Know-how for Horticulture™

Stop the rot - managing onion white rot in spring onions

Oscar Villalta
VIC Department of Primary Industries

Project Number: VG01096

VG01096

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the vegetable industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of Agrimm Technologies and Lincoln University.

ISBN 07341 1216 5

Published and distributed by:

Horticulture Australia Ltd

Level 1
50 Carrington Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399

E-Mail: horticulture@horticulture.com.au

HAL

Know-how for Horticulture™

Stop the Rot – Managing Onion White Rot in Spring Onions

Final Report
Horticulture Australia Limited Project VG01096
(30 October 2005)

Villalta et al
Department of Primary Industries, Victoria

Horticulture Australia Project VG01096 – 'Stop the rot – managing onion white rot in spring onions'

Project Leader: Oscar Villalta

Project Team
Oscar Villalta, Ian J. Porter, Denise Wite - Department of Primary Industries, Knoxfield, Victoria
Private Bag 15
Ferntree Gully Delivery Centre, VIC 3156 Australia
Phone: 03 9210 9222
Fax: 03 9800 3561
Email: oscar.villalta@dpi.vic.gov.au

Alison Stewart and Kirstin L. McLean – National Centre for Advanced Bio-Protection Technologies, Lincoln University, New Zealand
PO Box 84, Lincoln University
Canterbury, New Zealand
Tel: (64) (3) 325 3697
Fax: (64) (3) 325 3843

Purpose of project
The purpose of this project was to evaluate chemical and biological treatments for the control of the disease onion white rot on bunching onion crops. This was to provide vegetable growers with more control options and an integrated strategy for the sustainable control of this soil-borne disease and to better inform them of the most appropriate and effective use of chemical and biological treatments for disease management on their farms.

Acknowledgments
Horticulture Australia Limited (HAL), AUSVEG, the Federal Government, Department of Primary Industries, Victoria and contributions by Lincoln University and Agrimm Technologies, New Zealand.

October 2005

CONTENTS

1. Media Summary

The disease onion white rot is a serious problem in bunching *Allium* crops, which include spring onions and shallots, sometimes causing crop losses of up to 50% in eastern Australia. Before this project began vegetable growers used the fungicide procymidone to manage white rot, but complained that they were not getting good control. As a result of this research, growers now have a range of new options to control onion white rot.

By changing the timing and method of application (banding *vs* broadcast) of procymidone, control of white rot was 75% more effective than that obtained by growers. However, procymidone did not persist long enough in soil to effectively suppress late-season infections at high disease sites and recently procymidone was suspended from use in vegetable crops pending a review by the Australian Pesticides and Veterinary Medicines Authority. This project developed four new fungicide treatments that proved to be more effective and reliable than procymidone, and efficacy and residue data from this research will be used to support minor use permits for these products.

To compliment the conventional fungicide treatments two other methods of control were tested and developed. Treatment of soil with DADS (onion oil) tricked the fungus into germinating causing it to die out before spring onion crops were sown reducing the fungal population by up to 90%. This resulted in a 85% reduction in disease in the following spring onion crop at high disease sites. Because the amount of fungus in the soil was reduced the biological treatment *Trichoderma atroviride* C52, applied in the furrow with seed, was more effective reducing disease in soil treated with DADS than in untreated soil. DADS integrated with fungicide treatments resulted in almost complete disease control. Combining *T. atroviride* C52 treatments with post-planting sprays of fungicide was more effective reducing disease than using the biological treatment alone at high disease sites.

This project has successfully developed new treatments which can be used alone or as part of an integrated package by vegetable growers to control onion white rot of bunching onions in their farms. The project also provided valuable information that will assist vegetable growers to make informed decisions about the use of the biological control *Trichoderma* for managing onion white rot and soil health.

2. Technical Summary

Onion white rot is the most serious soilborne disease of bunching onion crops in eastern Australia. In this region, a survey found that this disease occur in approximately 194 hectares of land used for growing bunching onion crops in vegetable farms in Victoria, NSW, Tasmania and Queensland. Most of the land affected is located in the south and south-east of Melbourne, Victoria. The survey also found that this disease was responsible for crop losses of 5-50%, or higher, due to the high levels of sclerotia of *Sclerotium cepivorum* in soils (range 5-232/kg soil) and insufficient chemical protection provided by procymidone treatments. Petri dish experiments indicated that the lack of effective control with procymidone could not be attributed to the development of procymidone resistance in populations of *S. cepivorum*. However, survey data suggested that inadequate application of procymidone treatments was probably one the reasons for ineffective disease control with procymidone.

This project therefore conducted a series of field trials to evaluate new methods of applying procymidone treatments and identify alternative treatments to procymidone for control of white rot of bunching onions. Field trails also evaluated biological controls (*Trichoderma*) and a commercial formulation of the soil treatment diallyl disulphide or DADS (Alli-Up™, germination stimulant of sclerotia) for integrated control of white rot. Trials were conducted within commercial crops of spring onions grown in sandy loam/clay soils naturally infested with sclerotia of *S. cepivorum*. Petri dish experiments determined the range of soil temperatures favourable for disease development. This information and soil temperature data collected in the field were used to optimise the time of application of control measures in field trials.

Field trials demonstrated that the effectiveness of procymidone treatments for white rot control can be improved by improving its application. Results from two trials on fields where procymidone was 'ineffective' controlling white rot showed that two procymidone treatments reduced the incidence of plants with white rot by 75-76% of the untreated control, (20-40% plants diseased). Procymidone treatments were more effective in reducing disease incidence, compared to grower' practices, because sprays were strategically placed (banded) across the furrow with seed and plant-rows where protection against infection is needed. Although these and other trials showed that the effectiveness of procymidone treatments for disease control was improved by better application of treatments, its persistence in soil and plant was insufficient to provide the effective protection required throughout the growing season.

Field trials identified a number of fungicide treatments that can be used as alternatives to procymidone. Filan™ (boscalid) treatments were consistently more effective than procymidone treatments in controlling white rot and increasing yields at all field sites. At three field sites, for example, two applications of Filan™ reduced the incidence of plants with white rot by 90-99% of the untreated controls (15-30% of plants diseased). One spray with Folicur™ (tebuconazole) applied after sowing was as effective as Filan™ in controlling disease at three field sites. Two sprays with either Filan™, Amistar™ (azoxystrobin) or Bayfidan™ (triadimenol), applied to young plants approximately four and six weeks after sowing were more effective than procymidone treatments in controlling white rot. Commercial trials (treatments applied with boom sprayer) confirmed the excellent efficacies of Filan™ and Folicur™ for white rot control of spring onions.

Field trials showed that *Trichoderma* treatments alone were less effective than fungicide treatments in controlling white rot at high disease sites. At two field sites, for example, Filan™ treatments reduced the incidence of disease by 90-98% of untreated controls (28-29% diseased plants). *Trichoderma* C52 applied on formulated prills in the furrow with seed at sowing (Trichopel Ali 52™, 50kg prills/ha) gave a reduction in disease in the order of 50% of that of untreated plants at one of the two high-disease field sites. At both field sites, the biocontrol applied at sowing combined with one post-planting spray of Filan™ gave reductions in disease in the order of 58-75% of that of untreated controls. At a low-disease field site (11% plants diseased), *Trichoderma* treatments were as effective as two procymidone sprays in controlling disease.

Overall, results showed that *Trichoderma* C52 levels in sandy soils were below the required levels for effective biocontrol. Despite that, the results indicated that *Trichoderma* C52 was able to provide some degree of protection against early season infection when its levels were highest in the region of roots.

Field trials demonstrated that two applications of DADS were more effective than single applications in reducing the number of viable sclerotia in soil (90% reduction) and disease incidence. The two rates of DADS tested (5 and 10L/ha) were equally effective in reducing disease incidence. In two trials, for example, two applications of DADS applied at 5 L/ha caused reductions in the incidence of disease of 85-87% (20-34% plants diseased). Combining two applications of DADS with either 1 or 2 sprays of Filan™ resulted in almost completed disease control. At one field site, DADS integrated with Trichopel Ali 52™ gave levels of disease control comparable to those provided by DADS combined with fungicide treatments.

Controlled studies identified soils, chemicals and soil amendments that are compatible with *Trichoderma*. *Trichoderma* C52 grew to levels desirable for biocontrol ($>10^5$ cfu/g soil) in clay and black loam soils but required incorporation of pellets containing humic acids (Agrolig™) to grow to similar levels in sandy soils. *Trichoderma* growth was inhibited by nitrogen released from nitrogenous materials such as fertilizer (urea) and fresh chicken manure but it was compatible with low-nitrogen soil amendments (eg spent mushroom compost) and field rates of key fungicides used for white rot control (eg procymidone, boscalid). DADS was also detrimental (fungistatic) to *Trichoderma* growth in close contact. The information collected will be used to optimise the application of *Trichoderma* into soils and develop an integrated strategy for managing white rot and soil quality in vegetable farms.

Onion white rot on bunching onions and its control with the fungicide procymidone

Summary

Onion white rot is the most serious soilborne disease of bunching onion crops (eg shallots, spring onions, shives) in eastern Australia. In this region, a survey (fax, phone and visits to farms) estimated that this disease was present in at least 195 hectares of land used for growing bunching onions in commercial vegetable farms in Victoria, NSW, Tasmania and Queensland. Most of the land affected is located in the south and south-east of Melbourne, Victoria. The disease was not reported in vegetable farms in South and Western Australia. In farms in New South Wales, Tasmania and the Lockyer Valley, growers reported that white rot occurs in small areas or 'hot spots' within fields. The survey data does not include the hundreds of hectares of land infested with white rot that are used for growing bulb onions in Tasmania, Victoria and Queensland. The survey data revealed that onion white rot was responsible for crop losses of 5-50%, or higher, due to the high levels of sclerotia of *S. cepivorum* in soils (range measured 5-232/kg soil) and insufficient chemical protection provided by procymidone treatments.

Results from *in vitro* experiments showed that mycelial growth from thirteen isolates of *S. cepivorum*, collected from fields where procymidone was 'ineffective' controlling white rot, was significantly inhibited by the two lowest concentrations of procymidone tested (1 and 5 ppm/mL). These results indicated that the lack of effective disease control with procymidone was not due to the development of procymidone resistance in the populations of *S. cepivorum* tested. Survey data, however, suggested that inadequate application of procymidone treatments by growers was probable one the reasons for ineffective disease control with procymidone in commercial farms. Petri dish experiments verified the range of soil temperatures favourable for disease development. This information and soil temperature data collected in the field were used to optimise the time of application of procymidone treatments in field trials. Results from two trials on fields where procymidone was 'ineffective' controlling white rot showed that two appropriately timed and applied sprays of procymidone reduced the incidence of plants with white rot by 75-76% of the untreated control (20-40% plants diseased). Procymidone treatments were more effective in reducing disease incidence, compared to grower's practices, because sprays were strategically placed (banded) across the furrow with seed and plant-rows where protection against infection is needed. However, these and other trials showed that procymidone treatments did not persist long enough in soil and plant to provide the effective protection required throughout the season at high disease field sites.

Introduction

Sclerotium cepivorum Berk., causes the disease white rot on several *Allium* species. White rot of onion (*Allium cepa* L.) and bunching onions (*Allium fistoloson* L.) is a serious threat to the bulb onion and bunching onion industries worldwide and in Australia. White rot is now present in major onion-growing districts in the country. The disease severely reduced onion production in south-western Victoria, once Australia's major onion-producing area in the 60-80's. Prior to this study, there was no information available on the prevalence of white rot in major bunching onion growing districts in the country. In Victoria, vegetable growers reported that this disease has progressively increased over the years due to the frequent use of monocultures of spring onion crops in short rotations with other non-host crops (e.g. radish, endive, parsley). This has probably resulted in drastic increases of pathogen (sclerotia) populations, leading to high disease levels and therefore considerable yield losses.

Spring onion growers in Victoria have reported that in the past seed and soil treatments with the fungicide procymidone provided adequate control of white rot but, in recent years, procymidone treatments (soil surface and foliar sprays) have not provided consistently adequate control of white rot.

The decline of fungicide effectiveness has been attributed to resistance in the pathogen. Field isolates with resistance to dicarboximide fungicides (e.g. iprodione, vinclozolin, and procymidone) have been

reported in S. minor populations from peanut in Virginia, USA (Detweiler et al., 1983). Resistance to dicarboximates was not found in field isolates of S. cepivorum collected from a district with reported fungicide loss of effectiveness in New Zealand (A. Stewart, unpublished). However, it has been shown that S. minor and S. cepivorum have the capacity to develop resistance to dicarboximates in vitro (Entwistle 1983, Hubbard et al., 1997). Recent field and laboratory work in New Zealand indicated that the decline in the effectiveness of dicarboximide and triazole systemic fungicides used for white rot control could be attributed to enhanced microbial degradation of the chemicals in the soil and soil characteristics (Slade et al., 1992, Tyson et al., 1999).

Possible explanations for the inadequate control of white rot (bunching onions) with procymidone are (i) resistant strains of *S. cepivorum* have developed with continued fungicide use, (ii) fungicide applications are not being timed and applied properly and (iii) continue use of fungicide has increased the population of soil microbial populations that rapidly degrade the fungicide. Therefore this research conducted

- a survey to determine the distribution of onion white rot and levels of crop losses in bunching onion-producing districts of Australia.
- *in vitro* tests to determine the range of temperatures conducive to germination of sclerotia of *S. cepivorum* and mycelial growth.
- field monitoring of soil temperatures to define the periods of disease risk and therefore improve the time of application of control measures.
- collected isolates of *S. cepivorum* from fields where disease control with procymidone was reported as inconsistent and inadequate and conducted *in vitro* tests to determine their sensitivity to procymidone.
- two field trials to determine if better application of fungicides treatments at the right time could improve the efficacy of procymidone for white rot control on spring onions grown with three different compost amendments.

Materials and Methods

Survey

Sites details

Survey data was collected by fax and during visits to vegetable farms. Thirty-eight vegetable farms producing bunching onions in crop rotations with vegetables were surveyed in Australia, mainly from Victoria where most of the bunching onion production occurs. The properties were located in the Cranborne, Clyde and Heatherton areas. The presence of white rot and estimated area of land affected and yield losses were recorded at each site.

Population of sclerotia in soils

Levels of sclerotia of *S. cepivorum* in soil were determined from soil samples collected from six of the farms surveyed. Soil samples were collected from fields selected based on the history of procymidone use (2-10 yrs) and reported inconsistent control of white rot with procymidone. The size of the fields varied from 0.1 to 0.3 ha. Each field was divided into 10-16 sections and a composite soil sample collected from each section. Each composite soil sample consisted of five soil sub-samples collected with a soil core sampler from the top 10 cm of soil. The soil sub-samples were taken from separate locations within each section approximately 2 m apart to provide true replicates and later mixed well. Samples were stored in plastic bags at 5-10ºC until used. All soils assessed were sandy soils. Sclerotia were recovered from soil using a wet sieving method. One hundred grams of soil were wet-sieved through a 500 and 250 micron-screens. Sclerotia were collected from debri in the 250 microns screen. Soil moisture was calculated and then the dry weight of samples used to determine number of sclerotia per kg of dry soil. Viability of sclerotia was not measured.

Isolate sensitivity to procymidone

Isolates selection

Thirteen isolates were tested *in vitro* test for procymidone resistance. Single sclerotial isolates of *S. cepivorum* were obtained from soil samples collected from fields where disease control with procymidone was reported by growers as inconsistently inadequate and a history of procymidone overuse for disease management. Two of the isolates were collected from infected spring onion plants collected from commercial farms in Victoria and the Lockyer Valley in south Queensland. Sclerotia of *S. cepivorum* were isolated from soil using the wet-sieving method as described earlier.

Isolates preparation and incubation on amended plates

Sclerotial isolates were surfaced sterilised in a 1:1 v/v solution containing sodium hypochlorite (5%) and ethanol (90%) for 3 min and later rinsed in sterile distilled water for 3 min, and dried on sterile filter paper for 5 minutes in a laminar flow cabinet. Single (sclerotium) isolates were then placed onto potato-dextrose agar (PDA) drops with acromycin antibiotic. A 8-mm diameter agar disk containing actively growing vegetative young mycelium from PDA cultures of each isolate was plated on PDA amended with 0, 1, 5, 10, 50 and 100 µg per ml of a commercial formulation of procymidone (Sumisclex™ 500). Each concentration of the fungicide amended media was replicated four times. All plates were incubated at room temperature (15-21ºC) and colony diameters were measured every week for 3 weeks. Colony diameter was measured along x- and y-axes on each test plate. These two values were then averaged to provide a single measurement of colony growth for each plate. The experiment was conducted twice.

Data analysis

Analysis of variance was applied to the in vitro data using Genstat (6^{th} Edition). An F-test was conducted to determine whether observations from the two tests could be pooled. Analysis showed that results from the two experiments were consistent and therefore data was pooled. Data were transformed to log(x + 1) prior to ANOVA. Analysis of variance was applied to determine the effect of procymidone concentrations and length of incubation on growth of mycelium.

Growth of *S. cepivorum* at different temperatures

Production of sclerotia and mycelium

Two in vitro tests were conducted to determine the effect of temperatures on germination of sclerotia and growth of mycelium of *S. cepivorum*. A single sclerotium isolate of *S. cepivorum*, collected from a field in Victoria, was used to bulk up sclerotia used in the tests. A 8-mm diameter agar disk containing actively growing vegetative mycelium cut from the original culture were plated on fresh PDA plates. All plates were incubated at room temperature (15-21ºC) for several weeks until sclerotia was produced. Fifty plates yielded several thousands sclerotia. Sclerotia were collected from the plates and placed inside sandy soil in mesh bags (polyester stokings). The bags with soil and sclerotia were buried in the field for approximately 6 months from September to May to condition sclerotia and break their dormancy. Sclerotia were then recovered from soil by wet-sieving, dried and surface sterilised before use in the tests. Multiple sub-cultures of test isolate were made several days prior to inoculation of agar plates to provide sufficient mycelium for agar disks.

Inoculation and incubation of isolates

For sclerotia, a single sclerotium was placed on a PDA droplet (20 sclerotia/plate). Two plates with forty sclerotia were then incubated in each of the eight temperatures (5, 10, 13, 15, 18, 20, 25 and 30C) tested. The germination/viability of each sclerotia was measured on each plate after 7 and 14 days of incubation. After this period, plates were incubated at room temperature and then assessed for

germination and sclerotia production 7 days later. The number of sclerotia germinated was expressed as percentage of total germinated. For tests with mycelium, 8-mm diameter agar disk containing actively growing vegetative mycelium produced from PDA cultures of the same isolate were plated on fresh PDA plates. There were two plates for each temperature tested. The plates were incubated together with plates with sclerotia. Measurements of colony growth were conducted as described for sclerotia. Colony diameter was measured along x- and y-axes on each test plate. These two values were then averaged to provide a single measurement of colony growth for each plate. The experiment was conducted twice.

Recording of soil temperatures

Two Tinytag™ temperature loggers (Gemini data loggers) recorded soil temperatures at two field sites at one location (Cranbourne) in Victoria from June 2002 to June 2003. The temperature sensors (10 cm long x 5 mm thickness) were buried vertically into the top layer (10cm) of soil at the two fields separated by 1 km. One Tinytag™ logger also recorded soil temperatures at one field site in the Lockyer Valley, Queensland, from March to September during two years (2003 and 2004). The temperature records were compared and averaged for the locations in Victoria.

Data analysis

Results were analysed using an analysis of variance (ANOVA) to test the effects of temperature on sclerotial and colony growth. The appropriateness of an ANOVA for the data was checked by visual inspection of residual plots. Percentage data were arc-sin-transformed prior to ANOVA. Means were compared using LSD tests ($P \leq 0.05$). Analysis was conducted using Genstat (Edition 6).

Efficacy of procymidone treatments for white rot control

Field sites details

Trials were conducted within commercial crops of spring onion in two fields naturally infested with sclerotia of *S. cepivorum* at Cranbourne, Victoria. Details of trials and activities are presented in Table 1. The crops were grown using grower's methods.

Trial design

Two trials were set up to test a new method of applying procymidone for white rot control. In the first trial, a modified split-plot design was used with fungicide as the main treatment and composts (mulch) as the sub-treatments with five replicates. Procymidone was applied on plots with and without compost mulch. For the second trial, treatments (procymidone and untreated) were arranged in a randomised block designs with five replicates. Soil temperatures were measured with Tinytalk temperature loggers (Gemini data loggers) buried at depths of 10 cm and 15 cm in a plot in the middle of each trial. Measurements were taken at hourly intervals from the time of sowing through to harvest. Seed of cultivar 'Paragon' was planted with a high precision seeder using 50 k/ha of seed. Seed was planted on raised beds with triple rows of high density (eg 100-130 seedlings per linear meter). Overhead irrigation was applied when required. Slow release fertiliser (NPK) was applied when seedlings were 10cm height and later when required. The fields used were sandy soils with pH range 7.0-7.5. The main plot was 1 m wide by 10 m long on raised beds (0.3-0.4 m height) with three rows of high-density plants, each row of plants 20 cm apart.

Treatments

In the first trial, procymidone treatments were evaluated on plots unamended and amended with chicken manure compost (mulch) commonly used to increase soil fertility and prevent erosion of sandy soil and damage to young seedlings. Chicken manure was compare to spent mushroom compost and mature green compost and an unamended control. Composts sub-treatments were applied to each

plot (1 m wide by 5 m long) on raised beds (0.3-0.4 m height) with three rows of high-density plants per bed and each row 20 cm apart.
All composts were applied manually at the rate of 20 tonnes per hectare, all in a fresh weight basis. The composts formed an even layer of 5-10 mm thickness on the soil surface. The first application was made after sowing and the second when plants had 2 tillers or about 10 cm height. A typical composition analysis of the composts used is given in Table 2. Composted chicken manure (chicken excrement and pine shaving bedding) was provided by the grower. Certified compost soil conditioner was made of city green waste (MulchMaster™, Dandenong Vic), and spent mushroom compost (Mushroom farm compost) supplied by Organic Compost Pty Ltd, Victoria. In the second trial composted chicken manure was applied to all beds (mulch) with a farm spreader after sowing and repeated 3-4 weeks prior to harvest at the rate of 17-20 tonnes per hectare, in a fresh weight basis. Chicken manure formed an uneven layer of 5-10 mm thickness on the soil surface.

Spray program

The spray program used consisted of two sprays of procymidone applied at a rate of 1L ai/ha using 1000 L/ha of water. The first spray was applied at sowing and the second approximately 4-weeks later. In the first trial, sprays were applied with a knapsack fitted with three conical nozzles and calibrate to deliver the appropriate amount of fungicide using 1000L water/ha across furrows/rows with seed (soil surface sprays) and plants (stem base/foliar sprays) (Figure 1). In the second trial, sprays were applied with a grower's boom sprayer modified to deliver the appropriate amount of fungicide using 1000L water/ha also across furrow/rows with seed (soil surface sprays) and plants (stem base/foliar sprays). The modification included the use of 90º degree nozzles that allowed the use of high volumes of water and lowering the boom sprayer to ensure sprays were applied onto beds.

Measurements and data analysis

In the first trial, the density of sclerotia of *S. cepivorum* was determined from soil samples collected at 10 and 20 cm depths prior to sowing the crop. Five soil sub-samples were taken arbitrarily from each of six plots and later mixed and 100 g of the composite sample used from each plot for sclerotial counts. Sclerotia were extracted from the soil using the wet-soil sieving method.

Plant counts were made four weeks after sowing and thereafter the number of plants infected with white rot recorded in each plot every two weeks. All plants in a square meter within each plot were harvested and graded on the same day and bunches per plot counted and weighted together to determine yields/plot. The incidence of white rot was calculated based on the number of plants infected with white rot per square meter.

Data for percentages of plants with white rot and yields were transformed as required before subjected to analysis of variance (ANOVA) using Genstat (V6). Data for trial 1 was analysed as a modified split-plot design. The appropriateness of an ANOVA for the data was checked by visual inspection of residual plots. Means were compared using LSD tests (P = 0.05).

Results

Onion white rot on bunching onions

Thirty-eight vegetable farms producing bunching onions (spring onions, shives and shallots) were surveyed for the presence of onion white rot (Table 3). Sixteen of the total number of farms surveyed had fields or about 195 hectares of land infested with onion white rot (Table 3).

Eleven of these sixteen farms were located in the south and south-east of Melbourne (Cranbourne, Clyde, Devon Meadows, Heatherton and other locations). These eleven farms have approximately 189 hectares of land infested with white rot (Table 3). Fifty percent of the 189 hectares were reported as severely infested with white rot where disease control with procymidone (e.g. Sumisclex™) was inconsistent and inadequate.

The area of land infested with white rot in the eleven farms varied from small areas within fields (<0.3 acre) to entire fields. Growers reported that crop losses in fields infested with white rot ranged from 1 to 50%, or higher, for crops grown during periods conducive for disease development and treated with the recommended procymidone spray program. The main cultivars grown were Paragon, Straight leaf and Javelin. The survey also showed that onion white rot was also present in some fields used for producing bunching onions in vegetable farms in NSW, Tasmania and the Lockyer Valley Queensland. In these fields, white rot occurred only in small areas within fields (<0.1 acre) or as 'hot spots'. This land does not include the hundreds of hectares of land infested with white rot used for producing bulb onions in Victoria, Tasmania and the Lockyer Valley Queensland.

The mean number of sclerotia of *S. cepivorum* recovered from soils collected from six fields in Victoria ranged from 5 and 232 (range 2-552) sclerotia per kg of dry soil (Table 4). Disease control with procymidone was reported as inadequate in soils with more than 10 sclerotia per kg of dry soil.

Sensitivity of isolates of *S. cepivorum* to procymidone

On the media without procymidone, vegetative mycelium from all thirteen isolates of *S. cepivorum* filled the 85-mm petri dishes and produced sclerotia 7 and 14 days after incubation, respectively. seven days of incubation, none of the thirteen isolates showed any growth on the media amended with procymidone at any of the concentrations tested (data not shown). After 14 days of incubation, nine isolates began growing at slow rates on media amended with the two lowest concentration of procymidone tested. After 21 days of incubation, nine isolates grew to levels ranging from 6 to 23 mm on media amended with 1 mμ/ml of procymidone and five of these nine isolates grew to levels ranging from 1 to 7 mm in media with 5 mμ/ml of procymidone (Table 5). None of the isolates produced sclerotia in media amended with all concentrations of procymidone. Colony morphology on media containing procymidone was irregular in appearance and colour, and sometime individual sectors of the colony grew rather the whole colony indicating that procymidone was starting to break up in artificial media after 2 weeks of incubation.

Total variation in colony area recorded for the nine isolates 3 weeks after procymidone application was accounted for by isolate type, procymidone concentration, the length of incubation, and the interaction of concentration x isolate (Table 6).

Optimum soil temperatures for disease development

Germination of sclerotia

After 7 days incubation on artificial media, sclerotia of *S. cepivorum* germinated only at 13, 15, 18, 20 and 25ºC in experiment 1 and at 15, 18, 20 and 25ºC in experiment 2 (Table 7). In experiment 1, the percentage of sclerotia germinated was significantly higher (55-77.5%) at 18, 20 and 25ºC than at 13 and 15ºC (15-22.5%). After fourteen days of incubation, 90% of sclerotia had germinated at 13, 15, 18, 20 and 25ºC and 62.5% at 10ºC. In experiment 2, the percentage of sclerotia germinated was significantly higher (47.5-62.5%) at 18, 20 and 25ºC than at 15ºC (40%). After fourteen days of incubation, all sclerotia germinated at 13, 15, 18, 20 and 25ºC and 82.5% at 10ºC. Sclerotia did not germinate at 5 and 30ºC in both experiments. After a further 7 days of incubation at room temperature, all sclerotia from all temperatures had germinated and produced new sclerotia.

Mycelial growth

In the first experiment, mycelium of *S. cepivorum* completely filled the plates with media incubated at 10, 13, 15, 18, 20 and 25ºC after 7 days incubation (Table 7). Growth of mycelium was significantly lower (9-17 mm) at 5 and 30ºC than the rest of temperatures. Fourteen days after incubation, all cultures incubated at all temperatures, except 30ºC, completely filled the plates with media.
In the second experiment, mycelium of *S. cepivorum* completely filled the plates with media incubated at 15, 18, 20 and 25ºC after 7 days incubation (Table 7). Growth of mycelium was significantly

slightly lower (20-31mm) at 10 and 13ºC and lowest (5-10 mm) at 5 and 30ºC. Fourteen days after incubation, all cultures incubated at all temperatures, except 30ºC, completely filled the plates with media. After a further 7 days of incubation at room temperature, all cultures filled the plates and produced new sclerotia.

Soil temperatures in Victoria and Lockyer Valley

At one location in Victoria, soil temperatures began to fall below 20ºC from late February and gradually decreased from 20ºC to 10ºC during March, April and May (Figure 2). Soil temperatures were below 10ºC during June, July and most of August and gradually increased from 10C to 20C during September, October and November. In general, soil temperatures during December, January and February remained above 20ºC. At one location in the Lockyer Valley, soil temperatures began to fall below 20ºC in early May and fluctuated between 13ºC and 18ºC from mid-May to August (Figure 2).

Efficacy of procymidone treatments for white rot control

Trial 1

In this trial, the population density of *S. cepivorum* in soil averaged 186 sclerotia per kg of dry soil (range 92-270). Plants in all plots emerged evenly. There were no obvious symptoms of phytotoxicity in all plots treated with procymidone. Treatment means for percentage white rot and marketable yields (number bunches and fresh weights) are presented in Table 8. At harvest, untreated plots (no compost) had a mean white rot incidence of 18.8%. The fungicide treatments applied to plots with chicken manure and without any of the compost were highly effective in controlling white rot. These treatments significantly reduced the percentage of plants infected with white rot from 18.8% to 3.1% and 1.4% when compared to untreated plants. The chicken manure treatment was also effective in reducing disease. All other treatments were not effective in reducing white rot. The least effective of the treatments were those that had only green and spent mushroom composts applied to plots twice.

The number of marketable bunches for plots treated with chicken manure with and without fungicide were significantly higher than those of the untreated plots by an average of 6-9 more bunches per square meter. Fresh weights of spring onions for plots treated with two applications of chicken manure were significantly higher than those from untreated plots with fungicide. Split-plot analysis showed that all plots treated with two applications of procymidone had significantly lower percentages of plants infected with white rot than plots without fungicide. (Table 8). There were no significant interactions between fungicide and compost treatments.

Trial 2

Plants in all plots also emerged relatively evenly. There were no obvious symptoms of phytotoxicity in all plots treated with procymidone. Treatment means for percentage white rot and yields (number marketable bunches) are presented in Table 9. The fungicide treatment significantly reduced the percentage of plant infected with white rot from 43.6% to 10.4% when compared to plants without fungicide. The fungicide treatment effected a reduction in disease in the order of 85% of that of the untreated control. Yield of spring onions in fungicide-treated plots was significantly higher than those of untreated plots by about 10 more bunches per square meter.

Discussion

Onion white rot is becoming a limiting factor in the production of bunching onions in vegetable farms (sandy soils) in Victoria where most of this crop is grown in Australia. The disease is not currently a major problem in vegetable farms in NSW, Tasmania and the Lockyer Valley, Queensland. In Queensland and Tasmania, however, hundreds of hectares of land used for onion production are infested with onion white rot and this is also becoming a limiting factor for the production of *Allium* crops in these two states (Villalta 2005). Yield losses of bunching onions in field infested with white

rot commonly range from 5-50%, but in some seasons when soil conditions are favourable for disease development over 80% of plants may be killed. Losses of this kind have a devastating effect on grower incomes. Growers are being forced to rent adjacent land or move to another district to escape the disease, both options require additional capital investments.

This research investigated the reasons for procymidone (standard program) not providing adequate control of white rot on spring onions throughout the entire growing season. Firstly, it measured the levels of sclerotia in soil and found that in soils from six fields where growers applied procymidone and had high crop losses to white rot, the levels of sclerotia ranged from 5 to 232 sclerotia per kg of dry soil. Secondly, it investigated the sensitivity of populations of *S. sclerotium* to commercial dosages of procymidone. The mycelial growth of thirteen isolates of *S. cepivorum*, collected from fields where control with a full program of procymidone was reported as inadequate, was significantly reduced by the three lowest concentrations of procymidone tested (1, 5 and 10 ppm/mL). Higher procymidone concentrations tested completely inhibited mycelial growth of all isolates. The results indicated that concentrations of procymidone between below 10 ppm would reduce the growth of mycelium and prevent infection of roots and base of plants for at least 3 weeks after application or until a follow up application of procymidone is applied. These results also suggested that the isolates tested have not developed resistance to procymidone and therefore the decline in fungicide effectiveness cannot be attributed to the development of procymidone resistance in *S. cepivorum* populations. Recent field and laboratory work in New Zealand found that the decline in the effectiveness of dicarboximide and triazole systemic fungicides for white rot control could be attributed to enhanced microbial degradation of the chemicals in the soil and soil characteristics (Slade *et al*., 1992, Tyson *et al*., 1999).

The research then investigated whether inadequate application of procymidone treatments was the cause of lack of effective control of disease. First it verified the range of soil temperatures favourable for disease development to optimise the application of control measures. The range of temperatures found favourable for growth of an isolate of *S. cepivorum* agreed well with other patterns of pathogen development on bulb and garlic crops in other regions of Australia and the world (Mueller et al. 2004, Porter et al. 1991, Furllerton et al. 1995) The soil temperature range of infection for these crops is 10ºC to 25ºC, with the optimum being 15ºC to 18ºC. At soil temperatures above 25ºC or below 10ºC disease is considerably inhibited. Disease development is favoured by cool and moist soil conditions, typical of springtime weather patterns in Victoria, Tasmania and the San Joaquin Valley in California and New Zealand. In Victoria, Autumn and Spring were the periods the year when soil temperatures were most favourable for pathogen germination and disease development. Other results also indicated that disease can develop outside these two periods (eg. Summer) if soil temperatures persist within the range of infection, soil is wet and the population of sclerotia high. In the Lockyer Valley, Winter is the period of the year when soil temperatures were most favourable for pathogen germination and disease development.

The results from the two fungicide trials showed that the efficacy of procymidone can be improved by delivering sprays into the root zone, base of plants and plant rows where protection against white rot is required. In the first trial, two applications with procymidone banded across furrows with seed and plants rows (15 cm across), the first applied after sowing and the second 4-weeks later, provided good levels of protection during the entire growing season. In the second trial (commercial), two sprays of procymidone, also applied banded across furrow with seed and plants rows (boom sprayer 20-30cm), provide good levels of disease control, but yield losses in plots treated with procymidone were not commercially acceptable at harvest. Three applications of procymidone per crop were not tested at this stage due to fungicide resistance management strategies and risk of high levels of procymidone in leaves and stems (see fungicide residue data).

Results from both field trials indicated that procymidone did not last in soil and in the plant at the required levels for effective protection against white rot during the 12 weeks of the crop. The field performance of soil applied procymidone reported here suggests that this fungicide was not relatively persistent in the soil at two field sites. In soils with high levels of sclerotia, inadequate control of white rot with procymiodne would result in high yield losses. Research in New Zealand showed that a single application of procymidone at planting provided long-season control of white rot on bulb onions

(Fullerton et al. 1995). This and other research (Slade et al. 1992) indicated that procymidone is relatively stable in soil. Results obtained here suggest that procymidone would not persist in soil throughtout the season. Therefore, to obtain more effective levels of disease control with procymidone, it should be tested for early season disease control in combination with foliar sprays with other fungicides from other chemical groups to extend the protection to harvest. This includes the testing of different method of applying procymidone.

In one trial, two applications of composted chicken manure suppressed disease development probably due to the release of nitrogen (3% total nitrogen w/w) into the root zone. Chicken manure is used by growers to increase fertility of sandy soils and provide the extra nitrogen needed to finish off spring onion crops, however, its long-term use for vegetable production is unknown. Two alternative composts evaluated had lower levels of nitrogen and therefore were less effective for disease control and yield increases.

References

Detweiler, A.R., Vargas, J.M., Jr., and Danneberger, T.K. 1983. Resistance of Sclerotinia homoeocarpa to iprodione and benomyl. Plant Disease 67, 627-630.

Entwistle, A.R., 1983. Proc. 10th Int. Cong. Plant Prot.

Furllerton RA, Stewart A, Slade EA (1995). Use of demethylation inhibiting fungicides (DMIs) for the control of onion white rot (*Sclerotium cepivorum* Berk.) in New Zealand. New Zealand Journal of Crop and Horticultural Science 23, 121-125.

Hubbard, J.C., Subbarao, K.V., and Koike, S.T. 1997. Development and significance of dicarboximate resistance in Sclerotinia minor isolates from commercial lettuce fields in California. Plant Disease 81, 148-153.

Mueller S, Hembree K, Davis M (2004). Garlic White Rot Trial. Progress report for Garlic Industry, California.

Porter IJ, Maughan JP, Towers GB (1991). Evaluation of seed, stem and soil applications of procymidone to control white rot (*Sclerotium cepivorum* Berk.) of onions. Australian Journal of Experimental Agriculture, 31, 401-406.

Slade, E.A., Fullerton, R.A., Stewart, A., Young, H., 1992. Degradation of the dicarboximide fungicides iprodione, vinclozolin and procymidone in Patumahoe clay loam soil, New Zealand. Pesticide science 35, 95-100.

Tyson, J.L., Fullerton, R.A., Stewart, A., and O'-Calaghan, M. (1999). Changes in the efficacy of fungicide control of onion white rot. Proceedings of the Fifty-Second New Zealand Plant Protection Conference, pp171-175.

Villalta ON (2004). Report for Onion IAC 'Alli-up or DADS and possibilities for registration in Australia for the control of onion white rot', November 2004.

Table 1. Trials Summaries

Activity	Trial 1	Trial 2
Type of trial	small-plot replicated	large-plot commercial
Year	Spring 2002	Spring 2002
Location	Clyde	Cranbourne
Soil type	Sand loam	Sand loam
Spring onion variety	Paragon	Paragon
Fungicide application rates	procymidone 1L ai/ha	procymidone 1L ai/ha
Fungicide application dates	06/09/02, 10/10/02	05/09/03, 12/10/02
Sowing date(s)	05/09/02	01/09/03
Harvest date(s)	12/12/02	14/12/03

Table 2. Characteristics of compost materials used as soil amendments in field trial 1.

			% of total			
Compost	pH	Moisture content (%)	N	P	K	Ca
Composted chicken manure		19	3.5	1.7	1.4	2.6
Green waste compost	7.9-8.3	35-50	1.6	0.22	0.80	1.5
Mushroom compost	6.7-7.2	30-40	1.9	0.66	-	-

Figure 1. Application of soil surface and stem base sprays with procymidone

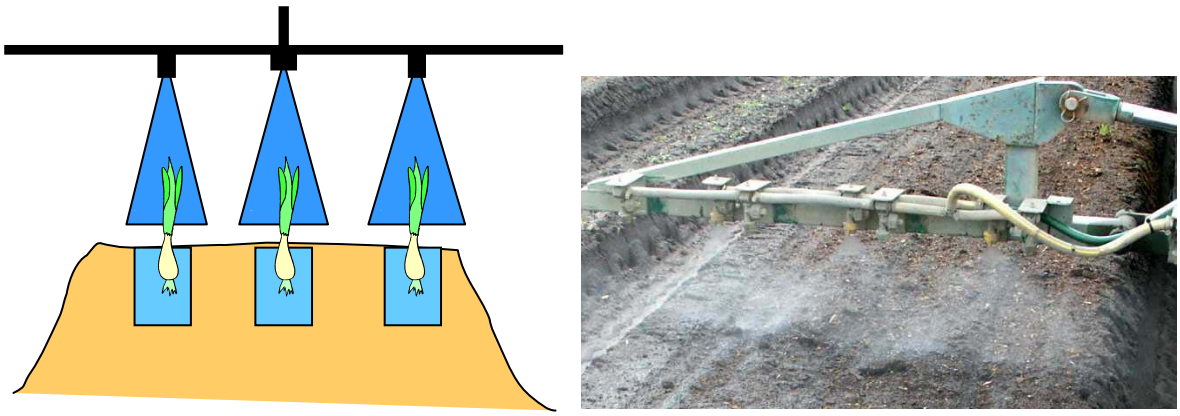

Table 3. Number of vegetable farms surveyed and estimated land area used for production of bunching onions (shallots, spring onions) infested with onion white rot.

State	No. of farms surveyed	Number of farms with OWR	Land infested with white rot (ha)
Victoria	21	11	189[a]
Queensland	4	2	<2
SA	2	0	
Tasmania	3	1	<2
WA	4	0	
NSW	4	2	<2
Total	38	16	195

[a] 50% of this land area was reported as severely infested with white rot where control with procymidone is difficult.

Table 4. Mean number of sclerotia of *S. cepivorum* per kg of dry soil collected from six fields in vegetable farms in Victoria where white rot control with procymidone was reported as inadequate.

Field	Location	Number of sub-samples	Mean no. sclerotia/kg soil[a]	Range	Fungicide control[b]
1	Clyde-North	12	232	90-552	inadequate
2	Fiveways	12	186	92-264	inadequate
3	Heatherton	12	180	80-240	inadequate
4	Cranbourne	10	92	22-178	inadequate
5	Devon Meadows	16	75	15-125	inadequate
6	Clyde	10	5	2-10	acceptable

[a] Extrapolated from samples of sclerotia extracted from 100g of soil.
[b] Disease control reported by spring onion growers using Sumisclex™ (soil surface and foliar applications).

Table 5 Mean colony diameter (mm) of *Sclerotium cepivorum* isolates incubated in potato-dextrose agar media amended with different concentrations of procymidone. Mycelial growth was recorded weekly for 3 weeks. Means of two tests with three replicate plates per fungicide dosage treatment per test.

Isolate	Location	Source	Concentration (mμ/ml)						
			0	1	5	10	50	100	
			Mean colony diameter (mm)						Sclerotia
V-7	Clyde, Vic	S. onion, soil	85[a]	0	0	0	0	0	No
V-8	Clyde, Vic	S. onion, soil	85	0	0	0	0	0	No
V-9	Fiveways, Vic	S. onion, soil	85	0	0	0	0	0	No
V-10	Fiveways, Vic	S. onion, soil	85	0	0	0	0	0	No
V-3	Heatherton, Vic	S. onion, soil	85	12	0	0	0	0	No
V-4	Heatherton, Vic	S. onion, soil	85	8	0	0	0	0	No
V-6	Cranbourne, Vic	S. onion, soil	85	7	0	0	0	0	No
V-11	Cranbourne, Vic	S. onion, soil	85	20	0	0	0	0	No
G-1	Gatton, Qld	S. onion, plant	85	19	3	0	0	0	No
G-2	Gatton, Qld	S. onion, plant	85	8	7	0	0	0	No
V-1	Clyde, Vic	S. onion, soil	85	6	5	0	0	0	No
V-2	Heatherton, Vic	S. onion, soil	85	8	1	0	0	0	No
V-5	Cranbourne, Vic	S. onion, soil	85	23	2	0	0	0	No

[a] Values are means of two tests for colony diameter (mm) for final assessment (3 weeks).

[c] Production of sclerotia in plates amended with procymidone. On unamended PDA, all isolates reached the edge of plates 4-5 days after plug with mycelium was plated out and produced sclerotia within 2 weeks.

Table 6. Statistical analysis of colony diameter (mm) for two experiments combined. All values for diameter growth were $\log_{nat}$-transformed before analysis.

Treatment	Level of significance ($LSD^{0.05}$)
isolate	<0.001 (0.11)
time of assessment	<0.001 (0.05)
dose	<0.001 (0.09)
dose x isolate	<0.001 (0.29)

Table 7 Mean percentage of sclerotia germinated in potato-dextro media (PDA) plates (drops) and mycelial colony diameter (mm) on full PDA plates of an isolate of *Sclerotium cepivorum* incubated at eight temperatures for 7 and 14 days.

Temperature	% Sclerotia germinated				Mycelial growth (mm)			
	Exp 1		Exp 2		Exp 1		Exp 2	
	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
5ºC	0.0	0.0	0.0	0.0	17.0b	39.0a	5.0e	30.0b
10ºC	0.0	62.5b	0.0	82.5b	39.0a	39.0a	20.0c	39.0a
13ºC	15.0c	90.0a	0.0	100.0a	39.0a	39.0a	31.5b	39.0a
15ºC	22.5c	92.5a	40.0b	100.0a	39.0a	39.0a	39.0a	39.0a
18ºC	65.0ab	95.0a	47.5a	100.0a	39.0a	39.0a	39.0a	39.0a
20ºC	77.5a	97.5a	62.5a	100.0a	39.0a	39.0a	39.0a	39.0a
25ºC	55.0b	92.5a	47.5a	100.0a	39.0a	39.0a	39.0a	39.0a
30ºC	0.0	0.0	0.0	0.0	9.5c	9.5b	10.0d	9.0c
$LSD^{0.05}$(P<0.001)	21.4	29.6	21.2	8.87	0.59	0.58	0.59	0.60

Figure 2. Soil temperatures in Vic and south Qld.

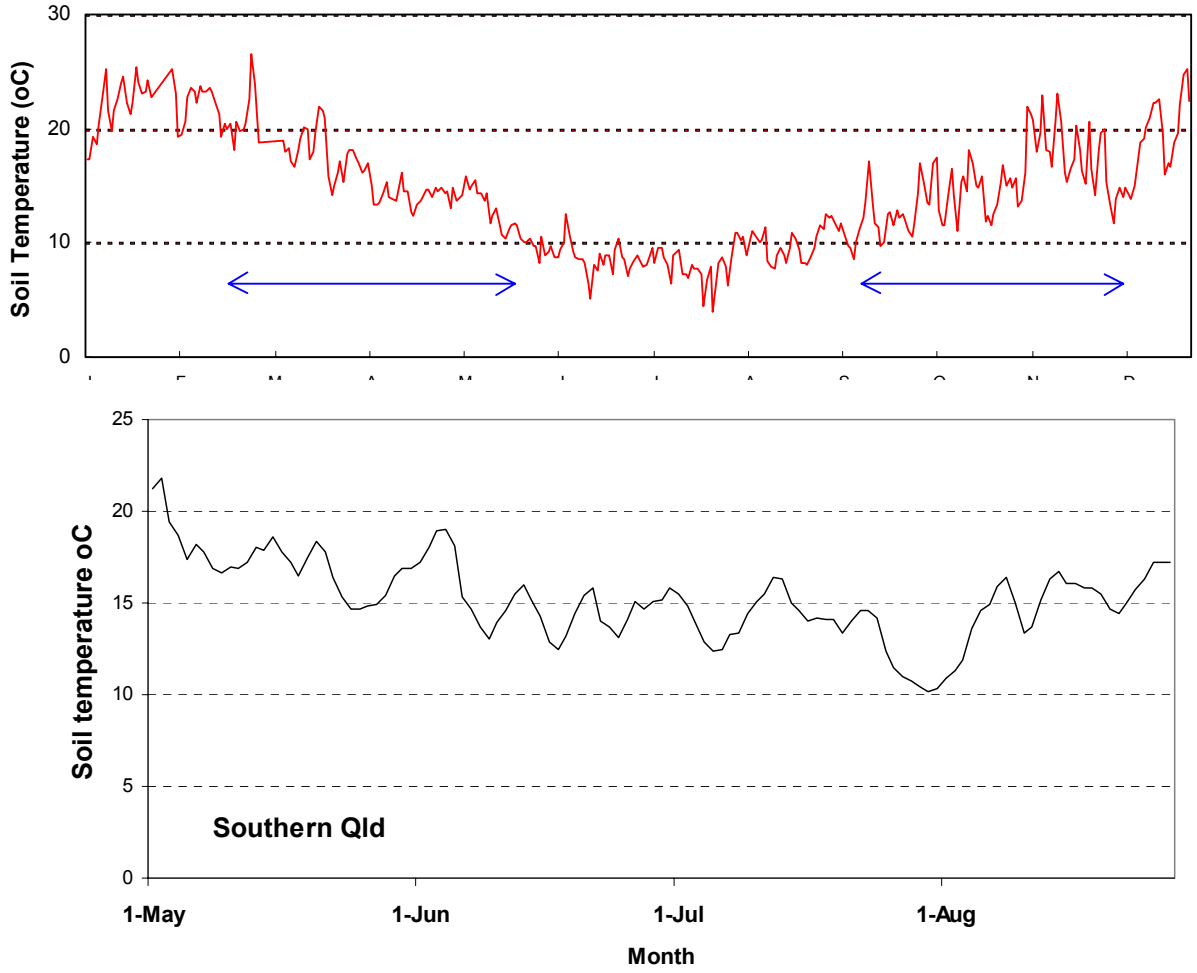

Table 8. Effect of composts and fungicide treatments on white rot and yield of spring onions grown during Spring in a trial conducted in a sandy loam soil at Cranbourne, Victoria. ANOVA results for disease incidence and number of marketable bunches were consistent for natural and transformed values therefore the raw data were analysed and presented.

Treatment	**% plants infected**	**No. marketable bunches**	**Fresh weight (kg)**
untreated control	18.8b	21.8a	4.4a
fungicide	3.1a	30.8c	4.0a
chicken manure	5.3a	28.8bc	5.5b
chicken manure + fungicide	1.4a	27.5bc	4.6b
compost	32.7c	17.6a	3.6a
compost + fungicide	21.1b	22.2a	3.8a
spent mushroom	25.7bc	23.3a	3.9a
spent mushroom + fungicide	16.7b	19.9a	3.5a
$LSD^{0.05}$ (*P* value)	8.55 (0.001)	5.36 (0.001)	1.04 (0.01)
Average fungicide	10.6	25.0	3.94
Average manures only	20.6	25.3	4.4
$LSD^{0.05}$ (*P* value) fungicide	4.49 (0.003)	ns	ns
$LSD^{0.05}$ (*P* value) compost x fungicide	ns	ns	ns

Table 9. Effects of fungicide treatments on white rot and yield of spring onions grown for 12 weeks during Winter-Spring 2002 at Cranbourne, Victoria. Treatments were applied with a growers' boom sprayer modified to deliver sprays onto beds using 1000L water/ha, the first spray applied after sowing as soil surface spray and the second as stem base/foliar spray using 1000g ai/ha of procymidone.

Treatment	**% plants with white rot**	**No. bunches(no./m2)**
untreated control	43.6a	18.6a
procymidone (two applications)	10.4b	28.0b
$LSD^{0.05}$ (*P* value)	29.16 (0.042)	4.86 (0.007)

Evaluation of fungicide treatments and application methods for control of white rot on spring onions

Summary

A series of field trials were conducted over three years (2003-2005) to compare the efficacies of procymidone treatments and new fungicide treatments for the control of onion white rot (*S. cepivorum*) on spring onion crops. The trials evaluated different fungicide rates and times and methods of applying fungicide. The trials were conducted within commercial crops of spring onions grown for 10-14 weeks in sandy soils naturally infested with sclerotia of *S. cepivorum* in vegetable farms in Victoria.

The results of field trials showed that procymidone treatments (seed, soil surface and stem base/foliar sprays), adequately applied and timed, were ineffective in controlling white rot in high disease field sites. Procymidone treatments applied at or after sowing and repeated mid-season did not persist long enough in soil and in plant to effectively suppress late season infections. Boscalid (Filan™, chemical group G) and tebuconazole (Folicur™, triazole fungicide) treatments were consistently more effective than procymidone treatments in controlling white rot on spring onion crops at all field sites. Either one or two applications with boscalid, applied after sowing as a soil surface spray and repeated as a stem base/foliar spray four weeks later, gave reliable and effective control of white rot. A single application with tebuconazole applied after sowing (soil surface spray) also gave reliable and effective control of white rot. These two fungicide treatments persisted long enough in soil and plant to provide effective protection against white throughout the growing season. Commercial trials (sprays applied with a boom sprayer) verified the excellent efficacies of boscalid and tebuconazole for white rot control. Azoxystrobin (Amistar™, strobilurin), triadimenol (Bayfidan™, DMI) and boscalid applied to young plants 4 and 6 weeks after sowing were more effective than procymidone treatments in controlling white rot.

Strategies for the use of the new fungicide treatments for white rot control on bunching onion crops are discussed. Fungicide residue data was collected for procymidone, boscalid, tebuconazole and azoxystrobin to support applications for minor use permits in Australia.

Introduction

From the early to mid 1980s, the dicarboximides vinclozolin and iprodione were commonly used for the control of white rot on onions (*Allium cepa* L.) overseas (Entwistle 1986). In Australia, these fungicides only protected the surface roots and most plants still developed disease before harvest (Merriman and Porter 1984). By mid 1980s, trials in New Zealand showed that these fungicides were not providing satisfactory control of onion white rot (Fullerton and Stewart 1991). Enhanced degradation (microbial degradation) of vinclozolin and iprodione in soil is considered the reason for failure of these two fungicides to control white rot (Walker 1987, Slade et al. 1992). During the mid 1980s, the systemic dicarboximide procymidone was found to be effective for white rot control when used as seed treatments combined with foliar sprays applied montly (x4) after emergence (Fullerton et al. 1995, Stewart and Fullerton 1991, Porter et al. 1991). Procymidone was also found to be effective against white rot when used as a single application applied either as a dispersable granule or as soil surface spray at planting. Slade et al. (1992) found that procymidone was more stable in soil than iprodione and vinclozolin. Results with procymidone in Australia were conflicting, seed treatment with procymidone were phytotoxic in a site in Victoria but not in another site in Tasmania (Porter pers comm., Wong and Maynard 1986). Effective control of white rot has been also obtained with the triazole tebuconazole when applied to the soil (Dennis, 1997) or to garlic cloves (Jackson et al 1997). Tebuconazole was phytotoxic causing seedling mortality when used as a seed treatment for onions in New Zealand (Fullerton et al. 1995). In order to reduce the risk of fungicide resistance and enhanced fungicide degradation, it is important to use fungicides from different chemical groups to control onion white rot.

In Australia, procymidone has been the only fungicide available to bunching onion growers for the control of white rot. Before the start of this project, spring onion growers in Victoria reported that this fungicide applied as soil surface and foliar sprays was not providing adequate control of white rot. Consequently a series of field trials were conducted to:

- compare the efficacies of procymidone treatments and alternative fungicide treatments for the control of white rot on spring onions,
- develop application methods for procymidone, without being phytotoxic, and other fungicide treatments to improve control of white rot on spring onions, and
- collect fungicide residue data for the most effective treatments to support minor use permits.

Materials and methods

Field sites and trials

The trials were carried out on sandy soils (pH range 6.5-7.5) in fields naturally infested with sclerotia of *S. cepivorum* in commercial vegetable farms at Cranbourne, Clyde and Heatherton, south and south-east of Melbourne. The field trials were set up within commercial crops of spring onions.

Seven small-plot replicated trials were conducted over 3 years to evaluate procymidone and other fungicide treatments for white rot control. Only four of the seven trials had sufficient disease on untreated plots to allow comparison of treatments. In addition, five large-plot replicated (commercial) trials were carried out to examine the efficacy of the most effective treatments identified in small-plot trials for disease control using growers' spray equipment. Only three of the five commercial trials had sufficient disease on untreated plots to allow comparison of treatments. Data from trials where disease levels were too low for comparing treatments are not presented here.

Fungicide treatments

The fungicide treatments used in the trials are listed in Table 1. Procymidone was used as the standard treatment, applied either as seed or fertilizer treatments and soil surface and stem base/foliar sprays (Figure 1). Other fungicide treatments were selected from those registered for white rot control on onions or reported as efficacious against sclerotial diseases. Details of trial activities are presented in Table 2. Fungicide treatments were applied when soil temperatures were favourable for disease development (Section 1). Seed and fertiliser treatments were applied as recommended by manufacturers. Other fungicide treatments were applied as soil surface sprays within the first 10 days after planting and thereafter as stem-base/foliar sprays at 4 and 6-weeks after planting. In small-plot replicated trials, sprays were applied using a knapsack at constant pressure and output using a timed application to deliver the appropriate amount of chemical using 1000L/water/ha followed by irrigation with overhead sprinklers. The knapsack was fitted with three cone jet nozzles to treat all three rows in one pass. The soil surface and stem-base sprays were applied in a narrow band 10-15 wide above each drill row furrow with seed or plant rows. In commercial trials, sprays were applied using a grower's boom sprayer modified to deliver the appropriate amount of fungicide with 1000L water/ha also applied across rows (25-30 cm) above each drill row furrow with seed (soil surface sprays) and plants (stem base/foliar sprays). The modification included the use of 90 degrees nozzles that allowed high volumes of water and lowering the boom sprayer to ensure sprays were applied to beds.

Experimental design

Experimental designs were set up as complete randomised blocks with four to six replications so that each treatment occurred once in each block. In small-plot replicated trials, each replicate consisted of a single bed/plot (1 m wide by 5-10 m long) of spring onions separated by 0.5 m bare soil between plots.

In large-plot replicated trials, each replicate consisted also of a single bed/plot (1 m wide by 10 m long) of spring onions separated by 0.5 m bare soil between plots. Each plot contained 3 rows of spring onions c. 1 cm apart. Beds were 1 m wide and 1.5 m centre to centre. Seed of spring onions were planted using a StanHay and GoldAcre precision seeders using approximately 50 k/ha of seed. Seed was planted on raised beds (0.3-0.4 m height) with plant densities ranging from 100-120 plants/linear meter. Overhead irrigation was applied when required. Slow release fertiliser (NPK) was applied when seedlings were 10 cm height and later when required. Two applications of composted chicken manure were applied either mixed into soil 2-3 weeks prior to sowing (Heatherton site only) or as topping spread onto beds after sowing and repeated 3-4 weeks before harvest to finish off the crop at the rate of 15-20 tonnes per hectare. The compost was applied using a farm spreader.

Measurements

Soil temperatures were measured with Tinytalk II temperature loggers (Gemini Data Loggers) at two depths (10 and 15 cm) in a plot in the middle of the trial. Measurements were taken at hourly intervals from the time of sowing through to harvest.

Emergence counts were made in all plots 2-3 weeks after sowing. At fortnightly intervals thereafter, all plots were inspected and white rot infected plants counted in a square metre/plot without removing plants. At harvest, the number of plants infected with white rot was measured in a square meter in each plot and then yields measured (number of marketable bunches, fresh weights, bunch length) approximately 12-14 weeks after sowing.

Spring onion samples were collected for fungicide residue analysis from four of the seven field trials carried out. The fungicides tested were procymidone, boscalid, tebuconazole and azoxystrobin. Samples of spring onions for each fungicide were removed at harvest from 3-4 replicate/plots and placed into an esky with ice. The samples were then cut into sections (leaves and bulbs) and later frozen within 24 hr of collection. Samples were submitted to various laboratories for analysis (Tables 10-13).

Data analysis

All data were analysed using analysis of variance using Genstat (Genstat 6). Data were transformed before analysis if required to stabilise the variance. Plants infected with white rot were expressed as a percentage of plants with white rot. The appropriateness of an ANOVA for the data was checked by visual inspection of residual plots. Where significant main or interaction effects were found then the mean values for treatments in each experiment were compared by Fisher's protected LSD tests (P = 0.05).

Results

Trial 1

Plants in all plots emerged relatively evenly. There was no obvious effect of seed or fertiliser treatments with procymidone on initial plant populations during the trial (data not shown). There were also no obvious symptoms of phytotoxicity in all plots treated with all other fungicide treatments. Treatment means for percentage white rot and yields (number marketable bunches and length of bunches) are presented in Table 3. At harvest, untreated plots had a mean incidence of white rot of 22%. Tebuconazole and boscalid were very effective in controlling white rot. The best treatments were those involving a single soil surface spray of tebuconazole applied after sowing and two applications of boscalid applied as soil surface spray after sowing and as a stem base/foliar spray approximately 4-weeks later. These two treatments significantly reduced the percentage of plants infected with white rot from 22.0% to 2.7% (tebuconazole) and to 2.4% (boscalid) when compared to plants without fungicide. All procymidone treatments did not provide commercially acceptable levels of disease control.

Yields of spring onions treated with boscalid and tebuconazole were significantly higher than those of untreated and procymidone treated plots by about 3-4 and 3-5 bunches, respectively.

Trial 2

Emergence of seedlings was reduced in plots with procymidone seed treatments combined with soil surface sprays. This was due to slow germination of seedlings in soil treated with procymidone in cool weather and wet soil conditions (data not shown). Some plants in these plots were stunted and yellow. Plants in other plots emerged relatively evenly. Treatment means for percentage white rot and yield (number marketable bunches) are presented in Table 4. Disease incidence at this site was low. At harvest, untreated plots had a mean incidence of white rot of 7.1%. All three rates of tebuconazole and boscalid tested were equally effective in controlling white rot. All procymidone treatments were also effective in controlling disease under low disease pressure.

Yields of spring onions in plots treated with all procymidone treatments, except the seed treatment alone, were significantly lower than those in other treatments.

Trial 3

Plants in all plots emerged relatively evenly. There were also no obvious symptoms of phytotoxicity in all plots treated with all fungicide treatments. Treatment means for percentage white rot and yields (number marketable bunches) are presented in Table 5. At harvest, untreated plots had a mean incidence of white rot of 15.1%. The best treatments were those involving a single soil surface spray of tebuconazole applied after sowing and two applications of boscalid applied as a soil surface spray after sowing and as a stem base/foliar spray approximately 4-weeks later. These treatments provided almost complete disease control. These two treatments significantly reduced the percentage of plants infected with white rot from 15.1% to 1.3% (tebuconazole) and to 0.5% (boscalid) when compared to plants without fungicide. The least effective treatments were those which had two applications of either procymidone or pyraclostrobin. Three applications of procymidone significantly reduced the percentage of infected plants from 20.2% to 6.3% when compared to plants that received only procymiodne applications. Plants treated with three applications of procymidone had more fungicide residue on leaves than those plants treated with only two applications of procymidone (Table 13). Two applications of either boscalid, BAS 516 (boscalid + pyraclostrobin), triadimenol or azoxystrobin, applied as stem base/foliar sprays at 4 and 6-weeks after sowing, were all equally effective in controlling white rot. These treatments resulted in a reduction in disease in the order of 60-66% of that of the untreated control. Combining one early season application of procymidone with two applications of either boscalid, BAS 516, triadimenol or azoxystrobin did not increase the levels of disease control provided by two applications of either boscalid, BAS 516, triadimenol or azoxystrobin.

The number of marketable bunches in tebuconazole and boscalid treated plots were higher than those in plots without fungicide and treated with the least effective treatments. However, these differences were not statistically different.

Trial 4

This trial compared the same fungicide treatments evaluated trial 3. Plants in all plots emerged also relatively evenly. There were also no obvious symptoms of phytotoxicity in all plots treated with all fungicide treatments. Treatment means for percentage white rot and yields (number marketable bunches) are presented in Table 6. Disease levels at this field site were lower than in trial 3. At harvest, untreated plots had a mean incidence of white rot of 6.1%. Under low disease-pressure, all treatments, except pyraclostrobin, were effective in controlling white rot. The best treatments were tebuconazole, boscalid, triadimenol, and procymidone combined with azoxystrobin and BAS 516. There were no significant differences in yields between all treatments.

Commercial Trial 1

Plants in all plots emerged relatively evenly. There were no obvious symptoms of phytotoxicity in all plots treated with tebuconazole (data not shown). Treatment means for percentage white rot and yield (number marketable bunches) are presented in Table 7. A single application of tebuconazole, applied after sowing as a soil surface spray, significantly reduced the percentage of plants infected with white rot from 15.5% to 2.3% when compared to plants without fungicide. This treatment provided a reduction in disease in the order of 85% of that of the untreated control.

Although not statistically different, the number of marketable bunches of spring onions in plots treated with tebuconazole were higher than those in untreated plots by about 3 more bunches per square metre.

Commercial Trial 2

Plants in all plots also emerged relatively evenly. There were no obvious symptoms of phytotoxicity in all plots treated with boscalid (data not shown). Treatment means for percentage white rot and yields (number marketable bunches) are presented in Table 8. Boscalid was highly effective in controlling white rot. Either one or two applications of boscalid, applied after sowing as a soil surface spray and repeated approximately 4-weeks later, significantly reduced the percentage of plants infected with white rot from 29.1% to 0.1% when compared to plants without fungicide. These treatments provided almost complete control of disease.

Yields of spring onions in plots treated with boscalid were significantly higher than those in untreated plots by about 6 more bunches per square metre.

Commercial Trial 3

Plants in all plots also emerged relatively evenly. There were no obvious symptoms of phytotoxicity in all plots treated with fungicide treatments (data not shown). Treatment means for percentage white rot and yields (number marketable bunches) are presented in Table 9. A single application of either boscalid or tebuconazole, applied as a soil surface spray after sowing, were very effective in controlling white rot. Boscalid gave the best control of disease. These treatments significantly reduced the percentage of plants infected with white rot from 14.9% to 3.2% (tebuconazole) and to 0.2% (boscalid) when compared to plants in untreated plots.

Yields of spring onions in plots treated with both fungicides were significantly higher than those in untreated plots by about 5 more bunches per square metre.

Discussion

The results showed that procymidone treatments, appropriately applied and timed, were not effective in controlling white rot in spring onion crops grown in sandy soils with moderate and high disease levels. The results obtained with procymidone in our trials were different to those obtained in trials with bulb onions (Fullerton et al. 1995, Porter et al. 1991). In these trials, seed treatments with procymidone suppressed early season infection and supplementary foliar sprays suppressed infections later in the season. Results obtained in trials carried by this project indicated that procymidone treatments (seed, soil surface, foliar) applied early in the season did not persist long enough in soil to suppress early and late season infections on short-season (10-14 weeks) onion crops in sandy soils. Procymidone treatments (seed and soil surface sprays) were phytotoxic and prevented seedling emergence when applied in cool weather. Procymiodne was suspended from use on some vegetable crops, including bunching onions, by APVMA in 2004 due to fungicide residue issues.

Results from our trials also showed that the triazole fungicide tebuconazole (Folicur™) and the new fungicide boscalid (Filan™), from group G fungicide, were both effective as post-planting treatments

for the control of white rot control on spring onions. These fungicide treatments are capable of providing commercially acceptable levels of protection against white rot on short-season onion crops (10-14 weeks) providing they are appropriately applied and timed. Fungicide treatments were applied in a narrow band across the furrow with seed and plants rows so that the appropriate amount of chemical is delivered into the root zone and base of plants using at least 1000L water/ha. Boscalid and tebuconazole treatments applied after sowing were very effective in suppressing early season infection. The results showed that these fungicide treatments when applied as soil surface and stem base/foliar sprays at the rates used in these trials were not phytotoxic to plants.

In commercial trials, a single application of boscalid applied after sowing as a soil surface had a lasting effect throughout the season indicating that boscalid had good persistence in sandy soils. Follow up applications with boscalid had also good systemic activity against disease when applied as stem base/foliar sprays. The long-term performance of boscalid in soils is unknown. Therefore boscalid should be used only during periods of high disease risk in fields known to be infested with sclerotia of S. *cepivorum* to minimise its use. Tebuconazole also had good persistence in sandy soils when applied after sowing as soil surface spray. This fungicide may have potential for use on 12-14-week or older crops due to its withholding period. For instance, it can used for crops sown during cooler weather and growing actively during periods of high disease risk (Spring).

Azoxystrobin (Amistar™), triadimenol (Bayfidan™) and boscalid also gave good levels of disease protection when applied as stem base/foliar applications 4 and 6 weeks after sowing after seedling had emerged. Results with these three treatments indicated that seedlings were unprotected in the first 4-weeks of the crop and this resulted in some early season infections. Therefore, fungicide treatments with boscalid or tebuconazole after sowing are important to provide early season protection against white rot for crops growing actively during periods favourable for disease development. To obtain the maximum level of disease control, both soil surface and stem base foliar sprays are required. Supplementary applications with azoxystrobin and triadimenol, applied 4 and 6 weeks after sowing, could provide the additional protection needed against disease until harvest in high disease sites. Integrating early season applications of boscalid with either azoxystrobin or triadimenol or tebuconazole with either boscalid, azoxystrobin or triadimenol would ensure that boscalid usage is minimise to one application per season/crop to prolong the field efficacy of this new efficacious fungicide.

In conclusion, the results showed that boscalid is an effective replacement for procymidone (Sumisclex™) for white rot control on bunching onions. Fungicide efficacy and residue data collected for boscalid supported an application for a minor use permit to use it to control white rot on bunching onions in Australia. Fungicide residue data was also collected for tebuconazole and azoxystrobin to support applications for minor use permits.

References

Dennis JJC (1997). Progress Towards an integrated control strategy for onion white root rot disease, including the sue of artificial germination stimulants. 2nd International Symposium on edible Alliaceae, November 1997 pp39 Adelaide, Australia.

Entwistle AR (Ed.) (1986). 'Proceedings of the Third International Workshop on *Allium* White Rot' 17-19 September, 1986 Wellesbourne, U.K. (T.M. Printing Associates Ltd: Warwickshire, UK) 305 pp.

Fullerton RA, Stewart A, (1991). Chemical control of onion white rot (*Sclerotium cepivorum* Berk.) in the Pukekohe district of New Zealand. New Zealand Journal of Crop and Horticultural Science, 19, 121-127.

Fullerton RA, Stewart A, Slade EA (1995). Use of demethylation inhibiting fungicides (DMIs) for the control of onion white rot (*Sclerotium cepivorum* Berk.) in New Zealand. New Zealand Journal of Crop and Horticultural Science, 23, 212-125.

Jackson KJ, Duff AA, O'Donnell We (1997). Tebuconazole (Folicur™) shows potential in the control of white rot (*Sclerotium cepivorum*) in garlic in sub-tropical Queensland, Australia. 2nd International Symposium on edible Alliaceae, November 1997 pp42 Adelaide, Australia.

Merriman PR, Porter IJ (1984). Control of white rot on dry bulb onions 1981-82 and 1982-83. Fungicide and Nematicide Tests 40, 146.

Porter IJ, Smith DJC (1986). Evaluation of fungicides for control of lettuce drop. Fungicide and Nematicide Tests 41, 96.

Porter IJ, Maughan JP, Towers GB (1991). Evaluation of seed, stem and soil applications of procymidone to control white rot (*Sclerotium cepivorum* Berk.) of onions. Australian Journal of Experimental Agriculture, 31, 401-406.

Slade EA, Fullerton RA, Stewart A, Young H (1992). Degradation of the dicarboximide fungicides iprodione, vinclozolin and procymidone in Patumahoe clay loam soil in New Zealand. Pesticide Science 35, 95-100.

Stewart A, Fullerton RA (1991). Additional studies on the control of onion white rot (*Sclerotium cepivorum* Berk.) in New Zealand. New Zealand Journal of Crop and Horticultural Science, 19, 129-134.

Walker A (1987). Further observations on the enhanced degradation of iprodione and vinclozolin in soil. Pesticide Science 21, 219-231.

Wong JAL, Maynard JR (1986). Application of procymidone to seed and fertilizer for the control of Allium white rot in bulb onions. In 'Proceedings Third International Workshop on Allium White Rot'. 17-19 September 1986, Wellesbourne, UK (Ed. A.R. Entwistle.) pp 86-91.

Table 1. Fungicide treatments applied in field trials for the control of onion white rot (*Sclerotium cepivorum*) of spring onions.

Treatment	Rate	Application method	Water L/ha
Untreated control	-	-	-
Procymidone (Sumisclex™, Sumitomo)	500g ai/L	seed treatment	20mL/kg seed
procymidone	500g ai/L	seed and fertiliser treatment	0.4mL/20g/m2
procymidone	1000g ai/ha	seed and soil surface spray	1000L
procymidone	1000g ai/ha	seed and stem base/foliar	1000L
procymidone	1000g ai/ha	seed, soil surface, stem/foliar	1000L
procymidone	1000g ai/ha	soil surface and stem base/foliar	1000L
procymidone	1000g ai/ha	soil surface and stem base/foliar	2000L
tebuconazole (Folicur™, Bayer)	430g ai/ha	applied to fertiliser	7mL/1000g
tebuconazole	500g ai/ha	soil surface spray	1000L
tebuconazole	300g ai/ha	soil surface spray	1000L
tebuconazole	700g ai/ha	soil surface spray	1000L
boscalid (Filan™, BASF, NuFarm)	500g ai/ha	soil surface	1000L
boscalid	500g ai/ha	soil surface and stem base/foliar	1000L
boscalid	300g ai/ha	soil surface and stem base/foliar	1000L
boscalid	900g ai/ha	soil surface and stem base/foliar	1000L
azoxystrobin (Amistar™, Syngenta)	150 ai/ha	stem base/foliar	500L
pyraclostrobin (Cabrio™, BASF)	200 ai/ha	stem base/foliar	500L
triadimenol (Bayfidan™, Bayer)	100 ai/ha	stem base/foliar	500L
BAS516(boscalid/pyraclostrobin, BASF)	400/200 ai/ha	stem base/foliar	500L

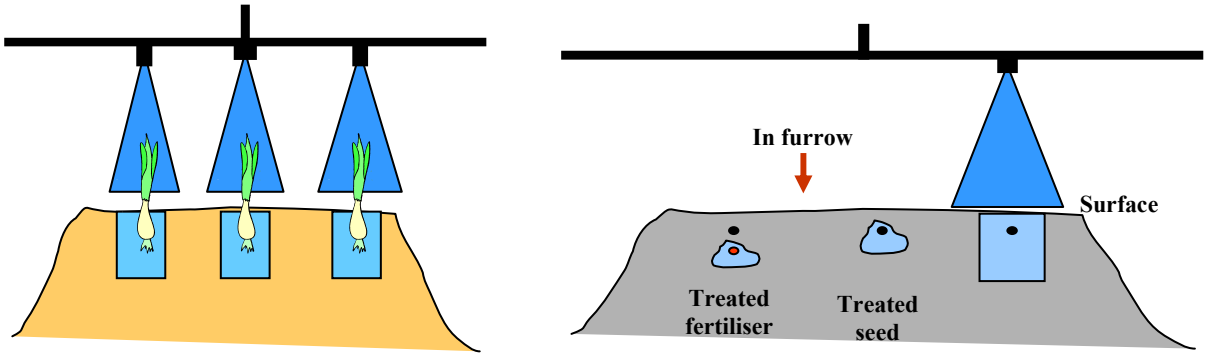

Figure 1. Method used to apply the various fungicide treatments. Left (stem base/foliar sprays; right (seed and fertilizer applications an soil surface sprays).

Table 2. Trials summaries

Activity	Trial 1	Trial 2
Type of trial	small-plot replicated	small-plot replicated
Year	Autumn-Winter 2003	Spring-Summer 2003
location	Clyde	Cranbourne
Soil type	Sand loam	Sand loam
Spring onion variety	Paragon	Paragon
Fungicide application dates	10/03/03, 03/04/03	02/09/03, 03/10/03
Sowing date(s)	06/03/03	29/08/03
Harvest date(s)	31/07/03	14/12/03

Activity	Trial 3	Trial 4
Type of trial	small-plot replicated	small-plot replicated
Year	Autumn 2004	Autumn 2004
location	Heatherton	Cranbourne
Soil type	Sand clay loam	Sand loam
Spring onion variety	Broad Leaf	Paragon
Fungicide application dates	27/02/04, 31/03/04,20/04/04	25/02/04, 31/03/04,20/04/04
Sowing date(s)	26/02/04	24/02/04
Harvest date(s)	05/05/04	20/05/04

Activity	Commercial Trial 1	Commercial Trial 2
Type of trial	large-plot replicated	large-plot replicated
Year	Winter-Spring 2004	Summer-Autumn 2004-05
location	Clyde	Cranbourne
Soil type	Sand loam	Sand loam
Spring onion variety	Paragon	Paragon
Fungicide application dates	26/06/04	27/12/04, 20/01/05
Sowing date(s)	24/06/04	20/12/04
Harvest date(s)	29/10/04	04/03/05

Activity	Commercial Trial 3
Type of trial	large-plot replicated
Year	Autumn 2005
location	Clyde
Soil type	Sand loam
Spring onion variety	Paragon
Fungicide application dates	07/03/05, 04/04/05
Sowing date(s)	01/03/05
Harvest date(s)	30/06/05

Table 3. Trial 1 - Effect of fungicide treatments on white rot and yield of spring onions grown during Autumn-Winter 2003 at Clyde, Victoria. Statistical analysis for disease incidence is based on $\log_{nat}$-transformed data (shown in parenthesis).

Treatment	% Plants with white rot (m^2)	Length bunches (cm)	Marketable bunches (no./m^2)
untreated control	22.0 (3.1)a	63.0	15.0ab
procymidone (seed)	14.7 (2.5)a	60.1	14.6ab
procymidone (seed, fertiliser)	15.8 (2.2)a	61.3	14.8ab
procymidone (seed and stem 1000g ai/ha)	13.0 (2.3)a	61.1	13.6ab
procymidone (seed and soil 1000g ai/ha)	10.1 (1.9)ab	60.7	15.4ab
procymidone (seed, soil and stem 1000g ai/ha)	16.8 (2.4)a	60.1	14.6ab
procymidone (soil and stem 1000g ai/ha)	13.2 (2.0)a	58.7	15.0ab
procymidone (soil 1000g ai/ha)	19.9 (2.9)a	59.6	13.2a
procymidone (soil and stem 1000g ai/ha)*	14.2 (2.4)a	59.5	14.2ab
tebuconazole (fertiliser)	6.0 (1.8)b	61.5	17.2bc
tebuconazole (soil 500g ai/ha)	2.4 (1.1)b	61.9	19.8c
boscalid (soil and stem 500g ai/ha)	2.7 (0.8)b	60.3	18.4c
$LSD^{0.05}$ (*P* value)	1.2 (0.029)	ns (0.719)	3.8 (0.025)

* 2000L water/ha used instead of 1000L/ha

Table 4. Trial 2 Effects of different rates of fungicides on white rot and yield of spring onions grown during spring 2003 at Cranbourne, Victoria. Statistical analysis for disease incidence is based on angular-transformed data (shown in parenthesis).

Treatment	% plants with white rot	Marketable bunches (no.)
untreated control	7.1 (14.7)c	13.7a
procymidone (seed)	2.8 (10.4)b	16.6b
procymidone (seed and soil 1000g a.i/ha)	1.3 (8.36)ab	8.9b*
procymidone (seed, soil and stem 1000g a.i/ha)	1.4 (7.73)ab	8.8b*
procymidone (soil and stem 1000g a.i/ha)	1.9 (8.58)ab	9.9b*
tebuconazole (soil 700g a.i./ha)	0.2 (6.11)a	15.4a
tebuconazole (soil 500g a.i./ha)	0.8 (7.59)ab	13.7a
tebuconazole (soil 300g a.i./ha)	0.2 (6.24)a	17.0a
boscalid (soil and stem 900g a.i./ha)	0.1 (5.74)a	14.7a
boscalid (soil and stem 500g a.i./ha)	0.2 (6.13)a	15.3a
boscalid (soil and stem 300g a.i./ha)	0.0 (5.88)a	15.7a
$LSD^{0.05}$ (*P* value)	3.6 (<0.001)	3.3(<0.001)

* low yield due to poor seedling germination (fungicide toxicity) in cool/moist soil conditions

Table 5. Trial 3 Effects of fungicide treatments on white rot and yield of spring onions grown during Autumn 2004 at Cranbourne, Victoria. ANOVA analysis of both raw and transformed (angular) data was consistent therefore the raw data were analysed and presented.

Treatment	Trial 3 Cranbourne	
	% plants with white rot (m^2)	Marketable Bunches (no./m^2)
untreated control	15.1b	16.6
procymidone (soil and stem 1000g ai/ha)	20.2a	15.2
procymidone (soil,stem,stem 1000g ai/ha)	6.3bc	19.2
procymidone + azoxystrobin*	6.8bc	20.0
procymidone + triadimenol*	15.1b	17.6
procymidone + boscalid*	6.4bc	20.4
procymidone + BAS516*	7.8bc	19.0
procymidone + pyraclostrobin*	14.6b	18.6
pyraclostrobin (stem and stem 200g ai/ha)	26.3a	16.6
azoxystrobin (stem and stem 150g ai/ha)	6.4bc	21.2
triadimenol (stem and stem 100g ai/ha)	5.9bc	19.2
boscalid (stem and stem 500g ai/ha)	5.3bc	19.0
BAS516 (stem and stem)	4.6bc	20.4
tebuconazole (soil 300g ai/ha)	1.3c	21.8
boscalid (soil, stem 500g ai/ha)	0.5c	21.0
$LSD^{0.05}$ (*P* value)	10.9 (<0.001)	ns (0.327)

* procymidone after sowing followed by two applications (stem/foliar) of other treatment

Table 6. Trial 4 Effects of fungicide treatments on white rot and yield of spring onions grown during Autumn 2004 at Heatherton, Victoria. ANOVA analysis of both raw and transformed (angular) data was consistent therefore the raw data were analysed and presented.

Treatment	Trial 4 Heatherton	
	% plants with white rot (m^2)	Marketable bunches (no./m^2)
untreated control	6.1a	14.7
procymidone (soil and stem 1000g ai/ha)	1.1b	16.5
procymidone (soil,stem,stem 1000g ai/ha)	1.3b	16.3
procymidone + azoxystrobin*	0.1c	15.8
procymidone + triadimenol*	2.6b	15.6
procymidone + boscalid*	1.3b	15.7
procymidone + BAS516*	0.2c	14.8
procymidone + pyraclostrobin*	1.8b	15.5
pyraclostrobin (stem and stem 200g ai/ha)	4.4ab	15.7
azoxystrobin (stem and stem 150g ai/ha)	1.9b	15.7
triadimenol (stem and stem 100g ai/ha)	0.7c	16.3
boscalid (stem and stem 500g ai/ha)	1.5b	15.8
BAS516 (stem and stem)	1.6b	15.5
tebuconazole (soil 300g ai/ha)	0.6c	16.8
boscalid (soil, stem 500g ai/ha)	0.6c	16.0
$LSD^{0.05}$ (*P* value)	1.8 (<0.001)	ns (0.781)

* procymidone after sowing followed by two applications (stem/foliar) of other treatment

Table 7. **Commercial Trial 1 - Effects of fungicide treatments on white rot and yield of spring onions grown during Winter-Spring 2004 at Clyde, Victoria.** Statistical analysis for disease incidence is based on angular-transformed data (shown in parenthesis). Treatments were applied with a boom sprayer modified to deliver sprays in a band across furrows with seed or plant rows using 1000L water/ha.

Treatment	Commercial Trial 1	
	% plants with white rot (m^2)	No. marketable bunches (no./m^2)
untreated control	15.5 (22.7)a	19.8
tebuconazole (soil 300g ai/ha)	2.3 (8.40)b	23.6
$LSD^{0.05}$ (*P* value)	5.9 (0.002)	4.8 (0.167)

Table 8. Commercial Trial 2 - Effects of fungicide treatments on white rot and yield of spring onions grown during Summer-Autumn 2004-2005 at Cranbourne, Victoria. Statistical analysis for disease incidence is based on angular-transformed data (shown in parenthesis). Treatments were applied with a boom sprayer modified to deliver sprays in a band across furrows with seed and plant rows using 1000L water/ha..

Treatment	Commercial Trial 2	
	% plants with white rot (m^2)	No. marketable bunches (m^2)
untreated control	29.1(32.4)a	16.8a
boscalid (soil 500g ai/ha)	0.1 (0.9)b	24.5b
boscalid (soil and stem 500g ai/ha)	0.1 (0.9)b	23.3b
$LSD^{0.05}$ (*P* value)	7.6 (<0.001)	3.4 (0.003)

Table 9. Commercial trial 3 - Effects of fungicide treatments on white rot and yield of spring onions grown during Autumn-Winter 2005 at Clyde, Victoria. Statistical analysis for disease incidence is based on angular-transformed data (shown in parenthesis). Treatments were applied with a boom sprayer modified to deliver sprays in a band across furrows with seed using 1000L water/ha.

Treatment	Commercial Trial 3	
	% plants with white rot (m^2)	No. marketable bunches (no./m^2)
untreated control	14.9 (22.4)a	16.2a
boscalid (soil 500g ai/ha)	0.2 (1.60)b	21.8b
tebuconazole (soil 300g ai/ha)	3.2 (8.50)b	21.6b
$LSD^{0.05}$ (*P* value)	7.0 (<0.001)	3.6 (0.011)

Fungicide residue data

Table 10. Field trial 3 and 4. Results for Azoxystrobin 2 applications per crop (150g a.i./ha using 1000L/ha water) applied with a knapsack (soil surface or stem base sprays) on spring onions grown in sandy soils in Victoria.

Sample	Trial		replicate	mg/kg
Spring onion leaves	3 Heatherton Vic	Sown: 24/02/04	1	<0.02
Spring onion leaves		Harvested: 21/05/04	2	<0.02
Spring onion leaves			3	<0.02
Spring onion bulb			1	<0.02
Spring onion bulb			2	<0.02
Spring onion bulb			3	<0.02
Spring onion leaves	4 Cranbourne Vic	Sown: 26/02/04	1	<0.02
Spring onion leaves		Harvested: 05/05/04	2	<0.02
Spring onion leaves			3	<0.02
Spring onion bulb			1	<0.02
Spring onion bulb			2	<0.02
Spring onion bulb			3	<0.02

The residues found in the samples were less than the limit of quantification (0.02mg azoxystrobin/kg). Analytical method used was Analytical Procedure 267A.00.
Laboratory: Novartis Animal Health Australasia Pty. Ltd.

Table 11. Field trials 3 and 4. Results for boscalid 2 applications per crop (500 g a.i./ha using 1000L/ha water) applied with a knapsack (soil surface or stem base sprays) on spring onions grown in sandy soils in Victoria.

Sample	Trial		replicate	mg/kg
Spring onion leaves	3 Heatherton Vic	Sown: 24/02/04	Pooled	0.05
Spring onion bulbs		Harvested: 21/05/04	Pooled	0.10
Untreated leaves			Pooled	<0.05
Untreated bulb				<0.05
Spring onion leaves	4 Cranbourne Vic	Sown: 26/02/04	Pooled	0.06
Spring onion bulbs		Harvested: 05/05/04	Pooled	0.06
Soil			Pooled	0.82
Untreated			Pooled	<0.05

Samples collected from 3 replicated plots (1 x 2.5 m) were pooled and 1 sub-sample analysed from each treatment for each component of plant.

LOQ = 0.05 mg/kg as rcvd. Analytical method used solvent extraction, cleanup, analysis by LCMS.
Laboratory: Hill Laboratories Limited, Hamilton New Zealand.

Table 12. Field trial 1, 3, 4 and 5. Results for tebuconazole 1 single soil surface spray per crop applied after sowing (300 g a.i./ha using 1000L/ha water) with a knapsack (trials 1, 3 and 4) on spring onions grown in sandy soils in Victoria.

Sample	Trials		replicate	mg/kg
Spring onion leaves	1 Cranbourne Vic	Sown: 25/03/03	1	0.20
Spring onion leaves		Harvested: 30/06/03	2	0.06
Spring onion leaves			3	0.07
Spring onion bulb			1	0.16
Spring onion bulb			2	0.11
Spring onion bulb			3	0.06
Soil			1	1.4
Soil			2	0.37
Soil			3	0.56
Spring onion leaves	3 Heatherton Vic	Sown: 24/02/04	1	<0.01
Spring onion leaves		Harvested: 21/05/04	2	<0.01
Spring onion leaves			3	<0.01
Spring onion bulb			1	<0.01
Spring onion bulb			2	<0.01
Spring onion bulb			3	<0.01
Spring onion leaves	4 Cranbourne Vic	Sown: 26/02/04	1	<0.01
Spring onion leaves		Harvested: 05/05/04	2	<0.01
Spring onion leaves			3	<0.01
Spring onion bulb			1	<0.01
Spring onion bulb			2	<0.01
Spring onion bulb			3	<0.01
Soil			1	0.022
Soil			2	0.010
Soil			3	0.038
Soil	1 commercial Vic	Sown: 24/6/04	Pooled	0.350
Soil		Harvested: 29/10/04	Pooled	<0.100
Spring onion leaves			Pooled	<0.100
Spring onion leaves			Pooled	<0.100
Spring onion bulb			Pooled	<0.100
Spring onion bulb			Pooled	<0.100

Trial 1: analysis conducted by SCL Vic, method OSJP17059

Trial 3, 4 and 5: analysis conducted by BayerCropScience BCRL, QUT. For trial 3 and 4 LOQ = 0.010 mg/kg and for trial 5 LOQ = 0.100 mg/kg.

Trial 5 was a replicated trial and 1 soil surface spray was applied after sowing with grower's boom spray using 1000L/ha of water. Samples collected from 3 plots (1 x 5 m) were pooled and from these 2 sub-samples used for analysis.

Table 13. Field trial 1, 3 and 4. Results for procymidone 2 and 3 applications per crop (2L/ha or 1000g a.i./ha using 1000L/ha water) applied with a knapsack as soil surface and stem base sprays on spring onions grown in sandy soils in Victoria.

Sample	Trial		replicate	mg/kg
Leaves x2	1 Cranbourne Vic	Sown: 25/03/03	1	<0.05
Leaves x2		Harvested: 30/06/03	2	<0.05
Leaves x2			3	<0.05
Leaves x3			1	<0.05
Leaves x3			2	0.27
Leaves x3			3	0.08
Leaves untreated			1	<0.05
Leaves untreated			2	<0.05
Leaves untreated			3	<0.05
Bulb x2			1	<0.05
Bulb x2			2	<0.05
Bulb x2			3	<0.05
Bulb x3			1	<0.05
Bulb x3			2	0.41
Bulb x3			3	0.12
Bulb untreated			1	<0.05
Bulb untreated			2	<0.05
Bulb untreated			3	<0.05
Soil x2			1	<0.1
Soil x2			2	<0.1
Soil x2			3	0.38
Soil x3			1	0.35
Soil x3			2	3.40
Soil x3			3	0.90
Soil untreated			1	<0.1
Soil untreated			2	<0.1
Soil untreated			3	<0.1
Whole plant x2	3 Heatherton Vic	Sown: 24/02/04	1	<0.05
Whole plant x2		Harvested: 21/05/04	2	<0.05
Whole plant x2			3	<0.05
Whole plant x3			1	0.13
Whole plant x3			2	0.05
Whole plant x3			3	0.08
Whole plant x2	4 Cranbourne Vic	Sown: 26/02/04	1	<0.05
Whole plant x2		Harvested: 05/05/04	2	<0.05
Whole plant x2			3	0.08
Whole plant x3			1	0.90
Whole plant x3			2	0.54
Whole plant x3			3	0.50

Analysis for all trials conducted by SCL Vic, method OSJP17059. LOD = 0.1 (soil) and 0.05 (plant material).

Comparison of fungicide and biological treatments for control of white rot on spring onions

Summary

Seven field trials were carried out over three seasons to compare the efficacies of fungicides treatments (procymidone and boscalid) and two commercial formulations of the biological control agent *Trichoderma atroviride* C52 (Trichople Ali 52™ and Trichoflow Ali 52™) for white rot control. Two of the seven trials evaluated five other biological products, prepared with a range of beneficial soil microbes, for their capacity to protect the roots of plants against white rot infection. The trials were conducted within commercial crops of spring onions grown for 10-14 weeks in sandy loam/clay soils naturally infested with sclerotia of *S. cepivorum* in vegetable farms in Victoria.

The results from field trials showed that boscalid (Filan™) and procymidone (Sumisclex™) treatments were more effective than biocontrol treatments in controlling white rot and increasing yields of spring onions at high disease field sites. At two field sites, Filan™ treatments reduced the incidence of disease by 90-98% of untreated controls (28-29% diseased plants). *Trichoderma* C52 applied on formulated prills in the furrow with seed at sowing (Trichopel Ali 52™, 50kg prills/ha) gave a reduction in disease in the order of 50% of that of untreated plants at one of the two high-disease field sites. At both field sites, the biocontrol applied at sowing combined with one post-planting spray of Filan™ gave reductions in disease in the order of 58-75% of that of untreated controls. At a low-disease field site (11% plants diseased), *Trichoderma* treatments were as effective as two procymidone sprays in controlling disease. Overall, results showed that *Trichoderma* C52 levels in sandy soils were below the required levels ($<10^5$ cfu g of soil) for effective biocontrol. Despite that, the results indicated that *Trichoderma* C52 was able to provide some degree of protection against early season infection when its levels were highest in the region of roots.

Trials showed that applying prills with spores of *Trichoderma* (Trichopel Ali 52™) below the seed at sowing was a more practical and effective method of delivering biocontrol treatments in the furrow than drenching the furrow or plants rows with solutions of biological treatments.

Disease levels were too low in the two trials that evaluate other biological treatments to determine their usefulness for white rot control.

Introduction

The development of alternative treatments to fungicides is needed for the integrated control of onion white rot on spring onions to ensure the efficacies of the few effective fungicides available for control of this disease are not lost through overuse. The use of fungal and bacterial antagonists to *S. cepivorum* offers the possibility for disease control with less chemical inputs and provides an environmetally sound control measure. *Trichoderma* species are among the soil microorganisms reported to provide biocontrol of *S. cepivorum* (Abd-EL-Moity and Shatla 1981, Kay and Stewart 1994). An isolate of *Bacillus subtilis* (Ehremberg) Cohn was also found to be effective inhibiting mycelial growth of *S. cepivorum* via antibiosis (Reddy et al 1992).

The biocontrol program at Lincoln University has tested promising isolates antagonistic to *S. cepivorum* in glasshouse and field trials (Kay and Stewart 1994; McLean and Stewart 2000). An isolate of *Trichoderma atroviride* (C52) provided the best control and subsequent formulation trials determined that a pellet formulation was optimum for fungal proliferation in soil (McLean *et al.* 2005). To register the pellet and a wettable powder formulation of *T. atroviride* in Australia, extensive field trials were required. In New Zealand, Trichopel Ali 52 provided control equivalent to the chemicals under low and moderate (<40%) disease pressure, but under high disease conditions, efficacy was lower (McLean *et al.* 2002). This result highlighted that additional research was required

to integrate Trichopel Ali 52 with existing disease management strategies for successful control of onion white rot.

A series of field trials were carried out to

- evaluate the effectiveness of the biological control agent *T. atroviride* C52, and other beneficial soil microbes, for protecting the roots and base of spring onion plants against white rot infection in sandy soils,
- develop application methods for two commercial formulations of *T. atroviride* C52 in spring onion cropping systems, and
- evaluate the usefulness of combining *T. atroviride* C52 with post-planting applications of fungicides for the integrated control of white rot on spring onions grown.

Materials and methods

Sites

The trials were carried out on sandy soils (pH range 6.5-7.5) on fields naturally infested with sclerotia of *S. cepivorum* in commercial vegetable farms at Cranbourne and Heatherton, south and south-east of Melbourne. The field trials were set up within commercial crops of spring onions.

Biological and fungicide treatments

Biological products and fungicides treatments and application rates used in the different field trials are listed in Table 1. Two commercial formulations of the biological control agent *T. atroviride* isolate C52 were tested in the trials. Spores of *T. atroviride* C52 were formulated in prills (Trichopel Ali 52™) and in wettable powder preparations (Trichoflow Ali 52™). Prills and wettable powder preparations of *T. atroviride* C52 were tested for levels of viable spores of *Trichoderma* prior to use in all trials. *Trichoderma* levels in prills ranged from 1 x 10^6 to 5 x 10^6 cfu gram of prills and in powder formulation from 1 x 10^8 to 2 x 10^8. In two field trials, the biological control agent *T. atroviride* C52 was compared to five other biological products prepared with a range beneficial soil microbes (Table 1). These biological treatments were prepared as wettable powder formulations therefore were applied as a drench to the open furrow before sowing and across plant rows. These biological products were also tested and were within the range specified on the labels. Details of the trial sites and dates of sowing and application of treatments are included in Table 2. Biological and fungicide treatments were applied when soil temperatures were favourable for disease development (see section 1).

Experimental design

Experimental designs were set up as complete randomised blocks with four to eight replications per treatment. Each replicate consisted of a single bed/plot (1 m wide x 5 m long) of spring onions separated by 0.5 m of bare soil between plots. Beds were 1.5 m centre to centre. Each plot contained 3 rows of spring onions c. 1 cm apart. Spring onion seed was planted using StanHay and GoldAcre precision seeders using approximately 50 k/ha of seed. Seed was planted on raised beds (0.3-0.4 m height) with plant densities ranging from 100-120 plants per linear meter. Overhead irrigation was applied when required. Slow release fertiliser (NPK) was applied when seedlings were 10cm height and later as required. Trials in Cranbourne and Clyde had two applications of composted chicken manure (17-20 tonnes per hectare fresh weight), the first spread onto beds 2-3 weeks after sowing and the second approximately 4 weeks later. Trials in Heatherton had also two applications of composted chicken manure, the first applied mixed into soils 2-3 weeks prior to sowing (20 tonnes per hectare fresh weight) and the second spread onto beds (10 tonnes per hectare fresh weight) approximately 4-3 weeks before harvest. The compost was applied using farm spreaders.

Formulated prills with spores of *T. atroviride* C52 were placed at the base of the planting furrow below the seed (1-2 cm) with StanHay and GoldAcre precision seeders. Post-planting treatments with *Trichoderma* C52 were applied as stem-base sprays across plant rows with a handheld sprayer (5L) using the wettable powder formulation of *Trichoderma* C52 and 300-400L of water/ha. These treatments were applied at approximately 4 and 8 weeks after sowing. Other biological treatments were applied as a drench to the open furrow before sowing and as stem base sprays at 4 weeks after sowing as previously described.

Fungicide treatments were applied as a soil surface sprays after sowing and as stem base/foliar sprays at approximately 4 weeks after sowing. The sprays were applied in a band across the furrow with seed and plant rows using a knapsack at constant pressure and output using a timed application to deliver the appropriate amount of chemical using 1000L of water per ha followed by irrigation with overhead sprinklers. The knapsack was fitted with three cone jet nozzles to treat all three rows in one pass. The soil surface and stem-base sprays were applied in a narrow band 10-15 wide across each drill row furrow with seed or plant rows.

Measurements

Soil temperatures were measured at hourly intervals with Tinytalk temperature loggers (Gemini data loggers) at depths of 10 cm and 20 cm in one plot in the middle of the trial. Five soil samples were selected arbitrarily from some of the plots to measure soil pH.

Soil samples were collected from the furrow with roots of young plants (4-8 cm depth) at different intervals after sowing and then *Trichoderma* colony levels determined twice per replicate sample (n=4/treatment) using a standard dilution plating method and selective medium.

Emergence counts were made in all plots 2-3 weeks after sowing. At fortnightly intervals thereafter, all plots were inspected and white rot infected plants counted in a square metre/plot without removing infected plants. At harvest, the number of plants infected with white rot was counted in a square meter in each plot and then spring onion yields (number of marketable bunches, fresh weights, bunch length) measured approximately 12-14 weeks after sowing.

Statistical analysis

Analysis of variance (ANOVA) was used to analyse data for all trials using Genstat (Genstat 6). Data were transformed before analysis when required to stabilise the variance. The plants infected with white rot were expressed as a percentage of plants with white rot. The appropriateness of an ANOVA for the data was checked by visual inspection of residual plots. Where significant main or interaction effects were found then the mean values for treatments in each experiment were compared by Fisher's protected LSD tests ($P \leq 0.05$).

Results

Trial 1 Clyde – Autumn 2003

Trichoderma species were not detected in soil of untreated plots (Table 3). Three weeks after sowing, the mean colony forming units of *T. atroviride* C52 in sandy soil of plots treated with Trichopel Ali 52 at sowing ranged from 5.4 x 10^4 to 7.4 x 10^4 cfu per g of soil. Seven weeks after sowing, *T. atroviride* C52 (2.1 x 10^2) was recovered only from plots treated with Trichopel Ali 52 (prills) at sowing and Trichoflow Ali (drench) applied to plant rows 4-weeks after sowing.

Plants in all plots emerged relatively evenly. There were no obvious symptoms of phytotoxicity in plots treated with boscalid and procymidone treatments. Treatment means for percentage of plants with white rot and yields (number marketable bunches and bunch lengths) are presented in Table 7. Two applications with boscalid, applied after sowing as soil surface spray and repeated 4-weeks later as stem base/foliar spray, were very effective in controlling white rot. These treatment significantly reduced the number of plants infected with white rot from 28.8% to 2.7% and increased the number of marketable bunches from 10.8 to 19.0 bunches/square metre when compared to the untreated control. The best biocontrol treatment was that involving an application of Trichopel Ali 52, applied at sowing at the rate of 50kg of prills per hectare in combination with a post-planting spray of boscalid applied approximately 4-weeks after sowing. This treatment resulted in a significant reduction in disease in the order of 60% of that of the untreated control.
There were no significant differences in yields (number of bunches and size of bunches) among plots treated with all biocontrol treatments and untreated.

Trials 2 and 3 Cranbourne and Clyde – Autumn and Spring 2003

The population density of the biocontrol agent *T. atroviride* C52 and other beneficial microbes tested in these two trial was not measured in soil, only in products prior to application.

Plants in all plots emerged also relatively evenly in both trials, except for plots treated with the fungicide procymidone in early Spring. There were obvious symptoms of phytotoxicity in plots treated with procymidone which reduced the number of emerged seedlings (data not shown). Treatment means for percentage of plants infected with white rot and yields for both trials are presented in Table 8. In the Autumn trial, two appropriately applied and timed applications of procymidone, the first applied after sowing (soil surface spray) and the second approximately 4-weeks after sowing (stem base/foliar spray), were very effective in controlling white rot in a low disease site in Autumn. This treatment significantly reduced the number of plants infected with white rot from 16.2% to 0.1% when compared to the untreated control. All biological treatments gave significant reductions in disease in the order of 35-72% of that of the untreated control. There were no significant differences in yields (number of bunches and size of bunches) between all treatments.

In the Spring trial, disease levels were too low to compare the same treatments evaluated in the previous Autumn. The number of marketable bunches harvested from plots treated with procymidone were lowest in plots treated with procymidone treatments due to phytotoxicity.

Trial 4 Heatherton – Spring 2003

Trichoderma species were not detected in soil from untreated plots (Table 4). Three weeks after sowing, the mean colony forming units of *T. atroviride* C52 in soil from plots treated with all rates of Trichopel Ali 52 at sowing ranged from 8.1 x 10^3 to 3.0 x 10^4 cfu per g of soil. In soils treated with two applications of *T. atroviride* C52 (Trichopel Ali 52 plus Trichoflow Ali 52), *Trichoderma* levels ranged from 5.7 x 10^3 to 2.8 x 10^4.

Although the field used for this trial had a history of high disease, white rot did not develop on any of the plots probably due to dry conditions encountered during Spring 2003 (Table 9). There were no significant differences in yields (number of bunches and weight and size of bunches) between all treatments.

Trial 5 Heatherton – Autumn 2004

Trichoderma species were not detected in soils of untreated plots (Table 5). The mean number of *T. atroviride* C52 cfu per g of soil in plots treated with three rates of prills of Trichopel Ali 52 applied at sowing ranged from 5.5 x 10^4 to 6.6 x 10^4 and 2.1 x 10^3 to 1.7 x 10^4 at 3 and 6 weeks after sowing, respectively. There were no significant differences in *Trichoderma* levels between the three rates of prills tested at 3 and 6 weeks after sowing. Nine weeks after sowing, *Trichoderma* was detected only in soil from plots treated with 50 and 70 kg/ha of prills at sowing.

Treatment means for percentage of plants with white rot and yields (number marketable bunches and bunch weights) are presented in Table 10. The best treatment was the fungicide procymidone applied as soil surface spray after sowing and as stem base/foliar spray approximately 4-weeks later. This treatment significantly reduced the number of plants infected with white rot from 11.4% to 2.2% when compared to the untreated control. Three treatments involving applications of three rates of prills of Trichopel Ali 52™ (30, 50 and 70 kg/ha) at sowing supplemented with one spray of procymidone provided significant disease reductions similar to that provided by two procymidone sprays. These combined treatments gave disease reductions in the order of 78% (30kg prills/ha), 83% (50% prills/ha) and 92% (70% prills/ha) of that of the untreated control.

There were no significant differences in yields (number of bunches and weight of bunches) between all treatments.

Trial 6 Heatherton – Autumn 2005

Trichoderma (species not identified) were detected in soil samples collected from untreated plots at the three times of soil sampling (Table 6). These levels were significantly lower than those levels measured in soil from plots treated with 50 kg/ha of prills of Trichopel Ali 5. The mean number of *T. atroviride* C52 cfu per g of soil in plots treated with Trichopel Ali 52 ranged from 1.1 x 10^3 to 6.6 x 10^3 at all times of testing.

Plants in all plots emerged relatively evenly. There were no obvious symptoms of phytotoxicity in plots treated with boscalid treatments. Treatment means for percentage of plants with white rot and yield (number marketable bunches) are presented in Table 11. The fungicide boscalid was very effective in controlling white rot at this site. The best treatments were those involving boscalid applied as a soil surface spray only after sowing and boscalid applied after sowing followed by a stem base/foliar spray approximately 4-weeks later. These two treatments significantly reduced the number of plants infected with white rot from 29.0% to 1.4% (one spray) and to 0.1% (two sprays) when compared to the untreated control. The biocontrol treatment applied at sowing at the rate of 50 kg of prill (Trichopel Ali 52™) per ha gave a significant reduction in disease in the order of 50% of that of the untreated control. The biocontrol applied at sowing combined with one spray of boscalid applied at approximately 4-weeks after sowing gave a disease reduction similar to that provided by one spray of boscalid applied also at approximately 4-week after sowing.
Yields (number of bunches) of spring onions from plots treated with boscalid were significantly higher (by 4-6 bunches/square metre) than those of the untreated control. Yields from plots treated with biocontrol treatments alone or combined with a post-planting spray of boscalid were also significantly higher (by 3 bunches) than those of the untreated control.

Trial 7 Heatherton – Autumn 2005

The population density of the biocontrol agent *T. atroviride* C52 in soil was not measured in this trial. Plants in all plots emerged also relatively evenly. Treatment means for percentage of plants infected with white rot and yield (number of marketable bunches) are presented in Table 12. Disease levels were too low at this site to compare treatments. There were no significant differences in yields (number of bunches) between all treatments.

Discussion

In all trials, boscalid and procymidone treatments were more effective than biocontrol treatments in controlling white rot and increasing yields of spring onions in sandy soils. In general, *Trichoderma* levels measured in sandy soils treated with two formulations of *T. atroviride* C52 (Trichopel Ali 52 and Trichoflow Ali 52) were probably not adequate for effective biocontrol of white rot on spring onions throughout the entire growing season (12-14 weeks). Despite that, *T. atroviride* C52 treatments alone significantly reduced disease by 80% at one low disease site (11% diseased plants) and by 24% and 50% at two high disease sites (28% and 29% diseased plants). These results indicated that *T. atroviride* C52 was able to give some degree of protection against infection in the region of roots of plants early in the season when its levels were highest in soil. Using an integrated approach combining early season applications of *T. atroviride* C52 (Trichopel Ali 52) with a post-planting spray of fungicide reduced disease by 78% and 93% at two low disease sites (11.4% and 16% diseased plants) and by 56% and 75% at two high disease sites (28% and 29% diseased plants). In New Zealand, Trichopel Ali 52 provided control equivalent to the chemicals under low and moderate (<40%) disease pressure, but under high disease conditions, efficacy was lower. Our results and those obtained in New Zealand with onions suggest that biocontrol applications should be integrated with other existing disease management strategies to obtain more effective control of this onion white rot.

The limited number of *Trichoderma* measurements taken from sandy soils over time in the different trials reported here are insufficient to make conclusions about the rhizosphere competence of *T. atroviride* C52. Nevertheless, the limited data obtained suggest that the levels of 'introduced' *Trichoderm*a into soil would be greater during the first 6 weeks after application and after this period *Trichoderma* levels would decline. Pot and field studies were conducted to evaluate the effect of different formulations on the survival, proliferation and rhizosphere competence of *Trichoderma atroviride* C52 in soil (McLean 2001, McLean et al 2005). In one of these studies, a pellet formulation maintained the concentration of *T. atroviride* C52 at 10^5 cfu per g soil for about 14-weeks, whereas soil-substrate and seed-coating formulations gave concentrations of 10^4 and 10^1 cfu per g soil, respectively (McLean et al 2005). *T. atroviride* C52 has been recovered from the distal parts of the roots, indicating that it was able to colonise the rhizosphere to at least 15cm depth in the soil (McLean et al 2005). A more detailed study with more measurements at different depths is required to determine the rhizosphere competence of *T. atroviride* C52 in sandy soils under standard cropping practices.

In our trials, nitrogenous materials were applied at least 3 weeks prior to sowing (composted chicken manure) and 2-3 weeks after sowing (composted chicken manure and fertilisers). Therefore spores of *Trichoderm*a had sufficient time to germinate and establish in sandy soil before nitrogen was released into soil. However, it is unknown whether *Trichoderma* spores and germinated spores (propagules) are being washed away in the sandy soils under regular irrigation. This would explain in part why there were no substantial differences in *Trichoderma* levels between soils unamended and amended (in-furrow application) with pellets containing humic acids or between soils treated with different rates of prills or with a single and dual applications of *Trichoderm*a C52. Results from a pot trial showed that humic acids have the potential to increase the growth of *Trichoderma* in sandy soil (see chapter 3). Formulation trials have determined that a pellet formulation of *Trichoderma atroviride* (C52) has been shown to be optimum for proliferation of this biocontrol and control of white rot in silt loam soils (McLean *et al.* 2005).

There were also no significant differences in yields (fresh weights and bunch length) between plots untreated and treated with the different biological treatments. Sandy soils are heavily fertilised and amended and these practices are probably hindering any possible gains arising from using *Trichoderma* in soils. The lack of increases in *Trichoderma* levels in soil, disease control and yields in plots treated with high levels of spores of *T. atroviride* may be due to the properties of sandy soils and effect of some crop management practices such as irrigation on spore retention and *Trichoderma* proliferation in sandy soils. These aspects of the research required further investigation.

Disease levels were too low in two trials conducted to determine the effectiveness of other biological products formulated with beneficial soil microbes for protecting the roots of plants against white rot infection in sandy soils. Nevertheless, results from these and other trials showed that applying prills with spores of *Trichoderma* below the seed at sowing is a more effective method of delivering biocontrol treatments into soil than drenching the furrow with seed or plants with liquid solutions.

In conclusion, appropriately applied and timed sprays of the new fungicide boscalid were more effective than biocontrol treatments in controlling white rot and increasing yields of spring onions in sandy soils. However, the use of an integrated approach combining applications of Trichopel Ali 52 at sowing with a post planting spray of boscalid also showed promise for providing effective control of white rot on spring onions at low disease field sites. More research is required to find means of modifying the soil environment to allow spores and propagules of *Trichoderma* to colonise sandy soils at higher levels so that the levels of protection against disease in the root zone are increased especially at moderate and high disease sites.

References

Abd-El-Moity TH, Shatla MN (1981). Biological control of white rot disease of onion (*Sclerotium cepivorum*) by *Trichoderma harzianum*. Phytopathologische Zeitschrift 100, 29-35.

Kay, S.J. and Stewart, A. (1994). Evaluation of fungal antagonists for control of onion white rot in soil box trials. Plant Pathology, 43:371-377.

McLean, K.L. and Stewart, A. (2000). Application strategies for control of onion white rot. New Zealand Journal of Crop and Horticultural Science, 28:115-122.

McLean, K.L., Hunt, J. and Stewart, A. (2001). Compatibility of the biocontrol agent *Trichoderma harzianum* (C52) with fungicides. New Zealand Plant Protection, 54:84-88.

McLean, K.L., Swaminathan, J., Hunt, J.S. and Stewart, A. (2002). Biological control of onion white rot in New Zealand. In, "Proceedings Seventh International Workshop on *Allium* White Rot, Coalinga, California, USA", (ed. M. R. MacDonald), 4-8th June 2002, pp. in press.

McLean, K.L., Swaminathan, J., Frampton, C.M., Hunt, J.S., Ridgway, H.J. and Stewart, A. (2005). Effect of formulation on the rhizosphere competence and biocontrol ability of *Trichoderma atroviride* C52. Plant Pathology, 54:212-218.

Reddy MS, Rahe JE, Levesque CA (1992). Influence on onion seed bacterization on germination and mycosphere microflora of *Sclerotium cepivorum* sclerotia. Canadian Journal of Microbiology 38, 1135-1143.

Stewart, A., Tyson, J.L., Fullerton, R.A., and Elliott, G.S. (2002). Integrated Disease Management of Onion White Rot in New Zealand. Proceedings of the 2nd Australasian Soilborne Diseases Symposium. p105-106.

Table 1. Description of biological products and fungicides and application rates used in the different field trials

Treatment	Active ingredient	Rate	Method of application
Companion™	*Bacillus subtilis* GB03	0.5 L/ha	drench furrow with seed/plant rows
Trichoflow Ali52	*Trichoderma atroviride* C52	0.5, 1.0 kg/ha	drench furrow with seed/plant rows
Trichopel Ali52	*Trichoderma atroviride* C52	30, 50 ,70 kg/ha	prills with spores at sowing with seed
TrichoShield™	*Trichoderma* spp and bacteria	1.0 kg/ha	drench furrow with seed/plant rows
Trichosoil™	*Trichoderma* spp	1.2 kg/ha	drench furrow with seed/plant rows
Tri-D25™	*Trichoderma* spp	1.0 kg/ha	drench furrow with seed/plant rows
PSP-Micro™	*Trichoderma* spp and bacteria	0.8 kg/ha	drench furrow with seed/plant rows
Sumixclex™	procymidone	1000g a.i./ha	1000L water/ha, banded across furrow with seed or plant rows
Filan™	boscalid	500g a.i./ha	1000L water/ha, banded across furrow with seed or plant rows

Table 2. Trials Summaries

Activity	Trial 1	Trial 2
Year	Autumn-Winter 2003	Autumn-Winter 2003
location	Clyde	Cranbourne
Soil type	Sand loam	Sand loam
Spring onion variety	Paragon	Paragon
Replicates	6	4
Fungicide application dates	10/03/03, 03/04/03	18/04/03, 23/05/03
Sowing date(s)	06/03/03	17/04/03
Harvest date(s)	31/07/03	13/08/03

Activity	Trial 3	Trial 4
Year	Spring 2003	Spring-Summer 2003
location	Clyde	Heatherton
Soil type	Sand loam	Sandy clay loam
Spring onion variety	Paragon	Broad Leaf
Replicates	6	8
Fungicide application dates	02/09/03, 25/09/03	24/09/03, 15/10/03
Sowing date(s)	29/08/03	11/09/03
Harvest date(s)	26/11/03	11/12/03

Activity	Trial 5	Trial 6 and 7
Year	Autumn 2004	Autumn 2005
location	Heatherton	Heatherton
Soil type	Sand clay loam	Sand loam
Spring onion variety	Broad Leaf	Paragon
Replicates	6	4 and 6
Fungicide application dates	27/02/04, 31/03/04	19/02/05, 31/03/05
Sowing date(s)	26/02/04	18/02/05
Harvest date(s)	05/05/04	16/05/05

Table 3. Mean number of *Trichoderma* colony forming units per gram of soil at 3 and 7 weeks after application in a sandy loam soil in a trial conducted during Autumn 2003 at Cranbourne, Victoria. Statistical analysis was based on log_{nat}-transformed data.

Treatment	*Trichoderma* cfu/g soil	
	3 weeks	7 weeks
untreated control	0.0a	0.0a
T. atroviride C52 50kg/ha (prills)	7.5×10^4b	0.0a
T. atroviride C52 50kg/ha prills+Trichoflow Ali C52	5.4×10^4b	2.1×10^2b

Mean values followed by the same letter do not differ significantly according to LSD value (P=0.05).

Table 4. Mean number of *Trichoderma* colony forming units per gram of soil at 3 weeks after application in a sandy loam soil in a trial conducted during Spring-Summer 2003 at Heatherton, Victoria. Statistical analysis was based on log_{nat}-transformed data.

Treatment	*Trichoderma* cfu/g soil
untreated control	0.0a
T. atroviride C52 30kg/ha prill	3.0×10^4c
T. atroviride C52 30kg/ha prill + Trichoflow Ali C52	5.7×10^3b
T. atroviride C52 50kg/ha prill	8.1×10^3b
T. atroviride C52 50kg/ha prill + Trichoflow Ali C52	1.6×10^4bc
T. atroviride C52 70kg/ha prill	8.2×10^3b
T. atroviride C52 70kg/ha prill + Trichoflow Ali C52	2.8×10^4c

Mean values followed by the same letter do not differ significantly according to LSD value (P=0.05).

Table 5. Mean number of *Trichoderma* colony forming units per gram of soil at 3, 6 and 9 weeks after application in a sandy loam soil in a trial conducted during Autumn 2004 at Heatherton, Victoria. Statistical analysis was based on log_{nat}-transformed data.

Treatment	*Trichoderma* cfu/g soil		
	weeks after planting		
	3	6	9
untreated control	0.0a	0.0a	0.0a
T. atroviride C52 30kg/ha prill	5.5×10^4b	2.1×10^3b	0.0a
T. atroviride C52 50kg/ha prill	5.8×10^4b	1.7×10^4b	7.1×10^3b
T. atroviride C52 70kg/ha prill	6.6×10^4b	4.6×10^3b	2.1×10^3b

Mean values followed by the same letter do not differ significantly according to LSD value (P=0.05).

Table 6. Mean number of *Trichoderma* colony forming units per gram of soil at 2, 4 and 6 weeks after application in a sandy loam soil in a trial conducted during Autumn 2005. Statistical analysis for week 4 and 6 data was based on log-transformed data.

Treatment	*Trichoderma* cfu/g soil		
	weeks after planting		
	2	4	6
untreated control	1.3×10^2a	1.6×10^2a	1.6×10^2a
T. atroviride C52 50kg/ha	1.1×10^3b	2.9×10^3b	1.2×10^3b
T. atroviride C52 50kg/ha plus Agrolig™	1.9×10^3c	6.6×10^3b	1.2×10^3b

Mean values followed by the same letter do not differ significantly according to LSD value (P=0.05).

Table 7. Trial 1 - Effect of *T. atroviride* C52 treatments alone and combined with fungicide on white rot and yields of spring onions grown during Autumn 2003 at Cranbourne, Victoria. Statistical analysis for disease incidence was based on square root transformed data (in parenthesis).

Treatment	% plants with white rot	Marketable bunches (no./m^2)	Bunch length (cm)
untreated control	28.8 (5.2)a	10.8a	57.3
T. C52 prills (sowing)	22.0 (4.2)ab	11.4a	60.9
T. C52 prills (sowing + drench 4 weeks after sowing)	21.5 (4.7)ab	13.6a	59.3
procymidone (after sowing + 4 weeks after sowing)	13.2 (3.0)b	15.0a	58.7
T. C52 prills (sowing + boscalid 4 weeks after sowing)	11.7 (3.2)b	14.6a	58.8
boscalid (after sowing + 4 weeks after sowing)	2.7 (1.0)c	19.0b	60.3
LSD$^{0.05}$ (*P* value)	1.9 (0.019)	4.5 (0.028)	ns (0.606)

Procymidone applied at 1000g a.i./ha and boscalid at 500g a.i./ha using 1000L water/ha

Table 8. Trials 2 and 3 - Effect of various biocontrol treatments on white rot and yields of spring onions grown in a loamy sand in a trial conducted during Autumn and Spring 2003 at Cranbourne, Victoria. ANOVA results for disease incidence were consistent for row and transformed data therefore the raw data were analysed and presented.

	Autumn trial			Spring trial	
Treatment	% plants with white rot	Marketable bunches (no./m^2)	Length of bunches (cm)	% plants with white rot	Marketable bunches (no./m^2)
Companion™	16.2a	17.8	57.2	1.2	14.4ab
untreated control	10.2b	20.0	57.3	1.4	19.0b
T. atroviride C52	9.3b	19.8	58.8	0.4	15.8ab
TrichoShield™	7.1b	23.2	59.1	0.1	18.2b
Trichosoil™	6.2b	18.5	59.8	0.2	16.2ab
Tri-D25™	4.6bc	21.2	58.7	0.4	21.2b
PSP-Micro™	4.4bc	22.8	54.2	0.5	18.8b
Procymidone*	0.1c	21.3	57.8	0.0	12.6a
LSD$^{0.05}$ (*P* value)	5.8 (<0.001)	ns (0.142)	ns (0.859)	0.89(0.02)	5.16 (0.043)

Procymidone was phytotoxic applied in cool weather early Spring, it reduced seedling emergence.

Table 9. Trial 4 - Effect of various biocontrol treatments on yields of spring onions grown on a sandy loam soil during Spring-Summer 2004 at Heatherton, Victoria.

Treatment	Marketable bunches (no./m^2)	Weight of bunches (kg)	Length of bunches (cm)
untreated control	10.5	2.5	65.9
T. C52 prill sowing 30kg/ha	10.0	2.4	66.2
T. C52 prill sowing 30kg/ha + drench TC52	9.0	2.1	63.3
T. C52 prill sowing 50kg/ha	8.0	1.8	62.2
T. C52 prill sowing 50kg/ha + drench TC52	9.0	2.1	65.6
T. C52 prill sowing 70kg/ha	7.8	1.9	67.9
T. C52 prill sowing 70kg/ha + drench TC52	7.9	1.8	62.3
procymidone (soil and stem 1000g a.i./ha)	10.2	2.3	63.9
LSD$^{0.05}$ (*P* value)	ns (0.186)	ns (0.249)	ns (0.372)

Table 10. Trial 5 - Effect of biocontrol and fungicide treatments on white rot and yields of spring onions grown in sandy soils in a trial during Autumn 2004 at Heatherton, Victoria. Statistical analysis for disease incidence was based on angular-transformed data (in parenthesis).

Treatment	% plants with white rot	Marketable bunches (no./m^2)	Weight of bunches (kg)
untreated control	11.4 (16.2)a	14.7	62.5
T C52 prill sowing 30kg/ha	6.9 (13.5)a	13.3	62.5
T C52 prill sowing 30kg/ha+drench T C52	2.2 (6.53)b	16.8	63.8
T C52 prill sowing 30kg/ha+procymidone (stem base)	2.4 (6.59)b	16.7	63.8
T C52 prill sowing 50kg/ha	8.3 (15.1)a	13.7	62.8
T C52 prill sowing 50kg/ha+drench C52	6.9 (12.5)a	15.2	62.8
T C52 prill sowing 50kg/ha+procymidone (stem base)	1.9 (6.24)b	16.2	63.2
T C52 prill sowing 70kg/ha	4.9 (10.2)a	14.7	62.8
T C52 prill sowing 70kg/ha+drench C52	3.3 (7.87)a	15.3	62.3
T C52 prill sowing 70kg/ha+procymidone (stem base)	0.8 (2.77)b	15.8	63.0
procymidone early (soil surface 1000g a.i./ha)	10.4 (14.9)a	14.8	63.7
procymidone late (stem base/foliar 1000g a.i./ha)	7.8 (13.5)a	14.3	64.5
procymidone full program (soil surface and stem base)	2.2 (7.11)b	16.3	63.3
LSD$^{0.05}$ (*P* value)	9.4 (0.034)	ns (0.164)	ns (0.458)

Table 11. Trial 6 - Effect of biocontrol treatments alone and integrated with fungicide on white rot and yield of spring onions grown in a sandy loam soil in a trial conducted during Autumn 2005 at Heatherton, Victoria. Statistical analysis for disease incidence was based on angular-transformed data (in parenthesis).

Treatment	%diseased plants	No. bunches
untreated control	29.0 (32.6)a	15.3a
T C52 prill 50kg/ha (at sowing)	14.4 (22.7)b	18.0b
boscalid 500g a.i./ha (late stem base)	8.1 (16.7)b	17.8b
C52 prill 50kg/ha+boscalid 500g a.i./ha (late stem base)	7.2 (16.3)b	18.7b
boscalid 500g a.i./ha (early soil surface spray)	1.4 (8.52)c	19.3bc
boscalid 500g a.i./ha (soil surface, and stem base)	0.1 (5.91)c	21.3c
$LSD^{0.05}$ (*P* value)	6.91 (<0.001)	2.88 (0.009)

Table 12. Trial 7 - Effect of biocontrol treatments alone and integrated with fungicides on white rot and yields of spring onions grown in a sandy clay loam in a trial conducted during Autumn 2005 at Heatherton, Victoria.

Treatment	**% plants with white rot**	**Marketable bunches (no./m^2)**
untreated control	1.2	22
T C52 prill at sowing 30kg/ha	1.6	21
T C52 prill at sowing 30kg/ha+late boscalid (stem base)	0.5	22
T C52 prill at sowing 50kg/ha	0.9	22
T C52 prill at sowing 50kg/ha+late boscalid (stem base)	0.7	23
T C52 prill at sowing 50kg/ha (plus Agrolig™ at sowing)	1.7	22
T C52 prill at sowing 70kg/ha	0.8	22
T C52 prill at sowing 70kg/ha+late boscalid (stem base)	1.3	22
boscalid after sowing (soil surface)	1.2	21
boscalid late application (stem base)	1.9	22
boscalid full program (soil surface and stem base)	0.0	23
$LSD^{0.05}$ (*P* value)	ns	ns

Compatibility of the biocontrol agent *Trichoderma atroviride* C52 with crop management practices in bunching onion production

Summary

A series of laboratory and glasshouse studies were conducted to determine the ability of *Trichoderma atroviride* isolate C52 to colonise different soils and its compatibility with chemicals and soil amendments used in bunching onion production. Two commercial formulations of *T. atroviride* isolate C52 (Trichopel Ali 52™ and Trichoflow Ali 52™) were used in the experiments. Four pot trials were carried out at Knoxfield, Victoria, and four *in vitro* experiments and five pot trials at Lincoln University, New Zealand.

The results of a pot trial showed that *T. atroviride* C52, applied on formulated prills (Trichopel Ali 52™) below the seed at sowing, grew to levels considered desirable for effective biocontrol (>10^5 cfu/g soil) in a black volcanic loam (Lockyer Valley) and in a clay loam soil (Werribee) but not in a sandy soil (Cranbourne). The results from another pot trial showed that placing pellets containing humic acids (Agrolig™, AgriChem) or pelletised composted chicken manure close to *Trichoderma* prills at sowing enhanced the growth of *Trichoderma* in sandy soils (>10^5 cfu/g soil). Results from other pot trials showed that *T. atroviride* C52 proliferated better when applied as a pellet (Trichopel Ali 52™) rather than as a wettable powder formulation (Trichoflow Ali 52™). This result could be specific to soil type, in that the soil used in the pot trial had a high sand content and it is likely that the *T. atroviride* spores/propagules were washed through the soil when applied as a wettable powder formulation.

Pot and *in vitro* experiments showed that the growth of *T. atroviride* C52 was inhibited by various concentrations of nitrogen from a fertilizer (urea) and fresh composted chicken manure. Another pot trial showed that *Trichoderma* was compatible with low-nitrogen organic amendments (spent mushroom compost, green certified compost, aged poultry manure). Therefore nitrogenous materials such as fertilisers and fresh composted chicken manure should not be applied close to applications of *Trichoderma*. The results from one of the pot trials indicated that different soil types may influence the interaction between *Trichoderma* and nitrogen in that *T. atroviride* C52 was less sensitive to field rate applications of nitrogen in river sand/silt compared with Patumahoe clay loam.

Results from *in vitro* tests indicated that diallyl disulphide or DADS was detrimental (fungistatic) to *T. atroviride* mycelial growth and spore germination in close contact. However, results from a pot trial showed that even when DADS was applied to soil two weeks prior to the application of the biocontrol treatment, *T. atroviride* growth was not inhibited. The results from another pot trial showed that *T. atroviride* C52 was compatible with field rate concentrations of three fungicides tested (procymidone, pyraclostrobin, boscalid). However, when fungicides were applied at rates greater than those recommended, *T. atroviride* populations were adversely affected only by pyraclostrobin.

The information collected will be used to optimise the application of *Trichoderma* into soil to ensure maximum colonisation of soils for effective biocontrol. The information will also enable the development of strategies for the use of *T. atroviride* C52 for managing onion white rot and soil health in vegetable farms.

Introduction

Trichoderma species are among the beneficial soil microorganisms reported to provide biocontrol of the disease white rot, caused *S. cepivorum* (Abd-EL-Moity and Shatla 1981, Kay and Stewart 1994). One of these microorganisms is *Trichoderma atroviride* isolate C52 which has been identified as a potential biological control agent of onion white rot due to its antagonistic activity against *S. cepivorum* mainly via nutrient competition in the root zone (Kay and Stewart 1994).

Pot and field studies have been conducted to evaluate the effect of different formulations on the survival, proliferation and rhizosphere competence of *Trichoderma atroviride* C52 in soil (McLean 2001, McLean et al. 2005). In one of these studies, a pellet formulation maintained the concentration of *T. atroviride* C52 at 10^5 cfu per g soil for about 14-weeks, whereas soil-substrate and seed-coating formulations gave concentrations of 10^4 and 10^1 cfu per g soil, respectively (McLean et al 2005). *T. atroviride* C52 has been recovered from the distal parts of the roots of onion plants, indicating that it was able to colonise the rhizosphere to at least 15 cm depth in the soil (McLean et al 2005).
To be able to provide effective protection against infection by mycelium of *S. cepivorum* in the field, *Trichoderma* biocontrol agents (good competitors) must be able to colonise the region of roots and soil around roots and the base of plants at levels considered optimal for biocontrol. It is therefore important to determine their ability to colonise different soils and compatibility with standard crop management practices so that its application can be optimised to maximise their growth in soil for effective biocontrol.

Overall aims

A series of *in vitro* experiments and pot trials were conducted in Australia and New Zealand to determine the compatibility of *T. atroviride* C52 with standard crop management practices in bunching and bulb onion production such as the use of sclerotial germination stimulants, organic amendments, fungicides and fertilizers.

Four pot trials in Victoria investigated:

- the ability of *T. atroviride* C52 to colonise different soils
- the effect of chicken manure and application method on *T. atroviride* C52 colonisation of soil
- the effect of adding soil amendments to sandy soil on *T. atroviride* C52 colonisation of soil

In vitro experiments and pot trials in New Zealand investigated:

- the effect of diallyl disulphide on *T. atroviride* C52 both *in vitro* and *in vivo*
- the compatibility of *T. atroviride* C52 with organic amendments in soil
- the compatibility of *T. atroviride* C52 with fungicides in soil
- the effect of nitrogen on *T. atroviride* C52 growth *in vitro* and *in vivo*

Methods and Results

Pot trials conducted at Knoxfield, Victoria

1. Effect of farm grade composted chicken manure on *Trichoderma* C52 colonisation of sandy soil

Brief Method

This pot trial was conducted using polystyrene boxes (40 x 30 x 14 cm) on glasshouse benches. Sandy soil and farm grade composted chicken manure were obtained from a commercial farm. The manure was either mixed with the soil before sowing or applied as topping (mulch) after sowing at the rate of 10 tonnes/ha. Prills with spores of *T. atroviride* C52 (10^6 cfu/g) were placed at the base of the planting furrow below (2 cm) spring onion seed (50 kg/ha) planted 1 cm below the soil surface on two furrows along the length of polystyrene boxes. Two soil sub-samples were collected from each of the two closed furrows with seed or growing seedling (depth 3 m) per replicate box (n = 4) at 2 and 6 weeks after planting. The four sub-samples/box were mixed and 10 g of this mixed soil used to determine *Trichoderma* colony numbers (n = 4) using a standard dilution plating method and *Trichoderma* selective medium. Microbial communities and *Trichoderma* species were measured on untreated soils also using dilution plating and selective media. Untreated soil was tested for the presence of *Trichoderma* before setting up the trial. *Trichoderma* species were not detected in untreated soils.

Main Results

Soil pH in untreated soil was 7.1 and in amended soil ranged from 6.6 to 6.8 (Table 1). The mean number of colony forming units of total fungi was significantly (P = 0.006) higher in sandy soil amended with (mixed) composted chicken manure than in untreated soil and soil covered (mulch) with the same manure. There were no significant differences in *Trichoderma* C52 levels between treatments 2 weeks after sowing. Six weeks after sowing, the mean number of colony forming units of *Trichoderma* C52 was significantly (P = 0.003) lower in sandy soil covered with composted chicken manure than in the other two treatments.

2. Ability of *Trichoderma* C52 to grow in different soils

Brief Method

A pot trial was conducted using 13cm diameter pots on glasshouse benches. Soils used were collected from commercial vegetable farms. Prills with spores of *T. atroviride* C52 (10^6 cfu/g) were placed at the base of the planting furrow (2 cm) and then spring onion seed were planted on two furrows across the diameter of the pot. Soil samples (one/pot) were collected from the closed furrow with seed or developing seedling (3 cm depth) after planting and then *Trichoderma* colony numbers determined twice per replicate sample (n = 4) using a standard dilution plating method and *Trichoderma* selective medium. Microbial communities and *Trichoderma* species were measured on untreated soils also using dilution plating and selective media. Untreated soil was tested for the presence of *Trichoderma* before setting up the trial. *Trichoderma* species were not detected in untreated soils. The composition analysis of soils used and levels of microbial communities are presented in Tables 2 and 3.

Main Results

Trichoderma levels were significantly (P = 0.003; P = 0.013) higher in black volcanic and clay loam soils than in sandy soil at 15 and 45 days after application (Table 3). In these two soils *Trichoderma* levels remained at 10^5 or higher for 45 days after sowing. Soil analysis showed that the black loam and clay loam soils had more organic matter and total nitrogen and carbon (%w/w) than the sandy soil and this probably contributed to the higher growth of *Trichoderma* in these two soils.

3. Effect of different rates of farm grade chicken manure on *Trichoderma* C52 growth in sandy soil

Brief Method

A pot trial was conducted using 13cm diameter pots on glasshouse benches. The sandy soil and farm grade composted chicken manure used were collected from a commercial vegetable farm. Different rates of chicken manure were mixed with soil before planting. Prills with spores of *T. atroviride* C52 (10^6 cfu/g) were placed at the base of the planting furrow (2 cm) and then spring onion seed were planted on two furrows across the diameter of the pot. Soil samples (one/pot) were collected from the closed furrow with seed or developing seedling (depth 3cm) after planting and then *Trichoderma* colony numbers determined twice per replicate sample using a standard dilution plating method and *Trichoderma* selective medium.

Main Results

Two days after sowing, *Trichoderma* levels were significantly (P<0.001) lower in sandy soil amended with 2.5%, 5% and 10% (w/w) of chicken manure than in untreated soil and soil amended with 1% manure (Table 4). Fifteen days after sowing, *Trichoderma* levels were significantly (P<0.001) higher in untreated soil than in sandy soil amended with all levels of chicken manure. Soil pH (n=4) measurements taken prior to sowing indicated that nitrogen levels (eg ammonium and nitrate) were

higher in sandy soils amended with manure than in untreated soil and this most likely inhibited the germination of spores of *Trichoderma* C52 in amended soils.

4. Effect of adding organic substrates to the furrow with seed on *Trichoderma* C52 growth in sandy soil

Brief Method

A pot trial was conducted using 13cm diameter pots on glasshouse benches. The sandy soil used was collected from a commercial vegetable farm. Amendments and rates used are presented in Table 5. Spores of *T. atroviride* C52 formulated in prills (10^6 cfu/g) were placed at the base of the planting furrow (2 cm depth) and then spring onion seed were planted (1 cm depth) on two furrows across the diameter of the pot. Soil samples were collected (one/pot) from the closed furrow with seed or developing seedling (3 cm depth) after planting and then *Trichoderma* colony numbers determined twice per replicate sample using a standard dilution plating method and *Trichoderma* selective medium.

Main Results

Trichoderma (species not identified) was detected in the untreated farm soil used for the pot trial at levels ranging from 2.6 to 3.9 x 10^2 cfu per g of soil (Table 5). One and fifteen day(s) after sowing, *Trichoderma* levels (C52 plus *Trichoderma* present in untreated soil) were significantly ($P = 0.009$; $P < 0.001$) higher in sandy soil amended (in-furrow application) with pellets containing humic acids or fully composted chicken manure than in soils unamended and amended with other organic substrates. *Trichoderma* levels in soil with in-furrow applications of humic acids and chicken manure ranged from 10^4 to 10^5 cfu g soil compared to 10^4 to 10^3 in other treatments at 1 and 15 days after sowing. Pellets containing humic acid and composted chicken manure were placed close to *Trichoderma* prills and dissolved slowly and this probably provided *Trichoderma* with an additional supply of nutrients to grow better in sandy soil.

In vitro **and pot trials carried out in New Zealand**

5. *Trichoderma atroviride* mycelial growth in media amended with DADS

Brief method

Trichoderma atroviride mycelial sensitivity to DADS was evaluated by spreading DADS at ½ field rate, field rate and twice field rate concentrations onto separate Potato dextrose agar (PDA) plates and centrally inoculating the plates with *T. atroviride* colonised mycelial disks (5 mm diameter). Radial growth was measured everyday until the colony reached the plate edge. Spore production was noted.

Main results

Diallyl disulphide (DADS) significantly reduced ($P<0.05$) *T. atroviride* mycelial growth on agar (Table 6). After 3 days, radial mycelial growth in the control treatment was 30 mm compared with 24, 28 and 15 mm at ½ field rate, field rate and twice field rate concentrations, respectively. In addition, all rates of DADS delayed spore development and maturity by 2 days compared with the control treatment.

6. *Trichoderma atroviride* spore germination in media with DADS

Brief method

A *T. atroviride* spore suspension was prepared from agar plates and diluted to 1 x 10^5 spores/ml using Potato dextrose broth (PDB) in 1.5 ml conical tubes. Diallyl disulphide was added to the broth at ½ field rate, field rate and twice field rate concentrations. The tubes were continually rotated for 24 h, following which spore germination was recorded using a haemocytometer.

Main results

Diallyl disulphide significantly reduced ($P<0.05$) *T. atroviride* spore germination at all rates tested (data not shown). After 24 h incubation, none of the spores incubated with DADS solutions at ½ field rate, field rate and twice field rate concentrations had germinated compared with 100% in the control. When the broth containing spores and the twice field rate concentration of DADS was diluted by ¼, ½ and ¾ with sterile distilled water (SDW), the spores germinated in the ½ and ¾ dilutions (48 and 81%, respectively) but not in the ¼ dilution.

7. Soil pot trial *Trichoderma atroviride* growth in soil with DADS

Brief method
Diallyl disulphide was applied at field rate to separate lots of soil contained in plastic pots at 8, 6, 4 and 2 weeks prior to inoculating the pots with Trichopel Ali 52 or Trichoflow Ali 52. Soil samples were taken at time 0 (directly after inoculation) and at weeks 1, 2, 4 and 6. Soil dilutions and plating on selective agar was used to determine the concentration of *T. atroviride* at each assessment time.

Main results

Trichoderma atroviride could not be recovered in the control treatments, therefore the trial was repeated.

8. Pot trial 2 Effect of diallyl disulphide on *Trichoderma a*troviride growth in soil

Brief method

A similar method was followed as described for pot trial 1, with the following modifications. A field rate concentration of DADS was applied to a soil and potting mix combination in separate plastic pots at 8, 6, 4 and 2 weeks prior to inoculating the pots with spores of *T. atroviride* C52 formulated in prills (Trichopel Ali 52) or wettable powder (Trichoflow Ali 52). Following *Trichoderma* inoculation, the soil/potting mix combination was covered with vermiculite. The potting mix and vermiculite were used to prevent the soil drying out and compacting. Soil samples were taken at time 0 (directly after inoculation) and at weeks 1, 2, 4 and 6 following inoculation. Soil dilutions and plating on *Trichoderma* selective agar (TSM-LU) (McLean *et al.* 2005) were used to determine the concentration of *T. atroviride* at each assessment time.

Main results

There was no significant difference ($P>0.05$) in *T. atroviride* growth when applied to DADS treated soil as Trichopel Ali 52 or Trichoflow Ali 52 (Table 7). At week 0, concentrations ranged from 4.6 x 10^4 cfu/g soil for the Trichopel control to 2.2 x 10^6 cfu/g soil for the Trichopel Ali 52 with DADS applied 6 weeks prior to the *Trichoderma* treatment. The *T. atroviride* concentration increased over time but not significantly ($P>0.05$) and at trial completion (week 6), concentrations ranged from 1.8 x 10^5 cfu/g soil (DADS applied 6 weeks before Trichoflow Ali 52) to 6.0 x 10^6 cfu/g soil (DADS applied 8 weeks before Trichopel Ali 52).

9. Soil pot trial - Compatibility of *Trichoderma atroviride* with organic amendments

Brief method

Poultry manure, spent mushroom compost and certified green compost were used as organic amendments. The poultry manure and the mushroom compost were applied as mulches (1 cm thick layer) to the surface of the sandy soil and the green compost was mixed into the sandy soil (20 tonnes/ha). Trichopel Ali 52 was added to the planting furrow following onion seed planting in 24 pots and Trichoflow Ali 52 was applied to an additional 24 pots.

Main results

The number of *T. atroviride* colony forming units for Trichopel Ali 52 and Trichoflow Ali 52 at trial set up in the soil amended with poultry manure, mushroom compost and green compost is shown in Table 8. Overall, organic amendments were not detrimental to *T. atroviride* growth, in some cases (poultry manure and mushroom compost) fungal growth was enhanced. In addition, formulation had an effect with greater *T. atroviride* proliferation when added to the soil as Trichopel Ali 52 (2.0×10^3 – 1.0×10^7 cfu/g soil) compared with Trichoflow Ali 52 (2.0×10^3 – 3.0×10^5 cfu/g soil).

10. Soil pot trial - Compatibility of *Trichoderma atroviride* with fungicides in soil

Brief method

Trichoderma atroviride compatibility with procymidone (Crop Care), pyraclostrobin (BASF), boscalid (BASF), and a combination of pyraclostrobin and boscalid was assessed in a soil pot trial. Soil and potting mix (3:1) was added to each of 20 pots. Trichopel Ali 52 was added to each of four planting holes in each pot. Fungicides were applied to the surface of the soil in each of two pots at field rate and twice field rate concentrations. Wooden toothpicks were inserted into the soil to mark the planting holes as no seed was planted and the soil was covered with vermiculite to prevent excessive drying of the soil. *Trichoderma atroviride* populations were determined before any fungicides were added (0), 2, 10 and 30 days after fungicide application using serial dilutions and plating on TSM-LU.

Main results

All fungicides reduced *T. atroviride* populations from 1.6×10^6 – 4.1×10^6 cfu/g soil to 4.0×10^4 – 2.2×10^5 cfu/g soil 2 days after initial application however, this was not significant (Table 9). After 10 days, the *T. atroviride* population had recovered to levels equivalent to the control (4.8×10^7 cfu/g soil) in all treatments although the *T. atroviride* population when exposed to the twice field rate application of the pyraclostrobin and boscalid combination, remained low (1.7×10^5 cfu/g soil) compared with the remaining fungicide treatments (1.1×10^6 – 5.1×10^7 cfu/g soil). After 30 days, *T. atroviride* had proliferated to levels equivalent to the control (1.4×10^8 cfu/g soil) in the presence of all fungicides except pyraclostrobin and the twice field rate concentrations of pyraclostrobin and boscalid combination. *Trichoderma atroviride* was the most tolerant of procymidone and boscalid as populations at both rates tested were equivalent to the control (8.1×10^7 and 9.2×10^7 cfu/g soil, respectively).

11. *In vitro* assay - Effect of nitrogen on mycelial growth of *Trichoderma atroviride*

Brief method

Nitrogen in the form of Urea was added in increasing concentrations (0.1, 0.2, 0.3, and 0.4%) to nitrogen assimilation media. Upon solidification in Petri dishes, the media was centrally inoculated with *T. atroviride* colonised mycelial disks (5 mm diameter). After 4 days, the colony diameter was measured and the mean colony area calculated.

Main results

Mycelial growth of *T. atroviride* was significantly inhibited by nitrogen when agar was spread with concentrations of 0.2% or greater (1.7 – 15.2 cm^2) compared with the control (42.2-52.3 cm^2) (Table 10). At the greatest nitrogen concentration of 0.4%, mycelial growth was significantly less ($P<0.05$) (2.7 cm^2) than at all other concentrations tested.

12. Soil pot trial - Effect of nitrogen on the growth of *Trichoderma atroviride*

Brief method
Nitrogen as Urea was applied at field rate and twice field rate concentrations to a combination of soil and potting mix (3:1) in plastic pots that had been inoculated with Trichopel Ali 52. Wooden toothpicks were inserted to mark the Trichopel Ali 52 inoculation sites and the soil was covered with vermiculite. *Trichoderma atroviride* populations were determined from soil samples taken at 2, 10 and 30 days after the application of nitrogen using serial dilutions and plating on selective agar.

Main results

The application of nitrogen to soil inoculated with *T. atroviride* as Trichopel Ali 52, significantly reduced fungal growth (data not shown). *Trichoderma atroviride* populations decreased from 1.4 x 10^6 cfu/g soil in the control treatment to 2.5 x 10^5 and 0 cfu/g soil in the field rate and twice field rate treatments, respectively 2 days after initial application. *Trichoderma atroviride* populations recovered in both the field rate and the twice field rate applications after 30 days (2.1 x 10^6 and 1.3 x 10^6 cfu/g soil) but populations were still significantly less than the control treatment (9.2 x 10^7 cfu/g soil).

13. *In vitro* - Effect of nitrogen on the growth of spores of *Trichoderma atroviride*

Brief method

A spore suspension of *T. atroviride* (1 x 10^5 spores/ml) was prepared in PDB. Separate samples of the spore suspension were exposed to nitrogen in the form of Urea at ½ field, field and twice field rates for 24 hours (4 replicate samples/treatment). After which, samples (10 µl) were stained with Lactophenol cotton blue (LPCB) and spore germination and germ tube length of 50 spores/replicate was measured using a light microscope. The mean germ tube length for each treatment was calculated.

Main results

T. atroviride spore germination was significantly less in the twice field rate concentration (16%) of nitrogen compared with all other treatments (100%) (data not shown). Germ tube length significantly decreased ($P<0.05$) with increasing nitrogen concentration. With the ½ field, field and twice field rates of nitrogen the mean germ tube length was 3.11, 3.07 and 0.25 mm, respectively. The germ tube length for the control treatment was 3.41 mm.

14. Soil pot trial - Effect of nitrogen on the growth of *Trichoderma atroviride*

Brief method

Nitrogen as Calcium ammonium nitrate (CAN) was applied to River sand/silt and Patumahoe clay loam soil in separate plastic pots at field and twice field rate concentrations 30 days after inoculation with Trichopel Ali 52. A second nitrogen application was applied 60 days after Trichopel application at field rate to half the pots that initially received a field rate application to simulate commercial

practice. A second nitrogen application (twice field rate) was also made to half the pots that initially received twice field rate nitrogen applications. Wooden toothpicks were inserted to mark the Trichopel Ali 52 inoculation sites and the soil was covered with vermiculite. *T. atroviride* populations were determined following initial soil application (0), 4 weeks later before nitrogen application(30 days), day 2 (32 days), 10 (40 days) and 30 (60 days) following the first nitrogen application and day 2 (62 days), 10 (70 days) and 30 (90 days) following the second nitrogen application.

Main results

In river sand/silt control treatment, the *T. atroviride* concentration increased from 6.0 x 10^4 to 1.7 x 10^5 cfu/g soil after 32 days, which was significantly greater ($P<0.05$) than the *T. atroviride* concentration in the nitrogen treatments (Fig. 1). The *T. atroviride* population in the control treatment was significantly greater ($P<0.05$) than all other treatments for the remainder of the trial. The fungal population in the nitrogen treatments which received two field rate applications and twice field rate application rates remained less than 1 x 10^5 cfu/g soil for the trial and were not significantly different ($P>0.05$) to one another (Fig. 1). The *T. atroviride* population in the nitrogen field rate application treatment was significantly greater ($P<0.05$) than the remaining nitrogen treatments after 60, 70 and 90 days (Fig 1), but was significantly less than the control ($P>0.05$) at all assessment times.

In Patumahoe clay loam soil, the *T. atroviride* population in the control treatment increased overtime and was significantly greater ($P>0.05$) than the fungal concentrations in any of the nitrogen treatments from 32 days after inoculation until trial completion (Fig. 2). In the nitrogen treated soil, the *T. atroviride* population ranged from 2.2 x 10^3 to 8.4 x 10^4 cfu/g soil (Fig. 2).

Discussion

A pot trial showed that *T. atroviride* C52, applied (spores on prills) at sowing with seed, can grow to levels desirable for effective biocontrol in black loam and clay loam soils but not in sandy soils. Another pot trial demonstrated that placing pellets (0.5% w/w) containing humic acids or fully decomposted chicken manure close to *Trichoderma* prills at sowing enhanced the growth of *Trichoderma* in sandy soils.

The sensitivity of *T. atroviride* to nitrogen was studied both *in vitro* and *in vivo*. *In vitro* experiments indicated that *T. atroviride* mycelium and spores were highly sensitive to nitrogen. Soil pot trial results showed that *T. atroviride* populations decreased (from 1.4 x 10^6 cfu/g soil to 0 - 2.5 x 10^5 cfu/g soil) following initial nitrogen application, but recovered after 30 days. An additional trial determined that repeated applications of nitrogen can restrict *T. atroviride* proliferation and that soil-type may influence how the fungal population responds to nitrogen. Another pot trial showed that using low rates of farm grade fresh composted chicken manure mixed with soil (0.5% w/w) or as mulch at the field rate of 10 tonnes/ha reduced the growth of *T. atroviride.* In conclusion, the pot and in vitro studies showed that *T. atroviride* is sensitive to nitrogen particularly at high concentrations. Therefore nitrogenous material such as fertilisers and composted chicken manure should not be applied close to applications of *Trichoderma* because nitrogen inhibits germination of spores of *Trichoderma*. It would be advisable to apply nitrogen at least 2-3 weeks before or after the application of *T. atroviride* to give *Trichoderma* time to establish and proliferate in the soil. Different soil types may influence the interaction between *T. atroviride* and nitrogen in that *T. atroviride* was less sensitive to field rate applications of nitrogen in river sand/silt compared with Patumahoe clay loam.

The incorporation of low nitrogen organic amendments including spent mushroom compost, certified green compost and fully composted aged poultry manure into the soil caused only a small decrease in *Trichoderma* concentration (from 7.3 x 10^4 – 4.8 x 10^6 cfu/g soil to 2.0 x 10^3 – 3.0 x 10^5 cfu/g soil) when *T. atroviride* was applied to the soil as liquid suspension (Trichoflow), irrespective of amendment type. The application of prills (Trichopel) to the amended soil resulted in an increase in fungal concentration for all amendments (from 4.4 x 10^2 – 1.2 x 10^3 cfu/g soil to 2.0 x 10^3 – 1.0 x 10^7 cfu/g soil). Pot trials with composts showed that *Trichoderma atroviride* is compatible with low-

nitrogen organic amendments tested, but as the amount of organic matter and nitrogen varies between products, it would be wise to test products before widespread use. The pot trial also indicated that *T. atroviride* proliferated better when applied as a pellet rather than as a wettable powder. This result may be specific to soil type, in that the soil used in the pot trial had a high sand content and it is likely that the *T. atroviride* propagules were washed through the soil when applied as a wettable powder formulation.

The compatibility of *T. atroviride* with chemicals used in crop disease management practices was assessed to develop an environmentally sustainable strategy for the control of onion white rot. *Trichoderma atroviride* mycelial growth and spore germination were significantly inhibited by the *S. cepivorum* sclerotial germination stimulant diallyl disulphide (DADS) at ½, 1 and 2 x field rate concentrations. However, in soil, *T. atroviride* populations in the Trichopel formulation remained high (10^4 – 10^7 cfu/g soil) over the 6 week trial. In general, products containing *T. atroviride* (Trichopel Ali 52 and Trichoflow Ali 52) can be used in conjunction with diallyl disulphide because DADS is applied to fallow soil many weeks prior to sowing. The results from the pot trial indicated that even when DADS is applied two weeks prior to planting, *T. atroviride* growth is not inhibited. While the *in vitro* results indicated that DADS was detrimental to *T. atroviride* mycelial growth and spore germination, *T. atroviride* was in direct contact with DADS in these assays, which didn't occur in the soil pot trial. In addition, the effect of DADS on *T. atroviride in vitro* appeared to be of a fungistatic nature, in that, the effect could be overcome with time (mycelial growth assay) or by diluting the concentration of DADS (spore germination assay).

Trichoderma atroviride as Trichopel was exposed to field rate and twice field rate applications of the fungicides procymidone, pyraclostrobin, boscalid and a combination of pyraclostrobin and boscalid in soil. Although all fungicides reduced the fungal population initially (from 1.6×10^6 – 4.1×10^6 cfu/g soil to 9.8×10^4 – 1.6×10^5 cfu/g soil), the population had recovered to levels equivalent to the control (4.8×10^7 cfu/g soil) after 10 days except for the twice field rate application of the pyraclostrobin/boscalid combination, where the population needed 30 days to recover. After 30 days, *T. atroviride* was most tolerant of procymidone and boscalid with fungal concentrations at both rates tested equivalent to the control, whereas fungal populations in the twice field rate applications of pyraclostrobin and the boscalid/pyraclostrobin combination were significantly less than the control. In conclusion, *Trichoderma atroviride* is compatible with all fungicides tested if applied at field rate concentrations. If fungicides were applied at rates greater than those recommended then *T. atroviride* populations would be adversely affected by pyraclostrobin and the combination of pyraclostrobin and boscalid.

References

Abd-El-Moity TH, Shatla MN (1981). Biological control of white rot disease of onion (*Sclerotium cepivorum*) by Trichoderma harzianum. Phytopathologische Zeitschrift 100, 29-35.

Kay, S.J. and Stewart, A. (1994). Evaluation of fungal antagonists for control of onion white rot in soil box trials. Plant Pathology 43:371-377.

McLean KL, Swanminatahn CM, rampton JS, Hunt JS, Ridgway HJ, Stewart A (2005). Effect of formulation on the rhizosphere competence and biocontrol ability of *Trichoderma atroviride* C52. Plant Pathology 54, 212-218.

McLean KL (2001). Biology Control of Onion White Rot Using *Trichoderma harzianum*. Canterbury, New Zealand: Lincoln University, PhD thesis.

Table 1. Mean number of *T. atroviride* C52 colony forming units fu per gram of soil sandy soil amended with composted chicken manure

Treatment	Mean number of cfu/g soil				
	Soil pH	Total Fungi	Total Bacteria	*Trichoderma*	
				Week 2	Week 6
untreated soil	7.1	5.1×10^4 b	2.9×10^8	7.6×10^4	9.0×10^3 a
with chicken manure mixed	6.8	2.7×10^5 a	4.9×10^7	5.9×10^4	8.7×10^3 a
with chicken manure mulch	6.6	7.5×10^4 b	2.0×10^7	3.6×10^3	3.1×10^3 b

Trichoderma species were not detected in untreated soils. Mean values followed by the same letter do not differ significantly according to LSD values (P = 0.05).

Table 2. Characteristics of soils used in pot trials.

Soil	Total ppm						% w/w
	K	Mg	S	Ca	P	N	Organic Matter
Clay loam	8900	2600	310	2300	680	4.4	4.4
Black loam	4500	23000	450	4400	2300	8.5	8.5
Sandy loam	390	520	190	4800	1800	0.12	2.3

Table 3. Mean number of *T. atroviride* C52 colony forming units per gram of soil at 1, 15 and 45 days after application in three different soil types.

Treatment	Before application of *Trichoderma*			*Trichoderma*		
	pH n=4	Total Fungi	Total Bacteria	Days after planting 1	15	45
black volcanic soil	7.4	1.7×10^4	3.0×10^7a	6.1×10^3a	9.4×10^5a	4.8×10^5a
clay loamy soil	7.3	3.4×10^4	5.1×10^6b	1.2×10^4b	1.2×10^6a	3.9×10^5a
sandy loamy soil	7.1	7.7×10^3	1.2×10^7c	5.2×10^3a	6.2×10^4b	4.9×10^4b

Trichoderma species were not detected in untreated soils. Mean values followed by the same letter do not differ significantly according to LSD values (P = 0.05).

Table 4. Mean number of *T. atroviride* colony forming units per gram of soil mixed with different levels of farm grade composted chicken manure before sowing.

			Mean number cfu/g soil	
Treatment	pH before planting 15 (days)	0	Day after planting 2	15
0% manure	7.7	7.6	4.3×10^3a	2.0×10^3a
1.0% manure	8.5	6.7	3.2×10^3a	7.4×10^2b
2.5% manure	8.5	6.4	2.9×10^2b	6.2×10^2b
5.0% manure	8.3	6.1	7.3×10^2b	4.7×10^2b
10% manure	8.5	6.2	1.3×10^2b	4.9×10^2b

Trichoderma species were not detected in untreated soil. Mean values followed by the same letter do not differ significantly according to LSD values (P=0.05).

Table 5. Mean number of *T. atroviride* C52 colony forming units per gram of soil amended (in-furrow applications) with various organic substrates.

		Day after planting	
	Treatment	1	15
Humic acids pellets	(0.5%$^{w/w}$,Agrolig™)	9.7 x 10^5a	1.1 x 10^5a
Chicken manure pellets	(0.5%$^{w/w}$, Dynamic Lifter™)	5.2 x 10^4a	1.3 x 10^5a
Sawdust compost	(0.5%$^{w/w}$, Argus™)	6.0 x 10^3b	2.7 x 10^3b
Humic acids liquid	(drench with 15$^{L/ha}$, SupaHumus™)	3.6 x 10^3b	2.2 x 10^3b
Composted chicken manure	(0.5% $^{w/w}$, farm grade)	4.1 x 10^3b	1.3 x 10^3b
Untreated control	(only *Trichodema* prills added)	6.9 x 10^4b	4.7 x 10^2c
Untreated	(*Trichoderma* was not added)	2.6 x 10^2c	3.9 x 10^2c

Mean values followed by the same letter do not differ significantly according to LSD value (P=0.05).

Table 6. Mean mycelial growth and spore germination of *Trichoderma atroviride* when exposed to varying concentrations of diallyl disulphide

Treatment	mycelial growth (mm)		spore germination (%)	
Control	30	a	100	a
½ x field rate DADS	24	b	0	b
1 x field rate DADS	28	a	0	b
2 x field rate DADS	15	c	0	b

Mean values followed by the same letter within each column do not differ significantly according to a Fisher's LSD test.

Table 7. Mean number of *Trichoderma atroviride* colony forming units per gram of soil in soils treated with DADS.

	Mean number of cfu/g soil					
Treatment	**Week 0**		**Week 2**		**Week 6**	
2 wk DADS, Trichopel	7.0 x 10^4	a	3.1 x 10^6	a	2.7 x 10^6	a
4 wk DADS, Trichopel	1.3 x 10^5	a	5.0 x 10^5	a	7.1 x 10^5	a
6 wk DADS, Trichopel	1.1 x 10^6	a	2.3 x 10^5	a	1.8 x 10^5	a
8 wk DADS, Trichopel	8.4 x 10^5	a	1.9 x 10^6	a	1.0 x 10^6	a
Control, Trichopel	4.6 x 10^4	a	5.8 x 10^5	a	7.6 x 10^5	a
2 wk DADS, Trichoflow	7.3 x 10^5	a	1.0 x 10^7	a	3.0 x 10^6	a
4 wk DADS, Trichoflow	1.2 x 10^6	a	7.7 x 10^5	a	2.8 x 10^6	a
6 wk DADS, Trichoflow	2.2 x 10^6	a	3.2 x 10^5	a	2.6 x 10^6	a
8 wk DADS, Trichoflow	1.1 x 10^6	a	3.1 x 10^6	a	6.0 x 10^6	a
Control, Trichoflow	1.3 x 10^5	a	1.2 x 10^6	a	2.4 x 10^6	a

Mean values followed by the same letter do not differ significantly according to a Fisher's LSD test.

Table 8. Mean number of *Trichoderma atroviride* colony forming units per gram of soil in sandy soil amended with different composts.

Treatment	Mean number of cfu/g soil Week 0		Week 8	
Trichopel, control	8.1×10^2	f	2.0×10^3	d
Trichopel, poultry manure	1.2×10^3	f	1.0×10^7	a
Trichopel, mushroom compost	4.4×10^2	f	2.0×10^5	bc
Trichopel, green compost	5.8×10^2	f	4.0×10^4	cd
Trichoflow, control	7.3×10^4	def	3.0×10^3	d
Trichoflow, poultry manure	1.6×10^5	d	1.0×10^4	d
Trichoflow, mushroom compost	4.8×10^6	a	3.0×10^5	b
Trichoflow green compost	3.9×10^5	c	2.0×10^3	d

Mean values followed by the same letter do not differ significantly according to a Fisher's LSD test.

Table 9. Mean number of *Trichoderma atroviride* colony forming units per gram of soil treated with different fungicide treatments

Treatment	Mean number of cfu/g soil Day 0		Day 2		Day 10		Day 30	
B500 x 1	1.6×10^6	e	2.2×10^5	e	1.8×10^6	e	3.6×10^7	b-e
B500 x 2	1.8×10^6	e	9.8×10^4	e	1.1×10^6	e	6.5×10^6	de
B510 x 1	2.8×10^6	de	1.3×10^5	e	4.9×10^6	de	9.2×10^7	a-c
B510 x 2	4.1×10^6	de	4.0×10^4	e	4.7×10^6	de	9.5×10^7	a-c
B516 x 1	3.1×10^6	de	1.8×10^5	e	4.7×10^6	de	1.1×10^8	ab
B516 x 2	2.8×10^6	de	1.3×10^5	e	1.7×10^5	e	7.8×10^6	de
Sum x 1	2.3×10^6	de	1.6×10^5	e	5.1×10^7	b-e	7.9×10^7	a-e
Sum x 2	1.9×10^6	e	9.8×10^4	e	1.8×10^7	c-e	8.1×10^7	a-d
Control	3.1×10^6	de	2.0×10^6	e	4.8×10^7	b-e	1.4×10^8	a

Key: B500 = pyraclostrobin, B510 = boscalid, B516 = pyraclostrbin and boscalid; x 1 = field rate, x 2 = twice field rate.
Mean values followed by the same letter do not differ significantly according to a Fisher's LSD test.

Table 10. Mean growth of *Trichoderma atroviride* when exposed to varying concentrations of nitrogen

Nitrogen concentration (%)	mean growth (cm^2)		range
0.00	46.9	a	42.2-52.3
0.10	43.9	a	39.0-48.6
0.20	13.1	b	10.1-15.2
0.30	7.4	b	4.6-10.2
0.40	2.7	c	1.7-3.4

Mean values followed by the same letter do not differ significantly according to a Fisher's LSD test.

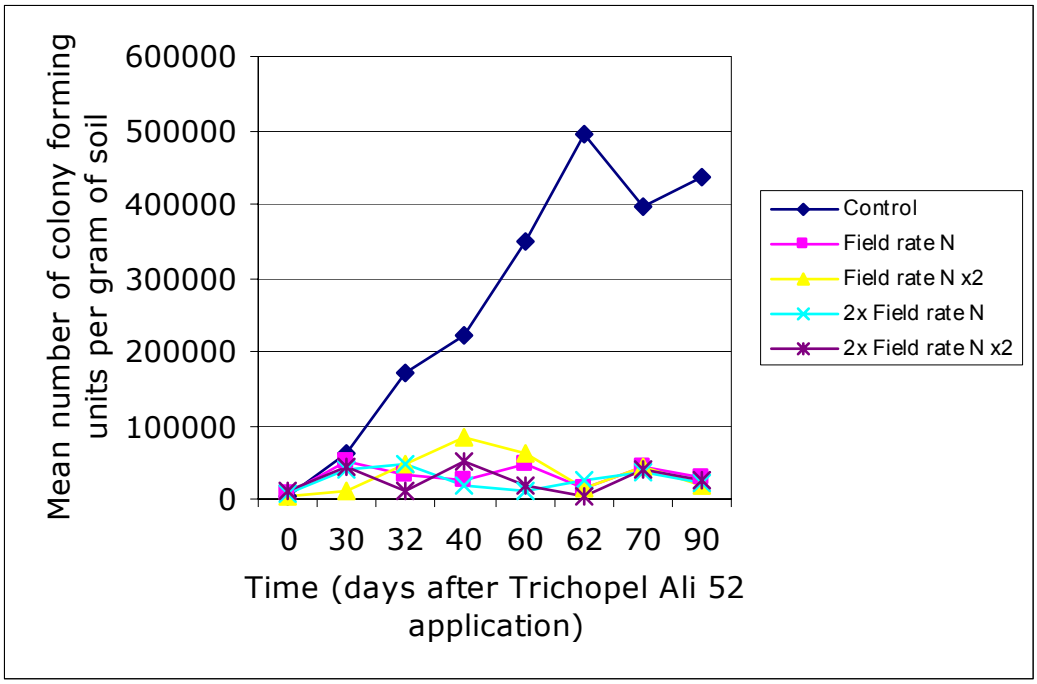

Figure 1. Mean number of *Trichoderma atroviride* colony forming units per gram of soil (river sandy/silt) amended with different field rates of nitrogen

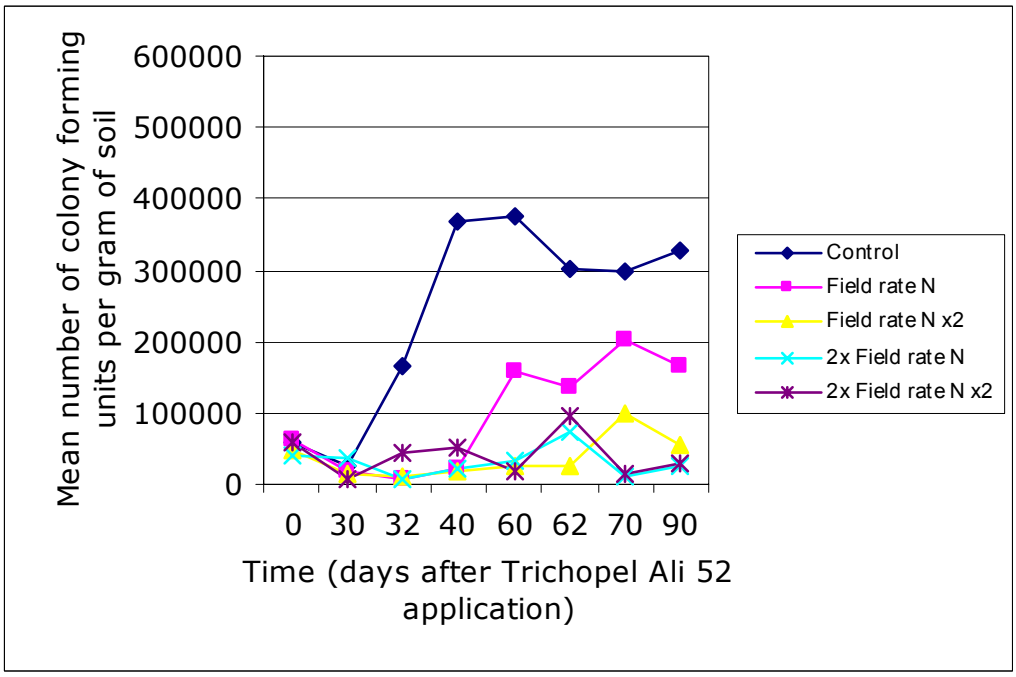

Figure 2. Mean number of *Trichoderma atroviride* colony forming units per gram of soil (Patumahoe clay loam) amended with different field rates of nitrogen

Evaluation of diallyl disulphide or DADS for the integrated control of onion white rot of bunching onions

Summary

Four field trials evaluated the effectiveness of the soil treatment diallyl disulphide or DADS (Alli-Up®, 78% DADS) for reducing the population of sclerotia of *S. cepivorum* in soil and white rot incidence on spring onion crops grown in sandy loam/clay soils in commercial vegetable farms. The trials evaluated single and dual applications of DADS injected into soils at 5 and 10L/ha using 500 and 1000 L/ha of water. The effect of combining DADS treatments with fungicide and biological treatments on white rot control was evaluated at three field sites. One trial also evaluated the efficacy of two applications of a formulation of garlic oil (ECOGuard™) injected into soil at 5 and 10L/ha in 500 L/ha of water.

The results of field trials showed that commercially acceptable levels of white rot control were obtained by treating naturally infested soils with synthetic DADS during two seasons (Autumn and Spring) for one year prior to sowing spring onion crops. At one field site, two applications using either 5L/ha or 10L/ha of DADS were more effective than single applications in reducing the population of sclerotia in soil (90% reduction) and disease incidence. For instance, two applications of DADS significantly reduced the percentage of plants infected with white rot from 34.4% to 5.94 (10L/ha) and to 2.62% (5L/ha) and increased the number of marketable bunches per m^2 from 10.7 to 16.3 (10L/ha) and to 14.0 (5L/ha) when compared to untreated controls. Both rates of DADS tested were equally effective in reducing disease levels.

The results of field trials showed that the control of white rot was more successful when DADS was integrated with fungicide and biocontrol treatments. Integrating DADS treatments with the fungicide boscalid (Filan™) resulted in almost complete control of disease. The biocontrol agent *T. atroviride* C52 (Trichopel Ali 52®) alone was not able to effectively protect the roots of growing plants against infection throughout the season at a high disease site. However, when it was applied to soil treated with two applications of DADS, the combined treatments gave disease reductions comparable to those provided by DADS treatments combined with fungicide.

Two rates of the organic garlic juice tested were not effective in reducing white rot in sandy soils. The low efficacy of this product was probably due to its low content of diallyl disulphide (8.7%) in the mixture with other related compounds.

The results reported here demonstrated that synthetic DADS was an effective soil treatment to reduce both sclerotial populations in soils prior to planting and white rot incidence on spring onion crops in sandy soils. DADS combined with appropriately applied and timed fungicide applications or early season biocontrol treatments have the potential to provide effective and sustainable control of white rot on bunching onion crops.

Introduction

Onion white rot, caused by *Sclerotium cepivorum*, is prevalent in many fields used for production of bunching onions in vegetable farms in Tasmania, Victoria, New South Wales and Queensland, Australia (Villalta unpublished data). In these fields, soils are infested with high of levels of sclerotia of *S. cepivorum*, resulting in crop losses to white rot ranging from 5 to 50%, or higher, if fungicidal control is insufficient throughout the growing season when soil conditions are favourable for disease development. Reducing the population of sclerotia of *S. cepivorum* in soil is vital to obtain improved and sustainable control of this disease in soils used for producing *Allium* crops in Australia.

A number of control methods have been investigated to eradicate sclerotia in soil including soil solarisation (Basallote-Ureba and Melero-Vara 1993, Porter and Merriman 1985), fumigants (Perez-Moreno et al 1996) and chemical stimulants of sclerotial germination (Coley-Smith and Parfitt 1986, Dennis 1997). Levels of sclerotia reduced and disease control varied with the method used and the time of the year with no single method offering complete control. Soil solarisation has proved useful reducing soil populations of sclerotia to very low levels in areas with appropriate weather conditions (Basallote-Ureba and Melero-Vara 1993) and has provided partial control in other areas such as SE Australia (Porter and Merriman 1985). Soil fumigants can give good control of white rot but their use is greatly limited in *Allium* crops due to environmental and economic issues (Perez-Moreno et al 1996, Porter pers. comm.). Promoting germination of sclerotia of *S. cepivorum* in soil by the application of germination stimulants such as diallyl disulphide (DADS) in the absence of *Allium* crops has demonstrated to be an effective method for reducing the population of sclerotia in soil, provided that DADS concentrations and environmental conditions are adequate (Crowe et al 1990, Porter at al, Dennis 1997).

In recent onion trials in Australia and New Zealand, a new formulation of synthetic DADS (Alli-UP®, UAP USA), has been shown to be an effective stimulant of germination of sclerotia of *S. cepivorum* when applied months prior to planting when soil conditions are most favourable for pathogen germination (HAL final report VG98140, Stewart et al 2000). In a trial in New Zealand, two applications of DADS (Alli-up®), the first applied in spring followed by a second in Autumn, at the rate of 10 litres/ha, reduced disease incidence from 42.2% to 12.2% with corresponding yield increases (Stewart et al 2000). In a trial in Tasmania, two applications of an experimental formulation of DADS, the first applied in Spring (5L/ha) followed by a second in Autumn (10L/ha), reduced disease incidence from 75.6% to 43.6% with corresponding onion yield increases (Dennis 1991). In the same trial, integrating the same treatments of DADS with the fungicide tebuconazole reduced white rot from 75.6% to 11.6% also with corresponding yield increases. These results clearly indicated that for more effective control of onion white rot, DADS soil treatments should be integrated with other treatments that protect the roots and base of plants against infection during the growing season.

Aims of research:

This project carried out four long-term field trials to evaluate the effectiveness of a commercial formulation of DADS (Alli-UP®), used alone and in combination with new fungicide and biocontrol treatments, for reducing the number of sclerotia of *S. cepivorum* in soil and white rot incidence on spring onion crops.

Materials and Methods

Sites

The four trials were carried out during 2002-2005 in field sites (sandy soils, pH range 6.2-7.5, 1.4-4.0% organic matter, 38-40% coarse sand and 7.5-10% silt) naturally infested with sclerotia of *S. cepivorum* in commercial vegetable farms at Cranbourne and Heatherton, south and south-east of Melbourne. The field trials were set up within commercial crops of spring onions.

Treatments

Description of the treatments and application rates used in the different field trials are listed in Table 1. The germination stimulant DADS (dially disulphide, 78%; related compounds 22%) was obtained from United Agri Products, USA and Elliott Chemical New Zealand. The organic garlic oil (dially sulphide, 4.75%; methylallyl disulphide 2.18% and diallyl trisulphide 47.09%) was obtained from Australian Botanical Products Pty Ltd. The organic formulated garlic oil or ECOguard™ (dially sulphide, 1.7%; diallyl disulphide 8.7%, diallyl trisulphide 33.9%, allyl-methyl trisulphide 5.7%, diallyl tetratsulphide 31.3) was obtained from EcoSpray Ltd and Kendon Australia).

Two commercial formulations of the biological control agent *T. atroviride* isolate C52 were tested in trial 1. Spores of *T. atroviride* C52 were formulated in prills (Trichopel Ali 52) and in wettable powder preparations (Trichoflow Ali 52™). Prills and wettable powder preparations of *T. atroviride* C52 were tested for levels of viable spores of *Trichoderma* prior to use in all trials. *Trichoderma* levels in prills ranged from 1 x 10^6 to 5 x 10^6 cfu gram of prills and in powder formulation from 1 x 10^8 to 2 x 10^8.

Experimental Design

At all sites, natural rain and irrigation provided sufficient moisture to maintain the soil at field capacity to maximise the effect of DADS in soil. Fields were irrigated before application of DADS treatments and after that maintained at field capacity for 3-months. DADS treatments were applied in Spring and Autumn (Table 2) when soil temperatures were favourable for germination of sclerotia (13-18ºC). Sites were weeds free before the DADS treatments. In the fields used for trials 1, 3 and 4, spring onions were harvested at least 12 months prior to the application of the first DADS treatment. In the field used for trial 2, spring onions were harvested 6 months prior to the application of the first DADS treatment.

The sandy soil from hilled beds (0.40-0.45 m height) was tilled to a flat bed (0.20-0.25 m height from ground level) with a cultivator and disked the day before the DADS treatment. DADS treatments were injected into soil at 5 and 10L/ha using two modified metham sodium rigs. Before injecting DADS, the rigs were calibrated to adjust the appropriate amount of DADS per volume of water per ha. The first rig had 9 tynes with injection hoses (spray nozzles) spaced 60 cm apart, all hoses with a nozzle fanning/injecting DADS solution at 45-60º and 20-30 cm depth using approximately 1000L water/ha (trials 1 and 2). The second rig had 2 tynes with injection hoses spaced 50 cm apart, the two hoses with nozzles injecting DADS also at 45-60º and 20-30 cm depth using approximately 500L water/ha (trials 3 and 4). The rigs had rollers which sealed the soil immediately after injecting DADS which was followed by 30 min of irrigation with sprinklers. The soil remained undisturbed for 3 months. No crops were grown in the field sites between DADS applications and the spring onion crop subsequently grown was destroyed.

Trials 2, 3 and 4 were arranged in randomized complete block designs with 5 and 6 replications. Trial 1 was arranged in a split-plot design with five main treatments (DADS rates and applications plus untreated check) and six sub-treatments (untreated check and fungicides and biological treatments). Details of the trial sites and dates of sowing and application of treatments and cultivars are included in Table 2. DADS treatments were applied to replicated plots approximately 10 m long x 14 m wide (trial 1), 10 m long x 3 m wide (trials 2 and 3) and 5 m long x 3 m wide (trial 4). All replicated plots were separated by a minimum of 0.5 m. Spring onions were sown in the fields 4 weeks (trials 1 and 2) and 6 months (trials 3 and 4) after the last application of DADS. Plots (beds) were prepared/hilled within the replicated plots untreated and treated with DADS applications. Each plot consisted of a single bed (1 x 10 m trial 1; 1 x 5 m trials 2 and 3; 1 x 2.5 trial 4) of spring onions separated by 0.5 m bare soil between plots. Each plot contained 3 rows of spring onions c. 1 cm apart. Beds were 1 m wide. Seed of cultivar 'Paragon' were planted with a GoldAcre precision seeder using approximately 10 k/ha of seed. Seed was planted on raised beds (0.3-0.4 m height) with plant densities ranging from 100-120 plants/linear meter. Slow release fertiliser (NPK) was applied when seedlings were 10 cm height and later when required. Two applications of composted chicken manure were applied as topping (approximately 10 t/ha) after sowing and repeated 5-6 weeks later. The manure was applied onto beds using a farm spreader.

Prills with spores of the biological control agent *Trichoderma atroviride* C52 (10^6 cfu/g prills, 50kg/ha, Trichopel Ali 52®) were applied below the seed at sowing with a modified seeder (trial 1) which delivered approximately 50 kg of prills/ha. A follow-up application of *Trichoderma* was applied 5 weeks after

sowing using a wettable powder formulation of *T. atroviride* C52 (10^8 cfu/g, Trichoflow Ali 52®). The solution was applied with a handheld sprayer (10L water) across spring onion rows using 300-400L of water/ha. Fungicide treatments were applied with a knapsack (1000L water/ha) in a band 15cm across the furrow with seed and plant rows. The first spray was applied after sowing (soil surface spray) and the second four weeks later (stem base/foliar sprays). Recommended control procedures for other fungal pathogens, weeds and insects were implemented by the growers. Irrigation was applied by growers as required.

Measurements

Soil temperatures were measured with Tinytalk temperature loggers (Orion Group) buried at two depths (10 and 15 cm) in one plot in the middle of the trial. Measurements were taken at hourly intervals from the time of sowing through to harvest. Soil temperatures when DADS was injected into soil ranged from 13-18ºC and remained between 10 and 20ºC for six weeks after application.

Density of sclerotia of *S. cepivorum* was determined from soil samples taken at 10 and 20 cm depths prior to sowing (trials 1, 2 and 3). Three soil sub-samples were taken arbitrarily along each of four plots per treatment at each site. Each soil sub-sample consisted of 100 g of soil taken from each of the two depths. The six soil sub-samples from each replicate were mixed and then a sample of soil from this composite soil sample used for counting sclerotia. Sclerotia were extracted from the soil using the wet-soil sieving method. For trial 1, a total of 300 g of soil were washed per each of four replicates. For trial 2 and 3, a total of 200 g of soil were washed per each of four replicates. Sclerotia recovered from soil were surface sterilised (3 min in 50:50 v/v 70% ethanol and 1.25% sodium hypochlorite followed by 3 min in sterile water) blotted dry and place on droplets of potato dextrose agar to test their viability. Sclerotia that germinated 3 weeks after plating and formed mycelial colonies and sclerotia were considered viable.The composite soil samples were also used to determine soil pH.

In trial 1, three soil samples were collected to determine the density of *Trichoderma* in soil at 3 and 6 weeks after sowing. Four soil sub-samples were collected from the furrow (4-8cm) with young plants and roots using a core borer along each of 4 plots per treatment. The soil sub-samples from each plot were mixed and 10 g of the composite sample used to determine *Trichoderma* colony levels (2 cfu counts per sample) using a standard dilution plating method and *Trichoderma* selective medium. Characteristic colonies of *Trichoderma* were counted 2 and 3 weeks after plating out.

Emergence counts were made in all plots 2-3 weeks after sowing. At fortnightly intervals thereafter, all plots were inspected and plants infected with white rot counted in a square metre/plot without removing infected plants. At harvest, the number of plants infected with white rot was counted in a square meter in each plot and yields measured (number of marketable bunches, fresh weights, bunch length) approximately 12-14 weeks after sowing. The plants infected with white rot were expressed as a percentage of plants with white rot.

Statistical Analysis

Analysis of variance (ANOVA) was used to analyse the data for all trials using Genstat v6. Data for trial 1 were analysed as a split-plot design. Data were transformed before analysis when required to stabilise the variance. The appropriateness of an ANOVA for the data was checked by visual inspection of residual plots. Where significant main or interaction effects were found then the mean values for treatments in each experiment were compared by Fisher's protected LSD tests (P = 0.05).

Results

Trial 1 Autumn-Winter 2003

The mean number of sclerotia recovered from soils of plots treated with one or two applications of DADS is presented in Table 3. About 70-83% of sclerotia recovered from soils were viable and the rest dead or contaminated by soil bacteria or fungi mainly *Penicillium* species. The mean number of sclerotia recovered from plots treated with two applications of both rates of DADS tested were significantly lower than those recovered from plots treated with a single application of both rates of DADS and untreated. The number of sclerotia recovered from plots treated with two applications of both rates of DADS ranged from 0 to 1.4 sclerotia per kg of dry soil compared to 11 to 21 in plots treated with a single application of DADS and 51.7 in untreated plots.

Trichoderma species were not detected in soil from untreated plots (Table 4). In soils from plots treated with Trichopel Ali 52 at sowing, the mean number of *T. atroviride* C52 cfu per g of sandy soil ranged from 3.7×10^5 to 5.9×10^5 and 7.3×10^3 to 6.5×10^4 at 3 and 6 weeks after application, respectively. There were no significant differences between levels of *Trichoderma* in soils treated with Trichopel Ali 52 at sowing and soils treated with both Trichopel Ali 52 at sowing followed by Trichoflow Ali 52 (drench) 4-weeks after sowing.

Plants in all plots emerged relatively evenly. There were no obvious symptoms of phytotoxicity in plots treated with DADS, boscalid, procymidone and Trichoderma treatments. Treatment and sub-treatment means for percentage of plants with white rot are presented in Table 5. At harvest, untreated plots had a mean incidence of white rot of 34.4% (range 23-75%). There was a significant difference between the plus and minus DADS main treatments. The mean percentage of plants infected with white rot for all plots treated with the two rates and two applications of DADS ranged from 0.8% to 6% compared to 14.1% in the untreated plots. There was a significant interaction between DADS application and the sub-treatments indicating that the treatment means needed to be compared separately for plots plus and minus DADS

All plots treated with DADS treatments had significantly lower percentage of infected plants than the untreated control when comparing plots with or without DADS across all main treatments. Two applications of either 5 or 10L/ha of DADS were more effective than single treatments of both rates of DADS in reducing the incidence of white rot. These treatments reduced the percentage of plants infected with white rot from 34.4% to 5.9 (10L/ha) and to 2.6% (5L/ha). When comparing sub-treatments within main treatments, two appropriately applied and timed applications of boscalid and procymidone, applied after sowing as soil surface spray and repeated 4-weeks later as stem base/foliar spray, were both equally very effective in controlling white rot in plots without DADS (34.4% disease). These treatments provided almost complete control of disease.When fungicide treatments were applied to soil/plants in plots treated with single or dual applications of both rates of DADS, the combined treatments also provided almost complete control of disease. These combined treatments significantly reduced disease levels when compared to plots that had DADS only, indicating that fungicide treatments reduced disease levels further. Best results were obtained in plots treated with dual application of DADS in combination with fungicide treatments.

The biocontrol treatments were not effective in reducing disease in plots without DADS treatments. However, when the biocontrol was applied to plots treated with all DADS treatments the combined treatments significantly reduced disease levels when compared to plots that had DADS only, also indicating that biocontrol treatments contributed to further reductions in disease levels. The best combined biocontrol-DADS treatments were those involving single or dual applications of 10L/ha of DADS followed by Trichopel Ali 52 at sowing and Trichoflow Ali 52 (drench) 4-weeks after sowing and single or dual applications of 5L/ha of DADS followed by Trichopel Ali 52 at sowing.

Treatment and sub-treatment means for yields (number marketable bunches and fresh weights) of spring onions are presented in Tables 6 and 7. At harvest, untreated plots had a mean number of marketable bunches of 10.7 per square metre. There was a significant difference between the plus and minus DADS main treatments for number of bunches. The mean number of marketable bunches for all plots treated with the two rates and two applications of DADS ranged from 13.7 to 15.5 bunches compared to 12.3 bunches

in the untreated plots. There was no significant interaction between DADS applications and the sub-treatments. In plots minus DADS, the best yields were those from plots treated with fungicide treatments (13-16 bunches/m^2). In plots plus 5L/ha of DADS, the best yields were those from plots treated with 2 sprays of procymidone or boscalid and 1 spray of procymidone or 2 sprays of boscalid for single and dual applications of DADS, respectively. In plots plus 10L/ha of DADS, there were no significant differences in yield between plots treated with DADS and DADS plus fungicide treatments.

Trial 2 Autumn 2003

The mean number of sclerotia recovered from soils of plots treated with two applications of DADS is presented in Table 3. The mean number of sclerotia recovered from plots treated with two applications of both rates of DADS tested were significantly lower than those recovered from untreated plots. The number of sclerotia recovered from DADS treated plots ranged from 30-45 sclerotia per kg of dry soil compared to 131.3 in untreated plots.

Plants in all plots also emerged relatively evenly. There were no obvious symptoms of phytotoxicity in plots treated with procymidone and DADS treatments. Means for percentage of plants with white rot are presented in Table 8. At harvest, disease in untreated plots was relatively low 12.9% (range 7-27%). The mean percentage of plants infected with white rot for all plots treated with two applications of DADS was 7.3% (5L/ha) and 3.9% (10L/ha) when compared to 12.9% in the untreated plots. The fungicide procymidone alone did not provide effective control of white rot at this field site. The best treatment was 10L/ha of DADS in combination with 2 sprays of procymidone.

There were no significant differences in yields (bunch numbers and bunch weight and length) between all treatments.

Trial 3 Autumn 2005

Levels of sclerotia recovered from soil samples collected from plots in this trial were low and variable and therefore could not be analysed (data not shown).

Plants in all plots also emerged relatively evenly. There were no obvious symptoms of phytotoxicity in plots treated with boscalid and DADS treatments. Means for percentage of plants with white rot and yield are presented in Table 9. At harvest, the mean percentage of plants infected with white rot was 20.1% (range 10-31%). The mean percentage of plants infected with white rot for plots treated with two applications of DADS at the rate of 5L/ha was 2.6% compared to 20.1% in the untreated plots. The DADS treatment resulted in a significant reduction in disease in the order of 87% of that of the untreated control. An experimental formulation of garlic juice tested at the rate of 5 and 10L/ha was not effective in reducing disease at this field site. A single appropriately applied and timed spray of boscalid provided complete disease control.

The number of bunches of spring onions for all the plots treated with DADS and fungicide treatments were significantly higher than those of the untreated control.

Trial 4 Autumn 2005

Disease levels were too low at this field site to allow comparison of treatments (Table 10).

Discussion

Commercially acceptable levels of white rot control were obtained by treating naturally infested fields sites with synthetic DADS during two seasons (Autumn and Spring) for one year prior to sowing spring onions on sandy soils. In two of three field sites, two applications of either 5 or 10L/ha of DADS were more effective than single applications of both rates in reducing the population of sclerotia in soil and white rot incidence at harvest. In the first site, a single application of DADS only provided disease reductions of about 49% (10L/ha) and 73% (5L/ha) compared to 82% (10L/ha) and 92% (5L/ha) obtained with dual applications, with corresponding yield increases. In the second field site, two applications of DADS (5L/ha) provided 87% of disease reduction. These results are consistent with reports that DADS (synthetic) soil treatments reduced the number of sclerotia in soil and incidence of white rot on garlic and onion crops (Coley-Smith JR, Parfitt D 1986, Dennis 1997, Hovious and McDonald). Both rates of DADS tested (5L/ha and 10L/ha) in our trials appeared to be equally effective in reducing the population of sclerotia in soil and incidence of white rot in sandy soils. In the third field site, two soil applications with the same rates of DADS did not provide the same levels of inoculum and disease reductions observed in the other two field sites. At this site, the first application of DADS occurred only 6 months after the last spring onion crop was harvested compared to at least 12 months in the other two field sites. It is possible that sclerotia were not fully mature by the time the first DADS treatment was applied and consequently the second application was not sufficient to promote germination of a large population of sclerotia present in the soil.

The number of viable *S. cepivorum* sclerotia per kilogram of soil ranged from 51 to 131 in most heavily infested sandy soils in commercial fields in the south of Melbourne. Crowe et al. (1980) found that sclerotial populations of over 1.5 sclerotia/kg soil could cause 40% disease on garlic and Tichelaar (1965) found that populations of 2-30 sclerotia/kg of mineral soil resulted in 25-40% infection. In the sandy soils, populations of 51 and 131 sclerotia/kg of soil (viability about 70-83%) caused 34.4% and 12.9% infection on short-season bunching onion crops. Utkhede et al. (1978) found that there was no clear relationship between population of sclerotia in soil and incidence of white rot on onion in Canada. This seems to be the case in at least one trial reported here. Some of the variability in disease levels that developed may have resulted from changes in weather and soil conditions and in the case of spring onions on the addition of nitrogenous amendments (composted chicken manure) at different times during the crops which could have inhibited the development of disease. This aspect of the research needs more detailed examination.

Two rates of organic garlic juice (formulated for foliar applications) tested were not effective in reducing white rot in sandy soils. This product has only 8.7% of diallyl disulphide and the rest are related sulphides (dially sulphide, 1.7%; diallyl trisulphide 33.9%, allyl-methyl trisulphide 5.7%, diallyl tetratsulphide 31.3). More work is required with this product to determine appropriate field rates to promote effective germination of sclerotia of *S. cepivorum* and its persistence in different soils.

Two appropriately applied and timed applications of procymidone were very effective in controlling white rot, in plots without DADS treatments, only at one of two field sites. On the other hand, the new fungicide boscalid provided consistently effective control of disease at two field sites. At these two field sites, when this efficacious new fungicide was applied to plots treated with either one or two pre-planting applications of DADS, it provided almost complete disease control. At one field site, the biological control agent *T. atroviride* C52, applied to untreated soil at sowing below the seed (Trichopel Ali 52) was not able to protect the roots of plants against infection throughout the growing season. Levels of *T. atroviride* C52 detected in the soil indicated that the density of this biocontrol agent was probably highest during the first 3-5 weeks of the growing season and therefore it could not effectively protect the crop against white rot infection in late season in a high disease site. When *T. atroviride* C52 was applied at sowing to soil treated with the different DADS treatments, the biocontrol was able to provide more effective disease control because disease pressure was reduced by DADS treatments. Supplementing the biocontrol application at sowing (Trichopel Ali 52) with a post-planting application (Trichoflow Ali 52) 4-weeks after sowing did not appear to increase the density of *Trichoderma* in soil or provide consistent reductions in disease levels when compared to single applications at sowing. In New Zealand, *T. atroviride* C52 (Trichopel Ali 52) provided control of white rot on onions equivalent to the chemicals under low and moderate (<40%) disease pressure, but under high disease conditions, efficacy was lower (McLean *et al.* 2002). This and our

results highlight that need to integrate Trichopel Ali 52 with other existing disease management strategies for successful control of onion white rot.

The results reported here demonstrate that synthetic DADS was an effective soil treatment to reduce sclerotial populations in soils prior to planting and white rot incidence on spring onion crops in sandy soils. Two applications of DADS, applied when soil temperatures are between 13-18ºC, will be required to obtain effective control of white rot in high-disease sites because of the persistence of DADS in soil and the staggered germination of sclerotia. In southern-Australia, the optimal soil temperatures for application of DADS occur during Autumn and Spring and in southern Queensland during Winter. DADS should be applied to soil at field capacity at the depth of 15-25 cm. After application, the soil should be rolled and if possible irrigation applied to form a seal to prevent escape of DADS outside the soil. A sensible integrated strategy for effective and sustainable control of white rot on bunching onions will be to use two pre-planting treatments with 5L/ha of DADS combined with either one or two appropriately applied and timed sprays of boscalid or with *T. atroviride* C52 (Trichopel Ali 52), applied at sowing, supplemented with a single post-planting application of boscalid. Efficacy data collected will be used to support minor permits or registration of synthetic DADS in Australia. At present, the use of synthetic DADS in Australia is being prevented by a costly registration process, a reliable supply of good quality product and cost of treatments.

Further research is needed to determine the effectiveness and soil persistence of synthetic DADS treatments under high disease pressure on different soils types and the lowest effective dosage for promoting germination of sclerotia as well as it efficacy against other soilborne pathogens. Future research should focus also on evaluating alternative soil treatments to synthetic DADS including products containing organic DADS and biofumigants for white rot control in Australia.

References

Basallote-Ureba MJ, Melero-Vara JM (1993). Control of garlic white rot by soil solarisation. Crop Protection 12, 219-223.

Coley-Smith JR, Parfitt D (1986). Some effects of diallyl disulphide on sclerotia of *Sclerotium cepivorum*: possible novel control method for white rot disease of onion. Pesticide Science 37, 587-594.

Crowe F, Hall DH (1980). Soil temperature and moisture effects on sclerotia germination and infection of onion seedlings by *Sclerotium cepivorum*. Phytopathology, 70, 74-78.

Dennis JJC (1997). Progress towards an integrated control strategy for onion white root rot disease, including the use of artificial germination stimulants. 2nd International Symposium on edible Alliaceae, November 1997 pp39 Adelaide, Australia.

Hovius MHY, McDonald MR (2002). Management of *Allium* white rot [*Sclerotium cepivorum*] in onions on organic soil with soil-applied diallyl disulphide and di-N-propyl disulphide. Canadian Journal of Plant Pathology, 24, 281-286.

McLean, K.L., Swaminathan, J., Hunt, J.S. and Stewart, A. (2002). Biological control of onion white rot in New Zealand. In, "Proceedings Seventh International Workshop on *Allium* White Rot, Coalinga, California, USA", (ed. M. R. MacDonald), 4-8th June 2002, pp. in press.

Merriman PR, Isaacs S, MacGregor RR, Towers B (1980). Control of white rot in dry bulb onions with artificial onion oil. Annals of Applied Biology, 96, 163-168.

Perez-Moreno L, Sanchez-Pale JR, Entwistle AR (1996). Effects of metham sodium, diallyl disulphide and thiocyanomethylbenzothiasol on *Sclerotium cepivorum* sclerotia in the Bajio region of mexico. Annals of applied Biology 128(suppl), 6-7.

Porter IJ, Merriman PR (1985). Evaluation of soil solarization for control of root diseases of row crops in Victoria. Plant Pathology 34, 108-118.

Stewart A, Tyson JL, Fullerton RA, Elliott GS (2002). Integrated disease management of onion white rot in New Zealand, 2nd ASDS Geelong 2002, pp105.

Tichelaar GM (1965). Witrot in zaai-uien. Groenten Fruit, 16, 2107.

Utkhede RS, Rahe JE, Ormrod DJ (1978). Occurrence of *Sclerotium cepivorum* sclerotia in commercial onion farms soils in relation to disease development. Plant Disease Reports, 62, 1030-1034.

Table 1. Treatments applied in field trials for the control of onion white rot (*Sclerotium cepivorum*) of spring onions.

Treatment	Rate	Application method	Water volume L/ha
Untreated control	-	-	-
78% diallyl disulphide (Alli-UP®, Unite Agri Products, USA)	5 and 10L/ha	Injected into soil (2 modified MS rigs used)	500 and 1000L/ha
procymidone(Sumisclex™, Sumitomo)	1000g a.i./ha	soil surface and stem base/foliar	1000L
boscalid (Filan™, BASF - NuFarm)	500g a.i./ha	soil surface and stem base/foliar	1000L
T. atroviride C52 (Trichopel Ali 52™)	50 kg of prills per ha	Prills applied with modified seeder below seed	-
Garlic granules (food grade)	100g/m^2	Spread by hand and rotary hoed into soil	-
Garlic oil (food grade)	Adjusted to 78% DADS	Injected into soil (modified MS rig)	500L/ha
Garlic Juice (ECOGuard®, EcoSpray Ltd, Kendon UK)	5 and 10L/ha	Injected into soil (modified MS rig)	500L/ha

Table 2. Trials Summaries

Activity	Trial 1	Trial 2
Year	2002-2003	2002-2003
Location	Clyde	Cranbourne
Soil type	Sand clay loam	Sand loam
Spring onion variety	Paragon	Paragon
DADS application(s)	06/09/02, 25/02/03	06/09/02, 25/02/03
Replicates	5	6
Fungicide application dates	21/04/03, 25/05/03	13/04/03, 23/05/03
Sowing date(s)	17/04/03	10/04/03
Harvest date(s)	13/08/03	17/08/03

Activity	Trial 3	Trial 4
Year	2004-2005	2004-2005
Location	Cranbourne	Heatherton
Soil type	Sand loam	Sandy clay loam
Spring onion variety	Paragon	Broad Leaf
DADS application(s)	19/03/04, 21/09/04	15/03/04, 17/09/04
Replicates	5	6
Fungicide application dates	31/03/05	
Sowing date(s)	05/03/05	04/04/05
Harvest date(s)	26/06/05	06/04/05

Table 3. Trials 1 and 2 - Mean number of sclerotia per kg of dry soil in plots treated with single or dual applications of DADS in two field trials at Cranbourne Victoria 2002-2003. Seventy five% of sclerotia recovered from soils were viable. Statistical analysis was based on log-transformed data (parenthesis).

Treatment	Trial 1		Trial 2	
	No. Sclerotia kg dry soil	SEM (natural)	No. Sclerotia kg dry soil	SEM (natural)
Control	51.7 (3.94)a	6.6	131.3(4.74)a	37.7
DADS 5 L/ha one application	11.4 (2.37)b	4.1	-	-
DADS 10 L/ha one application	21.6 (2.16)b	15.6	-	-
DADS 5 L/ha two applications	0.0 (0.00)c	0.0	45.5(3.70)b	9.3
DADS 10 L/ha two applications	1.4 (0.47)c	1.4	30.5(3.26)b	9.4
$LSD^{0.05}$ (P value)	1.3 (<0.001)		1.0 (0.032)	

Mean values followed by the same letter do not differ significantly according to LSD value (P=0.05).

Table 4. Trial 1 - Mean number of *T. atroviride* C52 colony forming units per gram of soil at 3 and 6 weeks after application in a sandy loam soil in a trial conducted during Autumn 2003 at Cranbourne, Victoria. Prills (Trichopel Ali 52) had 1.1 x 10^6 cfu of *T. atroviride* C52 per g of prills at sowing and Trichoflow Ali 52 1.8 x 10^8 cfu per g of wettable powder.

Treatment	*Trichoderma*cfu/g soil	
	weeks after planting	
	3	6
untreated control	0.0	0.0
T. atroviride C52 50kg/ha (prill)	3.7 x 10^5a	7.3 x 10^3a
T. atroviride C52 50kg/ha prills + Trichoflow Ali C52	5.9 x 10^5a	6.5 x 10^4a

Mean values followed by the same letter do not differ significantly according to LSD value (P=0.05).

Table 5. Trial 1 - Mean percentage of spring onion plants infected with white rot at harvest in a trial conducted during 2002-2003 at Cranbourne, Victoria. Statistical analysis is based on square root-transformed data (values are shown in parentheses). Sub-treatments means (fungicide, untreated, *Trichoderma*) are shown for plots treated with different applications and rates/ha of DADS soil treatments.

	Treatment[1]				
Sub-treatment	**untreated**	**DADS 5L/ha single**	**DADS 5L/ha dual**	**DADS 10L/ha single**	**DADS 10L/ha dual**
1 untreated control	34.4 (5.46)a[2]	9.32 (2.51)a	2.62 (1.18)a	17.7 (3.33)a	5.94 (2.40)a
2 procymidone 1 spray	0.45 (0.56)c	0.11 (0.17)c	0.38 (0.44)bc	0.11 (0.17)c	0.08 (0.14)c
3 procymidone 2 sprays	0.09 (0.15)c	0.57 (0.49)c	0.10 (0.16)c	0.61 (0.55)c	0.12 (0.17)c
4 boscalid 2 sprays	1.63 (0.84)c	0.23 (0.24)c	0.28 (0.36)c	0.42 (0.43)c	0.18 (0.21)c
5 *Trichoderma* C52 x1	20.8 (4.13)b	2.67 (1.30)b	0.28 (0.37)c	11.4 (2.38)b	4.43 (1.51)b
6 *Trichoderma* C52 x2	27.3 (4.92)a	5.09 (2.12)a	1.31 (0.98)ab	6.07 (1.83)b	0.59 (0.54)a
Average (treatment)	14.1 (2.68)	3.0 (1.14)	0.8 (0.58)	6.0 (1.45)	1.9 (0.83)
$LSD^{0.05}$ ($P < 0.001$)[3]	0.69	0.69	0.69	0.69	0.69
$LSD^{0.05}$ ($P < 0.001$)[4]	0.78 (treatment)				
($P = 0.05$)[5]	treatment x sub-treatment				

[1] Treatments with different letters are significantly different (P=0.05). Two rates of DADS applied into soil as a single (Spring 2002) and twin applications (Spring 2002 and Autumn 2003) of DADS.
[2] These letters show the differences between the sub-treatments within each the main treatments.
[3] The LSD value is the same for comparing mean percentage of plants with white rot between the sub-treatments within the untreated and DADS main treatments.
[4] The LSD value is for comparing mean percentage of plants with white rot between main treatments.
5 Analysis of interactions based on log_{10}-transformed data.

Table 6. Trial 1 - Mean number of marketable bunches of spring onion plants at harvest in a trial conducted during 2002-2003 at Cranbourne, Victoria. Sub-treatments means (fungicide, untreated, *Trichoderma*) are shown for plots treated with different applications and rates/ha of DADS soil treatments.

	Treatment[1]				
Sub-treatment	**untreated**	**DADS 5L/ha single**	**DADS 5L/ha dual**	**DADS 10L/ha single**	**DADS 10L/ha dual**
1 untreated control	10.7a[2]	14.5a	13.0a	13.5a	16.3a
2 procymidone 1 spray	13.0b	14.5a	16.0b	14.2a	16.8a
3 procymidone 2 sprays	16.0c	17.8b	14.7a	15.5b	15.3a
4 boscalid 2 sprays	12.0ab	17.5b	18.5c	16.5b	16.5a
5 *Trichoderma* C52 x1	11.5a	14.5a	15.3b	11.7a	12.5b
6 *Trichoderma* C52 x2	10.5a	14.2a	14.5a	12.8a	15.3b
Average (treatments)	12.3	15.5	15.3	13.7	15.4
$LSD^{0.05}$ ($P < 0.05$)[3]	1.9	1.9	1.9	1.9	1.9
$LSD^{0.05}$ ($P < 0.001$)[4]	1.84 (treatment)				
($P = 0.70$)	treatment x sub-treatment				

[1] Treatments with different letters are significantly different (P=0.05). Two rates of DADS applied into soil as a single (Spring 2002) and twin applications (Spring 2002 and Autumn 2003) of DADS.
[2] These letters show the differences between the sub-treatments within each of the main treatments.
[3] The LSD value is the same for comparing mean number of marketable bunches between the sub-treatments within the untreated and DADS main treatments.
[4] The LSD value is for comparing mean number of marketable bunches between main treatments.
There were not significant differences in bunch length between treatments or sub-treatments (data not shown); ranged from 58-60cm.

Table 7. Trial 1 - Fresh weight (kg) of marketable bunches of spring onion plants per plot at harvest. Sub-treatments means are shown for plots treated with different applications and rates/ha of DADS soil treatments.

	Treatment[1]				
Sub-treatment	**untreated**	**DADS 5L/ha single**	**DADS 5L/ha dual**	**DADS 10L/ha single**	**DADS 10L/ha dual**
1 untreated control	2.45a[2]	3.18a	3.22a	3.27a	3.78a
2 procymidone 1 spray	3.04b	3.22a	3.94b	3.35a	3.80a
3 procymidone 2 sprays	3.62c	3.77b	3.09a	3.71a	3.36a
4 boscalid 2 sprays	2.63a	3.93b	4.23c	3.82b	3.96a
5 *Trichoderma* C52 x1	2.67a	3.45a	3.37a	2.87b	2.74b
6 *Trichoderma* C52 x2	2.35a	3.47a	3.68b	2.75b	3.66a
Average	2.79	3.50	3.59	3.29	3.55
$LSD^{0.05}$ ($P < 0.05$)[3]	0.44	0.44	0.44	0.44	0.44
$LSD^{0.05}$ ($P < 0.001$)[4]	0.45 (treatment)				
(P = 0.60)	treatment x sub-treatment				

[1] Treatments with different letters are significantly different (P=0.05). Two rates of DADS applied into soil as a single (Spring 2002) and twin applications (Spring 2002 and Autumn 2003) of DADS.
[2] These letters show the differences between the sub-treatments within each of the main treatments.
[3] The LSD value is the same for comparing mean fresh weight of bunches between the sub-treatments within the untreated and DADS treatments.
[4] The LSD value is for comparing mean fresh weight of bunches between main treatments.

Table 8. Trial 2 - Effect of DADS treatments alone and combined with fungicide on white rot and yields of spring onions grown during Autumn 2003 in a trial conducted during 2002-2003 at Cranbourne,Victoria. Statistical analysis for disease incidence is based on angular-transformed data (shown in parenthesis).

Treatment	% plants with white rot	Bunches (no/m^2)	Weight (kg/m2)	Length (cm/bunch)
untreated control	12.9 (20.3)a	14.3	3.0	58.0
procymidone 2 sprays	10.0 (16.6)ab	15.8	3.5	59.1
DADS 5L/ha dual	7.3 (14.7)ab	16.1	3.5	59.2
DADS 5L/ha dual+2 procymidone sprays	3.5 (9.1)b	17.3	3.8	60.0
DADS 10L/ha dual	3.9 (10.8)b	17.3	3.8	59.3
DADS 10L/ha dual+2 procymidone sprays	1.5 (6.4)b	17.2	3.7	58.3
$LSD^{0.05}$ (P value)	6.5 (0.001)	ns (0.245)	ns (0.304)	ns (0.714)

Treatments with different letters are significantly different (P=0.05)

Table 9. Trial 3 Effect of DADS treatments alone and combined with fungicide on white rot and yield of spring onions grown during Autumn 2005 in a trial conducted during 2004-2005 at Cranbourne, Victoria.

Treatment	% plants with white rot	SEM	Bunches (no/m^2)	SEM
untreated control	20.1a	4.6	15.0a	0.7
Garlic juice (EcoGuard™) 10L/ha dual	15.0a	4.4	16.2a	1.4
Garlic juice (EcoGuard™) 5L/ha dual	11.9a	2.6	15.2a	0.5
DADS 5L/ha dual	2.6b	1.2	17.6b	1.0
DADS 5L/ha dual+1 spray boscalid	0.2b	0.2	19.8bc	1.0
boscalid 1 spray (stem-base/foliar)	0.0b	0.0	20.0c	0.8
$LSD^{0.05}$ (P value)	8.35 (<0.001)		2.20 (0.003)	

[1] Treatments with different letters are significantly different (P=0.05)

Table 10. Trial 4. - Effect of DADS treatments on white rot of spring onions grown during Autumn 2005 in a trial conducted during 2004-2005 at Cranbourne, Victoria.

Treatment	% plants with white rot
untreated control	6.5
Garlic juice (EcoGuard™, adjusted to 78% DADS)	3.2
DADS 5L/ha 2 applications	4.6
DADS 10L/ha 2 applications	6.0
Garlic Granules (100g/square metre, two applications)	4.1
Organic Oil (adjusted to 78%, two applications)	3.5

4 General Discussion

This project identified new chemical and biological treatments, and methods for their application, that will help vegetable growers to control onion white rot of bunching onion crops in their farms. The new fungicide Filan™ (boscalid, chemical group G), applied as a soil surface and stem base/foliar sprays, provided reliable effective control of white rot at all high disease field sites. Filan™ is therefore a suitable replacement for procymidone, suspended by APVMA in 2004, for the control of white rot on bunching onion crops. Folicur™ applied as a single soil surface application after sowing also consistently controlled white rot on 12-14-weeks old spring onion crops. The use of Folicur™ will be limited to one application per crop due to its withholding period of 90 days. This fungicide could be used in bunching onion crops grown for at least 14 weeks, typical of crops sown in Winter and harvested in late Spring in Victoria. Amistar™ and Bayfidan™ applied to emerged plants four andsix weeks after sowing were more effective than procymidone treatments in controlling white rot.

Filan™ is the only new fungicide produced in the last 10-15 years with excellent activity against the white rot pathogen. Although Filan™ is highly effective in controlling white rot, its long-term field performance is unknown. Therefore it would be sensible to restrict the use of Filan™ to strategic applications during the growing season when disease risk is high and integrated with other fungicides from different chemical groups to ensure its field efficacy is not lost too soon due to overuse. For instance, Amistar™ and Bayfidan™ could be used in combination with one early season application of Filan™ or Folicur™ to extend the level of disease protection until harvest at high disease field sites. Proper application of fungicides strategically placed in the root zone and base of plants with the right volume of water and at the right time is vital for effective disease control.

The biological control *T. atroviride* C52 applied on formulated prills (Trichopel Ali 52™) in-furrow at sowing showed promise for protecting the roots of growing plants against white rot infection at low disease pressure sites and in soils treated with DADS (<11% plants diseased). *Trichoderma* treatments on their own may not provide acceptable disease control in high-disease sites. Trichopel Ali 52™ therefore needs to be integrated with post-planting application of fungicide (eg Filan™) to ensure effective control of disease is obtained throughout the growing season. Consequently, a sensible strategy would be to apply Trichopel Ali 52™ at sowing to protect against early season infection but to supplement this with 1-2 foliar applications of fungicides, when required, to suppress late season infections. Levels of *Trichoderma* C52 measured in sandy soils were probably below the optimal levels required for effective biocontrol. Future research should therefore investigate the rhizosphere competence and population dynamics of *Trichoderma* in soils, especially in sandy soils to determine how depth *Trichoderma* grows and the effect of frequent irrigation on spore and propagules retention in soil. This research should identify means of modifying the sandy soil around the root zone to increase the levels of *Trichoderm*a colonisation and biocontrol.

Synthetic diallyl disulphide or DADS (80% diallyl disulphide, Alli-Up™) was very effective reducing the population of sclerotia of *S. cepivorum* in soil and disease incidence on spring onion crops. This soil treatment applied before planting can be a cost-effective soil treatment for white rot control on commercial spring and other bunching onion crops. DADS needs to be integrated with other control measures that protect the roots of growing plants from infection to obtain more effective disease control throughout the growing season. For instance, when DADS was combined with correctly applied and timed fungicide treatments (eg Filan™) or early season applications of the biocontrol agent *T. atroviride* (Trichople Ali 52™), effective and sustainable control of white rot was obtained. Commercialisation of DADS in Australia is being prevented by a reliable supplier of DADS, cost of treatments and costly registration process. When registered, DADS has the potential to provide an immediate increase in return per hectare but the benefits of the treatment will persist for several cropping seasons. Other chemical treatments readily available (eg dazomet, metham sodium) can reduce the population of sclerotia in soil but these can be too expensive and would require optimisation before their widespread use for white rot control in Australia. Therefore, the development of alternatives soil treatments to synthetic DADS is required to ensure growers have a variety of cost-effective soil treatments available for reducing sclerotial inoculum levels in soils. Biofumigants, organic *Allium* products containing DADS and nitrogenous soil amendments are among the potential soil treatments which have the capacity to kill sclerotia in soil. These also require further development before their widespread use for white rot control in Australia.

In summary, this project has demonstrated that control of white rot on bunching onion crops will be more effective when using an integrated disease management programme that incorporates a combination of different control strategies. These strategies include the use of DADS integrated with the new fungicide Filan™ and well-developed biocontrol treatments such as *T. atroviride* C52 (Trichople Ali 52™). The use of crop rotations with non-Allium crops and green manure crops with biofumigant activity should be encouraged to help growers to prevent the build up of sclerotia of *S. cepivorum* in their farms.

The project also developed valuable information that will assist vegetable growers to improve the time of application of control measures and make informed decisions about use of the biological treatment *Trichoderma* for managing onion white rot with less chemical input and soil health in vegetable farms.
In sandy soils, in-furrow incorporation of pellets containing humic acids (eg Agrolig™, AgChem) will be required to help *Trichoderma* to grow better in these soils with low levels of organic matter. *Trichoderma* growth will be inhibited by nitrogen released from fertilizers and fresh composted chicken manure. Therefore, these materials should not be applied for at least 2-3 weeks before and after sowing to allow *Trichoderma* spores/propagules to germinate and establish in soils. Field rates of Filan™ and low-nitrogen soil amendments can be applied to plots treated with *Trichoderma. Trichoderma* can be applied safely to soils several weeks after DADS was injected into soil.

5. Technology Transfer

Refereed Scientific Papers

1. McLean KL, Stewart A, Villalta O, Wite D, Porter IJ, Hunt J (2005). Optimising *Trichoderma atroviride* C52 for the control of onion white rot on bunching onions. (in prepation)

2. Villalta O, Wite D, Porter IJ, McLean KL, Stewart A, Hunt J (2005). Comparison of chemical and biological methods of controlling onion white rot on bunching onions in Australia. (in preparation)

Conference Abstracts

1. Villalta *et al* (2004) 'Integrated Control of Onion White Rot in Spring Onions'. 3rd Australian Soil Diseases Symposium, Barossa Valley, SA, pp 155-156.
2. Stewart *et al.* (2004) 'Optimising *Trichoderma* bio-inoculants for integrated control of soil-borne diseases', 3rd Australian Soilborne Diseases Symposium, pp 55-56.
3. Villalta et al. (2005) Alternative fungicide treatments to replace procymidone for control of white rot, 15th Australasian Plant Pathology Conference, Geelong September. pp219
4. Villalta et al. (2005) Optimising *Trichoderma* for the management of onion white rot control on spring onions, 15th Australasian Plant Pathology Conference, Geelong September. pp165
5. Villalta et al. (2005) Evaluating *Trichoderma* for integrated control of white rot on spring onions, 15th Australasian Plant Pathology Conference, Geelong September 2005. pp166
6. Villalta *et al* (2005). Evaluation of dially disulphide for integrated control of onion white rot on bunching onions. Australian Vegetable Industry Conference 2006 (submitted)

Industry publication

1. Report for Onion IAC 'Alli-up™ or DADS and possibilities for registration in Australia for the control of onion white rot', November 2004.

Extension articles/materials

1. 'Onion white rot – causing severe yield losses in spring onions'. National Onion Conference held at Yanco N.S.W. June 2002, pp78-80.
2. 'Integrated control for white rot in bunching onions'. Onions Australia vol 19, 2002, pp21.
3. Fact Sheet 'Onion White Rot – Vegetable matters of facts', VegCheque, Number 5, July 2003.
4. 'Progress Towards An Integrated Control Program for Onion White Rot of Spring Onions'. In 'Controlling Diseases of Spring Onions' Booklet, Cranbourne Victoria September 19, 2003.
5. Project Progress Report. Spring Onion Industry (Vic) Steering Committee Meeting, October 20, 2003.
6. Vegetable Matters-of-Facts 'Onion White Rot Control' in Diseases of Bunching Vegetables. In Booklet for seminars at Lonford, Wynyard and Forth Tasmania, February 26-27 2004.
7. Booklet 'Integrated Control Strategy for Onion White Rot Disease in Spring Onions and other Bunching *Allium* Crops' 2004. Results from first two years of research published and distributed nationally to growers, IDOs and industry people.
8. Article 'Research continues on white rot control', Onions Australia, Vol 21 November 2004, pp7-9.
9. Poster, Villalta et al (2004) 'Integrated Control of Onion White Rot in Spring Onions'. Australian Soil Diseases Symposium.
10. Optimising *Trichoderma* for the management of white rot on bunching onions. Poster
11. Evaluating *Trichoderma* for integrated control of white rot on bunching onions. Poster
12. Alternative fungicides to procymidone for control of white rot on bunching onions. Poster
13. Evaluation of diallyl disulphide (DADS) for integrated control of onion white rot on bunching onions. Poster
14. Control of Onion White (Root) Rot on Bunching Onions – Brochure, December 2005.

Grower extension activities / field walks / workshops

1. Seminar and field day notes 'Onion White Rot Project', Cranbourne Victoria 2 July 2002.
2. Seminar and field day notes 'Onion White Rot Project', Rochedale, Brisbane Qld 28 August 2002.
3. Seminar and field day notes 'Controlling Diseases of Spring Onions & Leeks', Cranbourne Victoria February 28, 2003.
4. Seminar and field day notes 'Progress Towards An Integrated Control Program for Onion White Rot of Spring Onions', Cranbourne Victoria September 19, 2003.
5. Seminar 'Onion White Rot Project' presented at the Vegetable Forum attended by HAL and vegetable growers and representatives. DPI Victoria Knoxfield, 12 August 2003.
6. Field trials walks, Cranbourne and Heatherton trials, Victoria 10 and 12 December 2003.
7. Project progress report, presented to Spring Onion Steering Committee, Cranbourne 17 March 2004.
8. Field trials walks, Cranbourne and Heatherton, Victoria 17 and 24 May 2004.
9. Spring Onion Grower Seminar Day, seminars presented by Australian and New Zealand project members. Amstel Golf club, Cranbourne, 17th June 2004.
10. Seminar 'Chemical and biological control of onion white rot in spring onions' presented at the Bunching Vegetable Workshop, Wanneroo, WA, 18 August 2004. Spring onion farms visited.
11. Seminar presented to onion growers attending the Annual Levy Payers Meeting held at Devonport, Tasmania on 15/11/04.
12. Spring onion growers observed the application of DADS treatments at two field sites in Vic (Cranbourne and Heatherton), September 2004.
13. Project progress report, presented to Spring Onion Steering Committee (7th meeting of project), Cranbourne 20/12/2004.
14. Seminar presented at the National Onion White Rot Workshop held at Devonport, Tasmania on March 2005.
15. Field trial walks conducted at field sites in Victoria during May-June 2005.

6 Recommendations

Recommendations to vegetable growers arising from this project have been summarised and distributed to growers nationally in the booklet 'Integrated Control Strategy for Onion White Rot Disease in Spring Onions and other Bunching *Allium* Crops' and the brochure 'Onion White Rot of Bunching Onions'. The publications outline a range of strategies that enable onion white rot to be managed in short-season onion crops in vegetable farms in Australia.

More successful control of white rot will be obtained when using an integrated management strategy that incorporates different treatments, strategies and tactics for disease control. In general, there are three key strategies that growers can use to obtain effective and sustainable control of white rot on bunching onion crops.

1. **The first strategy is to minimise the introduction and spread of the white rot pathogen within and between fields.**

- enforcing on-farm hygiene practices is the responsibility of individual growers.
- the build up of sclerotia of *Sclerotium cepivorum* in soil can be prevented by implementing crop rotations with non-*Allium* vegetable crops and break crops (e.g. green manure crops).

2. **The second strategy involves the use of chemical and biological treatments that protect the roots of growing plants against infection.** This project identified new treatments, and methods for their application, which can be used alone and integrated with other strategies for effective control of white rot.

- The new fungicide Filan™ (group G) can provide excellent control of white rot in high disease field sites. It is therefore a suitable replacement for procymidone, suspended by APVMA in 2004.
- The long-term field performance of Filan™ is unknown. Therefore it would be sensible to restrict its use to strategic applications during the year when disease risk is high (determined by soil temperatures). If possible it should be used integrated with other fungicides from different chemical group to ensure its field efficacy is not lost too soon due to overuse.
- Folicur™ (triazole), Amistar™ (strobilurin) and Bayfidan™ (DMI) showed excellent activity against white rot and therefore they should be used alone when possible or integrated with Filan™.
- The use of Folicur™ could be limited to one application per crop/season due to its withholding period.
- Proper application of fungicides strategically placed into the root zone and base of plants with the right volume of water and at the right time is vital for effective disease control.
- Fungicide residue data collected will support applications for minor use permits for Filan™, Folicur™ and Amistar™ in Australia.
- The biocontrol agent *T. atroviride* C52, applied on formulated prills (Trichopel Ali 52™) in-furrow with the seed at sowing showed promise for providing good early and late-season control of white rot at low disease sites and in soils treated with DADS (<11% plant diseased).
- *T. atroviride* C52 treatments on their own will not provide commercially acceptable disease control in high disease sites. Therefore, a sensible strategy to use would be to apply Trichopel Ali 52™ at planting to protect against early season infection but to supplement this with post-planting applications of fungicides (eg Filan™) to suppress late season infections.

3. **The third strategy is to reduce the population of sclerotia of *S. cepivorum* in soil.** This project developed a strategy, and application methods, for the use of synthetic DADS (80% diallyl disulphide, Alli-Up™) to reduce the population of *S. cepivorum* in soil and disease-pressure in soils used for growing bunching onions.

- Two applications of DADS, applied when soil temperatures are between 10-20ºC (Autumn and Spring in southern Australia; Winter in south Queensland), will be required during the year to obtain commercial levels of disease control.
- DADS treatments need to be integrated with other control measures that protect the roots of growing plants against infection to obtain more successful and sustainable disease control throughout the growing season.
- DADS is a cost-effective soil treatment to eradicate sclerotia in soils and reduce disease incidence on commercial spring onion crops and other *Allium* crops. DADS has the potential to provide an

immediate increase in return per hectare with the benefits of the treatment persisting for several cropping seasons.
- At present, however, commercialisation of DADS in Australia is being prevented by a reliable supplier of synthetic DADS, cost of treatments and a costly registration process.
- Although the soil treatments dazomet and metham sodium are readily available and can reduce the population of sclerotia in soil, methods for their application are not well developed and in most cases they would be too expensive for widespread use for white rot control.

The project developed valuable information that will assist vegetable growers to make informed decisions about the appropriate use of the biological treatment *Trichoderma* for managing white rot and soil health in vegetable farms.
- In sandy soils, for example, the use (in-furrow) of pellets containing humic acids (eg Agrolig™, AgChem) will be required to help *Trichoderma* to grow better in these soils with low levels of organic matter.
- *Trichoderma* growth is inhibited by nitrogen released from fertilizers and fresh composted chicken manure. Therefore, these materials should not be applied for at least 2-3 weeks before and after sowing to allow *Trichoderma* spores/propagules to germinate and establish in soils.
- Field rates of Filan™ and low-nitrogen soil amendments can be applied to soil treated with *Trichoderma.*
- *Trichoderma* can be applied to soils treated with DADS several weeks after it was injected into soil.

In summary, in the short and medium-term, onion white rot can be managed with new fungicide treatments and biological controls, when possible. For the long-term, the challenge remains to secure supply and registration of synthetic DADS for *Allium* industries in Australia. Therefore, the future of onion white rot research in Australia will be directed towards the development of cost-effective soil treatments to eradicate sclerotia of *S. cepivorum* and other important sclerotial pathogens of onions and vegetable crops from soils and development of integrated approaches for sustainable disease control. A new project 'Optimising soil treatments for integrated control of white rot and other diseases' has been developed to address some of these priorities.

Selecting the most cost-effective strategy for a given field still involves considerable guess work to estimate the potential level of disease-pressure in soils based on counts of sclerotia of *Sclerotium cepivorum*, previous cropping history and a range of other environmental factors). Therefore the development of methods to predict inoculum potential in soil (eg using PCR-specific probes for *S. cepivorum*) and the optimal time for fungicide applications (eg based on degree-hours) are two key research priorities. Such methods would enable growers to select the most appropriate and cost-effective control options (eg fungicide *vr* biological) for each field. Other research priorities include:

- demonstration programs in each state to facilitate uptake of effective white rot control strategies.
- investigate rhizosphere competence and population dynamics of *Trichoderma* in soils, especially in sandy soils, to identify means of modifying the soil around the root zone to increase the levels of *Trichoderm*a colonisation and disease protection.

7. Acknowledgments

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

- PIRVic DPI personal Craig Murdoch and Slobovan Vujivic for assistance with technology transfer activities, Peta Easton for technical assistance and Dr. Liz Minchinton for collection of survey data in NSW and SA.
- John Hunt of Agrimm Technologies Ltd for his valuable advice on biocontrol trial preparation and Rob Stanic for arranging supply of Trichopel Ali52 and other products for field trials.
- Doug Wilson and Paul Geister (NuFarm) for advice with fungicides and Elliott Chemical and Serve-Ag Research for supplying DADS for field trials.
- Peter DalSanto (AgAware Consulting) for advice with fungicides and processing minor use permits for fungicide treatments.
- The staff at the PIRVic (Soheir Salib), Department of Primary Industries, Knoxfield for their assistance in establishing and harvesting field trials and reviewing this manuscript.
- The spring onion growers in Victoria who graciously allowed trials on their farms and provided assistance in their establishment, maintenance and harvest.
- Agrochemical companies for providing samples of fungicides and other companies for supplying biological products for laboratory, glasshouse and field work.
- The authors thank the members of the Steering Committe, Rocky Lammatina, Tony Lammatina, Craig Arnott, Karl Raidell and others for their valuable advice to this project.