# Integrated Control Strategy for Onion White Rot Disease in Spring Onions and Other Bunching Allium Crops

Project VG01096 - two years project update research results



Project VG 01096 supported by AusVeg, HAL, DPI Victoria, Lincoln University NZ and Agrimm Technologies Pty Ltd NZ











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### Project VG 01096 'Stop the rot – managing onion white rot in spring onions'

This project began in July 2002. The overall objective of the project is to develop an integrated disease management programme in which chemical and non-chemical (eg biocontrols) control measures are used for a more sustainable control of onion white rot in spring onions (shallots) and other bunching *Allium* crops.

#### Acknowledgments

This project is funded by the National Vegetable Levy (AusVeg), Horticulture Australia (HAL), DPI Victoria, Lincoln University New Zealand and contribution by Agrimm Technology Pty Ltd New Zealand. We thank Soheir Salib for laboratory and field work. We thank Rocky & Tony Lamattina, Peter Butler, Greg Rankin and Shane Osborne (Mulgowie Qld) for allowing field trials on their properties and helping to maintain and harvest the trials. We also thank Elliott Chemicals Limited and Serve-Ag Research for supplying DADS (Alli-up<sup>TM</sup>) and Agrimm Technologies Pty Ltd New Zealand and Rob Stanic for supplying Trichopel Ali52<sup>TM</sup>.

#### Purpose of this publication

This report summarises the key outcomes from laboratory and field trials conducted during the first two years July 2002- July 2004) of project VG 01096 'Stop the rot – managing onion white rot in spring onions'.



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# Onion white rot in spring onions and other *Allium* crops in Australia

#### The onion white rot disease

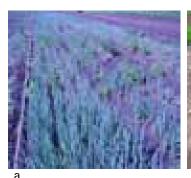
Onion white rot, caused by *Sclerotinia cepivorum*, can result in severe crop losses in Victoria despite the widespread use of fungicide sprays (procymidone). This is because intensive cropping has led to a build up of disease and high levels of inoculum (sclerotia) in the soil.

Sclerotia of the onion white rot pathogen (*S. cepivorum*) develop on infected onions.



### Survey data showed that:

- \* 60% of spring onion farms (30 surveyed) in Victoria have problems with onion white rot and inconsistent fungicide control, with crop losses ranging from 1 to 50%.
- \* The disease is also a problem in farms in southern Qld (Lockyer valley) and NSW.
- \* In the Lockyer Valley and Tasmania, onion (bulb and dry) crop losses to this disease can range from 10-30%.









Onion white rot in spring onions crops at Lockyer Valley Qld (a), NSW (b), Vic (c), and dry onions Lockyer Valley Qld (d)

#### Inconsistent fungicide control

Possible reasons for inconsistent fungicide control include:

- \* incorrect application and timing of fungicide sprays,
- \* low efficacy of fungicides in high disease pressure sites,
- continued use of the same fungicide has increased the populations of soil microorganisms that rapidly degrade the fungicide in soil, and
- \* the onion white rot pathogen can develop resistance to fungicide with overuse.

#### Fungicide resistance tests

Laboratory petri dish assays showed that low rates of the registered fungicide procymidone inhibited mycelial growth of *S. cepivorum* isolates collected from Victorian and Old farms (Lockyer Valley).



Mycelial growth of *S. cepivorum* inhibited by a low concentration of procymidone (left) compared to growth with no fungicide added (right).

#### **Improved Spray Application**

#### Strategic application of fungicide sprays

Effective fungicide application methods, appropriate volume of water and timing of sprays are essential for good control of the onion white rot in spring onions. In-furrow and soil surface spray treatments (eg plant-targeted applications) provide the best pattern and distribution of fungicide treatments for the control of onion white rot (Fig. 1). This strategic application allows the incorporation of fungicide treatments around the root zone where protection is needed.

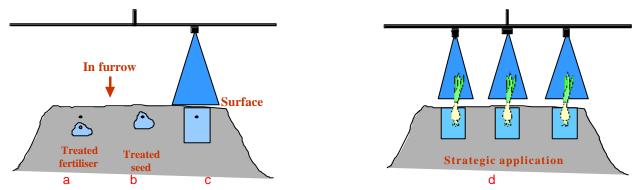
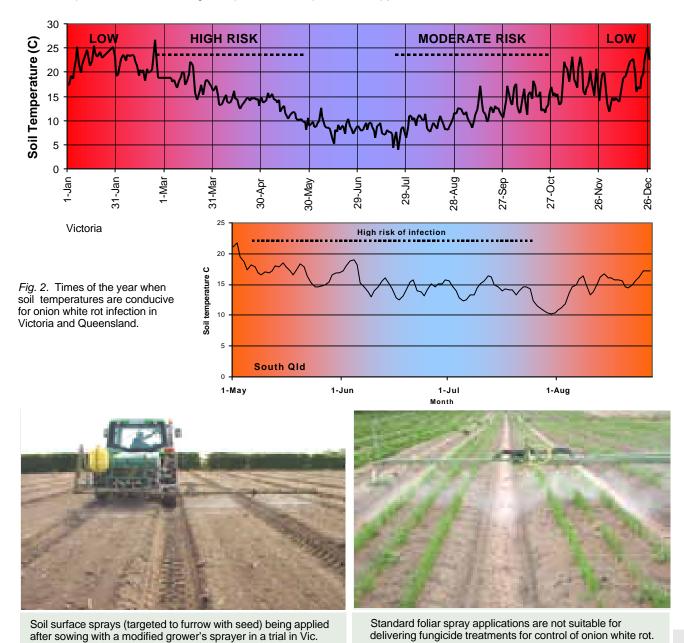


Fig. 1. Strategic application methods evaluated to incorporate fungicide treatments around the root zone where protection is needed.

a) fertiliser treated with fungicide b) seed treated c) soil treated d) plant treated



#### Strategic application of procymidone

A grower's sprayer was adjusted to deliver fungicide sprays onto the closed furrow with seed (soil surface sprays) and spring onion rows (plant-targeted). The modified sprayer was used in three replicated commercial trials to evaluate the effectiveness of procymidone treatments, applied using different product rates and water volumes, for controlling onion white rot in spring (2003) and autumn (2004) crops at Cranbourne Vic. Only one of the three trials had sufficient disease to allow comparison of treatments.



Strategic application of procymidone sprays after sowing

Table 1. Treatments in procymidone trial autumn 2004, Cranbourne Vic.

Fungicide treatment	procymidone Product rate	Spray volume
Untreated		
procymidone	2 L/ha	2000L water/ha
procymidone+azoxystrobin	2 L/ha	2000L water/ha

azoxystrobin applied using 500 L water/ha with the modified sprayer.

procymidone trial details	
Plot size: 1 x 10 m (n=5)	
Sown: 27/2/04	
First spray: 20/03/04	(procymidone)
Second spray: 19/04/04	(procymidone)
Third spray: 07/05/04	(azoxystrobin)
Harvested: 19/5/04	
Cultivar: Paragon	

#### Effect on disease control - Fig. 3:

Two sprays of procymidone, applied at week 3 and week 7 after sowing, significantly reduced disease by 77% when compared to an untreated control. The first spray was applied too late after sowing. This resulted in too many young seedlings becoming infected at a time of the year (early autumn) when seedlings grow fast. The azoxystrobin spray did not significantly reduce disease levels compared to plots treated only with 2 sprays of procymidone.

#### 

Fig. 3. procymidone trial

#### Effect on yield – Fig. 4:

Two sprays of procymidone resulted in significant increases in yield (20-35% or 5-8 more bunches/m²) compared to an untreated control. Yields were not affected by strategic applications of procymidone treatments (eg concentrated in root zone) in the other three trials conducted during mid-late spring and early autumn.

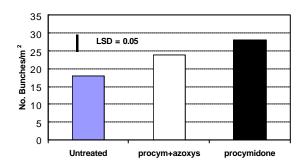


Fig. 4. procymidone trial

#### Comments:

Field trials using small replicated plots (see trial 3) showed that procymidone treatments (eg. concentrated in the root zone) can reduce seedling emergence during periods when growth is slow due to cool weather.

#### Evaluation of fungicide treatments for the control of onion white rot

#### Trial 1:

This trial evaluated the effectiveness of three fungicide treatments, applied using different application methods, for controlling onion white rot in spring onions in a high pressure site at Cranbourne in Victoria. Only the most promising treatments and application methods are presented.

Trial 1 details
Plot size: 1 x 5 m
Sown: 25/03/03
Sprayer: Knapsack
First spray: after sowing AS
Second spray: 3 weeks AS
Third spray: 6 weeks AS
Harvested: 30/06/03
Cultivar: Paragon

#### Effect on disease and yield trial 1 - Fig. 5.

Two sprays of the new fungicide BAS 510 (BASF-a.i.boscalid), the first applied as a soil surface application after sowing and the second as a stem base application 3 weeks after sowing, gave excellent early and late season disease control (90% disease reduction). One application of tebuconazole, applied in the furrow (treated fertiliser below seed) or as soil surface spray after sowing, also provided excellent early and late season disease control.

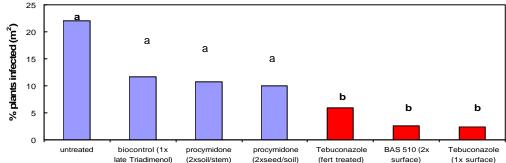


Fig. 5. Fungicide trial 1





Treated with fungicide (BAS 510) trial 1

#### Comments:

Tebuconazole is only registered in Tasmania for the control of onion white root rot on dry onions. Residues of tebuconazole were detected in spring onions harvested 12 weeks after being treated with one application of tebuconazole.

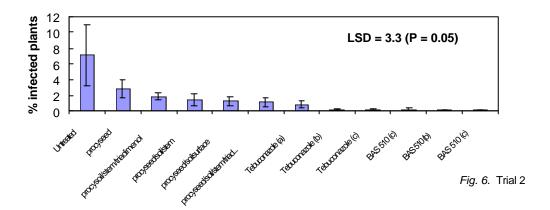
#### Trial 2:

This trial evaluated the effect of different rates of two promising fungicide treatments on control of onion white rot in a spring onion crop grown in a high pressure site at Cranbourne Vic during spring 2003. The trial also further investigated different methods of applying procymidone.

Trial 2 details
Plot size: 1 x 3 m
Sown: 29/8/03
Sprayer: Knapsack
First spray: 02/09/03
Second spray: 25/9/03
Third spray: 21/10/0
Harvested: 26/11/03
Cultivar: Paragon

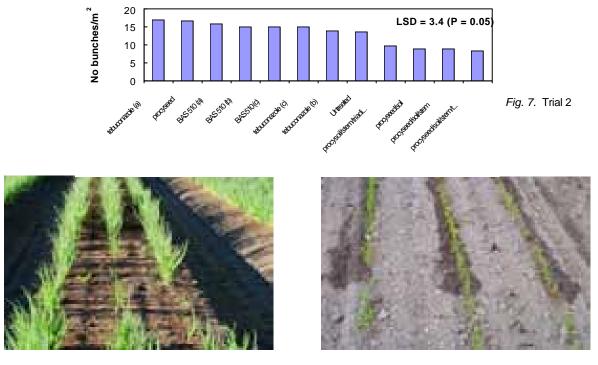
#### Effect on disease trial 2 - Fig. 6.

Two applications of the new fungicide BAS 510 were applied at three different rates. The first applied as a soil surface spray after sowing and the second as a stem base application at week 4. All BAS 510 treatments were very effective in controlling disease. Similarly, one application of tebuconazole at three different rates, was also very effective in controlling disease when applied as a soil surface spray after sowing. The integration of seed treatments (procymidone) with one or two sprays of procymidone or triadimenol, applied as soil surface and stem base applications, also gave good disease control.



#### Effect on yield trial 2 - Fig. 7.

Procymidone treatments (except the seed treatment alone) reduced seedling emergence and therefore resulted in significant reductions in plot yields. Germination of seedlings is slower in cool weather during winter and early spring. Under these conditions, procymidone treatments concentrated in the root zone are toxic to seedling germination.



Seedling emergence was reduced by strategic applications of procymidone (applied to root zone) in cool/wet weather conditions

#### Trials 3 and 4:

These trials evaluated the efficacy of promising new fungicide treatments for the control of onion white rot. The trials were conducted in a high and low disease pressure sites at Cranbourne and Heatherton, respectively, Victoria during autumn 2004. The trials also evaluated the effect of combining early season applications of procymidone with late season applications of fungicide treatments from different chemical groups on disease control.

Table 1. List of fungicide treatments in trials 3 and 4.

Treatments	Spray Program (*, consecutive sprays)
Nil	
procymidone 2L/ha 1000L water/ha	procymidone
procymidone 2L/ha 1000L water/ha	procymidone, procymidone
procymidone 1L/ha 1000L water/ha	procymidone, procymidone
procymidone 2L/ha 2000L water/ha	procymidone, procymidone
procymidone 2L/ha 1000L water/ha	procymidone,procymidone
procymidone+azoxystrobin	procymidone, procymidone, azoxystrobin
procymidone+pyraclostrobin	procymidone, procymidone, pyraclostrobin
procymidone+triadimenoI <sup>TM</sup>	procymidone, procymidone, triadimenol
procymidone+BAS 510	procymidone, procymidone, BAS 510
procymidone+BAS 516	procymidone, procymidone, BAS 516
azoxystrobin	azoxystrobin, azoxystrobin
pyraclostrobin	pyraclostrobin, pyraclostrobin
triadimenoI <sup>rM</sup>	triadimenol, triadimenol
BAS 510	BAS 510, BAS 510
BAS 516	BAS 516, BAS 516
Tebuconazole	tebuconazole
BAS 510* (soil surface and stem base applications)	BAS 510, BAS 510

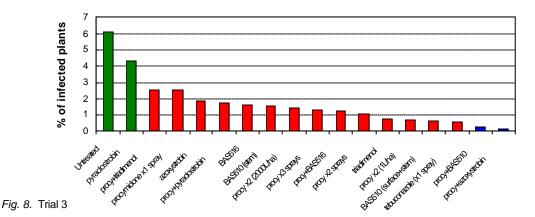
All procymidone and BAS 510\* treatments were applied as soil surface sprays after sowing and stem base sprays at week 4 and 6 after sowing using 1000L water/ha. Tebuconazole was applied as a soil surface spray using 1000L water/ha. All other fungicide treatments were applied as stem base/foliar spray using 500L water/ha starting at week 4 and 6 weeks after sowing.

Trials 3 details (Heatherton)
Plot size: 1 x 3
Sown: 24/02/04
Sprayer: Knapsack
First spray: 25/02/04
Second spray: 31/03/04
Third spray: 20/04/04
Harvested: 21/05/04
Cultivar: Paragon

Trials 4 details (Cranbourne)
Plot size: 1 x 3 m
Sown: 26/02/04
Sprayer: Knapsack
First spray: 27/02/04
Second spray: 31/03/04
Third spray: 20/04/04
Harvested: 05/05/05
Cultivar: Straight leaf

#### Effect on disease trial 3 - Fig. 8.

Under low disease pressure conditions (6% disease), all fungicide treatments, except pyraclostrobin, significantly reduced disease levels when compared to an untreated control. Two sprays of procymidone, applied after sowing (soil surface) and at week 5 after sowing (stem base), supplemented with one spray of azoxystrobin or BAS 510 applied at week 8 (stem base), gave the greatest disease control (95% disease reduction). Similarly, two sprays of BAS 510 or one spray of tebuconazole also resulted in excellent disease control (80-90% disease reduction). Two sprays of triadimenol, applied at week 5 and 8, gave as good control as 2 sprays of procymidone. There were no significant differences in plot yields between treatments.



#### Effect on disease trial 4 - Fig. 9.

Under conditions of higher disease pressure (25% disease), one spray of tebuconazole, applied after sowing, or two sprays of BAS 510, applied after sowing and at week 5 gave the greatest disease control (96% disease reduction) with corresponding yield increases. Two sprays of either BAS 510, triadimenol or azoxystrobin, applied at week 5 and 8 after sowing, gave as good disease control as three sprays of procymidone, Similarly, two sprays of procymidone, applied after sowing and at week 5 after sowing, supplemented with one spray of either azoxystrobin or BAS 510 applied at week 8, gave as good disease control as three sprays of procymidone.

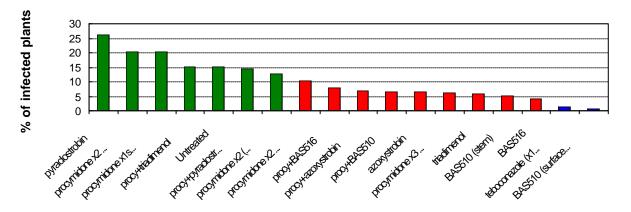


Fig. 9. Trial 4

#### Comments:

Residues were detected in spring onions treated with three sprays of procymidone. BASF (product bought by NuFarm) is planning national residue trials for BAS 510 in Australia.



Harvest of trial 4 showing bunches and infected plants in each plot

#### **Next step:**

The best fungicide treatments have been selected for further evaluation in a series of trials in Vic and south Qld during autumn/winter 2005. Efficacy and residue data (non-GLP) will be provided to grower's groups and chemical companies to promote efficacy trials in Australia.

#### Evaluation of DADS and other treatments for inoculum reduction in soil

Two long-term field trials evaluated the effectiveness of the pathogen germination stimulant DADS (dially-disulphide) for reducing sclerotial levels of *S. cepivorum* and improving control of onion white rot in two field sites (sandy soils) with high disease pressures at Cranbourne, Victoria, during spring 2002 – autumn 2003. DADS stimulates sclerotia to germinate in the absence of an *Allium* crop. Once germinated, sclerotia die out due to the absence of a host to infect.

#### Treatments evaluated:

DADS was evaluated as single and dual applications and applied to soil (soil temperatures 10-20°C) during periods of the year conducive for disease development in Victoria (autumn and spring). DADS was injected into soil (20-30 cm) using a modified MS rig at the rate of 5 and 10L product /ha and 1000L water/ha (large rig).

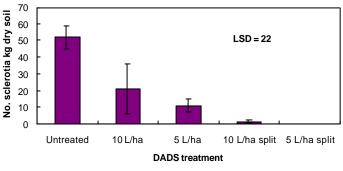




Modified prototype applicators injecting DADS into sandy soil.

#### Effect of DADS on inoculum levels - Fig 10 and 11.

Two applications of either 5 or 10L/ha of DADS, applied in spring 2002 and again in autumn 2003, were highly effective in reducing sclerotial inoculum levels (97% reduction) in a sandy soil kept fallow for a year (trial 1). In a sandy soil without a spring onion crop for 6 months, two applications of DADS were not effective in reducing inoculum levels (trial 2). In an on-going field trial in south Qld (black soil), one application of 10L/ha of DADS, applied in winter 2003, significantly reduced inoculum levels by 80% when compared to an untreated control (Fig 11).





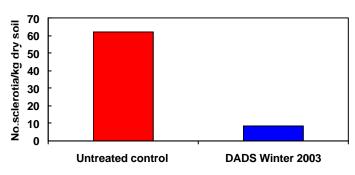
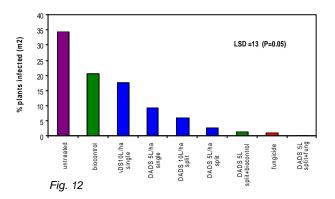
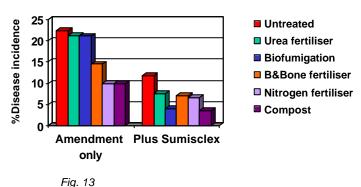


Fig. 11. Qld

#### Effect of DADS on disease - Fig. 12.

In trial 1 two applications of either 5 or 10L/ha of DADS significantly reduced disease levels by 80-90% when compared to the control. An integrated approach using DADS in combination with fungicides (procymidone or BAS 510) or biocontrol (*Trichoderma* C52) showed potential for enhancing disease control.





#### **Comments:**

In high pressure sites a minimum of two applications of DADS will be required to reduce inoculum levels below a threshold where disease can be controlled effectively by fungicide or biocontrol treatments. At the time of this report, registration of a commercial formulation of DADS (Alli-up<sup>TM</sup>) has been granted in the USA. Unfortunately, the original owner of Alli-up<sup>TM</sup> (UAP in USA) has stopped its production due to re-organisation of the company. UAP is re-assessing its interest in Alli-up<sup>TM</sup> and another two companies in USA are also interested in this product. Elliott Chemicals Limited (New Zealand) will not proceed with registration of this product in Australia until production of Alli-up<sup>TM</sup> resumes.

#### **Next step:**

Four long-term field trials were set up in spring 2003 to further evaluate the efficacy of DADS and alternative treatments containing DADS (eg organic garlic oils) for inoculum reduction in high disease pressure sites in Victoria (Cranbourne, Heatherton) and south Qld (Mulgowie). Soil is being treated during spring 2003, and autumn and spring 2004. The trials will be sown in March 2005. A smaller rig (500-600L water/ha) is being tested in Victoria.

#### Other treatments for inoculum reduction:

The project is also investigating the effectiveness of other treatments (eg solarisation and soil amendment) for reducing sclerotial inoculum in sandy soils. A solarisation treatment reduced disease incidence from 35% to 5% when compared to an untreated control. Solarisation is not as effective as DADS and can be used only for 2 months of year in Victoria. In preliminary trials, nitrogenous fertilisers and organic amendments (eg certified green compost) have shown some potential for reducing inoculum levels and improving disease control in sandy soils (Fig.13). These treatments require extensive research to optimise their potential as soil treatments for control of onion white.







Treated with two applications DADS

#### Evaluation of biocontrol treatments for the control of onion white rot

Four field trials have been conducted so far to evaluate the effectiveness of biocontrol treatments for suppressing the onion white rot disease in field sites with different disease pressures during spring and autumn (2002 - 2004) at Cranbourne and Heatherton, Victoria.

#### Biocontrol products and application methods:

The soil microorganism *Trichoderma atroviride* (isolate C52) has shown good antagonistic activity against *S. cepivorum* in the biocontrol program at Lincoln University New Zealand. Lincoln University is conducting studies to determine the compatibility of this biocontrol agent with farm practices (eg fertiliser, fungicides, soil amendments, DADS) used in spring onion production. Agrimm Technologies Pty Ltd has prepared this biocontrol agent in experimental formulations (eg pellets and drench) for use in the Australian trials. *Trichoderma* C52 was applied at sowing as pellets (10<sup>6</sup> cfu/g pellet) to the planting row below the seed by hand or using a Stanhay seeder calibrated to deliver various rates of product/ha. Other biocontrol products (sold as 'growth promoters') available in the market were also included in some of the trials. These products are formulated for application only as a drench.





The biocontrol agent Trichoderma C52 (formulated in prills (yellow) which are incorporated below the seed at sowing using a standard seeder with a fertiliser bucket

#### Treatments:

- *Trichoderma* C52 was evaluated as a single application (pellets at sowing at the rate of 40, 50 and 60 kg/ha) and as a dual application (pellets plus top-ups with a drench formulation 180 x 10<sup>6</sup> cfu/g applied at week 4 and 8).
- Trichoderma C52 was also evaluated as a single and dual application in combination with a late application of procymidone applied at week 5 (single) or 8 (dual).



A culture of Trichoderma C52

• Other biocontrol treatments were applied as a drench (by hand) into open furrows before seeding and as stem base applications (hand held sprayer) at week 4 and 6 after sowing.

#### Effect on disease control:

#### Trial 1 and 3 (spring 2002 and 2003):

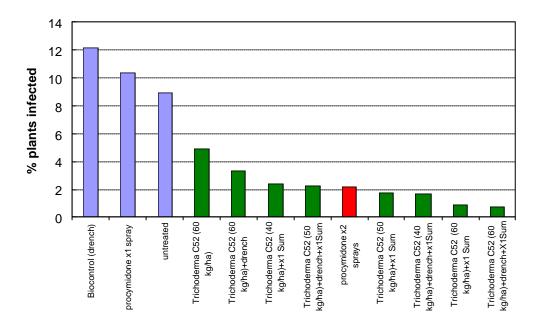
Disease levels were too low in these trials to allow comparison of treatments.

#### Trial 2 (autumn 2003):

None of the biocontrol treatments including *Trichoderma* C52, applied at the rate of 50 kg/ha in single or dual applications, reduced disease incidence when compared to the untreated control (30% disease). The low efficacy of *Trichoderma* C52 in this trial was probably due to the sub-optimal levels of *T. atroviride* detected (<10<sup>3</sup>/g soil) in the region of roots of spring onion plants.

#### Trial 4 (autumn 2004):

Only some of the treatments are shown. *Trichoderma* C52, applied at the rates of either 40, 50 or 60 kg/ha in single or dual applications and in combination with one spray of procymidone applied at week 4 (for single) and 8 (for dual) showed potential for reducing disease incidence when compared to an untreated control (77-88% disease control) under low disease pressure. The reduction was similar to that provided by two sprays of procymidone. Although not significant, plots treated with single applications of *Trichoderma* C52 (only at sowing), applied at the rate of 60 kg/ha, had lower levels of disease than the other treatments with single applications of *Trichoderma* C52 (eg 40 and 50 kg/ha) and another biocontrol treatment applied only as drench. Good levels of *Trichoderma atroviride* (10<sup>6</sup> - 10<sup>4</sup> cfu/g soil) were detected in the root zone of plants during the first 9 weeks of the crop.



#### Comments:

The combination of early season application of biocontrols with late season sprays of fungicides appears to be a promising strategy for the integrated control of onion white rot in spring onions in low disease pressure sites.

#### Next step:

The best biocontrol treatments will be further evaluated in field trials in Victoria and Qld during autumn 2005. Experiments are also being conducted to determine soil conditions (eg organic matter levels) that promote better biocontrol agent growth in different soils and compatibility of chemicals with biological control agents.



# Using beneficial microbes for plant disease control - onion white rot case study

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Our research group focuses on all aspects of biological control of plant diseases. Fifteen years ago, we recovered a strain of the fungus *Trichoderma atroviride* (C52) from vegetable growing soil in Pukekohe, South Auckland NZ. *Trichoderma* species are common in soil and are found all over the world. *Trichoderma atroviride* (C52) showed promise as a biological control agent of *Sclerotium cepivorum*, the causal agent of onion white rot. This fungus was then formulated as a pellet, wettable powder, solid substrate and prill by Agrimm Technologies Ltd to further evaluate the ability of *T. atroviride* to control onion white rot. Under low to moderate disease conditions, *T. atroviride* was able to control disease by 70-72% and three fold cost benefits through yield increases have been reported. However, under high disease pressure, only 40% disease control could be achieved. Therefore, under high disease pressure an integrated disease control programme is required to bring about satisfactory disease control.

Much of our current research is focused on gaining a greater understanding of the biotic and abiotic factors which influence biocontrol activity under field conditions as a means to enhance integrated control approaches. Compatibility studies with *T. atroviride* and diallyl disulphide (DADS), a synthetic onion oil used as a sclerotial germination stimulant in the off-season, indicated that they could be used in combination. Fungicide compatibility studies determined that *T. atroviride* was compatible with captan and procymidone seed treatments but not with thiram or benomyl seed treatments. *Trichoderma atroviride* was also compatible with foliar applications of fungicides. Research with fertilisers showed that *T. atroviride* was sensitive to nitrogen, particularly when concentrations greater than the recommended field rates were used.

The research conducted on the interactions of *T. atroviride* with the environment has enabled us to develop an integrated disease management programme. Diallyl disulphide can be used in the off-season to reduce the number of *Sclerotium cepivorum* sclerotia in the soil. At planting, *T. atroviride* can be applied to the planting furrow with onion seed treated with captan (not thiram) and/or procymidone granules. Foliar sprays for control of fungal diseases can be applied later in the season and any nitrogen applications should be applied at least four weeks after application of *T. atroviride* to allow the fungus to establish and proliferate in the root region and protect the roots from *S. cepivorum* infection.

Within New Zealand, *T. atroviride* has been commercialised as Vegevax *Allium* G. Further commercial onion trials are planned with different formulations of *T. atroviride*. *Trichoderma atroviride* (C52) is also being trialed against *Botrytis* diseases in fruit, vegetables and flowers, to determine its efficacy against foliar pathogens as opposed to soil borne pathogens. In addition, Lincoln University and Agrimm Technologies also have a *T. hamatum* (6SR4) strain that is commercially available for use against *Sclerotinia* lettuce drop and are currently working on bacterial seed treatments (*Bacillus* species) to be used against seedling damping-off.