80.65 (97.12)
LSD ($P < 0.05$)***	8.79	8.79

* Plus or minus Metham sodium with six different chemical or soil amendment sub-treatments.

** These letters show the differences between the sub-treatments within the main Metham sodium treatment.

*** The LSD value is the same for comparing mean percentage plants dead between the sub-treatments within the Metham sodium treatment and for comparing the same sub-treatment between the Metham sodium treatments.

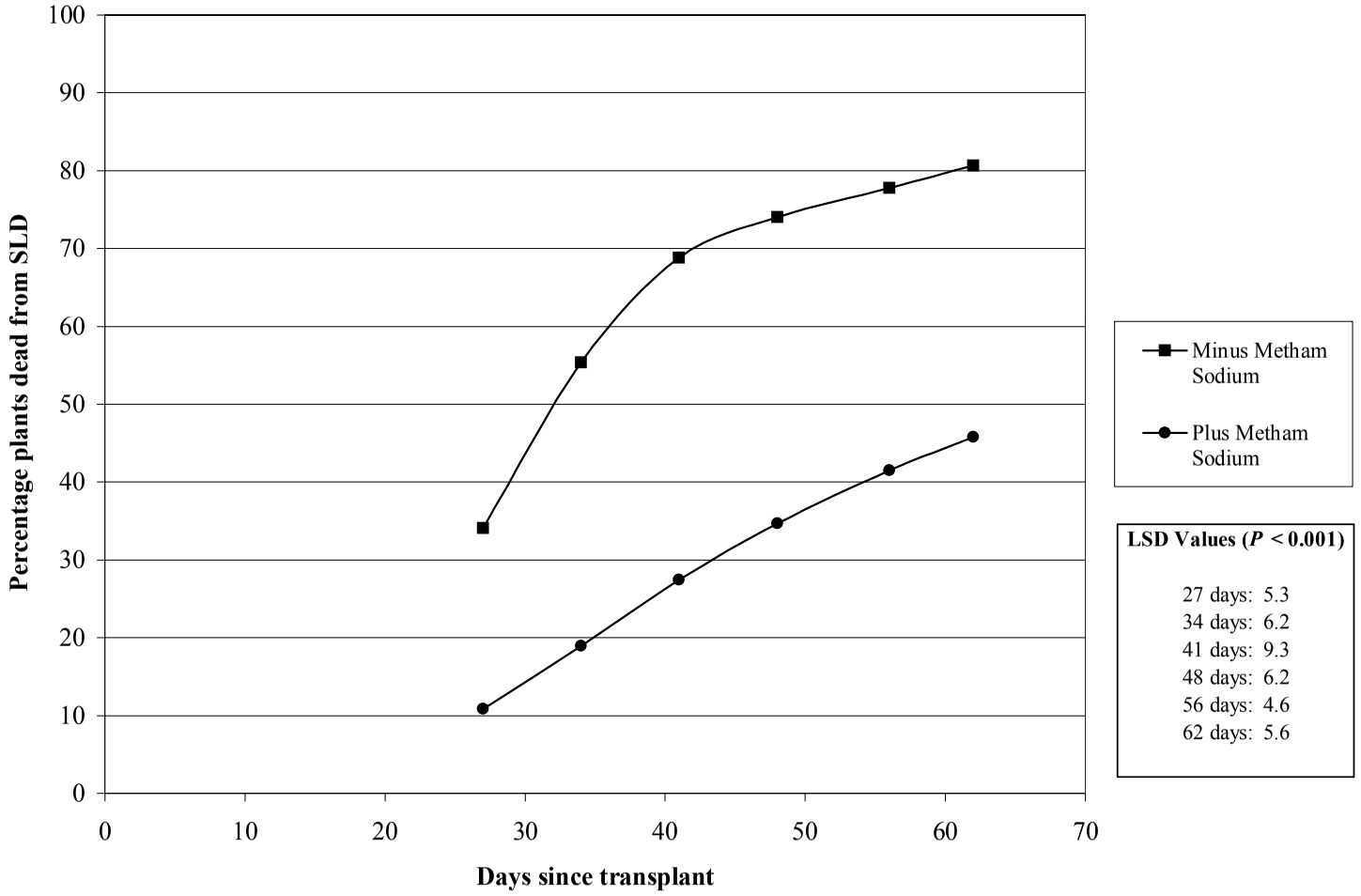

Figure 1. The percentage plants dead from SLD over time when fumigated with or without Metham sodium.

Soil pH and sclerote density

The initial, average pH ($CaCl_2$) of the soil samples before the addition of hydrated lime (sub-treatment 5) was 6.65. The average pH ($CaCl_2$) of topsoil and the root zone samples for these plots after application of hydrated lime to the surface of plots was 7.50 and 6.58, respectively (Table 2). For each of the eight replicated plots, the root zone soil samples had a consistently lower pH ($CaCl_2$) than those for the corresponding topsoil samples collected at the same time. The Metham sodium treatment had little effect on either the topsoil or the root zone soil pH (Table 3).

There was no significant difference ($P < 0.05$) in the number of sclerotes determined in the control plots that were fumigated with or without Metham sodium (Table 4).

Table 3. Average pH (CaCl2) for Limal treatment plots, six days after application of hydrated lime to the surface of plots. Average pH is shown for 4 replicate plots that were fumigated with Metham Sodium pre-plant and also for 4 replicate plots that were not fumigated. The average pH (CaCl2) is for all limal treatment plots

Treatment	Topsoil (0-2 cm)	Rootzone soil (2-17 cm)
Plus Metham Sodium	7.49	6.69
Minus Metham Sodium	7.51	6.46
Average pH (all plots)	7.50	6.58

Table 4. Mean number of Sclerotes per 100 cc of soil from the control plots (sub-treatment 6) that were fumigated with or without Metham sodium. Statistical analysis is based on the square-root transformed data (de-transformed means are shown in parantheses)

Treatment	Plus Metham Sodium	Minus Metham Sodium
T6 (untreated control)	7.5 (56.2)	12.8 (163.8)
LSD ($P < 0.05$)	7.68	7.68

Discussion

Disease incidence

The mean percentage plants dead from SLD at harvest was 97 per cent. This was very high compared with losses observed in commercial lettuce crops during the same growing season. The high disease level is most likely due to the inoculum being applied in a mycelial state rather than in the sclerote form. When the inoculum was prepared, no sclerotes had formed after 6 weeks of incubation. Thus the barley seed was applied with the inoculum providing an increase in the food source for *S. minor,* hence increasing pathogenicity.

Effect of Metham sodium on SLD

This study showed that pre-plant fumigation with Metham sodium provided significant control of SLD (Table 2). At harvest, the mean percentage plants dead from SLD in all of the Metham sodium treated plots was significantly lower than for the plots without Metham sodium (Table 2). These data supports those of Davis *et.al* (1997) who showed fumigation with a soil fumigant can provide control of SLD. However, observations on commercial properties indicate that the use of fumigation has very little effect on the control of SLD. Warton and Matthiesson (2000) reported enhanced biodegradation of Metham sodium at a Perth site where the fumigant had been used regularly for 10 years. This may explain why the Metham sodium appears to be having very little effect in controlling SLD on commercial lettuce properties.

The difference between the Media trial site and the commercial properties is that the soil had never been treated with Metham sodium previously. Hence, the result that fumigation with Metham sodium provides control of SLD.

In hindsight the sclerote data should have been collected at the beginning of the trial as well as at the end of the trial. This would have indicated if the inoculum levels were increasing over time in the plots that had been treated with or without Metham sodium.

Post-transplant control of SLD

There was no significant difference in the mean percentage plants dead between the different fungicide or amendment application sub-treatments within the minus Metham sodium plots (Table 2). This is most likely due to inoculum load being too high for the treatments to be effective and for differences between them to be measured. In general, it has been observed that some of these treatments can provide some control of SLD in a commercial setting.

The full fungicide treatment (sub-treatment 1) and the strategic application of fungicide (sub-treatment 3) gave the same level of control of SLD in the fumigated plots. Hence the application of Sumisclex® applied strategically in response to a disease threshold being reached (e.g. 5%) can provide adequate control and at the same time reduce the amount of fungicide applied to a crop. Only seven fungicide applications were made to the strategic fungicide treatment plots, compared to eight fungicide applications to full fungicide treatment plots (Table 1). However, the original plan for sub-treatment 3 was for only Sumisclex® to be applied to the plots. If this had been done, only 5 fungicide applications would have been made.

The minimal use of fungicides (sub-treatment 2) and the use of hydrated lime (sub-treatment 5) provided no significant control of SLD in the fumigated plots. The disease incidence levels in these plots were higher than for the plots that used a weekly (sub-treatment 1) or strategic application of fungicide (sub-treatment 3). This was unexpected, as the minimal use treatment still applied Sumisclex®. However, the crop only received two applications of Sumisclex® compared to the other treatments, which received three to five applications. This suggests that a minimum of three applications may be required for providing adequate control of SLD. The hydrated lime treatment failed to increase the pH of the topsoil above 7. A pH of 8.0 is required to stop sclerote germination of *S. minor* (C. Wilson *pers. comm.,* Section 3.5). The application of hydrated lime does not appear to be practical for Western Australian growers. It is a costly addition to the soils and due to the sandy nature of the soil it may leach through to the root zone. Conversely, results from trials in the eastern states (section 3.5) show a positive response with the addition of hydrated lime.

The compost treatment (sub-treatment 4) increased the disease incidence within the fumigated plots compared to all of the other sub-treatments. This is possibly due to the

environmental conditions created by the compost were ideal for the *S. minor* inoculum to continue to grow and infect lettuce seedlings over time.

In conclusion, the use of a strategic application of fungicides and the use of Metham sodium may provide control for SLD. This needs to be further investigated under commercial growing conditions and where *S. minor levels* are not so high.

3.2 Determination of the best practice for fungicide application to control *Sclerotinia minor* on crisphead lettuce in relation to application timing and frequency.

Introduction

Lettuce production in Western Australia is in the winter months (May to October). However, during the summer lettuce can be grown in southern areas where daily temperatures are cooler and the soil has a higher loam content. In Perth, most lettuce production occurs in the Wanneroo area on sand soil type (Karrakatta sand).

The pathogen of Sclerotinia Lettuce Drop (SLD) can either be *Sclerotinia minor* or *Sclerotinia sclerotiorum*, although *S. minor* tends to predominate. The lettuce industry relies on the regular use of fungicides to control the disease with varying degrees of success. In the previous experiment (Section 3.1) the use of cultural control measures did not reduce the severity of SLD. This experiment is a continuation of this work and investigates further the reduction in chemical application and the use of the soil fumigant Metham sodium.

Materials and methods

Site description and experimental design

The experiment was planted at the end of May 2001, at the Medina Research Station, 30 km south of Perth. This experiment was directly superimposed onto the previous year's trial (Section 3.1). The site had been artificially inoculated with sclerotia of *Sclerotinia minor* in July 2000 (Section 3.1). The site was left fallow from early October 2000 after the previous trial had been harvested. Beds were turned over by rotary hoe prior to the transplanting of lettuce for this experiment.

The experimental design was a split-plot with 12 treatments (2 main treatments by 6 sub-treatments) replicated four times. The plots were 1.2 m x 5.6 m with 42 lettuces planted in three rows. Between all treatments there were lettuce buffers (1.2 m x 1.6 m, with 12 plants). Row spacing in all plots including buffers was 40 cm within and between rows.

Overhead irrigation was applied daily during early to mid-morning throughout the trial. The herbicide Kerb® was applied as required, and prior to transplant. Superphosphate and trace elements were applied prior to transplant and additional fertilisers were applied as required.

Treatments

Main treatments

1. Metham Sodium applied twice (the metham sodium was applied over the plots that had metham sodium applied in 2000 (Section 3.1)).
2. Metham Sodium applied once (the metham sodium was applied over the plots that had no metham sodium applied in 2000 (Section 3.1)).

Soil was fumigated with Metham Sodium (sodium monomethyl dithiocarbamate) nine days prior to transplant. Metham Sodium was applied at 500 L/ha by boomspray and then incorporated with water.

Sub treatments

The scheduling of all fungicide applications and their relevant rates are shown in Table 5.

T1. Full fungicide

Bavistin® (carbendazim), Sumisclex® (procymidone) and Rovral® (iprodione) were applied in rotation weekly from transplant until crop maturity. Rovral® was removed from the rotation when plants became larger (after 28 days).

T2. Minimal fungicide

Fungicides and application rates were as above however the first application was not applied until a 5 per cent disease threshold was observed in at least one of the treatment 2 plots. The first spray was applied 35 days after transplant.

T3. Strategic applications

Sumisclex® was applied to all treatment 3 plots whenever 5 per cent of plants became infected in at least one of these plots. A total of 4 applications were applied to treatment 3 plots during the crop.

T4. Reduced full fungicide

Bavistin®, Sumisclex® and Rovral® were applied in rotation every 2 weeks from 7 days after transplant until crop maturity.

T5. Sumisclex® drenching of plots immediately after transplanting

After transplant, plots were drenched with Sumisclex® at 2500 L/ha. This was a high volume of water, 2.5 times the recommended rate.

T6. Control

Post-transplant control measures for SLD were not applied to these plots (i.e. no fungicides).

Measurements and data analysis

Disease incidence

The trial was scored weekly from transplant for disease incidence. The cumulative number of plants that had died from SLD in each plot was recorded. SLD infections were determined by the drooped appearance of outer leaves, combined with the subsequent collapse of the head in older plants, and/or the presence of white mycelium and/or black sclerotia underneath outer leaves near the crown region.

Soil pH

Soil samples (to a depth of 15 cm) were collected using a “pogo” stick at harvest along a W-pattern within each control plot. Five subsamples were collected per plot. Samples from control plots with the same Metham Sodium application history were bulked together and mixed thoroughly. The pH ($CaCl_2$) of soil samples was determined according to the methods of Piper (1950) and Loveday (1974).

Soil sclerote counts

At harvest, soil samples were collected from the untreated plots to determine the sclerote densities within the plots, and to see if there was a difference between the Metham sodium treatments. The number of sclerotia per 100 cc of soil was determined by the wet-sieving method of Adams (1979).

Enhanced biodegradation of Metham sodium

Three bulked soil samples were collected from; a) soil treated the most regularly with Metham sodium; and b) soil that had never been treated with Metham sodium on the research station. Samples were analysed by CSIRO, Floreat Park, Western Australia for enhanced biodegradation of Metham sodium.

Data analysis

Disease incidence data were analysed using Genstat. A split plot ANOVA was run using covariate data. The covariate data were the percentage plants dead from SLD at harvest of the first year trial. As *S. minor* can remain viable in the soil after harvest and re-infect tissue in the next crop, it was likely that the amount of disease in plots during the first year trial would have some influence on disease incidence in plots during the second year trial. Percentage data were arc-sin transformed prior to ANOVA. Separate analyses were run for each assessment date, and a disease incidence curve was plotted using detransformed treatment means.

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Table 5. Fungicide schedule showing application rates and date of application as days after transplant

Days after transplant	T1 full fungicide	T2 minimal fungicide	T3 strategic applications	T4 reduced full fungicide	T5 Sumisclex® drenching	T6 untreated control
Pre-plant	Sumisclex 1 mL/L	Sumisclex 1 mL/L	Sumisclex 1 mL/L	Sumisclex 1 mL/L	Sumisclex 1 mL/L	Sumisclex 1 mL/L
0					Sumisclex 1 mL/L at 2500 L/ha	
7	Rovral 1 mL/L at 1000 L/ha			Rovral 1 mL/L at 1000 L/ha		
14	Bavistin 0.5 mL/L at 800 L/ha					
21	Sumisclex 1 mL/L at 800 L/ha			Bavistin 0.5 mL/L at 800 L/ha		
28	Rovral 1 mL/L at 1000 L/ha		Sumisclex 1 mL/L at 1000 L/ha			
35	Bavistin 0.5 mL/L at 1000 L/ha	Sumisclex 1 mL/L at 1000 L/ha		Sumisclex 1 mL/L at 1000 L/ha		
42	Sumisclex 1 mL/L at 1000 L/ha	Bavistin 0.5 mL/L at 1000 L/ha				
49	Bavistin 0.5 mL/L at 1000 L/ha	Sumisclex 1 mL/L at 1000 L/ha	Sumisclex 1 mL/L at 1000 L/ha	Bavistin 0.5 mL/L at 1000 L/ha		
56	Sumisclex 1 mL/L at 1000 L/ha	Bavistin 0.5 mL/L at 1000 L/ha	Sumisclex 1 mL/L at 1000 L/ha			
63	Bavistin 0.5 mL/L at 1200 L/ha	Sumisclex 1 mL/L at 1200 L/ha	Sumisclex 1 mL/L at 1200 L/ha	Sumisclex 1 mL/L at 1200 L/ha		
70	Sumisclex 1 mL/L at 1200 L/ha	Bavistin 0.5 mL/L at 1200 L/ha				
77	Bavistin 0.5 mL/L at 1200 L/ha	Sumisclex 1 mL/L at 1200 L/ha		Bavistin 0.5 mL/L at 1200 L/ha		
84	Sumisclex 1 mL/L at 1200 L/ha	Bavistin 0.5 mL/L at 1200 L/ha				

Results

Disease incidence

The mean disease incidence at harvest for all treatment plots was 6.46 per cent (detransformed mean), and raw values per plot ranged from 0 per cent to 38.10 per cent. Mean disease incidence was much lower than that (97%, detransformed mean) found in the first year trial at harvest (Section 3.1).

When the data were analysed not using covariate data, there was a significant difference ($P < 0.02$) in the disease incidence at harvest between plots that had been fumigated twice and those that had been fumigated once. However, when the covariate data were included in the ANOVA, no significant difference ($P < 0.5$) was found between plots with different fumigation histories (Table 6). This was expected as all plots, had been fumigated prior to this trial. Variability between plots with different Metham sodium application histories could be accounted for by differences in disease incidence at harvest of the previous trial (Section 3.1) for plots with or without fumigation of Metham sodium.

Table 6. Mean percentage plants dead (adjusted with covariate data) from SLD after one or two years metham sodium treatment. Statistical analysis is based on angular transformed data (de-transformed means are shown in parentheses)

	Mean % plants dead (using covariate data)	Mean % plants dead (not using covariate data)
Metham Sodium (1 year)	29.31 (23.96)	20.41 (12.16)
Metham Sodium (2 years)	0.14 (0)	9.04 (2.47)
LSD ($P < 0.5$)	133.54	
LSD ($P < 0.02$)		7.71

There was no significant interaction ($P = 0.25$) between the main treatments of metham sodium application, and the sub-treatments of fungicide application (not adjusting the data for covariate). Thus the effect of the sub-treatments was similar for plots that had been fumigated twice with metham sodium and for those that had been fumigated once. The inclusion of covariate data in the ANOVA strengthened these results by a greater significant difference between the sub-treatments and the interaction between the main treatment and the sub-treatment was less significant. Therefore the data were then grouped and re-analysed ignoring the metham sodium history.

The full fungicide treatment (sub-treatment 1) and the minimal fungicide treatment (sub-treatment 2) were found to have significantly ($P < 0.001$) fewer plants dead from SLD at harvest than the untreated controls (sub-treatment 6) and the Sumisclex® drenching (sub-treatment 5) (Table 7). However, there was no significant difference between treatments 1 and 2.

Sub-treatments 3, 4 and 5 were not significantly different to the control (sub-treatment 6) (Table 7). The Sumisclex® drenching (sub-treatment 5) had the highest mean percentage plants dead at harvest from SLD compared to all other treatments, including the untreated control (sub-treatment 6) (Table 7). However, the difference between the drenching treatment and the control was not significant nor was the difference between the drenching and the fortnightly fungicide application (sub-treatment 4) (Table 7).

Table 7. Table of means (adjusted with covariate data) for percentage plants dead from SLD per plot at harvest. Statistical analysis is based on angular transformed data. De-transformed means (adjusted for covariate) are shown in parentheses. Values are derived from averages of eight replicate plots

Treatment	Mean % plants dead from SLD
T1 (Full fungicide)	9.47 (2.71) *a*
T2 (Minimal fungicide)	9.76 (2.87 *ab*
T3 (Strategic applications)	14.82 (6.54) *abc*
T4 (Reduced full fungicide)	15.22 (6.89) *bcd*
T6 (Untreated control)	18.22 (9.78) *cd*
T5 (Sumisclex® drench)	20.85 (12.67) *d*
Average	14.72 (6.91)
LSD (*P* = 0.001)	5.74

Figure 2 shows the disease incidence curve from SLD for sub-treatments 1 - 6 from day 28, when the first plant with SLD was recorded, through to harvest of the trial at day 84. This indicates that from day 28 until day 70, there were no significant differences in disease levels between the treatments. After day 70, significant differences between the treatments were detected.

Soil pH and sclerote density

The metham sodium history had no significant effect on the soil pH (Table 8). The small differences are within the bounds of natural soil variability. Sclerote density was much lower than expected.

Table 8. Soil pH and sclerote density based on history of metham sodium application

Treatment	pH (CaCl2)	Sclerote density (per 100 cc soil)
2 year Metham sodium	5.98	1
1 year Metham sodium	6.20	0

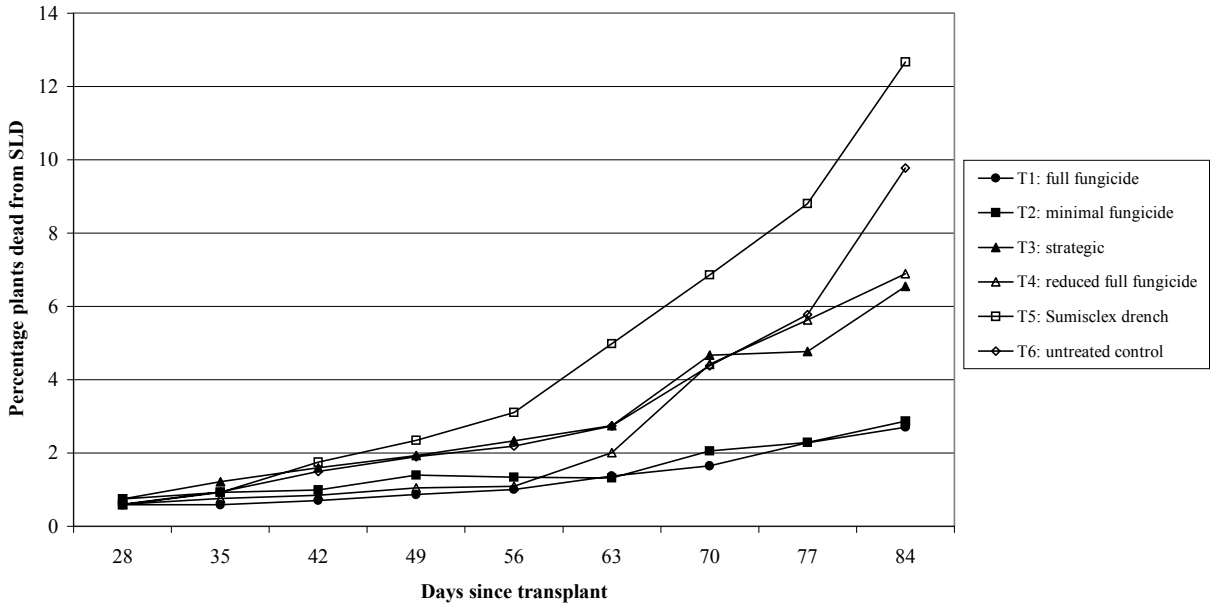

Figure 1. Disease incidence curve showing percentage plants dead from SLD for sub-treatments 1 - 6 from day 28 when the first plant with SLD was recorded through to harvest of the trial. LSD = 5.74, (P < 0.001).

Enhanced biodegradation of Metham sodium

Results from the soil samples collected and submitted showed that enhanced biodegradation was not occurring (Figure 3). After 24 hours, percentage MITC was 78.52 per cent (sample A) and 71.93 per cent (sample B). These results were similar for sample C (Medina soil never exposed to Metham sodium), which had 74.90 per cent MITC after 24 hours (Figure 3).

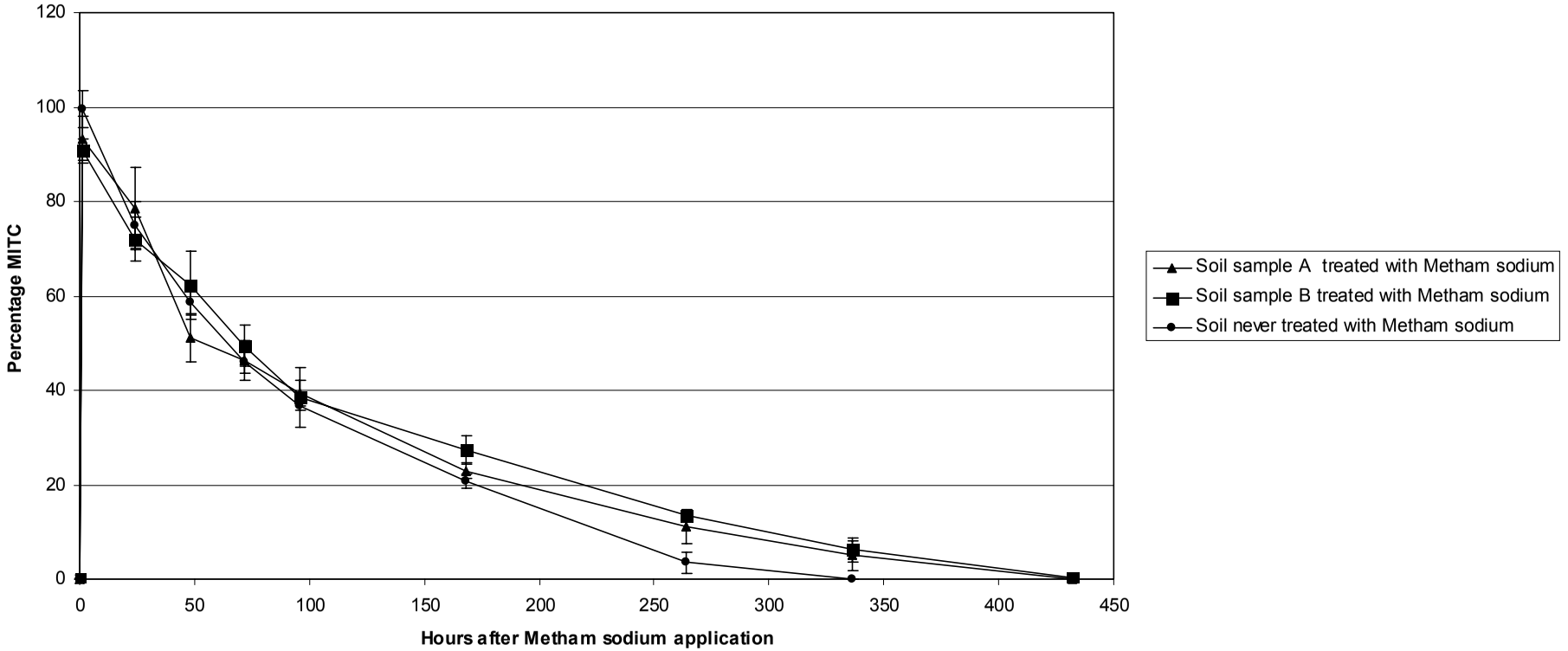

Figure 3. Demonstration of the breakdown of Metham sodium in soil samples collected from Medina Research Station. The soil was collected MITC (methyl isothiocyanate) is the breakdown product of Metham sodium. Samples A and B had both been fumigated with Metham sodium but had been collected from different locations within the experimental plots.

Discussion

Disease incidence

Disease incidence (6.46% detransformed mean) at harvest was comparable to the experiment conducted on a commercial property at Wanneroo (Section 3.3), 7.75 per cent (raw value). These values were much lower than that recorded the previous year on the same experimental site at Medina. The variability in disease levels between seasons is influenced by the weather conditions during the growing season, as well as those that influence sclerote survival and breakdown. Monthly average rainfall data and monthly average maximum temperature data from Medina Research Station for the 2000 and 2001 winter growing periods were compared. Data from 2001 were similar to the other years, indicating that conditions were favourable for disease development in the 2001 trials. This does not readily explain why the disease level was low for this experiment, but does suggest that for last years experiment the addition of the inoculum in a mycelial state rather than sclerote form created a higher than normal disease pressure.

The lack of significant variation in disease incidence between metham sodium application histories was expected, as all treatment plots were fumigated with metham sodium prior to transplant of the second year trial crop.

Soil pH and sclerote density

The soil pH for this experiment was very similar to that determined in the previous years experiment at the same site (Section 3.1). The variability shown is within normal ranges expected.

The number of sclerotes detected in the control plots was much lower than expected and did not account for the disease incidence determined. This indicates that the sampling technique needs to be revised. The pogo method for sclerote collection covers only a small surface area to a depth of 15 cm. A sampling method that collects soil over a larger surface area from a shallower soil profile might provide higher sclerote numbers, as sclerotes produced on lettuce during the season should remain in the first few cm of the soil profile. Sclerote numbers will vary greatly according to where the samples are collected and if they are near an infected or a healthy plant. No real comparison can be made between the soil samples collected during this work. A new sampling method is required to determine sclerote density in lettuce crops.

Enhanced biodegradation of Metham sodium

The lack of difference in the percentage of MITC after 24 hours, between the soil samples indicates that enhanced biodegradation of Metham sodium was not occurring at this site.

Post-transplant control of SLD

This study indicates that adequate control of SLD can be achieved with fewer fungicide applications. This experiment supports the findings of Section 3.1, that the strategic application of fungicides can reduce the number of plant deaths due to SLD.

The use of the standard practice for fungicide application (sub-treatment 1) and the minimal application of fungicides (sub-treatment 2) showed significantly fewer plant deaths than the untreated control plots (sub-treatment 6). However, the strategic application treatment (sub-treatment 3) was not significantly different to these other treatments.

The results indicate that Sumisclex® drenching of seedlings (sub-treatment 5) immediately after transplant was of no benefit. In reality, this treatment appeared to increase the number of plant deaths due to SLD significantly compared to all fungicide treatments. This result is contradictory to results (Section 3.5) from experiments

conducted in the Eastern States. The experiment found Sumisclex® drenching to be the most effective way of controlling SLD, (H. Pung, *pers. comm*.). This is most likely due to the different soil types where lettuce crops are grown. The soil in Western Australian production areas is sandier than in the Eastern States. The higher volumes of water used on the sandier soil types may leach the fungicide past the roots before it can be taken up by the plants.

The lack of large significant differences between the treatments is due to the low disease incidences detected in the experiment. However, it does indicate that the use of a soil fumigant combined with fungicide treatment will provide some control of SLD. The application of fungicides may be reduced by delaying spraying until plants are larger in low disease pressure situations. This level of control is dependent upon the disease pressure during the growing season. The fungicide regime used in sub-treatments 1 and 2 cannot be recommended to growers currently, as Bavistin is not registered for use on lettuce in Western Australia. This fungicide was used as a substitute for Benlate®, as manufacture of Benlate® was ceased in 2001. Bavistin® belongs to the same class as Benlate® and was therefore included in this experiment.

3.3 Determination of the effect of pre-plant Metham sodium fumigation on the incidence of SLD on a commercial lettuce crop

Introduction

Lettuce production in Western Australia is in the winter months (May to October). However, during the summer lettuce can be grown in southern areas where daily temperatures are cooler and the soil has a higher loam content. In Perth, most lettuce production occurs in the Wanneroo area on sand soil type (Karrakatta sand).

The pathogen of Sclerotinia Lettuce Drop (SLD) can either be *Sclerotinia minor* or *Sclerotinia sclerotiorum*, although *S. minor* tends to predominate. The lettuce industry relies on the regular use of fungicides to control the disease with varying degrees of success. There had been reports that the fumigant Metham sodium was not providing the control of SLD as expected. This simple experiment examined the difference in SLD levels between plots that had been fumigated with Metham sodium and those that had not been fumigated.

Materials and methods

Site description and experimental design

A commercial property specialising in lettuce production in Wanneroo, 26 km north of Perth, was used for this experiment. The soil type was Karrakatta sand. Crisphead lettuce, chinese cabbage and ryegrass have been grown on this property for the last 15 years. The soil fumigant, Metham sodium, had been applied at this site, at least once a year, for the past 15 years.

Two treatments were investigated; a) beds fumigated with Metham sodium prior to transplant and b) beds not fumigated with Metham sodium prior to transplant. Lettuce cv. "Oxley" was planted in June 2001. The plots were 6.65 m (3 beds) x 6.75 m with a total of 234 lettuce plants in three rows. Between all treatments there were buffers (1.1 m between the treatment beds and 9.2 m between the two main treatment plots). Row spacing in all plots including buffers was 35 cm within and between rows.

Overhead irrigation was applied daily during early to mid-morning throughout the trial. The herbicide Kerb® was applied as required, and prior to transplant. Superphosphate and trace elements were applied prior to transplant and fertilisers were applied as required.

Measurements and data analysis

Disease incidence and pathogen identification

The trial was scored weekly from transplant for disease incidence. The cumulative number of plants that had died from SLD in each plot was recorded. SLD infections were determined by the drooped appearance of outer leaves, combined with the subsequent collapse of the head in older plants, and/or the presence of white mycelia and/or black sclerotia underneath outer leaves near the crown region. In the absence of sclerotia, infected tissue was collected and incubated to promote mycelial growth, and plated onto PDA to check for sclerotia characteristics in culture.

Soil pH

Soil samples (at a depth of 15 cm) were collected using a "pogo" stick at three different times; a) at planting, b) mid crop and c) at harvest, along a W-pattern within each control plot. Five subsamples were collected per plot. Subsamples were bulked together from each plot and mixed thoroughly prior to determination of soil pH. The pH of soil samples was determined using $CaCl_2$ according to the methods described by Piper (1950) and Loveday (1974).

Soil sclerote counts

Soil samples for sclerote counts were collected at three different times; a) at planting, b) mid crop and c) at harvest from each control plot that either had Metham sodium applied or not. The number of sclerotia per 100 cc of soil was determined by the wet-sieving method of Adams (1979).

Enhanced biodegradation of Metham sodium

Two bulked soil samples were collected from; a) soil treated the most regularly with Metham sodium; and b) soil that had never been treated with Metham sodium on the grower's property. Samples were analysed by CSIRO, Floreat Park, Western Australia for enhanced biodegradation of Metham sodium by determining the percentage of methyl isothiocyanate (MITC), the breakdown product of Metham sodium over time.

Data analysis

No data analysis was done as the trial was not replicated. However, the results will still be discussed.

Results

Disease incidence

The mean percentage plants dead from SLD were compared (Table 1) for the plus and minus Metham sodium plots. Field observations during the season indicated that the majority of infections were occurring in the eastern bed. The distribution of infection within plots (i.e. across the 3 treatment beds) was compared at harvest. In general, the disease incidence for both treatments (with and without Metham sodium) was low. At harvest the percentage of deaths due to SLD was 8.2 per cent (plus Metham sodium) and 7.3 per cent (minus Metham sodium) (Table 9).

Pathogen identification

Only *S. minor* was found to be causing SLD in this experiment. Where sclerotia were obvious on infected plants, they were less than 2 mm in diameter, indicating the pathogen to be *S. minor* rather than *S. sclerotiorum*. Isolations from field plants with drooped leaves, but no obvious sclerotia, also yielded only *S. minor.*

Soil pH and sclerote density

Pre-plant fumigation with Metham sodium appeared to have no long term effect on soil pH at this experimental site. The pH of the soils in the two treatment plots was similar throughout the crop cycle (Table 10). Low numbers of sclerotes were detected on all sampling dates in both treatments (Table 10).

Enhanced biodegradation of Metham sodium

Results from the soil samples collected and submitted showed that enhanced biodegradation was occurring on the property where this experiment was planted (Figure 4). Twenty four hours after addition of Metham sodium to sample 1 the percentage MITC remaining was 2.57 per cent. However, for sample 2 (soil never exposed to Metham sodium) the MITC was still as high as 49.80 per cent (Figure 4). This explains why there were no differences in the disease severity between the Metham sodium treated plots and the non-treated plots.

Table 9. The number of dead plants from SLD at harvest for each of the three beds in each treatment plot

	Treatment	
Bed Number	**Plus Metham sodium**	**Minus Metham sodium**
1	0	1
2	4	6
3	15	10
Total	19 (8.2%)	17 (7.3%)

Table 10. Soil pH and sclerote density for soil with and without Metham sodium application at three different growth stages of a lettuce crop

Treatment	**pH ($CaCl_2$)**			**Sclerote density (per 100 cc soil)**		
	Seedling	**Mid-crop**	**Harvest**	**Seedling**	**Mid-crop**	**Harvest**
Plus Metham sodium	5.86	6.07	6.28	2	0	0
Minus Metham sodium	5.97	6.10	6.23	0	0	1

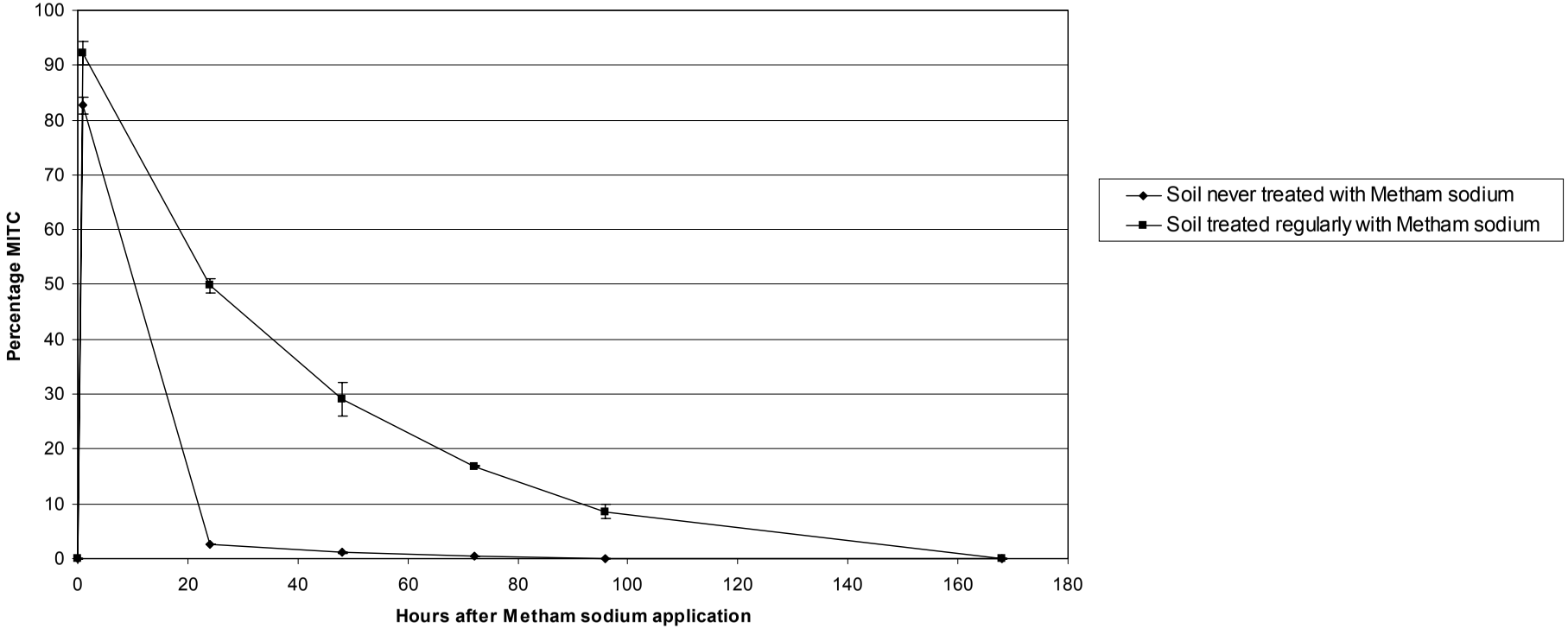

Figure 4. Demonstration of the breakdown of Metham sodium in soil samples collected from a commercial lettuce crop in Wanneroo. MITC (methyl isothiocyanate) is the breakdown product of Metham sodium.

Discussion

Disease incidence

The disease incidence in this experiment (7.3%) was low. In previous observations at this same site, losses of 50 per cent had been recorded for crops that had been planted earlier in the same winter growing season (C. Wood, personal observations). Losses due to SLD are often as high as 20-30 per cent for winter crops of Oxley. The incidence may have been low during this experiment due to the slightly warmer temperatures and lower than average rainfall received during the season. The fungus *Sclerotinia* is more pathogenic in cooler temperatures of less than 15°C.

The results of this experiment indicate that pre-plant fumigation with Metham sodium is not effective in the control of SLD caused by *S. minor*. These results contrast with a previous field experiment conducted at Medina Research Station (Section 3.1), where the application of Metham sodium had a significant affect on the disease incidence of SLD. This experiment needs to be repeated under conditions that are more conducive for SLD infection, as larger differences may show between Metham sodium treated and non-treated plots.

During the weekly assessment it was noted that most SLD infections developed in the eastern treatment beds. This suggests an inoculum gradient exists within the site or there is an inefficiency of spray equipment to reach plants in the eastern side of the lettuce bay.

Soil pH and sclerote density

The pH appeared to have increased slightly over time in both treatment plots. However, these increases in pH are quite small and may just be due to sampling error or natural soil variability.

The number of sclerotes detected in the soil sampling was low. This does correlate with the low disease incidence detected at the site. However, previous experience suggests that the inoculum level should be higher at this experimental site. This would indicate a problem with the sampling method used. The soil samples were collected with a 15 cm pogo stick, and therefore only a small soil surface area to depth ratio was sampled. As sclerotes are likely to build up near leaves and roots of infected plants, it would have been more appropriate to sample a larger surface area of the soil at a shallow depth. A new method for soil sampling is necessary to assess sclerote densities in lettuce crops. This method needs to account for the following; a) spatial variation in sclerote numbers as sclerotes will be clumped around infected plants, and b) surface area/depth ratio of the soil profile sampled.

Enhanced biodegradation of Metham sodium

The results from CSIRO indicated that enhanced biodegradation was occurring on this commercial property. The disease incidence levels at harvest also show this, as there were more diseased plants in the fumigated plots compared to the non-fumigated plots.

In conclusion, the soil fumigant, Metham sodium, may be useful for control of SLD in some situations. This is dependent upon the history of use and the soil type, as the product may have a reduced activity when used on soils where it has been applied regularly for many years.

3.4 Assessment of various fungicides and fertiliser applications for controlling *Sclerotinia minor* in crisphead lettuce crops

Introduction

Lettuce production in Western Australia is in the winter months (May to October). However, during the summer lettuce can be grown in southern areas where daily temperatures are cooler and the soil has a higher loam content. In Perth, most lettuce production occurs in the Wanneroo area on sand soil type (Karrakatta sand).

The pathogen of Sclerotinia Lettuce Drop (SLD) can either be *Sclerotinia minor* or *Sclerotinia sclerotiorum*, although *S. minor* tends to predominate. The lettuce industry relies on the regular use of fungicides to control the disease with varying degrees of success. The use of cultural control strategies such as crop rotation, the addition of soil amendments, is not used within the industry. Previous experiments (Section 3.1, 3.2 and 3.3) showed that control of SLD can be achieved through the use of the soil fumigant Metham sodium if enhanced biodegradation is not occurring within that soil, and that it may be possible to reduce the amount of fungicides applied during the lettuce crop. This experiment compares standard grower practices, with a reduced fungicide regime and the use of soil amendments that were successful n the control of SLD in the Eastern States.

Materials and Methods

Site description and experimental design

A commercial property specialising in lettuce production in Wanneroo, 26 km north of Perth, was used for this experiment. The soil type was Karrakatta sand. Crisphead lettuce, chinese cabbage and ryegrass have been grown on this property for the last 16 years. The soil fumigant, Metham sodium, had been applied at this site, at least once a year, for the past 16 years.

The experimental design was a Latinised alpha design with 10 treatments replicated six times. This allocated the 10 treatments within and across 2 long beds to account for possible between bed and within bed variability. The plots were 1.65 m x 1.35 m with 20 lettuces planted in four rows. Between all treatments there were lettuce buffers (1.32 m x 1.35 m, with 16 plants). Row spacing in all plots, including buffers, was approx 33 cm within and 34 cm between rows.

Overhead irrigation was applied daily during early to mid-morning throughout the trial. The herbicide Kerb® was applied as required, and prior to transplant. Superphosphate and trace elements were applied prior to transplant and additional fertilisers were applied as required.

Treatments

The scheduling of all fungicide applications and their relevant rates are shown in Table 11. The active ingredients for all of the fungicides are listed in the Appendix A. Unlike previous trials the fumigant Metham sodium was not used.

T1 Benlate® - sprayed weekly from transplant until crop maturity.

T2 Sumisclex® - sprayed weekly from transplant until crop maturity.

T3 Marvel® - sprayed weekly from transplant until crop maturity.

T4 Bayfidan® - sprayed weekly from transplant until crop maturity.

T5 Sumisclex® + Microgyp® (lime) - sprayed weekly from transplant until crop maturity. The Microgyp® was mixed with the fungicide spray.

T6 Current grower best fungicide practice - Benlate®, Sumisclex® and Rovral® were applied in rotation weekly from transplant until crop maturity.

T7 Reduced fungicide - Sumisclex® and Benlate® were applied in rotation weekly to all replicate plots once 5 per cent of plants (i.e. 1 plant per plot) became infected. This happened 31 days after transplant.

T8 High potassium nitrate (KNO_3) fertiliser application, no fungicides. A total of 900 kg/ha KNO_3 was applied to each treatment plot in 3 applications when lettuce were young (between 25 and 39 days after transplant). KNO_3 was applied dry, in 2 bands per plot.

T9 High potassium nitrate (KNO_3) fertiliser application plus current best practice fungicide.

T10 Untreated control.

Measurements and data analysis

Disease incidence

The trial was scored weekly from transplant for disease incidence. The cumulative number of plants that had died from SLD in each plot was recorded. SLD infections were determined by the drooped appearance of outer leaves, combined with the subsequent collapse of the head in older plants, and/or the presence of white mycelia and/or black sclerotia underneath outer leaves near the crown region.

Soil sclerote counts

Soil samples for sclerote counts were collected at three different times; a) at planting, b) mid crop and c) at harvest from each control plot that either had Metham sodium applied or not. The number of sclerotia per 100 cc of soil was determined by the wet-sieving method of Adams (1979).

Data analysis

Disease incidence data were analysed using Genstat. Data were analysed as a Latinised alpha design. Treatment means were compared using Fisher's least significance difference ($P < 0.05$).

Table 11. Fungicide spray schedule for the 10 treatments used in the experiment including fungicide rates and fertiliser applications

	Treatments									
Days after transplant	**T1 Benlate®**	**T2 Sumisclex®**	**T3 Marvel®**	**T4 Bayfidan®**	**T5 Sumisclex® + Microgyp**	**T6 Best fungicide practice**	**T7 Reduced fungicide**	**T8 High rate fertiliser**	**T9 High rate fertiliser + best practice**	**T10 Untreated control**
Pre-plant	Grower practice - 50 g/L KNO_3 + 5 g/L MAP	Grower practice - 50 g/L KNO_3 + 5 g/L MAP	Grower practice - 50 g/L KNO_3 + 5 g/L MAP	Grower practice - 50 g/L KNO_3 + 5 g/L MAP	Grower practice - 50 g/L KNO_3 + 5 g/L MAP	Grower practice - 50 g/L KNO_3 + 5 g/L MAP	Grower practice - 50 g/L KNO_3 + 5 g/L MAP	Grower practice - 50 g/L KNO_3 + 5 g/L MAP	Grower practice - 50 g/L KNO_3 + 5 g/L MAP	Grower practice - 50 g/L KNO_3 + 5 g/L MAP
4	Benlate 1 g/L at 800 L/ha	Sumisclex 1 mL/L at 800 L/ha	Marvel 1 g/L at 800 L/ha	Bayfidan 40 mL/100 L at 1200 L/ha	Sumisclex 1 mL/L at 800 L/ha Microgyp 2.5 kg/ha	Sumisclex 1 mL/L at 800 L/ha			Sumisclex 1 mL/L at 800 L/ha	
11	Benlate 1 g/L at 800 L/ha	Sumisclex 1 mL/L at 800 L/ha	Marvel 1 g/L at 800 L/ha	Bayfidan 40 mL/100 L at 1200 L/ha	Sumisclex 1 mL/L at 800 L/ha Microgyp 2.5 kg/ha	Rovral 1 mL/L at 1000 L/ha			Rovral 1 mL/L at 1000 L/ha	
18	Benlate 1 g/L at 800 L/ha	Sumisclex 1 mL/L at 800 L/ha	Marvel 1 g/L at 800 L/ha	Bayfidan 40 mL/100 L at 1200 L/ha	Sumisclex 1 mL/L at 800 L/ha Microgyp 2.5 kg/ha	Benlate 1 g/L at 800 L/ha			Benlate 1 g/L at 800 L/ha	
25	Benlate 1 g/L at 1000 L/ha	Sumisclex 1 mL/L at 1000 L/ha	Marvel 1 g/L at1000 L/ha	Bayfidan 40 mL/100 L at 1200 L/ha	Sumisclex 1 mL/L at 1000 L/ha Microgyp 2.5 kg/ha	Sumisclex 1 mL/L at 1000 L/ha		300 kg/ha KNO_3	Sumisclex 1 mL/L at 1000 L/ha 300 kg/ha KNO_3	
32	Benlate 1 g/L at 1000 L/ha	Sumisclex 1 mL/L at 1000 L/ha	Marvel 1 g/L at 1000 L/ha	Bayfidan 40 mL/100 L at 1200 L/ha	Sumisclex 1 mL/L at 1000 L/ha	Rovral 1 mL/L at 1000 L/ha	Sumisclex 1 mL/L at 1000 L/ha	300 kg/ha KNO_3	Rovral 1 mL/L at 1000 L/ha	

Treatments										
Days after transplant	**T1 Benlate®**	**T2 Sumisclex®**	**T3 Marvel®**	**T4 Bayfidan®**	**T5 Sumisclex® + Microgyp**	**T6 Best fungicide practice**	**T7 Reduced fungicide**	**T8 High rate fertiliser**	**T9 High rate fertiliser + best practice**	**T10 Untreated control**
					Microgyp 2.5 k g/ha				300 kg/ha KNO_3	
39	Benlate 1 g/L at 1000 L/ha	Sumisclex 1 mL/L at 1000 L/ha	Marvel 1 g/L at 1000 L/ha	Bayfidan 40 mL/100 L at 1200 L/ha	Sumisclex 1 mL/L at 1000 L/ha Microgyp 2.5 kg/ha	Benlate 1 g/L at 1000 L/ha	Benlate 1 g/L at 1000 L/ha	300 kg/ha KNO_3	Benlate 1 g/L at 1000 L/ha 300 kg/ha KNO_3	
46	Benlate 1 g/L at 1000 L/ha	Sumisclex 1 mL/L at 1000 L/ha	Marvel 1 g/L at 1000 L/ha	Bayfidan 40 mL/100 L at 1200 L/ha	Sumisclex 1 mL/L at 1000 L/ha Microgyp 2.5 kg/ha	Sumisclex 1 mL/L at 1000 L/ha	Sumisclex 1 mL/L at 1000 L/ha		Sumisclex 1 mL/L at 1000 L/ha	
53	Benlate 1 g/L at 1000 L/ha	Sumisclex 1 mL/L at 1000 L/ha	Marvel 1 g/L at 1000 L/ha	Bayfidan 40 mL/100 L at 1200 L/ha	Sumisclex 1 mL/L at 1000 L/ha Microgyp 2.5 kg/ha	Benlate 1 g/L at 1000 L/ha	Benlate 1 g/L at 1000 L/ha		Benlate 1 g/L at 1000 L/ha	
60	Benlate 1 g/L at 1200 L/ha	Sumisclex 1 mL/L at 1200 L/ha	Marvel 1 g/L at 1200 L/ha	Bayfidan 40 mL/100 L at 1200 L/ha	Sumisclex 1 mL/L at 1200 L/ha Microgyp 2.5 kg/ha	Sumisclex 1 mL/L at 1200 L/ha	Sumisclex 1 mL/L at 1200 L/ha		Sumisclex 1 mL/L at 1200 L/ha	-
67	Benlate 1 g/L at 1200 L/ha	Sumisclex 1 ml/L at 1200 L/ha	Marvel 1 g/L at 1200 L/ha	Bayfidan 40 ml/100 L at 1200 L/ha	Sumisclex 1 ml/L at 1200 L/ha Microgyp 2.5 kg/ha	Benlate 1 g/L at 1200 L/ha	Benlate 1 g/L at 1200 L/ha		Benlate 1 g/L at 1200 L/ha	

Results

Identification of pathogen

Sclerotinia minor was isolated from all plants collected during the four trials. No S. *sclerotiourum* was detected in any of the trials. Where possible, the plants were assessed in the field by the presence of sclerotia. The sclerotia were less than 2 mm in diameter, indicating that the pathogen was *S. minor* and not *S. sclerotiorum*.

Disease incidence

The mean percentage plants dead from SLD at harvest was 63.1 per cent (raw value), but individual plot values ranged from 15 per cent to 90 per cent.

At harvest, the following treatments had significantly lower ($P < 0.001$) disease incidence (Table 12) than the untreated control plots a) the weekly Sumisclex® applications (treatment 2), b) weekly fungicide applications plus extra KNO_3 fertiliser applications (treatment 9), c) weekly Sumisclex® + Microgyp® applications (treatment 5), d) weekly Benlate® applications (treatment 1), and e) current best fungicide practice (treatment 6).

Table 12 shows that the weekly application of Sumisclex® (treatment 2) resulted in a significantly lower level of disease ($P < 0.001$, 41.7 %) than the best practice treatment (treatment 6) and the weekly application of Benlate® (treatment 1) (61.7%).

Treatment 5 (weekly Sumisclex® + Microgyp® applications) was not significantly different to Treatment 2 (weekly Sumisclex® applications) in disease incidence at harvest (Table 12).

Disease incidence for the best practice fungicide applications (treatment 6) and this treatment with extra fertiliser applications (treatment 9) were not significantly different at harvest (Table 12). The use of high levels of potassium nitrate (muriated) fertiliser (treatment 8) by itself increased the amount of SLD in the plots and the disease incidence was not significantly different to the levels in the untreated control plots (treatment 10).

The weekly application of either Marvel® (treatment 3), or Bayfidan® applications (treatment 4) and the reduced fungicide application (treatment 7) did not reduce the disease incidence and these were not significantly different ($P < 0.001$) to the untreated control treatment 10) (Table 12). Treatment 3 was also not significantly different to Treatment 1 (Table 12), indicating that Marvel® is a comparable fungicide to Benlate®.

Table 12. Table of means for percentage plants dead from SLD per plot at harvest. Values are derived from averages of 6 replicate plots

Treatment	Percentage dead
T2 (Sumisclex®)	41.7 *a*
T9 (Best practice + high fertiliser)	49.0 *ab*
T5 (Sumisclex® + Microgyp®)	50.8 *abc*
T6 (Best practice)	58.7 *bcd*
T1 (Benlate®)	61.7 *cde*
T7 (Minimal fungicide)	71.4 *ef*
T3 (Marvel®)	72.1 *ef*
T8 (High fertiliser)	74.9 *f*
T4 (Bayfidan®)	75.2 *f*
T10 (Untreated control)	75.3 *f*
Average	63.07
LSD ($P < 0.001$)	11.89

Discussion

Disease incidence was relatively high in trial plots compared with the surrounding grower's crop (C. Wood, personal observations). It is suspected that reduced levels of mutual protection against physical damage within the lettuce crop resulted in higher disease incidence. The plants in treatment plots were more exposed than plants in the adjacent grower's crop, as unplanted buffer beds were placed either side of the beds containing treatment plots.

Also, it is likely that knapsack spraying of plots was not suitable for good disease control. Although plants received weekly sprays with fungicides (Table 11), high application pressures (> 1.0 bar) could not be achieved with the knapsack sprayer. This is a lower pressure than growers would use when spraying their crops. The equipment available to growers enables them to reach a pressure of two bars or greater and the droplet size is reduced to a fine mist. To prevent spray drift, when the fungicides were applied in this trial, a larger droplet size was selected. Misting may be more important for disease control than expected as the lower leaves will have some coverage. However, the majority of the fungicides used are systemic and the droplet size should not influence the uptake and efficacy of the fungicides if applied to run-off.

Due to the lack of significant difference between treatment 2 and 5, it appears that the addition of Microgyp® to the fungicide spray was of no benefit to the crop. This is contradictory to the findings of eastern states research where Microgyp® was found to be beneficial in reducing the pathogenicity of Sclerotinia by an increase in the host tolerance.

The use of high rate of potassium nitrate as a fertiliser did not appear to decrease the incidence of SLD (Table 12) as there was no significant difference between the disease levels for treatments 6 and 9. This treatment had been incorporated because previous observations by other research staff in DAWA had noted that an increase in plant health seemed to reduce the effects of SLD.

Due to the de-registering of Benlate® and the registration of Marvel® as its replacement, it was important to compare the efficacy of the two fungicides. The results show that there was no significant difference between them. Thus, Marvel® is comparable to Benlate®.

The weekly application of Sumisclex® gave the most significant control of SLD in this experiment. Despite this, weekly applications of Sumisclex® can not be recommended to growers for resistance management purposes.

These results indicate that standard grower practice of a weekly fungicide rotation is validated and is currently the best method to reduce crop losses due to SLD.

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SECTION 4.0

CONTROL STRATEGIES FOR THE MANAGEMENT OF SCLEROTINIA LETTUCE DROP AND LETTUCE BIG VEIN IN TASMANIA

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Summary

Lettuce drop caused by infections with *Sclerotinia minor* and lettuce big vein caused by infections with Mirafiori lettuce virus are major diseases of lettuce worldwide. Here we describe a series of field trials assessing conventional and novel management strategies using soil amendment approaches.

Of particular note is the use of soil surface applications of a highly alkaline material (finely powdered calcium hydroxide) to inhibit sclerotia germination of the lettuce drop pathogen and reduce infection at the collar region of lettuce plants. Laboratory assays indicated a pH greater than 8.0 significantly reduced germination of *S. minor* sclerotia. Under glasshouse conditions, applications of up to 10 t/ha calcium hydroxide successfully elevated the pH of the top 1-2 cm of the soil profile above 8.5 for at least 8 weeks without affecting the pH of soil within the transplant root zone. A linear relationship between rate applied and disease control was shown, with complete disease suppression at 10 t/ha. In field trials, a rate of 2.5 t/ha, maintained soil surface pH above 8.5 for 1-3 weeks. Use of soil conditioning polymers applied to the calcium hydroxide layer prolonged pH elevation through reduction in losses due to wind. At this rate, significant reductions in lettuce drop disease were shown (up to 85% disease reduction). Integration of calcium hydroxide treatment with a conventional procymidone based fungicide drench showed a significant synergistic effect further improving disease control. Integrated use of soil surface applied high pH materials such as calcium hydroxide with strategies such as fungicides, rotation and drip irrigation offer an opportunity for enhanced and sustainable disease control of *Sclerotinia minor* in lettuce.

Other studies showed compost, oats cover crops, and fumigation with metham sodium increases the incidence of LBV disease contrary to expectations. Compost amendments also exacerbated lettuce drop disease when used on a site with poor soil nutrition. Surveys of Tasmanian nurseries showed little evidence of disease within seedling stocks. A possible link between nursery hygiene and sporadic occurrences of LBV is discussed.

4.1 A study of soil amendment treatments for management of lettuce drop and lettuce big vein diseases

Introduction

Lettuce (*Lactuca sativa* L.) production provides an important component of the Australian fresh vegetable industry, having a 1999 gross value of A$89.1 M with export earnings of A$6.7 M over the same year (Archer *et al.* 2000).

Lettuce big vein disease (LBV) was initially attributed to infections with lettuce big vein virus (LBVV), but is now considered induced by infection with Mirafiori lettuce virus (MiLV; Lot *et al.* 2002; Roggero *et al*. 2003). Both viruses are spread by a soil-borne fungal vector, *Olpidium brassicae,* commonly found in lettuce production. LBV disease is prevalent wherever lettuce is cultivated (Subbarao, 1997) and results in significant yield and quality losses. Management of LBV is difficult as the virus can persist in soils with the fungal vector for extended periods in absence of the host. Furthermore, symptom expression is host and environmentally driven. Glasshouse grown seedlings are commonly symptomless and thus nursery producers may unwittingly spread infections if careful hygiene practices are not maintained.

Lettuce drop disease, caused by infection with *Sclerotinia* spp (Smith 1900), is reported from all lettuce-producing regions of the world (Subbarao, 1997) and poses a significant threat to lettuce production and export in Australia. The predominant species associated with lettuce drop in Australia is *S. minor* (Archer *et al*. 2000) as appears to be the case in many other regions including New Zealand (Hawthorne, 1975), Canada (Martin *et al.* /, 1991), New York (Jagger, 1920) and California (Abawi *et al*, 1985). *S. minor* has small (0.5-2.0 mm diameter) sclerotia that seldom produce apothecia, and thus field infection occurs predominantly by direct infection from germinating sclerotia (Jagger, 1920, Scannavini *et al*. 1993, Subbarao, 1997). Studies examining the importance of sclerotia from differing soil depths toward disease have shown that those within the top few centimetres of soil are responsible for the vast majority of infections, which typically occur at the root crown inducing a collar rot symptom (Patterson and Grogan, 1985, Scannavini *et al*. 1993, Melzer and Boland, 1994, Subbarao, 1997).

Management of lettuce drop disease is difficult. Commercial production in Australia relies heavily on the strategic use of fungicide drench, foliar sprays treatments and/or soil fumigants (Watson *et al*. 2000) with little use of other disease management strategies such as rotation or irrigation practices (Thaning and Gerhardson, 2001; Hao *et al*. 2003a). Reduced efficacy of fungicides (Martin *et al*. 1990; 1991) and consumer concerns over pesticides used in food crops suggest integrated disease management reducing reliance on chemical control is advisable for sustainable production.

Various studies examining optimal temperatures and soil moisture content for sclerotial survival, germination and infection have been done (Adams, 1987, Hao *et al*. 2003b, Huang *et al*. 1998). Management of soil-borne pathogens by raising or lowering cropping soil pH has been useful for certain diseases (de Rooster and Spiessens, 1999, Goto, 1985, Mayer and Shew, 1991, Murray *et al*. 1992; Voley, 1990) but *S. minor* is tolerant to the pH range required for successful lettuce cropping (Iliesen *et al*. 1988). In recent years studies of foliar and fruit pathogens of various crops have shown that strategic application of very alkaline materials onto leaf and fruit surfaces can inhibit spore germination and reduce disease losses (Heijne *et al*. 1998, Mora Alfaro, 2000, Washington *et al*. 1998) without impacting on plant productivity (Brown *et al*. 2000). As the majority of *S. minor* infections with occur within the top few cm of soil we proposed that a similar strategy could be used by applying a crust of alkaline material to the soil surface to inhibit sclerotial germination, and/or neutralise oxalic acid production by the pathogen and thus prevent or reduce infection at the collar region.

This report describes a series of experiments examining soil treatments for control of LBV and lettuce drop disease including the novel use of soil surface pH amendment and integration of such an approach with conventional fungicides or selected non-fungicide treatments.

Materials and methods

1. Amendment of soil surface pH for control of Sclerotinia minor infection in lettuce

Effect of pH on germination of *S. minor* sclerotia

Sclerotia of a Tasmanian isolate of *S. minor* obtained from diseased lettuce were harvested by sieving (4 mm^2 pores) a culture grown for 1 week on sterilised barley medium (300 g barley, 2.5 g gypsum, 400 ml deionised water).

Surface sterilised sclerotia were placed onto PDA plates (three per plate) which had been adjusted to pH 6.0, 7.0, 8.0, 9.0, 10.0 and 11.0 with 1M Tris-HCl (pH 6.0-8.0) and 1M borax (pH 7.0-11.0) buffers. Six replicates of each pH treatment were used including duplicates of each buffer in the overlapping pH range. Plates were incubated at 25°C for 7 days and sclerotia examined for evidence of germination and mycelia growth.

The experiment was repeated using liquid medium (PDB similarly adjusted to pH 6.0-11.0). Three replicates of each pH treatment (100 ml) were used and 10 surface sterilised sclerotia added to each flask. Flasks were incubated in an orbital shaker at room temperature for 1 week before sclerotia were removed and examined microscopically for evidence of germination. Data were analysed by ANOVA using Genstat (The Numerical Algorithms Group Ltd, Oxford, UK).

Evaluation of soil surface pH amendment on lettuce growth and lettuce drop disease under controlled conditions

Head lettuce plants were grown from transplants in individual pots containing topsoil sourced from a commercial lettuce farm in Cambridge, Tasmania. Varying amounts of finely powdered calcium hydroxide (Limil, David Mitchell Ltd, Box Hill, Victoria) were added onto the soil surface of each pot to give equivalent rates of 0, 2, 4, 6, 8, and 10 tonnes per hectare. Each treatment was replicated six times. Plants were grown under glasshouse conditions until maturity where visual appearance, head diameter, fresh and dry weight and foliar nutrient analysis (calcium, phosphorus, magnesium and Kjeldahl nitrogen) were assessed to ascertain any treatment effects on lettuce growth and quality. At harvest soil pH (1:5 in water) was measured in soil sampled from 0-2, 3-5, 8-10 and 10-12 cm depths.

A second glasshouse experiment replicated the above six soil treatments with the inclusion of pathogen inoculum (50 sclerotia/100 g soil) placed in a band within the top 2-3 cm of soil. Each treatment was replicated eight times. Diseased plants were noted and isolations from diseased tissue used to confirm pathogen identity. Data were assessed by ANOVA and linear regression using Genstat (The Numerical Algorithms Group Ltd, Oxford, UK).

Evaluation of soil surface pH amendment and fungicide treatments on lettuce drop under commercial field conditions

A field trial assessing the efficacy of soil surface pH amendment integrated with conventional fungicide treatments was established on a commercial farm at Don, Tasmania on a well buffered red ferrosol soil (pH 6.9) in late summer (30 March). Treatments were a) untreated control; b) fungicide drench (Sumisclex 275 flocol liquid fungicide, a.i. 275 g/L procymidone, Sumitomo Chemical Australia Pty Ltd) applied to transplant trays at 2 kg a.i./1000L/ha immediately before transplant; c) fungicide drench

and three fungicide foliar sprays (Sumisclex 275) applied at 1 kg a.i./1000 L/ha at 1, 2 and 3 weeks after transplant; d) three foliar sprays only; e) fungicide drench with powdered calcium hydroxide (Limil) broadcast onto soil surface at 2.5 t/ha immediately after transplanting; f) Limil only; g) fungicide drench with crushed lime broadcast at 10 t/ha; and h) crushed lime only. Each treatment was replicated six times in plots 8.7 m x 1.88 m which contained 100 plants in three rows. Plots were assessed for incidence of disease at weekly intervals for the first 5 weeks and then fortnightly until harvest (12 July). Soil samples (100 cm^3 of top 1 cm soil) were taken from four plots each of untreated, Limil, and crushed lime treatments at fortnightly intervals and assessed for pH (measured 1:5 in water). At harvest, the number of marketable lettuce in each plot was assessed. Data were analysed by ANOVA using Genstat (The Numerical Algorithms Group Ltd, Oxford, UK).

Phytotoxicity of soil stabilising compounds and their ability to enhance pH stability of Limil applications

A small pot trial was established to assess the efficacy of two soil conditioning polymers, poly vinyl acetate (PVA) and polyacrylyamide (PAM) to stabilise broadcast Limil applications and maintain elevated soil surface pH. Lettuce seedlings were transplanted into 40 x 30 cm trays containing topsoil from a commercial lettuce farm in Cambridge, Tasmania. Limil was broadcast (2.5 t/ha) on the soil surface. Pots then either received no additional treatment, spray applications of PVA solution (5, 10 and 10 kg a.i./ha equivalent) or PAM solution (20, 40 and 60 L/ha equivalent). Spray treatments liberally covered both soil and plants. Pots were placed on exposed benches out of doors. Soil samples (0-2 cm depth) were collected from treatment trays and assayed for pH at 1-4 weeks following application. Plants were grown to maturity and assessed for evidence of any disorders associated with treatment.

Evaluation of soil surface pH amendment (with and without soil conditioner treatments), compost and green manure treatments on lettuce drop under commercial field conditions

This field trial examined the interaction of Limil soil surface treatments with other (non-fungicidal) treatments aimed at increasing soil organic matter and microbial activity. The trial was established on the Cambridge lettuce farm at a site that had not been cropped for two years with a poorly buffered duplex sandy soil (pH 4.7). *S. minor* inoculum (50 sclerotia/100 g top 5 cm soil) was applied to the trial site in mid summer (December) three months prior to transplanting lettuce. The trial was established in a factorial design with eight replicates of the following treatments, a) compost (5 t/ha composted chicken manure and straw applied 8 weeks prior to transplanting and rotary hoed into plots), b) broadcast Limil (2.5 t/ha applied at transplanting), c) broadcast Limil (2.5 t/ha applied as two split application of 1.25 t/ha at transplanting and 14 days after transplanting) and d) oats green manure crop planted 8 weeks prior to transplanting lettuce, sprayed with herbicide and rotary hoed 1 week prior to transplanting. Plots were 1 x 3 m (100 plants in three rows/plot) with 1 m buffers. Within the Limil treatments, a split plot assessment of the efficacy of PVA soil conditioner (applied at 1 g/m2 as a 100 ml/L solution directly following Limil broadcast) to minimise windblown losses of Limil was included. PVA sprays were applied to half of the plots receiving Limil treatments (both rates).

Plots were inspected at weekly intervals and diseased plants noted. Soil samples were taken from eight plots at planting, two and nine weeks post planting and assessed for pH (1:5 soil:water). At harvest, lettuce fresh weights assessments were made from individual treatment plots. Data were analysed by REML using a log transformation with Genstat (The Numerical Algorithms Group Ltd, Oxford, UK).

2. Disease surveys of lettuce seedling nursery stocks

Lettuce transplants (100 per variety, two varieties per supplier) were sourced from the two major Tasmanian suppliers. Seedlings were planted into sterilised potting mix and grown on open benches to maturity. The incidence of LBV disease symptoms was noted and observations of general plant health were made.

In addition, health surveys of newly planted bays of lettuce at a commercial property in Margate, Tasmania were conducted. The site was only recently cultivated to lettuce (two years cropping) and prior lettuce rotations at the survey site showed little or no LBVV presence. Thus we assumed little soil inoculum of LBV or its fungal vector was present.

3. Soil treatments for management of LBV and lettuce drop diseases, and effects of treatments on insect pests and slug damage

Evaluation of soil treatments for expression of disease in lettuce due to lettuce big vein virus (LBVV) and Sclerotinia minor

Two field trials were established to assess the effect of soil treatments on incidence and/or expression of disease caused by infections with LBVV, *S. minor*, and any other diseases noted within the trial. Both trials were established on a commercial lettuce farm in Cambridge, Tasmania at sites with known history of LBVV and *S. minor* induced disease. Both trials were planted concurrently with disease-free lettuce seedlings on adjacent sites following a commercial lettuce crop (double cropping) in order to maximise likelihood of disease incidence. The first trial assessed the effects of a) metham sodium fumigant treatment, b) incorporation of 10 t/ha compost (mix of composted pine bark and chicken manure), c) soil surface application of 1.25 t/ha calcium hydroxide (Limil) and included an untreated control. The second trials assessed the effects of a) growth and incorporation of a 6 week old oat cover crop, b) incorporation of 10 t/ha compost, c) soil surface application of 1.25 t/ha calcium hydroxide (Limil) and an untreated control.

Treatments for both trials were overlayed in a factorial manner to assess all possible interactive effects. Plots were 8 m x 3 planting rows with a 2 m buffer between plots.

Disease incidence was assessed fortnightly for 2 months from trial establishment until harvest.

Data were analysed by Analysis of Variance or REML using Genstat (The Numerical Algorithms Group Ltd, Oxford, UK). Some data were $\log_e$ transformed to achieve normality.

Evaluation of soil surface liming for insect deterrence and comparisons with commercial slug bait treatment for minimising slug damage on lettuce

Following the success of the soil surface liming treatments for reducing *Sclerotinia minor* infections further trials were conducted to assess whether the treatment had additional pest management benefits.

It is known that many insect pest species are deterred by light reflectance in the ultra violet range (Harpaz, 1982). A small trial was established on a commercial lettuce farm to assess whether soil surface applied limil, a bright white material, would affect the incidence of pest insects. Treatments consisted of two 10 m^2 plots each of 2.5 t/ha calcium hydroxide (Limil) applied 2 weeks after transplanting (cv. Greenaway) and untreated control. Clear plastic sticky traps (used to avoid any artificial attraction of insects) were positioned within each plot and replaced fortnightly over a period of two months. Aphids, thrips and total insect counts were done during the trapping period.

A field trial was established on a commercial farm in Cambridge, Tasmania in the 1998/99 season to assess the efficacy of various treatments in reducing damage to head lettuce by slug feeding. All initial treatments were applied two weeks following transplanting lettuce seedlings and included a) slug pellets (Baysol, Methiocarb 20 g/Kg, 100 pellets/m^2), b) cone application of 1 tonne / ha calcium hydroxide (Limil) to the soil surface c) two applications of 1 tonne / ha calcium hydroxide to the soil surface, (2 and 4 weeks following transplanting), and d) an untreated control. Each plot consisted of 5 m x 3 rows (containing approx. 160 lettuce plants) with a 1 m buffer planted with lettuce between plots. Treatments were replicated four times with plots were arranged in a Latin square to allow for possible edge effect gradients. Only the central 5 metres of each plot were assessed. Fertilisation, irrigation and fungicide applications followed normal commercial practice, however no insecticides were applied.

A pre-treatment slug damage visual assessment was conducted to determine background damage levels. The post-treatment assessment was conducted 6 weeks after transplanting. Assessments scores were obtained using the following scale, 0 – no visual damage; 1-1 hole per leaf, slight damage; 2-2-4 holes per leaf, moderate damage; 3-large loss of leaf area, severely cropped plant.

Data were analysed by ANOVA or regression using Genstat (The Numerical Algorithms Group Ltd, Oxford, UK).

Results

1. Amendment of soil surface pH for control of Sclerotinia minor infection in lettuce

Effect of pH on germination of *S. minor* sclerotia

The pH of the buffered solution or solid medium had a significant effect ($P < 0.001$) on sclerotial germination. With the exception of the borax buffered pH 8.0 broth treatment (germination rate of 33%), all treatments below pH 9.0 encouraged efficient sclerotial germination (66-100%). In contrast treatments of pH 9.0 and above restricted sclerotial germination to below 34 per cent (Figure 1). From these data we established a theoretical pH threshold level of 8.5 for likely disease suppressive effect.

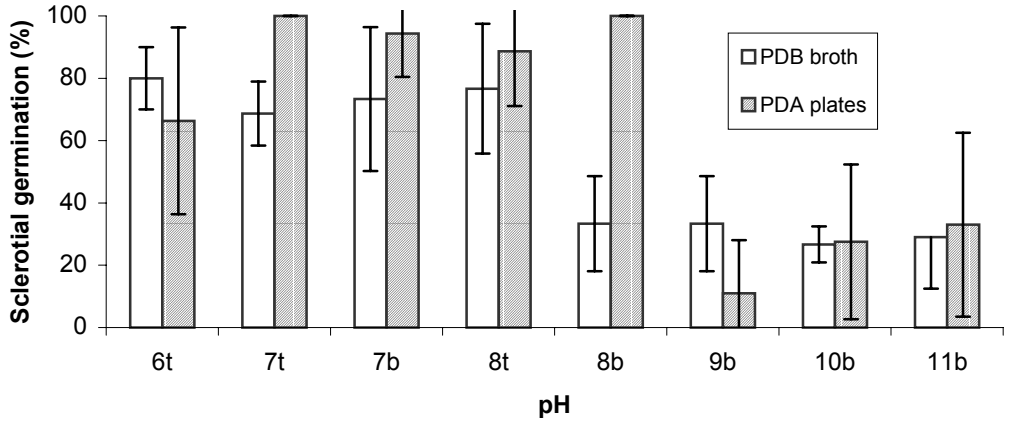

Figure 1. Effect of agar and liquid potato dextrose media pH on germination of *S. minor* sclerotia (t and b following pH value indicates Tris-HCL or borax buffer respectively).

Evaluation of soil surface pH amendment on lettuce growth and lettuce drop disease under controlled conditions

Mineral analysis of the soil used in the glasshouse experiments is given in Table 1. Assessment of soil pH at the conclusion of the trial indicated that only the top 1-2 cm of the soil was influenced by addition of calcium hydroxide (Limil) (Figure 2). Eight weeks post application, all Limil treatments had maintained an elevated surface pH of approximately 8.5 or above.

The addition of Limil to the soil surface had a significant positive effect on lettuce fresh weight but not on dry weight nor head diameter although a positive trend was indicated (Table 2). No treatment effects on foliar nutrient levels were noted (Table 2). Nitrogen and magnesium levels were deficient across all treatments, resulting from no supplemental fertiliser applications to treatment pots. No symptoms of disorders were noted associated with treatment.

In the presence of the pathogen a linear relationship ($y = -3.39x + 33.6$; $R^2 = 0.8841$) between the amount of calcium hydroxide added and the reduction in disease incidence was shown (Figure 3). Complete disease suppression was obtained (and predicted) at the 10 t/ha rate.

Table 1. Mineral analysis of the field soil used in glasshouse experiments

pH	OC[a]	NO_3	S	PO_4	K	Ca	Mg	Na	Cl	Cu	Zn	Mn	Fe	B	Con[a]	CEC[a]
6.3	2.3	55	11	275	1.22	11.1	3.72	0.54	20	1.9	3.6	5	130	1.68	0.16	16.6

[a]OC = organic carbon; Con = electrical conductivity; CEC = cation exchange capacity.

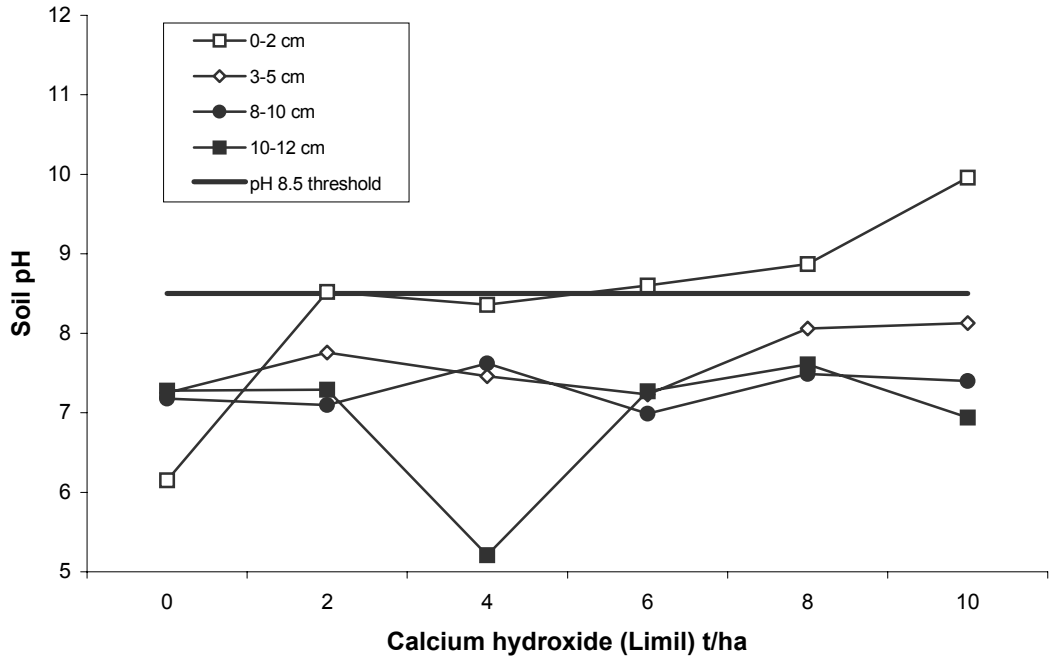

Figure 2. Effect of the addition of various rates of calcium hydroxide (Limil) onto the soil surface on the pH through the soil profile eight weeks after application.

Table 2. Effect of soil surface applied calcium hydroxide (Limil) treatments on fresh and dry weight of lettuce and head diameter and foliar nutrient analysis of lettuce grown under glasshouse conditions

Treatment (t/ha)	Fresh weight (g)	Dry weight (g)	Head diameter (cm)	N (Kjeldahl)	P	Mg	Ca
0	136.65 a	8.35	3.75	2.63	0.46	0.21	1.12
2	167.48 ab	9.43	4.75	2.08	0.47	0.19	0.97
4	183.20 bc	9.40	5.75	2.09	0.46	0.26	0.74
6	185.09 bc	9.32	5.92	2.18	0.47	0.19	0.93
8	182.52 bc	9.13	6.33	2.50	0.42	0.16	1.01
10	206.17 c	10.05	6.67	2.18	0.44	0.25	0.92
Normal Range				*2.8-3.0*	*0.35-0.6*	*0.3-0.7*	*0.8-2.0*
P (0.05)	0.006	0.644 (ns)	0.243 (ns)	0.12 (ns)	0.32 (ns)	0.54 (ns)	0.79 (ns)
SED	18.872	0.964	1.676	0.196	0.257	0.572	2.597

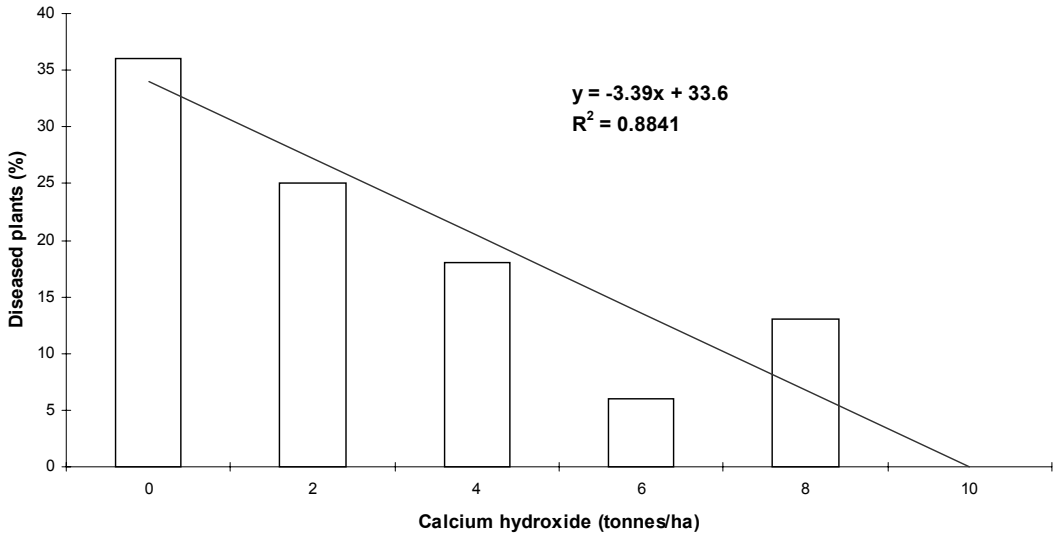

Figure 3. Effect of the addition of various rates of calcium hydroxide (Limil) onto the soil surface on the incidence of lettuce drop under glasshouse conditions.

Evaluation of soil surface pH amendment and fungicide treatments on lettuce drop under commercial field conditions.

Significant reductions ($P < 0.001$) in the level of lettuce drop disease at harvest in comparison to the untreated control were shown (Figure 4, Table 3). The best treatments, fungicide drench and 3 foliar sprays (86.2% disease reduction), fungicide drench and broadcast Limil (70.3%), and fungicide foliar sprays only (65.3%) did not differ significantly in their level of disease suppression. Fungicide drench and crushed lime treatment

(49.0%) had significantly less disease than the untreated control but did not differ from other treatments except the drench and foliar spray combination. Limil alone (33.9%), fungicide drench alone (33.5%), and crushed lime alone (21.8%) treatments did not differ significantly from the untreated control.

Evaluation of soil pH showed Limil treatments maintained soil pH greater than 8.5 for the first 3 weeks of the trial (and above pH 8.0 for the remaining 11 weeks). While the crushed lime treatment also increased pH (compared to untreated control), this never exceeded pH 8.5 at the soil surface (Figure 5).

Table 3. Effect of fungicide treatments and soil surface amendments on final harvest losses due to lettuce drop disease at the Don field site

Treatment	Disease loss (%)		Disease reduction (% of control)
Drench + foliar sprays	5.5	a	86.2
Drench + Limil	11.8	ab	70.3
Foliar sprays	13.8	ab	65.3
Drench + Crushed lime	20.3	bc	49.0
Limil	26.3	bcd	33.9
Drench	26.5	bcd	33.5
Crushed lime	31.2	cd	21.8
Untreated control	39.8	d	-
P	< 0.001		
sed	7.866		

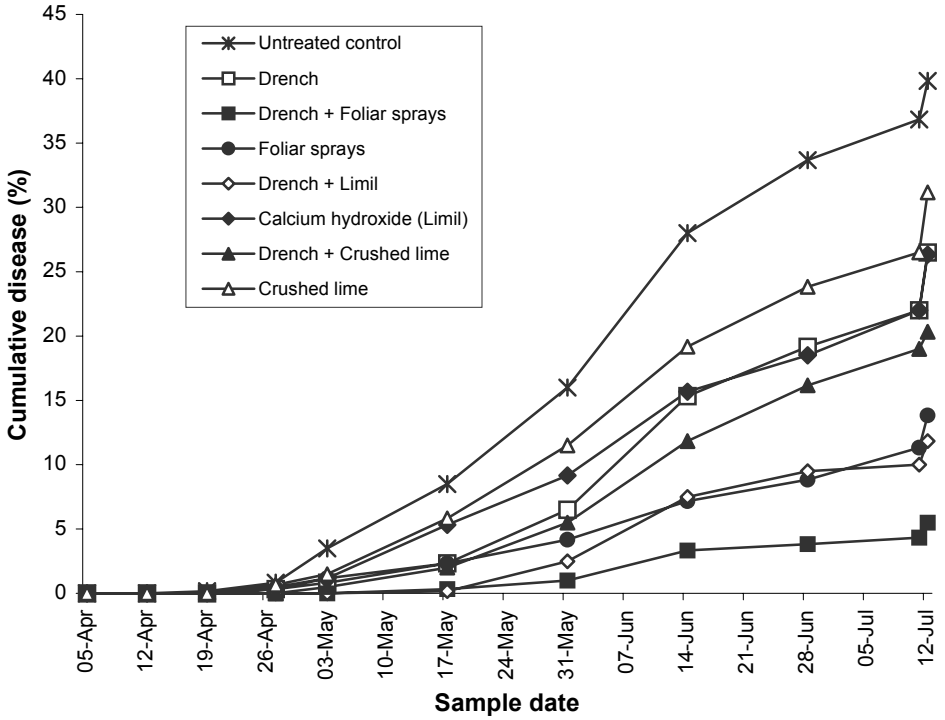

Figure 4. Cumulative incidence of lettuce drop disease following fungicide and soil surface pH amendments in a field trial at Don, Tasmania.

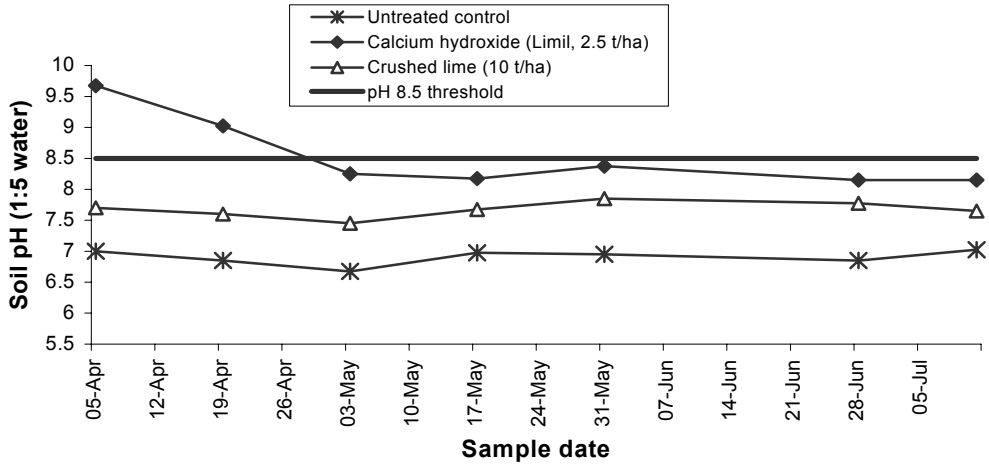

Figure 5. Effect of soil surface amendment treatments on the pH of the top 1 cm of the soil profile throughout the life of the trial crop (Don field site).

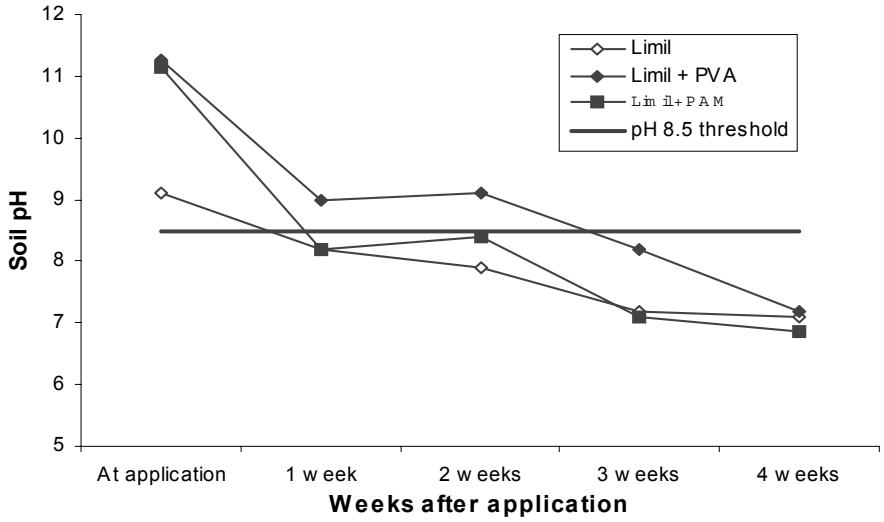

Figure 6. Comparison of application of PVA and PAM soil conditioner sprays onto broadcast Limil on maintenance of elevated soil surface pH (top 0-2 cm).

Phytotoxicity of soil stabilising compounds and their ability to enhance pH stability of Limil applications

No visible disorders were noted on any plants associated with any treatment. At these rates, PVA treatment prolonged the elevated soil surface pH above the threshold level of pH 8.5 for 2 weeks longer than the control (Limil only) and PAM treatments (Figure 6).

Evaluation of soil surface pH amendment (with and without soil stabiliser treatments), compost and green manure treatments on lettuce drop under commercial field conditions

Significant effects of calcium hydroxide ($P = 0.001$) and compost treatments ($P < 0.001$) on disease incidence were shown (Figure 7, Table 4). Use of Limil at 2.5 t/ha significantly decreased the incidence of lettuce drop disease (85% reduction in number of diseased plants). Two applications of 1.25 t/ha Limil gave 53 per cent a reduction in disease but this was not significantly different from control. Application of the soil conditioner PVA to half of the Limil plots appeared to enhance the pH soil surface elevation (Figure 8) resulting in 10 per cent fewer diseased plants however this difference was not significant. Surprisingly, soil amendment with compost (5 t/ha) significantly increased disease by 1100 per cent ($P < 0.001$). The oats green manure treatment showed a similar trend with an average increase in disease of 325 per cent, but this was not significantly different from the control ($P = 0.752$).

Significant differences in harvested fresh weight was shown for compost ($P < 0.001$, 174% increase in fresh weight), and Limil treatments ($P < 0.001$, 10% yield reduction for both Limil rates), and for interactions between compost and oat green manure treatments ($P = 0.02$), and compost, oats and Limil treatments ($P = 0.05$; Table 5).

Table 4. Effect of individual treatment factors on incidence of lettuce drop disease at the Cambridge field site

Treatment	Mean	Back-transformed Mean	
No Compost	-4.487	0.0113	a
Compost	-2.084	0.1244	b
P (Compost)		< 0.001	
No Limil	-2.391	0.0915	a
Limil (1.25 t/ha two applications)	-3.142	0.0432	ab
Limil (2.5 t/ha single application)	-4.232	0.0133	b
P (Limil)		0.001	
No Oats	-3.881	0.0206	
Oats	-2.690	0.0679	
P (Oats)		0.752	

NB. All possible treatment interactions were assessed and all failed to show significant differences at 5 per cent level and are not presented.

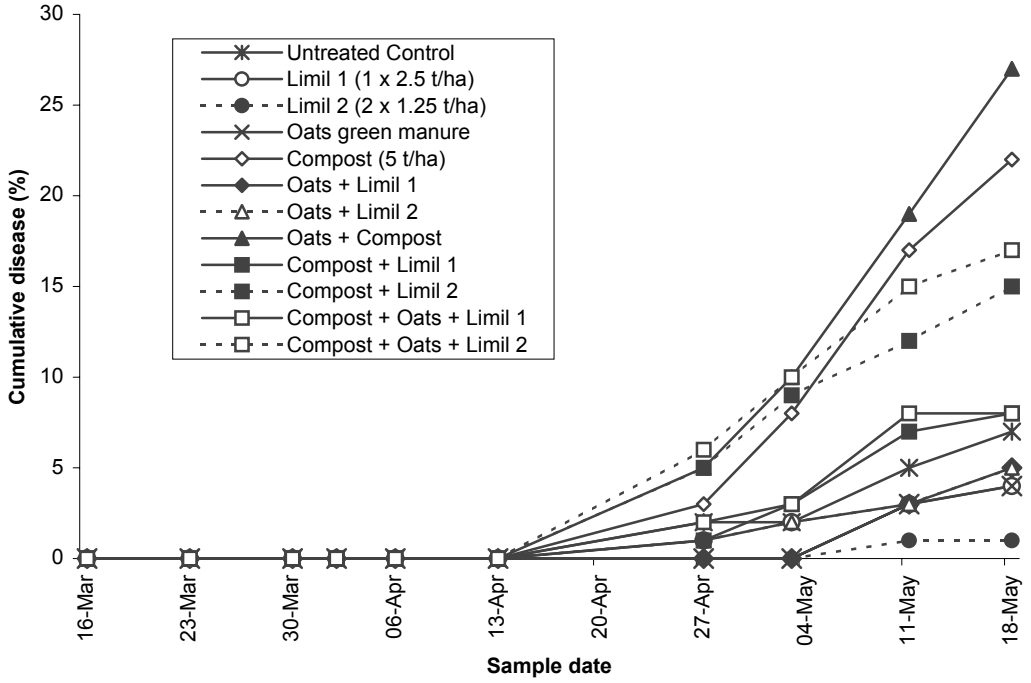

Figure 7. Cumulative incidence (%) of lettuce drop disease in a field trial at Cambridge, Tasmania.

Table 5. Interactive effects of compost amendment, oats green manures and Limil addition to fresh weight of harvested lettuce at the Cambridge field site

Compost	Oats	Limil	Fresh weight (g)	
Y	Y	nil	881.4	a
Y	Y	1.25 t/ha (x2)	855.9	ab
Y	N	nil	813.2	abc
Y	Y	2.5 t/ha	811	bc
Y	N	2.5 t/ha	766.4	c
Y	N	1.25 t/ha (x2)	702.5	d
N	Y	nil	551	e
N	N	nil	472.8	ef
N	Y	1.25 t/ha (x2)	451.3	f
N	N	2.5 t/ha	434.4	f
N	Y	2.5 t/ha	433.8	f
N	N	1.25 t/ha (x2)	431.7	f

P (compost)	< 0.001
P (Limil)	< 0.001
P (compost x oats)	0.020
P (compost x Limil x oats)	0.050

NB. All other possible treatment interactions were assessed and all failed to show significant differences at 5 per cent level.

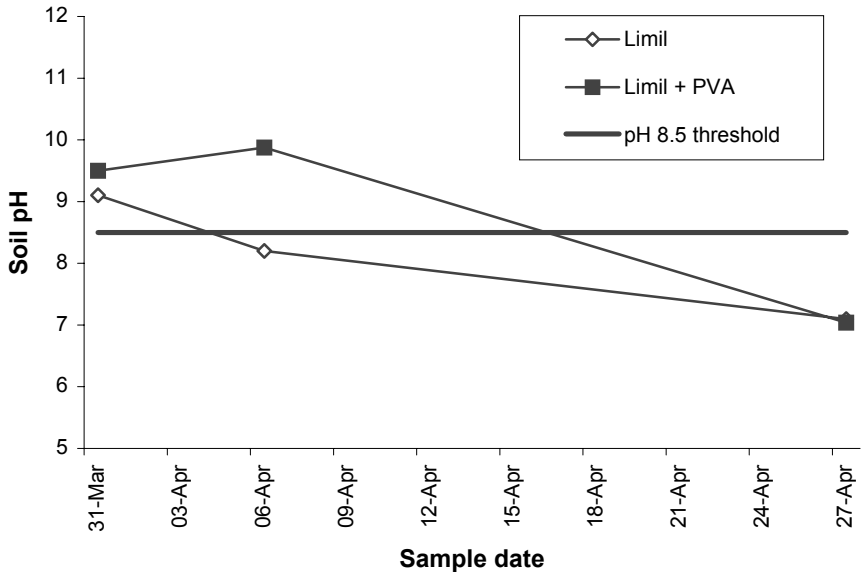

Figure 8. Effect of spraying PVA soil conditioner on broadcast Limil on maintenance of elevated soil surface pH (top 0-2 cm) at the Cambridge field trial site.

2. Disease survey of lettuce seedling nursery stocks

None of the lettuce plants sourced from either nursery showed any evidence of LBVV infection.

In the health surveys of newly planted bays of lettuce, one planting line of cv. Del Rio (approximately 500 plants) had high incidence of LBV (> 75%) restricted in distribution to the planting stock of this cv. from a single supplier planted at one date. Other lines (of various cultivars, from both suppliers) showed no or negligible LBVV. Given prior health status and the distribution pattern, one could be fairly certain that the infection arose from the planted seedlings and not from a field infection event.

The supplier of the infected line is known to recycle planting boxes on occasion whilst the nursery from which no infections were observed always uses new boxes. This indicated LBVV may be a sporadic problem within at least one of the Tasmanian nurseries and given the sporadic nature suggests it may possibly be linked to occasional contaminated planting boxes returning from commercial properties.

3. Soil treatments for management of LBV and lettuce drop diseases, and effects of treatments on insect pests and slug damage

Evaluation of soil treatments for expression of disease due to lettuce big vein virus (LBVV) and Sclerotinia minor

Significant treatment effects on disease incidence were noted in both trials. Of note, the limil surface crust was disrupted in both trials by tip hoeing for mechanical weed control 1 week after application. This was associated with a loss of elevated pH at the soil surface and may have affected disease suppression ability.

Assessment of plants expressing symptoms of LBVV disease in trial 1 the compost ($P < 0.001$) and the fumigant ($P < 0.001$) treatments both significantly increased the

incidence of diseased plants, whilst the limil treatment had no effect. No interactive effects between treatments were found (Table 6). In trial 2 the compost treatment was shown to again significantly increased disease ($P < 0.001$), as did the oats treatment ($P = 0.006$) and also in this trial the limil treatment ($P = 0.025$). A significant interaction between oats, compost and limil was also shown ($P = 0.009$; Table 7).

For *S. minor* infections, in the first trial a significant deleterious effect of compost was shown ($P = 0.002$) with no other treatment or interaction proving to have a significant effect (Table 8). In the second trial no significant effects were demonstrated, however the compost treatment had a similar deleterious trend (Table 9).

Other disease noted within both trials included lettuce necrotic yellows virus (LNYV) and tomato spotted wilt virus (TSWV). No treatment in either trial had an effect on the incidence of TSWV and too few LNYV infected plants were noted for analyses to be conducted.

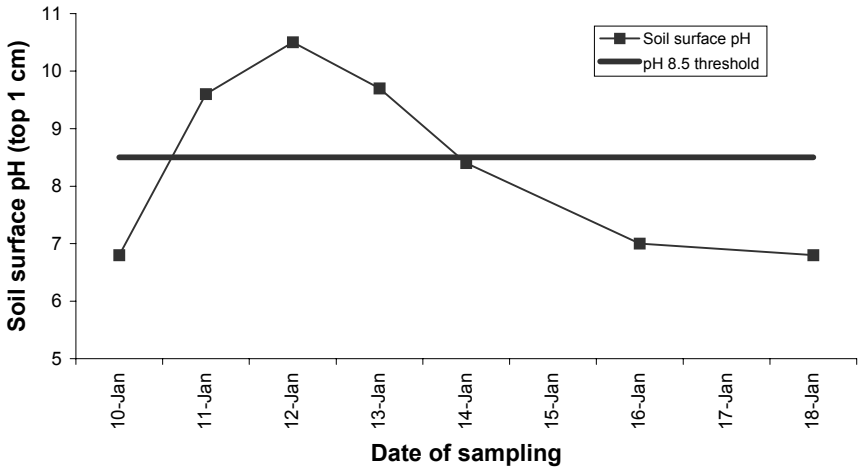

Figure 9. Soil surface pH (top 1 cm) in Limil treated plots (trial 1). Limil was applied on 11 January and plots tip hoed on 15 January disturbing the Limil crust.

Table 6. Effect of soil treatments on incidence of LBV disease symptoms (trial 1)

Treatment	Mean disease rating	Treatment	Mean disease rating	Treatment	Mean disease rating
Compost	8.31 a	Fumigant	7.98 a	Limil	7.24
No compost	5.83 b	No fumigant	6.17 b	No Limil	6.91
P	< 0.001	P	< 0.001	P	0.425
P (compost.fumigant)		0.881			
P (compost.limil)		0.318			
P (limil.fumigant)		0.195			
P (compost.fumigant.limil)		0.920			

Parameter estimates followed by the same letter subscript are not significantly different (P < 0.05).

Table 7. Effect of soil treatments on incidence of LBV disease symptoms (trial 2)

Treatment	Mean disease rating	Treatment	Mean disease rating	Treatment	Mean disease rating
Compost	2.148 a	Oats	2.183 a	Limil	2.138 a
No compost	2.025 b	No oats	1.99 b	No Limil	2.035 b
P	< 0.001	*P*	0.006	*P*	0.025
P (compost.oats)		0.024			
P (compost.limil)		1.000			
P (limil.oats)		1.000			
P (compost.oats.limil)		0.009			

Parameter estimates followed by the same letter subscript are not significantly different (P < 0.05).

Table 8. Effect of soil treatments on incidence of lettuce drop disease (trial 1)

Treatment	Mean disease rating	Treatment	Mean disease rating	Treatment	Mean disease rating
Compost	-2.56 a	Fumigant	-2.67	Limil	-2.63
No compost	-2.68 b	No fumigant	-2.57	No Limil	-2.61
P	0.002	*P*	0.095	*P*	0.531
P (compost.fumigant)		0.792			
P (compost.limil)		0.483			
P (limil.fumigant)		0.153			
P (compost.fumigant.limil)		0.498			

Parameter estimates followed by the same letter subscript are not significantly different (P < 0.05).

Table 9. Effect of soil treatments on incidence of lettuce drop disease (trial 2)

Treatment	Mean disease rating	Treatment	Mean disease rating	Treatment	Mean disease rating
Compost	2.588	Oats	2.608	Limil	2.635
No compost	2.669	No oats	2.649	No Limil	2.622
P	0.069	*P*	0.655	*P*	0.752
P (compost.oats)		0.138			
P (compost.limil)		0.583			
P (limil.oats)		0.206			
P (compost.oats.limil)		1.000			

Evaluation of soil surface liming for insect deterrence and comparisons with commercial slug bait and treatment for minimising slug damage on lettuce

No disease was observed in the lettuce plants within the trial plots. Relatively few aphids and thrips were trapped at each assessment period within the treatment plots and thus data was pooled for all traps and individual aphid or thrips species were not separately analysed. Analysis of trapped insects (total insects, total aphids and total thrips counts) showed no differences between Limil treated and untreated control plots (Table 10) thus failing to indicate any insect pest inhibitory effect as a result of increased light reflectance from treated plots.

Table 10. Total insects trapped over soil surface applied calcium hydroxide squares during trapping period

Treatment	Aphids	Thrips	Total insects
2.5 t/ha $Ca(OH)_2$	81	9	910
Untreated control	40	26	875
P	0.24 (n.s.)	0.17 (n.s.)	0.90 (n.s.)

In the slug trial, the pre-treatment assessment of seedling damage showed some slug feeding damage occurring across the trial in a random manner.

The post treatment assessment showed significant differences between treatments in the level of slug damage observed ($P < 0.001$). Slug pellets and the double application of calcium hydroxide had significantly less feeding damage than other treatments. The single calcium hydroxide treatment also had significantly less damage than the untreated control (Table 11).

Table 11. Comparision of soil surface pH amendments with conventional slug pellet treatment form control of slug damage in lettuce

Treatment	α parameter estimate	β parameter estimate
Methiocarb slug pellets (100 pellets/m^2)	-0.918 a	-0.2529 c
2 t/ha $Ca(OH)_2$ (split application)	-1.241 a	-0.0744 c
1 t/ha$Ca(OH)_2$	-1.839 b	-0.2129 b
Untreated control	-2.323 c	-0.4171 a

Parameter estimates followed by the same letter subscript are not significantly different ($P < 0.05$).

DISCUSSION

Amendment of soil surface pH for control of Sclerotinia minor infection in lettuce

In this study we have demonstrated the efficacy of soil surface amendment with the strongly alkaline material calcium hydroxide (Limil) for suppression of lettuce drop disease. We have also demonstrated that calcium hydroxide soil surface treatments do not induce injury to the plants even when seedlings are initially covered with the material by broadcast application post transplanting.

The use of such a management strategy was complementary to conventional procymidone fungicide drench treatments with significant synergistic activity shown. Similarly we would expect the treatment to be compatible with most non-fungicidal control strategies, some of which (e.g. biological control) can be difficult to integrate with conventional fungicide systems (Adams and Wong, 1991). For example, the use of sub

surface drip irrigation (Subbarao, 1997) should improve the results we present here, as the frequency of wetting of the calcium hydroxide layer accelerates the reaction with carbon dioxide to form calcium carbonate with a subsequent reduction in pH.

In vitro assays suggested exposure to pH greater than 8.0 significantly reduces the germination capacity of *S. minor* sclerotia. On the agar medium, mycelia produced from those sclerotia on which germination was observed at or above pH 9.0 grew in a manner that avoided contact with the medium. This suggested a soil surface pH threshold of > 8.0 would be required for substantial inhibition of *S. minor* germination capacity. We have arbitrarily chosen pH 8.5 as an indicative threshold level for the field studies.

The glasshouse trial suggested rates of 10 t/ha or greater would provide the best level of disease suppression. However, we felt such rates were not feasible on a regular basis as the addition of such quantities of hydrated lime despite being an additional cost, would also be likely to have an adverse effect on the soil pH profile following cultivation over time (33). In our trials we used a rate of 2.5 t/ha calcium hydroxide to reflect a more feasible commercial level. A means of effectively increasing treatment rate whilst maintaining total added volumes to a minimum may be to apply calcium hydroxide in localised spots surrounding the individual lettuce transplants rather than broadcast and could be examined in future trials.

The disease progress curves from the two trials indicate disease was first detected two (Don) and four (Cambridge) weeks after transplanting. If Limil amendments are only effective above the suggested pH threshold, it appears early suppression of sclerotial germination leads to reduced disease. Furthermore, extension of elevated surface pH may be useful in provide a greater disease suppressive effect. In the glasshouse study, pH elevation appeared to be more stable than seen in the field trials. Much of the loss of effect in the field would have been due to wind losses of the applied powder, and the frequency of irrigation and rainfall. In an attempt to manage wind loss of applied materials soil conditioner polymers (PVA and PAM) used for soil erosion control (Khattab *et al*. 2002, Lee *et al*. 2001, Peterson *et al*. 2002, Yu *et al*. 2003) was trialed. PVA appeared to be superior to PAM in stabilising soil surface pH at the rates used. The application of PVA soil conditioner to the calcium hydroxide layer assisted in aggregating the crust and reducing losses (noted visually) leading to a slightly prolonged elevated soil surface pH. However, in this trial, while an interesting trend was observed, use of PVA did not give a significant improvement in disease control.

The attempt to provide supplemental Limil as two split applications in order to prolong elevated soil surface pH failed, most likely because the individual rates were too low. However repeated application at higher rates, if these proved sustainable and cost effective, may be useful in prolonging the protective effect providing later applications did not result in residual product on the harvested produce.

While soil type and local environment (e.g. strength of wind and aspect) may also play a role in the efficiency of calcium hydroxide treatments, reassuringly disease suppression was demonstrated at both of the trial sites demonstrating the robustness of the technique. Soil surface pH declined below pH 8.5 at around the same rate at both sites (within 1-5 weeks post application) although the final soil surface pH remained higher (above 8.0) at the Don site compared to the Cambridge site (7.0).

Crushed lime is a cheaper bulk material than Limil and used routinely by agriculturalists for managing soil acidity. However, it did not have sufficient reactivity to raise soil surface pH above the threshold level of 8.0 and had a reduced effect on disease management.

This work was conducted with lettuce transplants which is the industry standard method for head lettuce production in Tasmania. For this technique, this had the advantage of utilising plants with established root systems below the band of elevated pH activity that may have been important in avoiding negative effects on plant growth. It would be useful

to ascertain whether direct seeded lettuce could also be treated in this manner without adverse affect.

We have demonstrated the potential for elevated soil surface pH treatments to reduce lettuce drop disease and successful integration of such treatments with conventional fungicide treatments. This approach may also be useful for organic vegetable producers where synthetic fungicides are not available. Future studies could focus on means of prolonging elevated pH (choice of product, rates, stabilisers and application technologies), and integration with other conventional disease management tools.

Disease survey of lettuce seedling nursery stocks

The surveys have indicated the nurseries supplying Tasmanian lettuce producers generally produce quality disease free seedlings. It appears the majority of LBVV infections observed in Tasmanian production result from infections from contaminated soils. Soil fumigation is not routinely undertaken in Tasmania.

Evidence for the occasionally batch of LBVV infected seedlings was shown. The nursery from which this batch was obtained does recycle planting boxes which could be a source for sporadic infection (often boxes used for planting in the filed are held down to avoid being blown away by dumping a small quantity of soil in them, which is emptied before return to the nursery. Ineffective cleaning of these boxes could pose a source of infection. The other nursery surveyed always used new boxes.

Evaluation of soil treatments for expression of disease due to lettuce big vein virus (LBVV) and Sclerotinia minor

The two field trials demonstrated interesting effects of soil treatments in expression of LBVV and *Sclerotinia minor* induced disease.

Prior to our study we suspected that increasing soil organic matter content through application of composts and/or a green manure cover crop would increase the soil microbial community leading to enhanced pathogen inoculum decay and increased soil microbial competition. We therefore expected a reduction in disease following such treatments as has been found in other pathosystems including sclerotinia (Boulter *et al*. 2002; Ferraz *et al*. 1999; Kurle *et al.* 2001).

Similarly the soil fumigation treatment was expected to reduce total soil microbial activity (including that of the pathogens and virus vector) and thus was expected to result in disease suppression. (Walsh 1998)

Contrary to expectations, the compost treatments significantly increased expression of both LBVV (both trials) and *S. minor* (trial 1 only and also in limil trial) induced disease. Furthermore, fumigation, oats green manure, and calcium hydroxide (trial 2 only) treatments also increased LBVV induced disease, while these treatments had no effect on *S. minor* infections.

Further studies would be necessarily to elucidate the mechanisms of disease elevation by these soil treatments but we can speculate on possible effects that could explain the observations.

As we did not have a diagnostic test for the LBV disease causal agent, we relied on expression of disease symptoms for scoring infection. However it is known that some environmental factors such as temperature and light can influence disease expression (Walsh 1994). It is therefore unclear whether the resulting differences observed were due to enhanced infection events, or enhanced expression of disease symptoms with control treatments becoming infected but not expressing disease.

The significant increase in LBV and lettuce drop disease incidence with the addition of compost was surprising, but consistent across trials. Previous studies have shown the general trend for disease suppression with addition of composted organic matter in a number of pathosystems (Dittmer *et al*. 1990, Kowalchuk *et al*. 2003, McKellar and Nelson, 2003) including *Sclerotinia* spp. (Boulter *et al.* 2002, Ferraz *et al.* 1990, Huang *et al.* 1997). These have been attributed to the enhancement of competitive microorganisms. In comparison, few studies have shown that addition of composted organic matter can exacerbate disease (Nasira *et al.* 2003). Similarly, oat green manure crops have been previously shown to reduce the incidence of disease caused by *Sclerotinia* spp. (Kurle *et al.* 2001, Thaning and Gerhardson, 2001). In our study LBV disease was exacerbated by the oats treatment, but contrary to prior published work no significant effect of the oat green manure on lettuce drop disease was shown. We believe the absence of effect for lettuce drop was most likely due to limited period for oat growth (6 weeks growth giving plants of 10-15 cm at desiccation) prior to transplanting lettuce.

In our study, the trial sites had a sandy duplex soil with poor levels of organic matter, relatively low pH (typically 5.0-6.0) and supplemental fertilisers were not used. The effect of compost application on fresh weight yield was in one trial visually obvious in the field. Compost and oats green manure would have would affect soil nutrient availability by either directly supplying organic inputs and improved soil structure. Fumigation would have also increased soil fertility by releasing organic nutrients from soil microflora. The calcium hydroxide treatment would have supplied additional calcium and/or increased the pH in the upper soil profile (esp. after tip hoeing) and possibly altered soil structure.

It is possible that nutrient effects could have altered expression of LBV disease (although this has not been previously documented). Alternatively, the additional nutrients and calcium could have allowed greater plant growth and root development (observed in some trials) , increasing the availability of infection sites and perhaps thus exacerbating disease incidence. This latter explanation might also account for the increased *S. minor* infections observed following compost treatment (which would have supplied the greatest quantity of organic input of the treatments).

It is also possible that the compost treatment could have increased the water holding capacity of the soil that could have affected the fungal vector mobility. The rapidity of water drainage from a soil has been shown to affect LBVV infection levels (Westerlund *et al.* 1978). Lastly, the optimal pH level for the fungal virus vector is noted between pH 6 and 8, and thus the calcium hydroxide treatment could have increased the competitiveness of the *Olpidium* zoospores (Garbi and Verhoyen, 1994).

Prior studies have shown that soil fumigants (including metham sodium) are effective against spread of LBV disease (White, 1980; Walsh, 1998), although other studies have shown some fumigants provide little disease suppression (Campbell *et al.* 1980). The lack of effect of metham sodium fumigation in this trial perhaps indicates inefficient fumigation was obtained.

The lack of effect of soil surface applied calcium hydroxide on *S. minor* infections which has been demonstrated in other trials, can probably be attributed to the mechanical tip hoeing activity shortly after application which disrupted the soil surface crust and led to a rapid decay of soil surface pH (fig 2). This the elevated pH effect was not maintained for a sufficient period for disease control. The requirement for mechanical weed control could be a limitation to the commercial use of soil surface calcium hydroxide treatments.

Evaluation of soil surface liming for insect deterrence and comparisons with slug bait and copper silicate treatments for minimising slug damage on lettuce.

Slugs are a serious problem for fresh lettuce production. In addition to the direct damage they cause, which can be severe especially if affected as seedlings, feeding damage decreases cosmetic appeal of the produce reducing quality and value. Furthermore,

lettuce retailers have a zero tolerance for presence of slugs (or other insects) within sale produce. Detection of slugs within harvested produce can lead to sales losses.

Conventional treatments include the application of slug pellets shortly after transplanting. These appear to work effectively in reducing slug damage and presence, however care in the use of slug baits is required to ensure bait decay before harvest as contamination of harvested produce with slug bait would be unacceptable.

Following the success of the use of soil-surface applied calcium hydroxide for Sclerotinia disease control, we wanted to look at the effect of this treatment on losses due to slugs feeding. This trial demonstrated the efficacy of conventional treatments slug pellets and elevation of soil surface pH using calcium hydroxide treatments for minimising damage due to slugs in lettuce. The level of control using the double split rate of calcium hydroxide was equivalent to that given by slug pellets and thus may offer an alternative to slug baits. It is probable that these two treatments may have an additive effect, and thus where slugs are a major problem combined pellet and soil surface treatment may be useful.

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SECTION 5.0

EPIDEMIOLOGY AND CONTROL STRATEGIES FOR LETTUCE BIG-VEIN

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Summary

Most batches of lettuce seedlings taken over an 18 month period from a vegetable nursery were infested with lettuce big-vein disease (LBVD) with an up to 31 per cent incidence. Using lettuce seedlings in bait tests, contamination was detected at the nursery in potting mix composted for different periods and dirt from under the benches, and at the bark suppliers site in this ingredient of the potting mix and waste 'bark' from the ground. In a field experiment in which lettuce seedlings from the infested nursery were inoculated with infested roots or left uninoculated before transplanting into subplots on land with no history of lettuce planting, disease progress followed a sigmoid curve with the former but an almost straight line with the latter. However, significant clustering of symptomatic plants was found only in the subplot with the uninoculated plants. Leaf symptoms of LBVD were more severe in lettuces infested later, while symptoms in those infested earlier were obvious initially but then became milder. The disease impaired formation of hearts: the proportion of symptomatic plants that lacked hearts was 24-36 per cent when leaf symptoms first appeared 5-7 weeks after transplanting but 14-16 per cent after 8-9 weeks. When leaf symptoms first appeared at 5-6 weeks, there was a fresh weight loss of 14-15 per cent for heads (all plants) and 39 per cent for hearts (excluding plants without hearts). When leaf symptoms first appeared 7 weeks after transplanting, there was no significant yield loss for heads and only a 14 per cent loss for hearts. At 8-9 weeks, there were no significant yield losses for heads or hearts.

In five field experiments on land infested with lettuce big-vein disease (LBVD) at different locations, the incidence of LBVD in partially resistant lettuce breeding line LE169 was always diminished significantly compared to its incidences in other crisphead lettuce genotypes. Also, the fresh weight yield of LE169 was always significantly greater than those of more susceptible genotypes. When different genotypes were grown on virgin land where infested introduced lettuce seedlings were the only source of the disease, LBVD incidence was again significantly diminished and yield significantly greater with LE169. When black plastic mulch was spread on the surface of LBVD-infested soil and LE169 was deployed along with more susceptible lettuce genotypes, presence of mulch and partial resistance both diminished LBVD incidence significantly on their own, but combining the two suppressed spread the most. The mulch diminished soil moisture and increased soil temperature, one or both of which decreased activity of the viruliferous zoospores of its *Olpidium brassicae* vector resulting in less virus transmission to lettuce roots.

5.1 Lettuce Big-vein disease: sources, patterns of spread and losses

Introduction

Lettuce big-vein disease (LBVD) is associated with a complex of two viruses, *Lettuce big-vein virus* (LBVV) and *Mirafiori lettuce virus* (MiLV). Both are vectored by the soil-borne root infecting fungus *Olpidium brassicae* but only MiLV causes the typical symptoms of the disease in lettuce (*Lactuca sativa*) (Lot *et al*. 2002; Vetten and Walsh 2003). Symptoms of LBVD are obvious clearing of the leaf lamina alongside the veins, giving the typical enlarged vein appearance from which its name is derived, a stiff upright appearance of the leaves with ruffled margins, and plant stunting. Overall yields are decreased and quality impaired when incidences of LBVD are high in lettuce crops (Zink and Grogan 1954; Ryder 1979; Patterson *et al.* 1986; Bos and Huijberts 1990). However, published quantitative data on yield and quality losses from LBVD are lacking.

LBVD is a widespread problem for lettuce production in Australia. In south-west Australia, more than 10,000 tonnes of lettuce are produced each year for domestic consumption and export. The irrigated land used to grow lettuce in the Swan Coastal Plain, is farmed intensively and sometimes without crop rotation resulting in widespread infestation with LBVD. Crops of the lettuce cv. Oxley, which is extensively grown in winter, often have a > 90 per cent incidence of the disease (Kumar *et al*. 2000). These high incidences are not observed in lettuce grown in the summer months as expression of symptoms is dependent on cool air temperatures and low light intensities (Westerlund *et al*. 1978; Walsh 1994).

This paper reports tests done to examine the extent of infestation with LBVD in batches of lettuce seedlings collected at different times of year from a vegetable nursery and to identify the source of contamination. Also, a field experiment was done on land with no history of lettuce planting to examine the pattern of spread of LBVD and quantify the effect of symptom appearance in plants of different ages on yield and quality losses from the disease. Brief reports of parts of this work were provided as abstracts (Latham *et al.* 2001; Latham and Jones 2003).

Materials and methods

Occurrence in nursery seedlings

To determine the incidence of LBVD in lettuce seedlings over different seasons at a vegetable nursery, one tray of seedlings was collected every 6-12 weeks over an 18 month period. Each tray contained 100 lettuce seedlings 4-6 weeks old that were ready for transplanting. All seedlings were transplanted into pots containing sterilised potting mix and grown for 6-8 wks on free-draining wire benches in a glasshouse where temperature fluctuated between 19^{o}C (day) and 17^{o}C (night). Each plant was inspected weekly for presence of LBVD symptoms.

Bait tests

To identify the source of the LBVD infestation at the vegetable nursery, the following samples were collected: two 5 kg bags of lettuce potting mix consisting of (1:1) 'pine bark':peat (Tests 1 and 2), a 5 kg bag of potting mix from each of 4 different stages of composting (Test 3), and a 5kg bag of dirt from under the benches where the lettuce seedlings were grown (Test 4). The following samples were collected from the site of the nurseries' supplier of 'pine bark': three 5 kg bag samples of 'pine bark' at different stages of weathering and one of waste 'pine bark' from the ground (Test 5). Samples were also obtained of the imported peat ingredient of the mix, which came from peat bogs in Canada or Ireland, by purchasing bags of each of them (Tests 6, 7 and 8). In all bait tests, soil from a known LBVD-infested site at Wanneroo, Perth was used as an infested control and steam-sterilised potting compost as an LBVD-free control. Because lettuce seedlings

grew poorly in the Canadian peat (which dried out rapidly) in Tests 6 and 7, a 1:1 mixture of this peat with steam sterilised potting mix was used instead in Test 8. For each test, ten pots were filled with soil from each type of sample and sown with 4 seeds of lettuce cv. Oxley.

Three additional tests were done on samples from the contaminated vegetable nursery. Four 50 celled recycled seedling trays were collected and 4 identical seedling trays purchased new as a 'clean' control. The trays were filled with sterilised potting mix and sown with lettuce cv. Oxley (1 seed/cell) (Test 9). One hundred pelleted seeds and 100 un-pelleted seeds of cv. Oxley were germinated in new 50 celled seedling trays filled with sterilised soil (1 seed/cell) (Expt 10). Four, 50 celled seedling trays were sown with lettuce cv. Oxley (1 seed/cell). Two trays each were left to soak overnight in tubs containing water from the nursery dam or tap water (Test 11). At 4 weeks the seedlings from Tests 9, 10 and 11 were transplanted into pots.

All plants in tests 1-11 were grown on free-draining wire benches in a temperature controlled glasshouse where temperature fluctuated between 19^{o}C (day) and 17^{o}C (night). All were inspected weekly for characteristic symptoms of LBVD. After 8 weeks, leaf samples were collected from all plants in Expts 6, 7 and 8 and tested by enzyme-linked immunosorbent assay (ELISA) using LBVV-specific antiserum to detect presence of LBVV.

Field experiment

Trays with seedlings of lettuce cv. Oxley were obtained from a vegetable nursery known to be LBVD-contaminated. Six hundred, 6 week-old seedlings were left overnight with their roots soaking in a slurry of macerated roots from lettuce plants infested with both LBVD and *O. brassicae*. Another 600 seedlings were left unsoaked. The 600 inoculated seedlings were transplanted into one half of a large irrigated plot at a site at Medina Research Station with no known past history of lettuce production, and the 600 uninoculated seedlings into the other half. The dimensions of the 2 subplots were 7 m by 30 m and a bare earth buffer of 3 m width separated them. Plants were inspected weekly for characteristic symptoms of LBVD. On each occasion when symptoms were first seen, plants were marked individually by placing a colour coded stake next to them. For each date, the percentage of symptomatic plants was calculated and disease progress determined.

Just before harvest the two subplots were subdivided into 1.2 m^2 quadrats. Within each quadrat, the numbers of plants tagged on each assessment date were counted. At harvest for each assessment date, 50 asymptomatic plants from the uninoculated subplot and 50 symptomatic plants from the inoculated sub-plot were selected at random and harvested by cutting off at the base of the stem. Numbers of plants with and without hearts were recorded. For each plant, fresh weight yields were determined separately for heads and then for the hearts of those that formed them. After weighing heads, plants were inspected externally for symptoms. Finally, hearts were cut in two halves and inspected internally. A sensitivity ranking of 1-5 for leaf symptoms of LBVD (1, symptomless, 5, very severe) was then assigned to each plant.

Enzyme-linked immunosorbent assay

Lettuce leaf samples were extracted (1 g/20 mL) in phosphate buffered saline (10 mM potassium phosphate, 150 mM sodium chloride), pH 7.4, containing 5 mL/L of Tween 20 and 20 g/L of polyvinyl pyrrolidone and tested using polyclonal antiserum to LBVV (047 32 supplied by Prof. J. Vetten, Braunschweig, Germany) by double antibody sandwich ELISA as described by Clark and Adams (1977). Each sample extract was tested in duplicate wells in microtitre plates and appropriate diseased and healthy lettuce leaf samples were included in paired wells as controls. The substrate used was 0.6 mg/mL of *p*-nitrophenyl phosphate in 100 ml/L of diethanolamine, pH 9.8. Absorbance values (A_{405nm}) were

measured in a Multiskan plate reader (Labsystems, Finland) and values more than 10 times those of healthy leaf sap were considered to represent infected plants.

Statistical analysis

Yield data for lettuce head and fresh weights for pairs of asymptomatic and symptomatic plants were subjected to t-tests. Spatial pattern of counts of infested plants within the quadrats was identified using Spatial Analysis by Distance Indices (SADIE) as described by Thackray *et al.* (2002). For a random arrangement of the observed counts amongst the given sample units, the expected value for the index of aggregation (I_a), an index of the degree of clustering for the whole area sampled is one, while $I_a > 1$ indicates aggregation of counts into clusters (Thackray *et al.* 2002).

Results

Occurrence in nursery seedlings

Infestation with LBVD was found in lettuces grown from seedlings from 7/10 trays collected at different times of year (Table 1). Incidences of LBVD in seedlings from individual infested trays ranged from 2 per cent in cv. Raider seedlings from September 2000, to 31 per cent in cv. Magnum seedlings from September 1999. Occurrence of LBVD was unrelated to seasonal conditions, with high incidences occurring in both winter and summer.

Table 1. LBVD incidence in lettuce seedlings collected from a seedling nursery over an 18 month period. A tray of 100 seedlings was collected on each occasion. After collection, the seedlings were grown at 19°C (day) and 17°C (night) with a 12-h photoperiod for 8 weeks, and LBVD symptoms recorded

Date of collection (month/year)	Cultivar	LBVD infestation (%)
9/99	Magnum	31
12/99	Magnum	31
3/00	Magnum	0
6/00	Oxley	12
7/00	Oxley	7
8/00	Oxley	6
9/00	Raider	2
2/01	Raider	0
4/01	Marksman	20
6/01	Oxley	0

Bait tests

In the different bait tests, highest incidences of contamination were always detected in the infested control soil but none was ever detected in the uninfested control soil (Table 2). In Tests 1-3, infestation with LBVD was detected in potting mix collected from the nursery and was still present after 9 weeks of composting. Infestation was also detected in dirt collected from under the benches at the nursery (Test 4). In addition, it was found at the site of the supplier of this ingredient in 'pine bark' samples from 6-8-week-old compost piles and in waste 'bark' from the ground (Test 5). No contamination was detected in the peat ingredient of the potting mix sourced either from Canada or Ireland (Tests 6-8). No LBVD was detected in tests involving the nursery trays (Test 9), the pelleted seed (Test 10), or the dam water (Test 11).

When a leaf sample from each bait plant in Tests 6-8 was tested for presence of LBVV by ELISA, the virus was always detected in some plants growing in the infested control soil but in none from any of the other soils (Table 2). Thus, the ELISA tests confirmed the results obtained by visual assessment for LBVD. However, visual assessment for symptoms had underestimated the extent of infection because LBVV was detected in more pots and bait plants by ELISA than by visual assessment for LBVD.

Patterns of spread

LBVD first developed 3-4 weeks after transplanting in both subplots. In the uninoculated subplot, appearance of symptoms then continued at a slow, almost constant rate until harvest when 15 per cent of the plants had LBVD (Fig. 1). In contrast in the inoculated subplot, appearance of symptoms followed a sigmoid curve reaching a 91 per cent incidence at harvest time. In the uninoculated subplot, cumulative counts of symptomatic plants were significantly clustered at final assessment (I_a = 1.772, P < 0.05) but not before this. With non-cumulative counts only those for the penultimate assessment were significantly clustered (I_a = 2.161, P = < 0.01). In the inoculated sub-plot, analysis of cumulative and non-cumulative counts did not reveal any significant clustering around foci on any assessment date.

Yield and quality loss

Although early LBVD appearance resulted in severe symptoms initially, these then faded somewhat. The final severity ranking declined to 2.3 when symptoms first developed after 5-6 weeks (Table 3). In contrast, when symptom development was delayed until 9 weeks, they remained severe, giving a 4.3 ranking. In plants in which LBVD first appeared 5 or 6 weeks after transplanting, there was a significant decrease in head fresh weight (14-15%), which was associated with a substantial loss of heart weight (39%) on those that produced hearts, 24-34 per cent not producing them. However, when LBVD symptoms first appeared 7 weeks after transplanting, although there was no significant decrease in head weight, heart weight for plants with them decreased by 14 per cent while 36 per cent had no hearts. Neither head nor heart weight was diminished significantly when plants developed symptoms 8 or 9 weeks after transplanting but 14-16 per cent of them had no hearts.

Table 2. Bait tests to determine the source of LBVD at a vegetable seedling nursery

	No. of plants with LBVD/total[A]	No. of pots with LBVD/total
Unsterilised potting mix		
Test 1		
Potting mix from nursery	3/32	1/10
Infested soil (control)	21/26	10/10
Uninfested soil (control)	0/30	0/10
Test 2		
Potting mix from nursery	4/39	2/10
Infested soil (control)	21/28	9/10
Uninfested soil (control)	0/37	0/10
Composting stage of potting mix		
Test 3		
Potting mix composted for 3 weeks	2/30	1/10
Potting mix composted for 5 weeks	5/31	2/10
Potting mix composted for 7 weeks	0/32	0/10
Potting mix composted for 9 weeks	1/26	1/9
Infested soil (control)	19/27	8/10
Uninfested soil (control)	0/30	0/10
Waste soil from nursery		
Test 4		
Soil from under nursery benches	2/30	2/9
Infested soil (control)	19/27	8/10
Uninfested soil (control)	0/30	0/10
'Pine bark' component from supplier		
Test 5		
2-3 week pile	0/24	0/10
2-3 week pile	0/28	0/10
6-8 week pile	5/27	2/10
Waste from floor	6/29	4/10
Infested soil (control)	11/24	7/10
Uninfested soil (control)	0/27	0/10
Peat component of potting mix		
Test 6 [B]		
Irish peat	0/37 (0/37)	0/10 (0/10)
Canadian peat	0/19 (0/19)	0/10 (0/10)
Infested soil (control)	20/33 (30/33)	9/10 (10/10)
Uninfested soil (control)	0/39 (0/39)	0/10 (0/10)
Test 7 [B]		
Irish peat	0/40 (0/40)	0/10 (0/10)
Canadian peat	0/10 (0/10)	0/10 (0/10)
Infested soil (control)	11/32 (22/32)	8/10 (10/10)
Uninfested soil (control)	0/40 (0/40)	0/10 (0/10)

	No. of plants with LBVD/total[A]	No. of pots with LBVD/total
Test 8 [B]		
Irish peat	0/37 (0/37)	0/10 (0/10)
1:1 Canadian peat:uninfested soil	0/37 (0/37)	0/10 (0/10)
Infested soil (control)	15/30 (23/30)	(9/10) (10/10)
Uninfested soil (control)	0/38 (0/38)	0/10 (0/10)

A Ten pots of each treatment were sown with four lettuce cv. Oxley seeds.

B A leaf sample was taken from each bait plant 8 weeks after sowing and tested with LBVV antiserum using ELISA. Figures in parentheses are numbers of plants/10 (second column) or pots/10 (third column) in which LBVV was detected by ELISA.

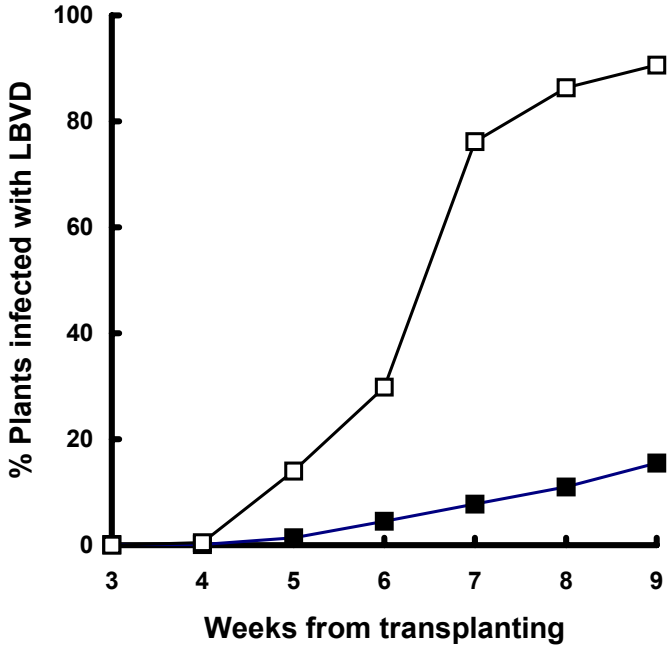

Figure 1. Disease progress curves for LBVD in plots of lettuce with (□) or without (■) inoculation of seedlings before transplanting.

Table 3. Effect of LBVD on yield and quality of lettuce cv. Oxley plants that developed symptoms at different times after transplanting. Numbers of pairs of plants compared were 32 (weeks 5 and 7), 38 (weeks 6 and 9) and 42 (week 8)

Time to LBVD appearance (weeks)		Head fresh weight (kg)	Heart fresh weight (kg)[A]	Per cent plants without hearts	Symptom severity[B]
5	Infested	1.60	0.58	34	2.3
	Healthy	1.88	0.96	0	1
	P	< 0.001	< 0.001		
	lsd	0.106	0.107		
6	Infested	1.64	0.61	24	2.3
	Healthy	1.90	1.00	0	1
	P	< 0.001	< 0.001		
	lsd	0.124	0.094		
7	Infested	1.83	0.80	36	2.7
	Healthy	1.86	0.93	0	1
	P	ns.	0.046		
	lsd	-	0.125		
8	Infested	1.88	0.86	16	3.2
	Healthy	1.95	0.90	0	1
	P	n.s.	ns		
	lsd	-	-		
9	Infested	1.92	0.90	14	4.3
	Healthy	1.94	0.93	0	1
	P	ns	ns		
	lsd	-	-		

[A] **Fresh weight for plants that produced hearts only.**

[B] **LBVD severity rating: 1, symptomless, 5, very severe.**

Discussion

In Europe, spread of LBVD from vegetable nurseries is reported due to reliance on purchased lettuce transplants rather than direct seeding. The source of infestation at nurseries was attributed to viruliferous *O. brassicae* resting spores in dirt remaining on inadequately cleaned, recycled seedling trays (Walsh 1992). LBVD is not expressed in lettuce seedlings from nurseries until after transplanting, so such distribution is inadvertent. In our studies, the 'bark' ingredient of the potting mix was identified as a source of LBVD-infestation. Presumably it became contaminated with infested dirt from the ground at the 'bark' suppliers' site. How this dirt first became contaminated is unknown. Possibly, virulifeous resting spores of the vector came from previous cultivation of diseased lettuce in the vicinity or they might have arrived on the wheels of incoming vehicles or machinery arriving from LBVD-contaminated farms, or through the inadvertent introduction of infested soil. The thermal death point of *O. brassicae* resting spores is between 55 and 60^{o}C for 10 minutes (Campbell and Grogan 1963). To help ensure that healthy seedlings are produced, nurseries should sterilise their potting mixes at such temperatures and improve overall hygiene (Campbell and Grogan 1963). At the LBVD-contaminated nursery in Perth, the potting mix was composted but not sterilised before use. Also, hygiene was inadequate there, eg. LBVD-infested dirt was found under the benches. Sterilisation of potting mixes and strict hygiene measures were both included with others in an integrated disease management strategy described specifically for LBVD in vegetable seedling nurseries (Latham and Jones 2001; Jones 2003).

Planting of batches of lettuce seedlings unknowingly infested with LBVD and *O. brassicae* means that the first crop of lettuce on virgin land is diseased and that the soil becomes infested. This has been happening in Perth as metropolitan horticultural properties are sold for building land and lettuce production moves to new land further from the city centre. Due to the persistent nature of *O. brassicae* resting spores, once land is infested, LBVD is extremely difficult to control (Campbell *et al.* 1980; Falk 1997). Infestation of new sites can be avoided by planting only healthy lettuce transplants and taking precautions not to introduce dirt or soil from infested land. Both are important control measures within an integrated disease management strategy for LBVD designed specifically for uninfested land (Latham and Jones 2001; Jones 2003).

In the uninoculated subplot in our field experiment, the increase in numbers of symptomatic lettuces was at an almost constant rate. Here, infection was from resting spores contaminating the nursery potting mix. The SADIE analysis identified significant clustering only at the penultimate and final assessments suggesting that secondary spread was very limited. Thus, the slow rate of spread may be attributed to gradual infection of new lettuce plants as resting spores germinated over time producing a near monocyclic pattern (Thresh 1983). However, once lettuces grew large and their roots approached each other, there was scope for secondary spread of LBVD by zoospores, which would explain the clustering eventually found. In the inoculated subplot, the increase in numbers of symptomatic plants followed a sigmoid curve. Although some infections will have been derived from resting spores in the potting mix, the most plausible explanation for the curve found is that most were from multiple new infections occurring in the roots at the time when the inoculum was applied. Staggered systemic movement from roots infected simultaneously by zoospores from the applied inoculum would seem to fit the type of curve obtained with most symptom appearance occurring 6-7 weeks afterwards. A polycyclic infection pattern (Thresh 1983) would not explain the data, as significant clustering would be expected, which did not occur reflecting swamping by simultaneous multiple infections from the applied inoculum such that only 9 per cent of plants remained healthy.

Zink and Grogan (1954) reported that in a lettuce crop 52 per cent of plants with LBVD, compared to 13 per cent of healthy plants, were not harvested due to 'immaturity', which presumably meant failure to form hearts. Failure to form hearts due to LBVD has also been referred to as 'bushiness' (Ryder 1979). Bos and Huijberts (1990) found that inoculation with LBVD before transplanting caused a significant decrease in head yield whereas it was not decreased significantly in plants inoculated later. Our findings provide the first quantitative data published on the magnitude of yield and quality losses from LBVD. In the field experiment, head yield loss was diminished by 14-15 per cent when leaf symptoms first appeared 5-6 weeks after transplanting but not subsequently. With lettuces with hearts, yield losses for hearts were 39 per cent when symptoms appeared after 5-6 weeks and 14 per cent when they appeared after 7 weeks, but absent subsequently. The greater yield losses recorded for hearts than heads result from poor heart formation due to LBVD, and not from failure to produce them because plants without them were excluded from the heart data sets. Thus, with early symptom expression not only did more lettuces fail to produce hearts than with later symptom expression but also in those that produced them, heart formation was poorer. In contrast, symptoms were most severe with late infection. The magnitude of the yield and quality losses found here warrant a re-appraisal of the importance of LBVD in Australia and elsewhere. Considerable economic benefits are likely to arise from adopting integrated disease management tactics to control the disease (Latham and Jones 2001; Jones 2003).

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5.2 Deploying partially resistant genotypes and plastic mulch on the soil surface to suppress spread of lettuce big-vein disease in lettuce

Introduction

Lettuce big-vein disease (LBVD) occurs widely in lettuce (*Lactuca sativa*) crops in regions of the world with temperate or Mediterranean-type climates. It diminishes yield and its symptoms impair lettuce quality and depress market value. They consist of vein banding giving the typical 'big-vein' appearance accompanied by crinkling of leaves. Symptom development is favoured by cool growing conditions (Zink and Grogan 1954; Ryder 1980; Bos and Huijberts 1990; Latham *et al.* 2004). A complex of two viruses is associated with LBVD, *Lettuce big-vein virus* (LBVV) and *Mirafiori lettuce virus* (MiLV). MiLV rather than LBVV causes the typical symptoms of LBVD (Kuwata *et al*. 1983; Roggero *et al*. 2000; Vetten and Walsh 2003). The chytrid fungus, *Olpidium brassicae,* transmits both viruses, its motile zoospores transmitting LBVD to the roots of healthy plants. In contaminated soil, resting spores of *O. brassicae* retain their ability to transmit LBVD for up to 20 years in the absence of susceptible hosts, so crop rotation is not a control option. Environmental factors that influence transmission of LBVD by *O. brassicae* include soil moisture, temperature, light intensity and pH. Moisture is a critical factor, as saturated soil is needed for zoospore release (Campbell and Grogan 1963; Westerlund *et al*. 1978; Campbell 1985; Walsh 1994; Lot *et al*. 2002). Because of the long persistence of LBVD inside viruliferous resting spores present in infested soils, its narrow host range and the localised spread to roots by zoospores, alternative hosts are relatively unimportant as LBDV reservoirs for lettuce or persistence of the disease in soil at contaminated sites (Jones 2003).

Diverse types of cultural and chemical control measures are available to diminish spread of LBVD in lettuce grown on infested land (e.g. Tomlinson & Faithfull 1980; Campbell *et al*. 1980; White 1980, 1983; Walsh 1992; Latham and Jones 2001; Latham *et al*. 2001). Cultural control includes both phytosanitary and agronomic measures, and involves seed, seedlings, soil, water, machinery, alternative hosts, hygiene, planting date, site selection, etc (Jones 2003). With chemical control, fumigation of contaminated land is expensive, does not eliminate resting spores completely and may leave toxic residues. Also, although methyl bromide is the most effective soil fumigant, it is now banned on environmental grounds (White 1980, 1983). No biological control measures are available. In general, crisphead lettuce cultivars are the most susceptible to LBVD, butterhead types are intermediate and cos types are the least susceptible. Immunity in *Lactuca virosa* is being used to breed LBVD-resistant lettuce (Bos and Huijberts 1990; Walsh 1992). Lettuce selections with tolerance (Patterson *et al*. 1986) or partial resistance (PR) (Bos and Huijberts 1990; Ryder and Robinson 1995) are available.

LBVD is a widespread problem in commercial lettuce producing areas of Australia. In south-west Australia, the lettuce production system on the Swan Coastal Plain is intensive and often uses irrigated land that is contaminated with LBVD, sometimes without crop rotation. Also, winter plantings of the most widely grown genotype (cv. Oxley) often have > 90 per cent of plants with symptoms of the disease (Kumar *et al*. 2000). In the past, widespread LBVD occurrence and a tolerant domestic consumer resulted in farmers choosing to 'live with the disease' rather than attempting to control it. However, this situation has now changed because of a trend towards greater sophistication of the local consumer and the need for a product without any disease symptoms for export to south-east Asia.

Recently, knowledge of the epidemiology of the disease and data from field experiments evaluating the effectiveness of control measures were used to devise comprehensive

integrated disease management (IDM) tactics against LBVD in lettuce grown in different situations (Jones 2003). This paper describes work done to improve current IDM tactics for LBVD in lettuce grown on infested land. Our research evaluated the effectiveness at suppressing LBVD spread of (1) deploying Australian crisphead lettuce genotypes with PR, and (2) laying plastic mulch on the surface of raised beds watered by overhead sprinklers to restrict soil moisture and increase soil temperature, thereby diminishing movement of vector zoospores between roots. Brief summaries of this work were reported as abstracts (Latham and Jones 2002, 2003; Latham *et al.* 2001).

Materials and methods

Procedures common to all field experiments

For Expts 1-8, details of years, sites, planting dates, replications, and numbers of lettuce genotypes grown are in Table 1. The sites were all commercial lettuce growing farms in the north of the Perth metropolitan region on the Swan Coastal Plain. Seed of the lettuce breeding lines used were supplied by a commercial seed company (Yates Pty Ltd) that had selected them previously for possible PR. Except for Expt 3 which was on virgin land, all of the sites had cropping histories of continuous or annual plantings of lettuce over the last 7-15 years, and the land used was infested with viruliferous resting spores of *O. brassica*. The soil types were a mix of Bassendean and Karrakatta sands and the experiments were all watered and fertilised *via* overhead sprinklers. The experiments were all rigorously hand weeded.

Lettuce seedlings were from a commercial vegetable seedling nursery in Perth, where they were grown in the open on benches in trays containing a non-sterilised potting mix of peat and pine bark. They were then transported to the different experimental sites. Twenty lettuce seedlings at the four leaf stage were transplanted into each plot and placed 40 cm apart within each row. All plants were inspected at 1-2 week intervals to record those with symptoms typical of LBVD. In other studies, LBVD was detected in lettuce seedlings from this nursery source (Latham *et al.* 2004).

Experiments 1-6

Experiments 1-6 examined the responses of different local lettuce breeding lines and cultivars of crisphead lettuce to LBVD. Different planting times and locations were used and plots were arranged in randomised block designs. Lettuce cv. Oxley was used as a susceptible control in each experiment. Each plot was 1.6 m wide by 2.0 m long, and the soil within it was mounded and divided into four rows, 30cm apart along its length. Expts 1 and 2 each contained 11-12 genotypes and consisted predominantly of cultivars. The genotypes planted in each were the same, except that cv. Titanic was not included in Expt 2. At harvest, all plants from each plot were cut off at ground level and weighed together to determine fresh weight yields/plot, which were then converted to yield/plant. Expts 3-6 were smaller, only containing cv. Oxley and four breeding lines. Except in Expt 4, which was not harvested, at harvest all plants were cut off at ground level and fresh weight determined for each plant individually.

Experiments 7 and 8

Expts 7 and 8 were planted with lettuce cv. Oxley and four breeding lines. A split-plot design was used with plastic mulch as the main treatment and lettuce genotype as the sub-treatment. Thus each genotype was present either with or without black plastic mulch. Each plot was 1 m wide by 4 m in length with the soil mounded. The black polyethylene mulch was laid to cover entire plots just before transplanting and its margins were buried to hold it in place. Soil temperature was measured using Tinytalk II Temperature Data Loggers (Orion Group). The data loggers were buried at depths of 10, 20, and 30 cm in the centres of 2 plots with mulch and 2 without. Measurements were taken automatically at hourly intervals from time of lettuce transplanting through to harvest. Overall means were calculated from hourly readings of maximum, minimum and

average daily temperatures. At harvest all plants were cut off at ground level and their fresh weights determined individually.

Statistical analysis

Data for percentages of plants with LBVD from Expts 1-8 were transformed to angles and subjected to analysis of variance (ANOVA) using GENSTAT Version 5.3.1. A split-plot analysis was used in Expts 7 and 8. Area under disease progress curve (AUDPC) values were calculated as described by McKirdy and Jones (1997) and subjected to ANOVA. Fresh weight data from experiments 1-3 and 5-8 were subjected to ANOVA.

Table 1. Details of field experiments

Expt No.	Year	Location and Site No.	Planting date	No. of replications	No. of genotypes
Genotype evaluation					
1	2000	Wanneroo (1)	26 May	4	12
2	2000	Yanchep (2)	26 May	4	11
3	2001	Yanchep (3)	31 May	5	4
4	2001	Carabooda (4)	3 July	5	4
5	2001	Wanneroo (1)	3 July	5	4
6	2001	Wanneroo (5)	1 August	5	4
Genotype evaluation x plastic mulch					
7	2001	Yanchep (6)	31 May	5	4
8	2001	Yanchep (6)	3 July	5	4

Results

Experiments 1 and 2

In Expts 1 and 2, LBVD was first seen in young lettuce leaves 4 wks after transplanting. Differences in incidence of LBVD were highly significant between genotypes, both for AUDPC and at final assessment (Table 2). In Expt 1, incidence increased rapidly (Fig. 1), reaching > 50 per cent in seven cultivars. Cultivars Assassin, Del Oro, Oxley and Titanic were ranked as highly susceptible (HS) with > 80 per cent incidence, the six other cultivars and breeding line LEC 8550 as susceptible with 20-80 per cent incidence, and breeding line LE169 as partially resistant (PR) with < 20 per cent incidence. There was less spread of LBVD overall in Expt 2 than in Expt 1, with final incidence not exceeding 48 per cent in any genotype, and no symptomatic plants whatsoever appearing in LE169. Because of these lower incidences, defined susceptibility categories were less clear, e.g. cvs Assassin and Del Oro had < 25 per cent incidences which did not confirm their previous HS rankings. However, cv. Oxley and LE169 again stood out at the two extremes as regards high and low incidences respectively.

There were highly significant differences between the fresh weight yields for different genotypes in both experiments (Table 2). The divergence in yields was greatest in Expt 1 ranging from 1.11kg/head in LE169 to only 0.52 kg/head in cv. Assassin, while cv. Oxley yielded 0.75kg/head. Yields were lower overall in Expt 2, but those of cv. Del Oro and LE169 were significantly greater than those of cv. Oxley. Yield of LE169 exceeded that of cv. Oxley by 48 per cent (Expt 1) and 16 per cent (Expt 2). Thus LE169 proved the best genotype, not only for PR to LBVD but also for yield.

Experiments 3-6

As Expt 3 was on virgin land, the LBVD was introduced with the seedlings transplanted from the nursery. It first appeared after 4 wks in cv. Oxley. With this scenario, final LBVD incidence only reached 12 per cent in cv. Oxley and 0.3 per cent in both LE169 and LE138xLE170 (Table 3). Both at final assessment and with AUDPC, incidence of LBVD was significantly smaller with LE169, LE170 and LE138xLE170 than in cv. Oxley. Yields were significantly greater than those of cv. Oxley for LE169 and LE170, but not for LE138xLE170. Yield of LE169 exceeded that of cv. Oxley by 11 per cent.

In Expt 4, LBVD first appeared after 4 weeks. At final assessment, incidence reached 57 per cent in cv. Oxley and 32 per cent in LE169, and the latter incidence was significantly smaller than the former (Table 3). There were no significant differences between AUDPC values. In Expt 5 symptoms again appeared after 4 weeks. Although incidences at final assessment reached > 90 per cent in all 4 genotypes grown, spread of LBVD was slower in LE169 and LE138xLE170 (Fig. 2). AUDPC values for these 2 genotypes were significantly smaller than those for cv. Oxley and LE170. As in Expt 3, LE169 and LE170 produced significantly greater yields than the other 2 genotypes, with that of LE169 exceeding that of cv. Oxley by 17 per cent. In Expt 6, LBVD first appeared after 5 weeks and incidence at final assessment was 93 per cent in cv. Oxley but only 47 per cent in LE169. The AUDPC and final incidence values for LE169 and LE138xLE170 were significantly smaller than those for the other 2 genotypes. Yield of LE169 was significantly greater than those of the other 3 genotypes, exceeding that of cv. Oxley by 39 per cent.

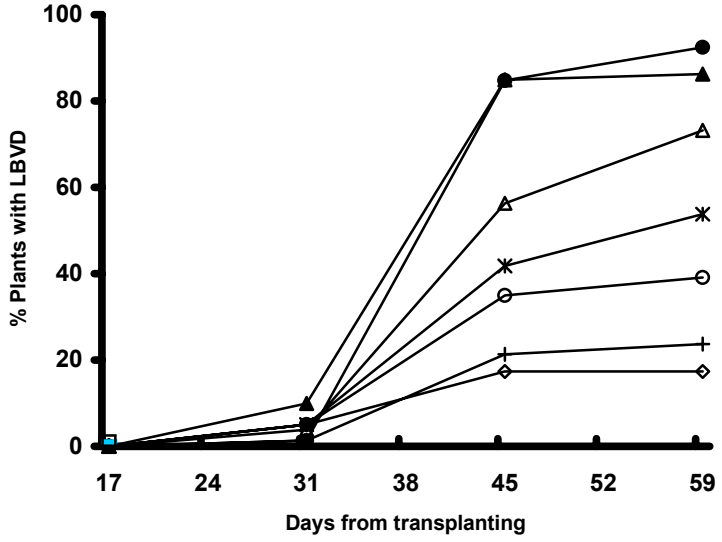

Figure 1. Disease progress curves for percentages of plants with LBVD in 7 genotypes of lettuce in Expt 1. Oxley (●), Titanic (▲), Magnum (△), LEC8550 (⋆), Greenway (○), Veronica (+) and LE169 (◊).

Table 2. Statistical analysis of LBVD incidence and yield data from Expts 1 and 2. Final percentage incidence determined by visual assessment for LBVD. All percentage LBVD data were transformed to angles before analysis. Values in parentheses are detransformed final percentages. HS, highly susceptible; S, susceptible; PR, partially resistant

Genotype	AUDPC	Per cent plants with LBVD - after 59 days		Fresh weight /head (kg)	Ranking
	Expt 1				
LE169	378	22.1	(14)	1.11	PR
cv. Veronica	474	28.0	(22)	0.71	S
cv. Jacqueline	644	41.2	(43)	0.68	S
cv. Del Rey	741	31.0	(27)	0.76	S
cv. Greenway	797	38.5	(39)	1.00	S
LEC 8550	997	47.3	(54)	0.82	S
cv. Magnum	1326	59.4	(74)	0.69	S
cv. Pacific	1467	56.5	(70)	0.58	S
cv. Assassin	1720	77.4	(95)	0.52	HS
cv. Del Oro	1780	68.8	(87)	0.84	HS
cv. Oxley	1843	78.3	(96)	0.75	HS
cv. Titanic	1862	71.7	(90)	0.84	HS
P	< 0.001	< 0.001		< 0.001	
df	33	33		33	
lsd	412	16.3		0.14	
	Expt 2				
LE169	0	1.8	(0)	0.77	-
cv. Veronica	137	20.1	(12)	0.60	-
cv. Jacqueline	449	34.8	(33)	0.59	-
cv. Del Rey	82	15.0	(7)	0.59	-
cv. Greenway	471	27.4	(21)	0.74	-
LEC 8550	44	8.2	(2)	0.72	-
cv. Magnum	382	28.4	(23)	0.58	-
cv. Pacific	223	23.7	(16)	0.57	-
cv. Assassin	186	20.4	(13)	0.60	-
cv. Del Oro	287	29.4	(24)	0.79	-
cv. Oxley	560	43.9	(48)	0.66	-
P	< 0.001	< 0.001		< 0.001	
df	29	29		28	
lsd	257	16.5		0.11	

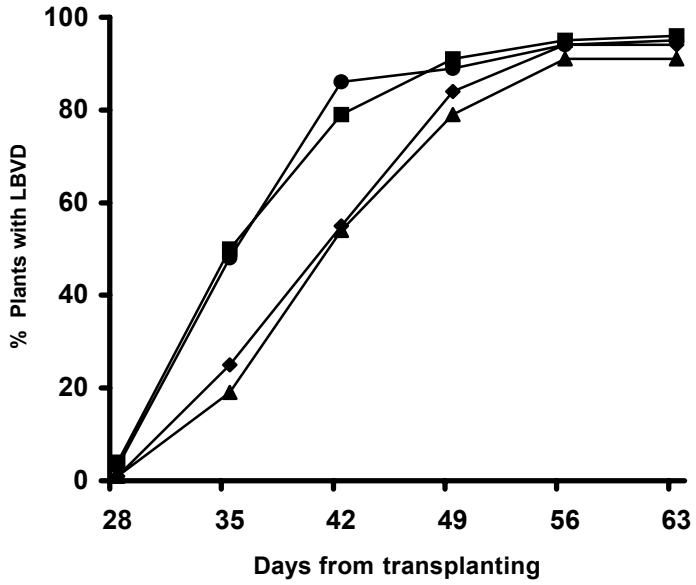

Figure 2. Disease progress curves for percentages of plants with LBVD in lettuce cv. Oxley (●), LE169 (◆), LE170 (■), and LE138xLE170 (▲) in Expt 5.

Table 3. Statistical analysis of LBVD incidence and yield data from Expts 3-6. Final percentage incidence determined by visual assessment for LBVD after 61 days (Expt 3), 63 days (Expts 4 and 5) and 54 days (Expt 6). All percentage LBVD data were transformed to angles before analysis. Values in parentheses are detransformed percentages

Genotype	AUDPC	Per cent plants with LBVD		Fresh weight/ head (kg)	AUDPC	Per cent plants with LBVD		Fresh weight/ head (kg)
	Expt 3				Expt 4			
LE169	10	3.0	(0.3)	1.67	585	34.3	(32)	-
LE170	35	9.2	(3)	1.72	623	41.9	(45)	-
LE138xLE170	17	3.0	(0.3)	1.45	548	45.5	(51)	-
Oxley	196	20.2	(12)	1.51	836	49.4	(57)	-
P	0.039	0.005		< 0.001	ns	0.035		-
df	12	12		387	12	12		-
lsd	138	9.4		0.07	-	9.9		-
	Expt 5				Expt 6			
LE169	2127	79.0	(96)	1.40	701	43.5	(47)	1.04
LE170	2533	82.3	(98)	1.47	1181	67.7	(86)	0.82
LE138xLE170	2007	74.2	(92)	1.00	1142	52.1	(63)	0.88
Oxley	2532	78.5	(96)	1.20	2164	74.3	(93)	0.75
P	< 0.001	ns		< 0.001	< 0.001	< 0.001		< 0.001
df	12	12		321	12	12		206
lsd	217.5			0.07	341.5	12.0		0.08

Experiments 7 and 8

In Expt 7, LBVD first appeared after 2 weeks and at final assessment the incidence reached was 72 per cent in plots of cv. Oxley without mulch (Fig. 3a). When cv. Oxley was protected with mulch, final incidence was 55 per cent. The smallest final incidences were in LE169 either with (11%) or without (18%) mulch. There was a significant decrease in LBVD incidence due to presence of mulch (Table 4). Final incidence and AUDPC values for LE169 were both significantly smaller than those for the other 3 genotypes. There was no interaction between mulch and genotype. Yields were significantly increased by presence of mulch, by 17 per cent overall. Yield of LE169 was significantly greater than that of cv. Oxley, exceeding it by 13 per cent.

In Expt 8, which was transplanted one month after Expt 7 at the same site, LBVD first appeared after 4 weeks. Greatest incidences at final assessment were in plots without mulch and smallest ones in those with mulch. The extremes were 72 per cent in plot of LE169 with mulch and 93 per cent in those of LE138xLE170 without mulch (Fig. 3b). At final assessment there was a significant decrease in overall LBVD incidence due to presence of mulch (Table 4). Also, LE169 had a significantly smaller AUDPC value than those of the other 3 genotypes. There was no interaction between mulch and genotype. Presence of mulch increased maximum, minimum and mean soil temperatures at depths of 10, 20 and 30 cm by 1.2-2.8^{o}C, 1.9-2.3^{o}C and 1.4-1.9^{o}C respectively, the divergence in temperatures declining with increasing depth (Table 5). When monthly mean data were compared instead of overall means, the divergence in soil temperature between plots with and without plastic mulch remained similar regardless of month. Yields were significantly increased by presence of mulch, by 149 per cent overall (Table 4) but that of LE169 was not significantly different from that of cv. Oxley.

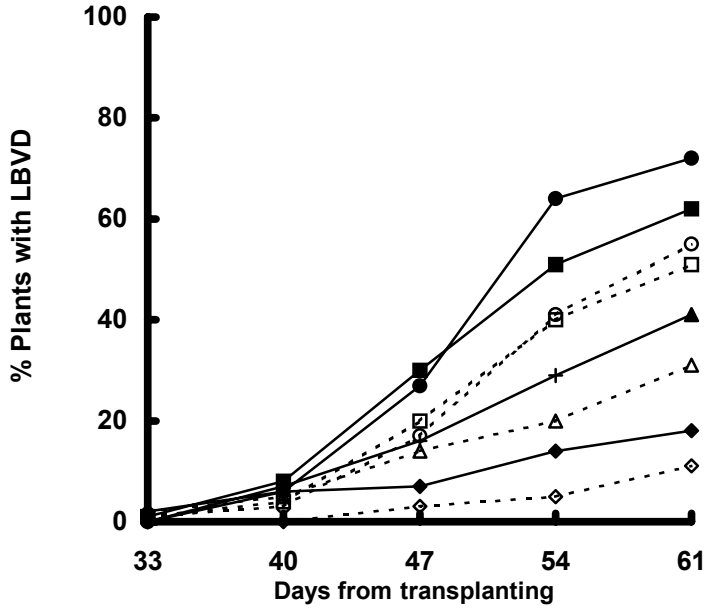

Figure 3a. Disease progress curves for percentages of plants with LBVD in four lettuce genotypes grown with or without plastic mulch in Expt 7. Oxley - mulch (●), + mulch (○); LE169 - mulch (◆), + mulch (◊); LE170 - mulch (■), + mulch (□); LE138xLE170 – mulch (▲), + mulch (Δ). Broken line in (a) = with mulch.

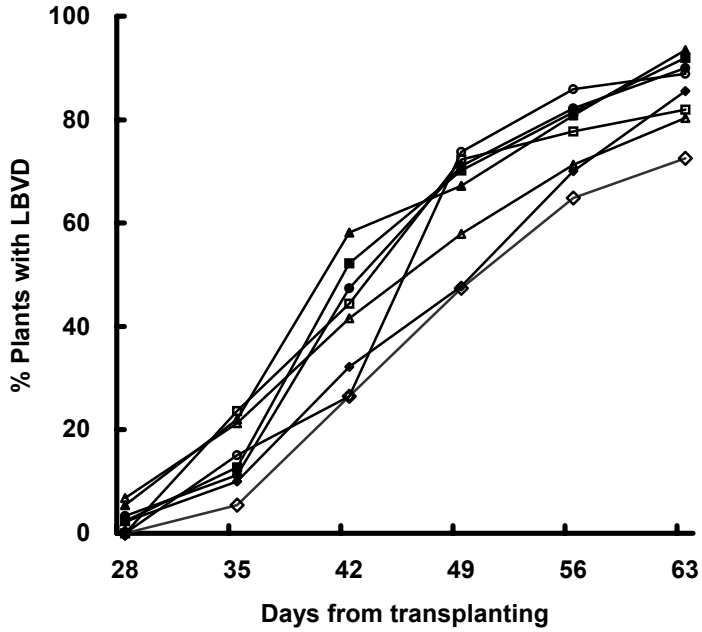

Figure 3b. Disease progress curves for percentages of plants with LBVD in four lettuce genotypes grown with or without plastic mulch in Expt 8. Oxley - mulch (●), + mulch (○); LE169 - mulch (◆), + mulch (◊); LE170 - mulch (■), + mulch (□); LE138xLE170 – mulch (▲), + mulch (Δ). Broken line in (a) = with mulch.

Table 4. Statistical analysis of LBVD incidence and yield data from Expts 7 and 8. Final percentage incidence determined by visual assessment for LBVD after 61 (Expt 7) and 63 (Expt 8) days. All percentage LBVD data were transformed to angles before analysis

Treatment	AUDPC	Per cent plants with LBVD	Fresh weight/head (kg)
Expt 7			
- mulch	640	44.0 (48)	1.20
+ mulch	4360	36.6 (36)	1.40
LE169	179	21.5 (13)	1.43
LE170	738	49.3 (57)	1.39
LE138xLE170	452	36.9 (36)	1.13
Oxley	782	53.5 (65)	1.26
Level of significance (lsd, df)			
Mulch	0.02 (159, 4)	0.007 (4.0, 4)	0.023 (0.16, 4)
Genotype	< 0.001 (180, 24)	< 0.001 (7.5, 24)	< 0.001 (0.08, 24)
Genotype x mulch	ns	ns	ns
Expt 8			
- mulch	1734	73.5 (92)	0.56
+_ mulch	1478	62.7 (79)	1.38
LE169	1331	64.3 (81)	1.00
LE170	1689	68.0 (86)	1.12
LE138xLE170	1775	71.0 (89)	0.75
Oxley	1631	69.1 (87)	0.99
Level of significance (lsd, df)			
Mulch	ns	0.03 (8.9, 4)	< 0.001 (0.16, 4)
Genotype	0.008 (251, 22)	ns	< 0.001 (0.12, 22)
Genotype x mulch	ns	ns	ns

Table 5. Temperatures at three depths in LBVD-infested soil with or without a covering of black plastic mulch in Expt 7. Mean temperatures were calculated from hourly readings for full trial duration (59 days) in two plots of each type.

Depth of data logger	Maximum temperature °C	Minimum temperature °C	Average temperature °C
Black plastic			
10 cm	21.8	7.7	14.3
20 cm	18.7	9.5	14.2
30 cm	17.3	11.0	14.4
Bare earth			
10 cm	19.0	5.4	12.4
20 cm	16.9	7.7	12.8
30 cm	16.1	9.1	13.0

Discussion

In 7 irrigated field experiments planted in winter on infested land, deploying lettuce breeding line LE169 consistently decreased the incidence of LBVD. The difference in incidence between LE169 and the other genotypes was more pronounced when the conditions favoured less spread (e.g. Figure 1), even to the extent of no LBVD being found in LE169 (Expt 2). When conditions were optimal for spread, onset of symptoms and rate of spread were delayed with LE169 but its final incidences approached those in susceptible genotypes (e.g. Figure 2). These results resemble those of Bos and Huijiberts (1990) and Ryder and Robinson (1995) who evaluated lettuce genotypes in the field and found that some North American and European crisphead and cos lettuce types exhibited PR behaviour like that of LE169. When we used virgin land instead of infested land (Expt 3) and the source of inoculum was lettuce transplants some of which already had LBVD, final incidences were much smaller than those in the other experiments but LE169 still exhibited PR.

Decreased moisture and increased temperature diminish activity of *O. brassicae* zoospores in the soil and so decrease LBVD spread (Pryor 1944; Thompson and Doolittle 1942; Westerlund *et al.* 1978). In lettuce production in Australia, black is the predominant colour used for plastic mulch. Lamont (1999) found that, at depths of 5 and 10 cm, black mulch increased the mean daily temperature beneath it by 2.8 and 1.7°C respectively compared to the corresponding temperatures at these depths in uncovered soil. This order of increase under black plastic mulch resembles the increase of 1.9°C that we found when measurements were taken at a depth of 10 cm. We did not measure soil moisture. Deploying black plastic mulch on the soil surface significantly diminished the incidence of LBVD in two field experiments. Whether the decreased zoospore activity leading to this effect resulted from diminished soil moisture, increased soil temperature or both was not established. However, diminished soil moisture seems the more likely cause because of the overriding requirement for sufficient moisture for the zoospores to swim through if they are to move from root to root successfully. Reflective plastic mulches are deployed to decrease landing rates of insect vectors and thereby diminish virus spread (e.g. McLean *et al*. 1982; Jones 1991) but, to the best of our knowledge, use of plastic mulch to control spread of a soil-borne virus with a fungal vector is novel.

In Expts 1-6, the yield increase in lettuce head weight from deploying LE169 on infested land was often considerable. It was 17-48 per cent greater than that of cv. Oxley in field experiments where LBVD spread was substantial (Expts 1, 5 and 6) and 16 per cent greater where there was less spread (Expt 2). Where virgin land was used and the only

LBVD source was the seedling transplants, the increase in yield was 11 per cent (Expt 3). These yield increases differ from what was found in North American bred crisphead lettuce cv. Pacific with PR which failed to outyield susceptible cv. Salinas when grown on infested land (Bos and Huijberts 1990). Plastic mulches on the soil surface can increase vegetable yields, sometimes 2-3 fold, by increasing soil temperature and decreasing soil moisture (Lamont 1999). When a combination of lettuce with PR (LE169) and plastic mulch were used together on infested land, yield more than doubled in one experiment (Expt 8). However here the increased yields were derived from improved growing conditions rather than LBVD control as the yields of LE169 and cv. Oxley were the same (Expt 8). In another similar experiment (Expt 7) in which conditions were less conducive for spread (Fig. 3) increased yields came from PR as well as mulch. We did not compare the yields of the different lettuce genotypes used in any field experiments with entirely healthy lettuce seedlings transplanted into uninfested land. Therefore, whether cv. Oxley growing in the complete absence of any LBVD would outyield LE169, or *vice versa*, was not determined.

The results suggest that when growing lettuce on infested land under overhead irrigation, combining together lettuce genotypes with PR, like LE169, with laying black plastic mulch on the soil surface provides an effective way of decreasing LBVD spread and loss of marketable yield. Because combining them was less effective when conditions were more conducive for spread, they should always be included with other control measures (such as manipulating planting date, avoiding poorly drained land, decreasing irrigation inputs, removing old crop debris, roguing, etc.) in an IDM strategy devised specifically for LBVD-infested land (Latham and Jones 2001; Jones 2003).

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Appendix A

Table 1: List of fungicides used in field experiments and plate testing experiments to control SLD in Western Australia. Their current registered status is shown including the chemical class they belong to and mode of action. Mode of action: S = systemic and P = protectant. Registration of each fungicide for similar pathogens is shown where: B = Botrytis sp.; M = *Monilinia* sp.; SH = *S. homeocarpa*; SM = *S. minor;* and SS = *S. sclerotiorum*. WHP = with holding period for lettuce or other leafy vegetable crops (e.g. broccoli, cauliflower and cabbage). NS = fungicide not suitable for use in lettuce due to excess residues, and N/A = WHP not available for lettuce or leafy vegetables. *Fungicides chosen for in vitro efficacy tests. ***Disclaimer***: All formulations with the same active ingredients were not tested due to time/funding constraints, and this does not represent the preference of a particular formulation over another.

Product	Active ingredient	Chemical Class	Mode of action	Registered for SLD in WA	Registered on lettuce in WA	Registered for other pathogens	WHP for leafy vegetables
Currently registered fungicides							
*Benlate®, *Marvel®	benomyl	A	S	Y	Y	B, M, BB, S	5
*Rovral®	iprodione	B	P	Y	Y	B, M, BB, D, S	7
*Sumisclex®	procymidone	B	S	Y	Y	B, M, BB, D, S	2
Non-registered fungicides							
*Bavistin®,	carbendazim	A	S	N	N	B, M, BB, D	n/a
*Bayfidan®	triadimenol	C	S	N	N	D	7
*Amistar®	azoxystrobin	K	P	N	N	B, S	1
*Chlorothalonil®,	chlorothalonil	Y	P	N	N	B, M, BB, D	7
*Mancozeb plus®	sulfur/mancozeb	Y	P	N	Y	B, M, BB, D	14