FINAL REPORT

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HDC Project FV 358

Onion: Pot experiment to examine the suppression of Fusarium basal rot of onion using compost colonised with *Trichoderma viride*

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Commercial - In Confidence

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Grower Summary

Headline

- Under high disease pressure, compost colonised with *Trichoderma viride* S17A and incorporated in soil at 25% reduced Fusarium in onion plants by 48% compared with the soil control

Background

In onion growing, Fusarium basal rot is becoming a greater problem in the UK and other parts of Northern Europe and is likely to increase with global warming. US-bred short-day varieties of onion that show resistance to Fusarium basal rot may not be resistant to European strains of the pathogen *Fusarium oxysporum* f.sp. *cepae*, and do not have the same attributes of susceptible long-day varieties. The only approved (off-label) fungicide for Fusarium of onion is a seed treatment with thiabendazole + thiram; however this treatment is aimed at preventing damping-off of seedlings and not basal rot. A HortLINK project (HL0176) has shown that that *Trichoderma viride* S17A can colonise a wide range of composts. Control of Allium white rot in the field has been achieved by broadcasting and incorporating Trichoderma colonised compost into soil artificially infested with sclerotia of the pathogen, *Sclerotium cepivorum*. The Trichoderma can persist in the soil and control white rot from one year to the next, potentially avoiding the need for repeated applications. Broadening the efficacy of Trichoderma colonised compost to also control Fusarium basal rot would enhance the economics of a single treatment.

There are increasing amounts of compost available (currently around 3 million tonnes/year in the UK). Commercial trials in the above LINK project showed that screened compost could be applied within the onion planting row at rates of 3–5 tonnes/ha using a modified set planting machine. At such rates, the *Trichoderma* colonised and degraded compost can provide useful amounts of plant nutrients, particularly P and K.

A Defra funded project at Warwick HRI has developed a PCR-based method for distinguishing formae speciales of *Fusarium oxysporum*. Cultures obtained from diseased onions in the UK have been identified as *F. oxysporum* f.sp. *cepae* and *Fusarium proliferatum,* when compared with resulting onion disease symptoms and molecular taxonomy of isolates deposited in fungal culture collections elsewhere. This has enabled

isolates confirmed as *F. oxysporum* f.sp *cepae* to be available for the pathogenicity bioassay used for this pot experiment.

Objectives and expected deliverables

- Obtain isolates of the pathogen *Fusarium oxysporum* f.sp. *cepae* from onion crops.
- Produce composts colonised with *Trichoderma viride*.
- Determine the suppressive effects of composts inoculated with *Trichoderma viride* on basal rot of onion in pot bioassays.
- Monitor the populations of Trichoderma and Fusarium propagules in the compost amended soil and non-amended soil.
- Disseminate results to the onion industry and make recommendations for future pot and field-scale experiments.

Summary of the project

A pot bioassay experiment was set up to examine the effect of incorporating green waste compost, with and without colonised Trichoderma, into soil on Fusarium in onion plants. The soil and soil/compost mixtures were infested with a chlamydospore inoculum of *Fusarium oxysporum* f.sp. *cepae*. Soil and soil/compost mixtures without the Fusarium chlamydospore inoculum were also prepared. The following treatments were compared:

- Control, no compost amendment added to soil
- Compost incorporated in soil at 25% and 40% by volume
- Compost, colonised with *Trichoderma viride* S17A, incorporated at 25% and 40%
- Compost, colonised with *Trichoderma hamatum* L4, incorporated at 40%.

Plant deaths due to Fusarium were recorded during a 19-week growing period, and as basal rot symptoms at harvest. The weight of symptomless onions at harvest was also recorded.

Main conclusions

1. Under high disease pressure, compost colonised with *Trichoderma viride* S17A and incorporated in soil at 25% reduced Fusarium in onion plants by 48% compared with the soil control. This compares with a 26% reduction in white rot previously found in a pot experiment with the same treatment, although white rot control in the field with the same treatment was 59-100%.
2. After growing in Fusarium infested soil, average weight of onions without disease symptoms was higher following incorporation of 25% *T. viride* S17A-colonised compost than in the soil control.

3. Compost without added Trichoderma and incorporated in soil at 25% had no effect on Fusarium disease symptoms or plant weight compared with the soil control.
4. Compost without added Trichoderma and incorporated in soil at 40% increased Fusarium; this effect of was offset by the presence of *T. viride* S17A in the compost.
5. Results for *T. viride* S17A were slightly better than for *T. hamatum* L4 although the differences were not significant.
6. There were no Fusarium disease symptoms in any of the treatments without the Fusarium chlamydospore inoculum.
7. There were no differences in average plant weight at harvest between treatments without the Fusarium chlamydospore inoculum.
8. Following application of Trichoderma-colonised compost to soil, the Trichoderma propagule count in the soil remained at a high level throughout the glasshouse pot experiment, although the decline was greater than that observed in the field, possibly due to the warmer and less favourable conditions for Trichoderma survival in the glasshouse.

Financial and environmental benefits

- Potential for control of both Fusarium and white rot using a single treatment of *Trichoderma virdie* S17A colonised compost.
- The pot bioassay developed is an effective method for preliminary testing the efficacy of treatments against Fusarium of onion, before testing in the field.
- Substitution of inorganic fertilisers, particularly P and K, by application of compost.
- Suitable for conventional and organic production.

Action points for growers

- Further development work on a range of Trichoderma isolates in pot experiments and in the field is needed to establish which isolates are the best. Work is currently being conducted on semi-commercial scale production of Trichoderma colonised compost, and within planting row compost application.
- High rates of compost or organic matter application immediately before an onion crop, in the absence of Trichoderma, should be avoided since this appears to encourage Fusarium, possibly by increasing soil moisture availability or by volatiles released from the compost stimulating Fusarium chlamydospore germination. This mechanism requires further investigation since it may also provide an opportunity for longer term Fusarium disease control, and has implications for the application of high rates of onion waste compost at short or long intervals before onion cropping.

- Where compost is applied to onions, inorganic fertiliser rates, particularly P and K, should be reduced.

Science Section

Introduction

In onion growing, Fusarium basal rot is becoming a greater problem in the UK and other parts of Northern Europe and is likely to increase with global warming. US-bred short-day varieties of onion that show resistance to Fusarium basal rot may not be resistant to European strains of the pathogens *Fusarium oxysporum* f.sp. *cepae* and *Fusarium proliferatum*, and do not have the same attributes of susceptible long-day varieties. The only approved (off-label) fungicide for Fusarium of onion is a seed treatment with thiabendazole + thiram; however this treatment is aimed at preventing damping-off of seedlings and not basal rot. *Trichoderma* species have been used successfully to suppress diseases caused by *Fusarium oxysporum*, including onion and narcissus basal rots [1–4]. A HortLINK project (HL0176) has shown that that *Trichoderma viride* (S17A) can colonise a wide range of composts. Control of Allium white rot in the field has been achieved by broadcasting and incorporating Trichoderma-colonised compost into soil artificially infested with sclerotia of the pathogen, *Sclerotium cepivorum*. The colonised composts have given reproducible control of white rot in the field, and more reliable control than using either the compost or *Trichoderma* in isolation, or with tebuconazole (Folicur) treatment of sets. The *Trichoderma* can persist in the soil and control white rot from one year to the next, potentially avoiding the need for repeated applications. Broadening the efficacy of Trichoderma-colonised compost to also control Fusarium basal rot would enhance the economics of a single treatment.

Composted green waste provides a cheap and effective method of obtaining sufficiently high *Trichoderma viride* inoculum levels in soil. There are increasing amounts of compost available (currently around 3 million tonnes/year in the UK). Commercial trials in the above LINK project showed that screened compost could be applied within the onion planting row at rates of 5 tonnes/ha using a modified set planting machine. At such rates, the Trichoderma colonised and degraded compost can provide useful amounts of plant nutrients, particularly P and K, thus reducing the requirement for expensive inorganic fertilisers.

A Defra funded project at Warwick HRI has developed a PCR-based method for distinguishing formae speciales of *Fusarium oxysporum*. Cultures obtained from diseased onions in the UK have been identified as *F. oxysporum* f.sp. *cepae* and *Fusarium proliferatum,* when compared with resulting onion disease symptoms and molecular taxonomy of isolates deposited in culture collections elsewhere. This has enabled isolates confirmed as F*. oxysporum* f.sp *cepae* to be available for the pathogenicity bioassay used for this pot experiment.

A pot bioassay was developed from a pot test used for basal rot of narcissus (*F.*

oxysporum f.sp. *narcissi*) using a chlamydospore inoculum [5].

Commercial Objectives

01 Obtain isolates of the pathogen *Fusarium oxysporum* f.sp. *cepae* from onion crops.

02 Produce composts colonised with Trichoderma.

03 Determine the suppressive effects of composts inoculated with Trichoderma on basal rot of onion in pot bioassays.

04 Monitor the populations of Trichoderma and Fusarium propagules in the compost amended soil and non-amended soil.

05 Disseminate results to the onion industry and make recommendations for future pot and field-scale experiments.

Materials and methods

Organisms

Fusarium oxysporum f.sp. *cepae* – The isolate was obtained from an onion infected with Fusarium basal rot obtained from Lincolnshire in 2008. A culture was retrieved from the infected onion using selective Komada's medium. Comparison of DNA of the retrieved isolate with other *F. oxysporum* isolates confirmed that it was most similar to Dutch isolates of *F. oxypsporum* f.sp. *cepae*. A culture of *Fusarium oxysporum* f.sp. *cepae* grown on potato dextrose agar (PDA) was used to produce a mycelial and conidial suspension for inoculating sterilised talc. The suspension contained 2.3×10^8 colony forming units (cfu)/ mL and 4 mL were added to 20 g talc. The talc was kept for 6 weeks at 20 °C to allow chlamydospores to develop. The prepared talc inoculum contained 2.9×10^7 colony forming units (cfu)/g.

Trichoderma – Two isolates originally obtained from white rot infected onions were used, L4 (*Trichoderma hamatum*) and S17A (*Trichoderma viride*). Spore suspensions were obtained by adding 20 mL of sterile distilled water to three-week old PDA plate cultures; a sterile spatula was used to dislodge the spores. Spore suspension (5 mL) was added to 320 mL glass jars containing 150 g sterile rye grain (Sylvan Spawn Ltd, Peterborough, UK) and incubated for 14 days at 20 °C before use.

Composts

Four batches of green waste compost were obtained from three composting sites: Jack Moody Ltd (Wolverhampton), Organic Recycling Ltd (Peterborough), Simpro (Gaydon, Warwickshire). The composts were prepared from parks and gardens wastes including prunings and leaves, and fruit and vegetable wastes in turned windrows for 3-9 months. The green waste composts (30 L of each) were inoculated with the *Trichoderma* grain inoculum

at 1 % w/w. The inoculum was allowed to colonise the green waste compost for 14 days at 20 °C. Composts were analysed for pH, electrical conductivity, and moisture content. The levels of *Trichoderma* were determined the in spawn inoculum and in the samples of green waste composts before and after addition of the Trichoderma inoculum, and after colonisation. Suspensions of compost were plated on to PDA + tetracyclin + chlorotetracyclin.

Pot experiment

Compost (Organic Recycling) colonised with *Trichoderma* isolate S17A was incorporated at 25 and 40 % v/v into sieved (5 mm) sandy loam soil (Kirton, Lincolnshire). The same rates of compost without Trichoderma inoculum, and the 40 % rate of compost with *Trichoderma* isolate L4, were also added to soil. Batches of the soil/compost mixtures and soil alone were inoculated with the Fusarium talc inoculum at either 1 or 2 g talc/kg. These rates were equivalent to 2.9×10^4 and 5.8×10^4 chlamydospores/g soil or soil/compost mixture and were based on previous tests with basal rot of narcissus [5]. Batches of the soil/compost mixtures and soil without the Fusarium inoculum were prepared as negative controls. To test the efficacy of the *Trichoderma* S17A colonised compost against Allium white rot, samples of the 25% and 40% Trichoderma-compost rate treatments, as well soil, were infested with *Sclerotium cepivorum* sclerotia at 8 sclerotia/ g.

Square pots ($70 \times 70 \times 80$[deep] mm Optipots, LBG Ltd, Evesham, Worcestershire) were filled with the soil-compost mixtures or soil. A single untreated onion set (cv Hercules, Elsoms Seeds Ltd, Spalding, Lincolnshire) was planted in each pot. There were four replicate plots with each plot containing 10 pots. All plants received watering with a nutrient solution (2N:1P:4K) every 14 days. The pots were assessed weekly for the presence of *Fusarium*, which was scored as dead plants with visible pinkish-white mycelium. At the end of the experiment (19 weeks) harvested bulbs were assessed for Fusarium symptoms. Bulbs with no visible external symptoms were weighed and then cut in half to determine if any internal rotting was present. The glasshouse heating and ventilation set points were 15 °C and 17 °C respectively; shading was drawn over the compartment when the solar radiation exceeded 400 W/m^2.

The populations of Trichoderma propagules in the soil were determined at the start of the experiment and in the soil and soil/compost mixtures at the end of the experiment as previously described. The population of Fusarium propagules in the different treatments was determined at the end of the experiment by plating suspension on to PDA + chlorotetracyclin. Three replicate samples were taken from each treatment and three replicate plate counts were made for each sample.

Results

Composts and Trichoderma counts

The rye grain spawn inoculum used for inoculating the green waste composts contained 6.2 × 10^6 colony forming units (cfu) of Trichoderma /g substrate for isolate S17A and 3.6 × 10^6 cfu/g substrate for isolate L4. Compost obtained from J. Moody was drier than the other the other sources, and with ORL compost (batch 1) had a lower pH value than the Simpro compost and ORL compost (batch 2) (Table 1). This may explain the higher *Trichoderma* counts obtained in J. Moody and ORL (batch 1) composts. *Trichoderma* isolates S17A and L4 produced similar propagule counts in ORL compost (batch 1b), which was used for the pot experiment.

Table 1. Analysis of composts and Trichoderma propagule count in the composts (in colony forming units per g compost) before and after inoculation with *T. viride* S17A spawn and after colonisation. Each value is the mean of three replicate samples

Compost source	**Moisture % w/w**	**pH**	**EC mS/cm**	***Trichoderma* propagules, cfu/g**		
				Initial*	Inoculated**	final
J. Moody	35	7.36	2.15	0	6.5 × 10^4	4.2 × 10^7
ORL (1a)	47	7.64	1.51	2.7 × 10^3	1.2 × 10^5	3.0 × 10^7
ORL (1b)					5.2 × 10^3	1.5 × 10^7
ORL (1b)					(L4) 6.9 × 10^3	(L4) 6.7 × 10^7
ORL (2)	47	8.77	2.12	0	8.8 × 10^3	1.1 × 10^6
Simpro	48	8.72	1.38	0	6.3 × 10^4	2.0 × 10^5

* Initial background level of naturally occurring *Trichoderma* species in compost
* *Trichoderma viride* isolate S17A unless indicated as *Trichoderma hamatum* isolate L4.

Fusarium in pot experiment

Emergence of plants from sets was at least 95% and was not significantly affected by the treatments. Plant deaths due to Fusarium started to occur about 4 weeks from the start of the experiment. Initially these appeared as blight symptoms with affected plants showing yellowing and wilting leaves (Fig. 1). The occurrence of typical basal rot symptoms started about 8 weeks after the start of the experiment, with plants showing pinkish white mycelium around the base of the bulb, as well as the above leaf symptoms (Fig. 2).

Figure 1: Healthy plants and plants showing Fusarium blight symptoms

Figure 2: Healthy bulbs and bulbs with Fusarium basal rot symptoms

Inoculation of soil with 1 g/kg of the talc Fusarium chlamydospore inoculum resulted in high disease pressure with 40% plant deaths due to Fusarium at the end of the experiment. Compost colonised with *Trichoderma viride* S17A and incorporated in soil at 25% reduced Fusarium in onion plants by 48% compared with the soil control (Fig. 3). After growing in soil at this level of Fusarium infestation, average weight of onions without disease symptoms was higher following incorporation of 25% *T. viride* S17A-colonised compost than in the soil control (Fig. 4). Compost without Trichoderma and incorporated in soil at 25% had no effect

on Fusarium disease symptoms or plant weight compared with the soil control. Compost without Trichoderma and incorporated in soil at 40% increased Fusarium; this effect of was offset by the presence of *T. viride* S17A in the compost. Results for *T. viride* S17A were slightly better than for *T. hamatum* L4 although the differences were not significant.

Inoculation of soil with 2 g/kg of the talc Fusarium chlamydospore inoculum resulted in a higher disease pressure with an average of 45% plant deaths due to Fusarium at the end of the experiment, except the 40% compost without Trichoderma treatment (77% plant deaths). There were no other significant differences between treatments at this level of chlamydospore inoculum. There were no Fusarium disease symptoms in any of the treatments without the Fusarium chlamydospore inoculum. There were no differences in plant weight at harvest between treatments without the Fusarium chlamydospore inoculum (average plant weight 59 g).

White rot symptoms occurred in only 12.5% of the *S. cepivorum* infested soil control treatment. It was therefore not possible to determine if there were any significant effects of the Compost + *T. viride* S17A treatments on white rot (5% and 7.5% diseased plants for 25% and 40% compost respectively). The low level of white rot was possibly due to the conditions in the glasshouse which were kept warm to encourage the development of Fusarium.

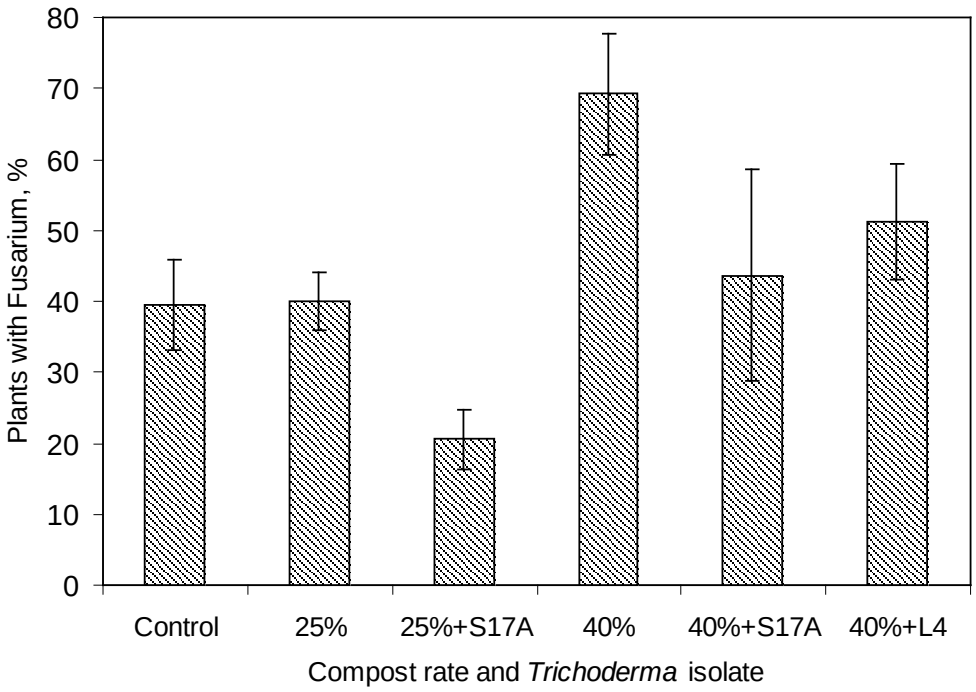

Figure 3: Effect of compost and *Trichoderma* isolates in infested soil on the percentage of plants with Fusarium symptoms. Each value is the mean of 4 blocks of 10 replicate pots

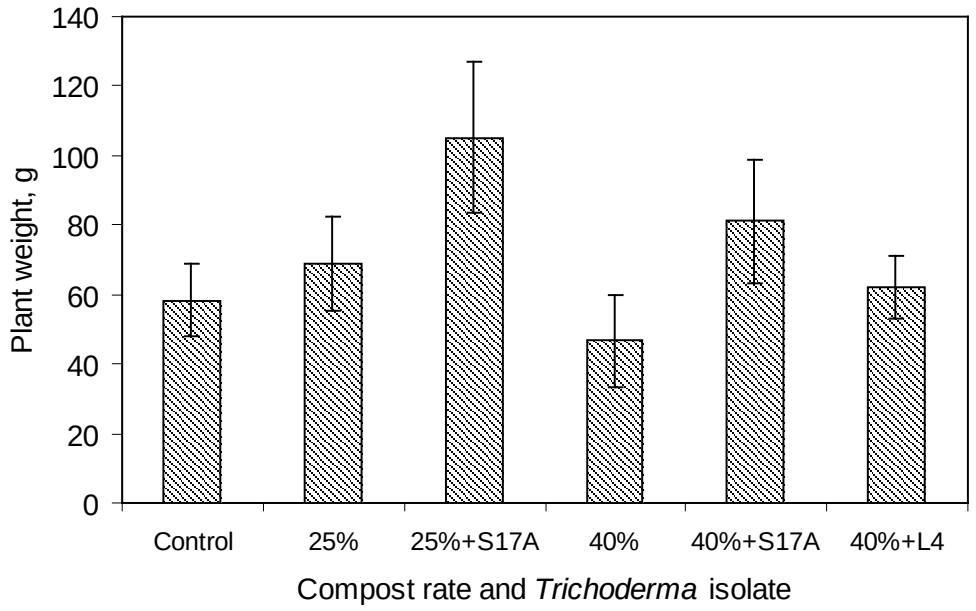

Figure 4: Average weight of symptomless plants grown in Fusarium infested soil

Trichoderma and Fusarium propagule counts at the end of the pot experiment

At the start of the experiment, the Trichoderma propagule counts in the 25 and 40% *Trichoderma* S17A compost treatments were about 4×10^6 and 6×10^6 cfu/g soil. By the end of the experiment, these respective figures had declined to about 8×10^5 and 2×10^6, i.e. to about one third of the original level.

The Trichoderma propagule counts at the end of pot experiment were higher in the *Trichoderma* S17A and L4 compost treatments than in the soil control and 25 and 40% compost treatments, in which the Trichoderma count was below the detection limit of 1×10^3 cfu/g soil (Table 2). The Trichoderma propagule count was higher following amendment of soil with the 40% Trichoderma compost than with 25% Trichodema compost. The Trichoderma counts in the S17A and L4 treatments were similar. In the soil control, 40% compost treatment, and 25% compost treatments with and without Trichodema S17A, the Trichoderma count was unaffected by the Fusarium inoculum (0, 1 or 2 g/kg soil). However, in the 40% compost + Trichoderma S17A or L4 treatments, the Trichoderma count at the end of the experiment was higher in the soil/compost mixtures containing Fusarium inoculum (Table 2).

The Fusarium propagule count at the end of the pot experiment remained below the detection limit in the treatments that were not inoculated with Fusarium chlamydospores (Table 3). In soil and soil/compost mixtures inoculated with Fusarium chlamydospores, the

Fusarium propagule count in the different treatments was similar, but was below the detection limit in the 40% compost + *Trichoderma* L4 treatment.

Table 2. Trichoderma propagule counts at the end of the pot experiment, cfu/g soil or soil/compost mixture. Each value is the mean of three replicate samples and three replicate plates per sample

Compost rate and *Trichoderma* isolate	**Initial Fusarium inoculum, g/kg soil or soil/compost**		
	0	1	2
Control	$< 1 \times 10^3$	$< 1 \times 10^3$	$< 1 \times 10^3$
25%	$< 1 \times 10^3$	$< 1 \times 10^3$	$< 1 \times 10^3$
25% + S17A	2.1×10^5	9.4×10^5	7.5×10^5
40%	$< 1 \times 10^3$	$< 1 \times 10^3$	$< 1 \times 10^3$
40% + S17A	6.5×10^4	1.4×10^6	2.1×10^6
40% + L4	5.8×10^5	3.4×10^6	1.2×10^6

Table 3. Fusarium propagule counts at the end of the pot experiment, cfu/g soil or soil/compost mixture. Each value is the mean of three replicate samples and three replicate plates per sample

Compost rate and *Trichoderma* isolate	**Initial Fusarium inoculum, g/kg soil or soil/compost**		
	0	1	2
Control	$< 1 \times 10^3$	1.4×10^3	3.6×10^3
25%	$< 1 \times 10^3$	4.9×10^3	2.0×10^3
25% + S17A	$< 1 \times 10^3$	5.2×10^4	$< 1.0 \times 10^3$
40%	$< 1 \times 10^3$	1.1×10^3	2.0×10^3
40% + S17A	$< 1 \times 10^3$	1.1×10^4	1.7×10^3
40% + L4	$< 1 \times 10^3$	$< 1 \times 10^3$	$< 1.0 \times 10^3$

Discussion

The pot bioassay developed in this project was successful in producing Fusarium disease symptoms on onion plants in the form of blight and basal rot. The concentrations of inoculum used (2.9×10^4 and 5.8×10^4 chlamydospores/g soil) resulted in high to very high levels of disease and lower rates of inoculum may give disease levels more typical of those found in the field.

The results of this work have shown that *Trichoderma viride* S17A colonised compost can suppress Fusarium blight and basal rot of onion caused by *Fusarium oxysporum* f.sp. *cepae*, at a rate of application in soil of 25% v/v. Although the level of white rot was very low in all treatments in this experiment, work in project HL0176 has shown that the same treatment can suppress white rot of onions in pot experiments by 26%. In the field, the same treatment suppressed white rot by 59-100%. For both diseases, application of compost at

the same rate without Trichoderma had no influence on disease incidence. High rates of compost (40% v/v in soil) encouraged Fusarium disease, possibly by increasing soil moisture availability or by volatiles released from the compost stimulating Fusarium chlamydospore germination. This mechanism requires further investigation since it may also provide an opportunity for longer term Fusarium disease control, and has implications for the application of high rates of onion waste compost at short or long intervals before onion cropping.

Following application of Trichoderma-colonised compost to soil, the Trichoderma propagule count remained at a high level through the pot experiment, although the decline (about two thirds) was greater than that observed in the field over a one-year period. This may be due to the warmer conditions in the glasshouse which were less favourable to Trichoderma survival than field conditions.

There was no evidence for a decline in the Fusarium propagule count of the soil following amendment with Trichoderma S17A colonised compost. This indicates that the Trichoderma is preventing infection of the onion plants rather than depleting the soil of Fusarium inoculum. A similar trend has been observed in project HL0176, where Trichoderma colonised compost did not reduce the soil population of *Sclerotium cepivorum* sclerotia, but white rot disease in onion plants was suppressed.

A semi-commercial-scale system for producing Trichoderma-colonised compost has been developed at Organic Recycling Ltd following project HL 0176. A converted onion set planter has been used for applying compost within the onion planting row at application rates of 2–5 tonnes/ha. Preliminary tests with compost application at 5 tonnes/ha using these methods have shown an increased soil Trichoderma population and a 30% reduction in white rot under very high disease pressure. An economic analysis conducted as part of project HL0176 indicated that compost applied within the planting row at 5t/ha would break-even with a fungicide and inorganic fertiliser regime, if the nutrient value of the compost was also considered. Compost applied across the entire bed with at 30t/ha would also be economically viable compared with the conventional regime, if a single compost application controlled disease in more than one season.

Previous work has also shown a suppressive effect of *Trichoderma* species on Fusarium basal rot of onion and narcissus [1-4], although none used compost as a delivery method. The same technology for producing Trichoderma-colonised compost in this project has also been used in project FV 352 for producing compost colonised with the Koppert product Trianum (*Trichoderma harzianum* T22). A New Zealand Trichoderma product Tenet (*Trichoderma atroviride*) has also been shown to suppress white rot and Fusarium basal rot in onion. The efficacy of a range of isolates of *Trichoderma* species and related species (*Gliocladium*, *Clonostachys*) using compost as a delivery method should be compared.

Projects HL0176 and HL0167 examined the use of onion sets and seed as a delivery method for Trichoderma. The inoculum could also be applied to a pellet seed coat or as 'blank' pellets which are added to pelleted seed before sowing. The use of pellets, made from screened compost or other nutrient sources, and application through a fertiliser applicator could be used as an alternative delivery method.

Work in project HL0176 demonstrated that Trichoderma S17A colonised compost resulted in better onion plant growth than compost without added Trichoderma, even in the absence of disease. This was probably due to the release of plant nutrients from the compost, since Trichoderma species are known to be effective in degrading organic matter such as cellulose and lignin [6].

Conclusions

1. Under high disease pressure, compost colonised with *Trichoderma viride* S17A and incorporated in soil at 25% by volume reduced Fusarium in onion plants by 48% compared with the soil control (Fig. 1). This compares with a 26% reduction in white rot previously found in a pot experiment with the same treatment, although white rot control in the field with the same treatment was 59-100%.
2. After growing in Fusarium infested soil, average weight of onions without disease symptoms was higher following incorporation of 25% *T. viride* S17A-colonised compost than in the soil control (Fig. 2).
3. Compost without added Trichoderma and incorporated in soil at 25% had no effect on Fusarium disease symptoms or plant weight compared with the soil control.
4. Compost without added Trichoderma and incorporated in soil at 40% increased Fusarium; this effect of was offset by the presence of *T. viride* S17A in the compost.
5. Results for *T. viride* S17A were slightly better than for *T. hamatum* L4 although the differences were not significant.
6. There were no Fusarium disease symptoms in any of the treatments without the Fusarium chlamydospore inoculum.
7. There were no differences in average plant weight at harvest between treatments without the Fusarium chlamydospore inoculum.
8. Following application of Trichoderma-colonised compost to soil, the Trichoderma propagule count in the soil remained at a high level throughout the glasshouse pot experiment, although the decline was greater than that observed in the field, possibly due to the warmer and less favourable conditions for Trichoderma survival in the glasshouse than in the field.

Recommendations for further pot and field experiments

1. The effect of adding *Trichoderma viride* S17A colonised composts to soil at rates below 25% on Fusarium should be investigated since 25% was more effective than 40% due to the stimulating effect of the compost on Fusarium.
2. The possibility of alternative delivery methods for Trichoderma into soil (pelleted seed, nutrient pellets) should be investigated since these could be applied at sowing or with a fertiliser applicator.
3. The efficacy of *T. viride* S17A in controlling Fusarium should be compared with other isolates of *Trichoderma* species and related species (*Gliocladium, Clonostachys*) in products such as Trianum, GlioMix, Tenet, and Endofine.
4. Tests should be conducted with other isolates of *F. oxysporum* f.sp. *cepae* and *Fusarium proliferatum* at similar and lower chlamydospore infestation levels used in this pot experiment.
5. The efficacy of *Trichoderma* S17A in controlling Fusarium in field experiments should be tested on Fusarium infested land.
6. The effect of Trichoderma S17A colonised compost on onion crops should be tested in the absence of Fusarium and white rot diseases since the plant nutrients should be more readily available from the Trichoderma degraded composts than from unamended green waste composts.

Technology transfer

Reducing onion disease risks. HDC News No. 156, September 2009, p.10.

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References

1. Beale, RE, Pitt D (1990) Biological and integrated control of Fusarium basal rot of Narcissus using *Minimedusa polyspora* and other micro-organisms. Plant Pathology 39: 477 – 488.

2. Coskuntuna A., Ozer N. (2008) Biological control of onion basal rot disease using *Trichoderma harzianum* and induction of antifungal compounds in onion sets following seed treatment. Crop Protection 27: 330 – 336.
3. Rajendran K, Ranganathan K (1996) Biological control of onion basal rot (*Fusarium oxysporum* f.sp. *cepae*) by combined applications of fungal and bacterial antagonists. J. Biol. Control 10: 97-102.
4. Rod J (1984) Antagonistic effects of some fungi on fungal pathogens causing storage rots of onion (*Allium cepa* L.). Ceska Mykologie 38: 235-239.
5. Bowes, S.A., Edmondson, R. N., Linfield, C. A. & Langton, F. A. (1992) Screening immature bulbs of daffodil (Narcissus L) crosses for resistance to basal rot disease caused by Fusarium oxysporum f. sp. narcissi. Euphytica 63, 199-206.
6. Haddadin MSY, Haddadin J, Arabiyat OI, Hatter B (2009) Biological conversion of olive pomace into compost by using *Trichoderma harzianum* and *Phanerochaete chryosporium*. Bioresource Technology 100: 4773-4782.

