Project title	Optimising field-scale control of Fusarium basal rot and white rot of onion using Trichoderma amended substrates and pellets, and onion residues
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Project leaders:	Dr Ralph Noble
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Key staff:	Andreja Dobrovin-Pennington
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Signed on behalf of: East Malling Research

Signature:……..…….…… Date: 9 March 2012

Dr Christopher Atkinson
Deputy Chief Executive and Senior Programme Leader

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CONTENTS

GROWER SUMMARY

Headline

Potential new biological treatments are evaluated for the control of Fusarium and white rot in onions.

Background and expected deliverables

White rot is still a major problem in the UK bulb and salad onion industry, and Fusarium basal rot of onion is an increasing problem, and is likely to increase further with predicted climate change.The only approved fungicide for Fusarium of onion is a seed treatment with thiram, which is aimed at controlling seedling blight rather than basal rot. All commercial onion varieties are susceptible to white rot, and varieties that show resistance or tolerance to Fusarium basal rot do not have the same quality attributes of susceptible varieties. *Trichoderma* species have been used successfully to suppress diseases caused by *Fusarium oxysporum*, including onion basal rot. The main problem has been the development of a cheap delivery method that can achieve sufficiently high *Trichoderma* inoculum levels in soil.

Summary of the project and main conclusions

Pellet seed development

- Pelleted onion seed with spores of the biocontrol agents HDC F39 and HDC F41 was produced by Incotec/Elsoms. High populations (>10^6 cfu/g pellet) of propagules were detected in the HDC F39 and HDC F41 treated pellets respectively.

Pot Experiments

- HDC F35 amended compost ora dip and/or drench treatment of HDC F41 suppressed Fusarium by 25-60% in two pot bioassays. HDC F42 which was only used in the first bioassay, also suppressed Fusarium by 50%.
- HDC F39 and HDC F40 suppressed Fusarium by 25% in the second pot bioassay but not in the first.
- HDC F35 amended compost or a dip and/or drench treatment of HDC F39 suppressed white rot by 40-60% in two pot bioassays. HDC F40 or HDC F38 granules, or drench treatments of HDC F37 or HDC F42, which were only used in one of the pot bioassays, suppressed white rot by 30-50%.

- An HDC F41 drench treatment suppressed white rot by 30% in the second pot bioassay but not in the first.
- HDC F35 granules or HDC F36, HDC F43 or HDC F44 drench treatments were ineffective against Fusarium or white rot. HDC F38 granules or a HDC F37 drench were also ineffective against Fusarium.
- Unamended compost suppressed white rot by 25-30% but had no effect on Fusarium
- A fungicide (thiabendazole + Folicur) set treatment controlled Fusarium and white rot in the first pot bioassay but not in the second.

Field Experiments

- Preliminary field tests in 2009 and 2010 showed that screened green waste compost amended with HDC F35 could be applied along the planting row with a converted onion set planter at an application rate of 7 tonnes/ha.
- Composted onion waste was amended with grain inoculum of HDC F35 and then broadcast at 50 tonne/ha.
- The above application methods achieved some reduction in white rot compared with unamended soil and increased the soil *Trichoderma* propagule population at harvest by times 20 compared with the natural background soil population; however, this was less than that obtained in the pot experiments.
- Application of HDC F39 as a drench or granule application one month after sowing or planting resulted in a zero to times10 increase in soil *Trichoderma* propagule population at harvest and reduced white rot in one out of two field sites but had no significant effect on Fusarium on one field site.

Financial benefits

None at this stage.

Action points for growers

None at this stage.

SCIENCE SECTION

Introduction

White rot caused by the fungus *Sclerotium cepivorum* is still a major problem in the UK bulb and salad onion industry due to the lack of very effective fungicide treatments. Fusarium basal rot of onion, caused by various races of *Fusarium oxysporum* f.sp. *cepae* and *Fusarium proliferatum*, is an increasing problem, and is likely to increase further with predicted climate change. The only approved fungicide for Fusarium of onion is a seed treatment with thiram, which is aimed at controlling seedling blight rather than basal rot.Onion varieties that show resistance to basal rot do not necessarily have resistance to all of the causal Fusarium pathogens, or the same quality attributes and daylength requirements of susceptible varieties. All commercial bulb and salad onion varieties are susceptible to white rot.

Trichoderma species have been used successfully to suppress diseases caused by *Fusarium oxysporum*, including onion basal rot[1, 2, 3]. The main technical problem has been the development of a cheap delivery method that can achieve sufficiently high Trichoderma inoculum levels in the root zone soil. Applying *Trichoderma* species or other biocontrol agents in a low cost organic carrier material could potentially lead to a high soil population and effective disease control. Application of Trichoderma inoculum to primed seed has also been developed in project HL0167[4] and Elsoms/Incotec have seed pellet technology. Pellets could be used as a delivery method of Trichoderma and other microbial biopesticides into the soil at sowing.

Onion waste compost controlled white rot when incorporated at 10% in silt and sandy loam soils in pot experiments, but was not tested at less than 25% in the field, at which rate it was effective[5,6]. Composting of onion waste is now widely practised in the industry but the rate and timing of application for white rot control needs to be optimised for different soil types.

Project Aims

- Evaluate control of Fusarium basal rot and white rot of onion using substrates and/or pellet carriers amended with specific *Trichoderma* isolates and other commercial biopesticides in pot experiments.
- Test the best Trichoderma substrate and pellet carriers within planting row applications in the field, and establish any independent effects of the substrate carriers on the crop.
- Determine the minimum effective rates and optimum application timing of onion waste compost for controlling white rot in fields of different soil types, and establish the

independent effects of the compost application on disease biocontrol, nutrition, and soil moisture availability to the onion crop

- Compare disease control achieved with the biological methods, chemical fungicide treatments, and combinations of biological and fungicide treatments.

Materials and methods

Preparation of Trichoderma inocula, amended substrates and pellet carrier systems with Trichoderma isolates and monitoring survival in soil (Milestone 1.1)

Colonisation of different organic substrates by Trichoderma *species and other biocontrol agents*

A series of small-scale laboratory tests were set up to determine whether HDC F35 and other biocontrol agents could be grown on compost or other organic substrates, which could then be used to amend to soil to control disease.

Samples of inoculum of HDC F35 were prepared by Sylvan on different substrates: wet and dry rye grain, dry millet and a synthetic substrate (biodac). The inocula were added at 1% w/w to 100g of two green waste composts: Organic Recycling (ORL) and J Moody, and incubated in jars at 18ºC for two weeks.

Further samples of HDC F35 inocula prepared by Sylvan, and the commercial products HDC F39, HDC F41 and HDC F42 were used to inoculate ORL green waste compost (batch 2) in 20L containers at 1% w/w inoculum. The containers were left for two weeks at 18ºC before assessment of biocontrol agent populations by plating a suspension on a selective agar containing a bactericidal antibiotic (chlorotetracyclin).

The growth of HDC F35 and four other biocontrol products or isolates (HDC F38; HDC F36; HDC F37; Kinsealy Research Ireland *Trichoderma harzianum* T5) was tested on the following organic substrates: green waste compost, paper pulp waste, straw, and woodfibre. The inocula were added at 1% w/w to the organic substrates and incubated at 18ºC for 2 weeks. The colonised substrates containing about 10^6 cfu/g were then placed in a 10mm layer in the bottom of jars and covered with a 40 mm layer of uninoculated substrates. This enabled the growth of different isolates to be compared on an equal basis, rather mixing different levels and types of inoculum throughout the substrates. The propagule populations

in the substrates were then determined after a further two weeks at 18ºC by plating a suspension on selective agar.

Preparation of pelleted seed containing biocontrol agents

Pelleted salad onion seed was prepared by Incotec with the following pellet components:

- HDC F39
- HDC F41
- The above pellets with thiram + metalaxyl-M fungicides (these are the standard fungicides used in pelleted onion seed).

HDC F39 and HDC F41 were used due to the availability of spore inoculum (HDC F35 does not sporulate profusely and was therefore unsuitable for pelleting). The population of propagules in the pellets was determined as previously described. The germination of 100 seeds of each batch was tested on moist filter paper after 11 days by Elsoms.

Effects of substrate and pellet carrier systems amended with Trichoderma isolateson Fusarium basal rot and white rot of onion in pot bioassays (Milestone 1.2)

Two greenhouse onion pot bioassays in 2010 and 2011 were used to determine the effects of different biocontrol agents and application substrates on Fusarium and Allium white rot compared with fungicide treatments.

2010 Pot bioassay treatments

Biocontrol agents/Soil amendments

1. Control (soil)
2. Compost 25% v/v
3. Compost + HDC F35 25% v/v
4. Compost + HDC F41 25% v/v
5. Compost + HDC F39 25% v/v
6. Compost + HDC F42 25% v/v
7. HDC F41 dip + drench treatment (2%, 20g in 1L)
8. HDC F39 dip + drench treatment (0.3%, 3g in 1L)
9. HDC F43 dip + drench treatment (2%, 20g in 1L)
10. HDC F42 + drench treatment (2%, 20g in 1L)
11. HDC F44 dip + drench treatment (0.1%, 1g in 1L)
12. Fungicide dip + drench treatment (thiabendazole + Folicur tebuconazole, 0.5% w/w)

Pathogens

1. Control (no pathogen)
2. *Fusarium oxysporum* f.sp. *cepae* chlamydospores in talc (high) 4.88 x 10^6 colony forming units/kg soil
3. *Fusarium oxysporum* f.sp. *cepae* chlamydospores in talc (low) 4.88 x 10^5 cfu/kg soil
4. *Sclerotium cepivorum* sclerotia (high) 1.6 ×10^4 sclerotia / kg soil.
5. *Sclerotium cepivorum* sclerotia (low) 1.0×10^4sclerotia/ kg soil.

The compost (ORL) was prepared as described in the previous section. The onion sets were dipped in the respective biocontrol or fungicide treatments for 15 minutes before planting. After planting, the soil around each set was drench with 5 mL of the respective biocontrol treatment.

2011 Pot bioassay treatments

Biocontrol agents/Soil amendments

1. Control (soil)
2. Compost 25% v/v
3. Compost + HDC F35 25% v/v
4. HDC F35 dried grain (prepared by Sylvan)(0.3g applied at planting)
5. HDC F40 (granular powder, 0.2g applied at planting)
6. HDC F39 (drench, post planting)
7. HDC F41 (2%, 20g in 1L drench, post planting)
8. HDC F38 (granules, 0.2g applied at planting)
9. HDC F37 (0.3%, 3g in 1L drench, post planting)
10. HDC F36(0.3%, 3g in 1L drench post planting)
11. Fungicide dip + drench treatment (thiabendazole + Folicur tebuconazole, 5% w/w)

Pathogens

1. Control (no pathogen)
2. *Fusarium oxysporum* f.sp. *cepae* chlamydospores in talc (high) 6.3 x 10^7 colony forming units/kg soil
3. *Sclerotium cepivorum* sclerotia (high) 1.6×10^4 sclerotia / kg soil.

Sets of the fungicide treatment were dipped for 15 minutes and dried before planting. The biocontrol drench treatments were applied in 7 mL to the soil around the set after planting.

Each bioassay was conducted with sets of the onion cultivar Stur BC20 supplied by Elsoms. Sets were potted in FP7 Optipot pots, placed in saucers and watered from below. The first bioassay was conducted with a silt soil from Kirton, set up in April 2010 and harvested in September 2010. Each bioassay was conducted on benches in open-sided poly-tunnels. There were four replicate blocks with 10 plants of each biocontrol × pathogen treatment (5 plants of each biocontrol treatment with no pathogen) in each block. The second bioassay was conducted with a sandy loam from Wellesbourne, set up in March 2011 and harvested in August 2011. There were eight replicate blocks with eight plants of each biocontrol × pathogen (4 plants with no pathogen) in each block. The number of diseased plants was recorded at weekly intervals and at harvest. The weight of 'healthy' plants at harvest was recorded. The populations of biocontrol isolate propagules in the soils at the end of the pot bioassays was determined by dilution plating suspensions of soil on selectiveagar.

Develop large-scale methods for producing Trichoderma inoculum, substrate and pellet carriers and pelleted seed amended with Trichoderma isolates (Milestone 2.1)

Preliminary work was conducted during 2009 and 2010 to examine the large scale production of Trichoderma amended composts.

Rye grain spawn of HDC F35 was produced by Sylvan and added at 0.2% w/w/ to 25 tonne batches of green waste compost or onion waste compost (composted and matured for 9 months). The grain inoculum of HDC F35 had a *Trichoderma* propagule population of 5×10^7 cfu/g, and the initial propagule population in the inoculated compost was 1×10^5 cfu/g. The compost temperature was 15-30ºC for three weeks. Samples were then assessed for propagule populations as previously described.

Optimise within planting-row substrate and pellet applications (Milestone 2.2)

Application of Trichoderma amended composts

Preliminary work was conducted during 2009 and 2010 to examine the large scale application of Trichoderma amended composts in the field.

Screened (20 mm) green waste compost and onion waste compost amended with biocontrol agent HDC F35 were prepared as previously described. The green waste compost was applied with a converted set planter at 7 t/ha to three sites and the onion waste compost applied at 50 t/ha by broadcasting at two sites. Onions from sets (cv. Red Baron) were grown on the green waste compost amended sites and onions (cv. Red Baron) and shallots

(cv. Matador) from seed were grown on the sites amended with onion waste compost. On each site, control areas without compost application were planted with the same cultivars. Before harvest, 2 m row lengths in five replicate plots in compost treated areas and in untreated control areas were assessed for disease (white rot and/or Fusarium) and soil samples taken and assessed for *Trichoderma* propagule populations as previously described.

Application of HDC F39 to field sites

Two formulations of HDC F39 were applied to six different sites about one month after sowing or set planting in 2011. HDC F39 was applied at 3 g in 10L per square metre of growing area. HDC F40 granules were applied at 10 g per square metre of growing area and watered in with 10L water. Control (untreated) plot areas were also watered with 10L. On each site there were three replicate plots of each treatment. Before harvest, 2 m row lengths in each replicate plot in HDC F39 treated and untreated control areas were assessed for disease (white rot and/or Fusarium) and soil samples taken and assessed for *Trichoderma* propagule populations as previously described.

All soils and composts used in the pot and field experiments were analysed for pH, electrical conductivity (EC) and organic matter.

Results and discussion

Preparation of Trichoderma inocula, amended substrates and pellet carrier systems with Trichodermaisolates and monitoring survival in soil (Milestone 1.1)

Colonisation of different organic substrates by Trichodermas *and other biocontrol agents*

The dry rye grain and dry millet inocula produced the highest *Trichoderma* counts (Table 1). The counts were higher in the ORL compost than in the J Moody compost; the ORL compost had a lower pH (7.0) and moisture content (33.6%) than the J Moody compost (8.0 and 39.1%).The *Trichoderma* propagule count in the HDC F35 dried rye and fresh rye grain inocula were similar but milling the dried inoculum significantly reduced the colony count (Table 2). Unlike the fresh inoculum, the dried inocula did not need cold storage.

After two weeks in the compost, only the HDC F35 inoculum showed any increase in colony counts, the populations of the other biocontrol agents were similar or lower than those at the time of adding to the compost (Table 2).

Table 1: Population of *Trichoderma* HDC F35 produced on different inocula and added to two green waste composts. Each value is the mean of three replicates (±S.D.)

Inoculum/Compost	ORL	J Moody
wet rye grain	1.7 (±0.3) x 10^6	1.8 (±0.5) x 10^5
dry rye grain	1.2 (±0.5) x 10^7	2.1 (±1.3) x 10^6
dry millet	6.0 (±1.0) x 10^6	4.6 (±4.1) x 10^6
wet biodac	1.3 (±1.0) x 10^4	< 10^3
dry biodac	1.2 (±0.2) x 10^5	2.9 (±1.0) x 10^5

Table 2: Population of *Trichoderma* species and other biocontrol agents in different inocula and in amended and non-amended compost. Mean of three replicates (±S.D.)

Species/ Isolate	Inoculum	Inoculum cfu/g	Compost cfu/g
None	None	-	< 10^3
HDC F35	fresh rye grain	4.5(±0.9) x 10^7	-
HDC F35	dried rye grain	4.9 (±1.8) x 10^7	4.5 (±0.6) x 10^8
HDC F35	milled, dried rye grain	1.0 (±0.5) x 10^5	-
HDC F39	spores	1.2 (±0.2) x 10^9	5.1(±4.1) x 10^6
HDC F41	spores	9.7 (±2.2) x 10^7	4.4 (±2.3) x 10^6
HDC F42	spores	5.9 (±1.3) x 10^8	1.2 (±1.0) x 10^7

After two weeks, the uninoculated paper waste contained a high natural level of *Trichoderma* spp. None of the *Trichoderma* inocula increased this population (Table 3). Inoculation of woodfibre with *Trichoderma* inoculum also did not increase the natural background *Trichoderma* population. HDC F35 and *T. harzianum* T5 were the only isolates to show significant growth on compost. All of the *Trichoderma* isolates grew on straw with the highest *Trichoderma* counts obtained from HDC F35 and *T. harzianum* T5 (Table 3).

Table 3: Total colony forming units in different organic substrates following incubation over a layer of the same substrate containing different *Trichoderma* species. Each value is the mean of three replicates. (±S.D.).

Species/ Isolate	**Inoculum**	**Trichoderma cfu/g**
None	compost	4.5 (±1.0) x 10^4
	straw	2.0 (±1.0) x 10^4
	paper waste	5.6 (±1.0) x 10^6
	woodfibre	8.5 (±1.0) x 10^3
HDC F35	compost	3.6 (±1.0) x 10^5
	straw	1.4 (±1.0) x 10^7
	paper waste	1.2 (±1.0) x 10^6
	woodfibre	3.0 (±1.0) x 10^4
HDC F38	compost	2.0 (±1.0) x 10^3
	straw	6.0 (±1.0) x 10^5
	paper waste	6.0 (±1.0) x 10^5
	woodfibre	2.0 (±1.0) x 10^3
HDC F36	compost	$<10^3$
	straw	5.0 (±1.0) x 10^5
	paper waste	1.3 (±1.0) x 10^6
	woodfibre	1.0 (±1.0) x 10^3
HDC F37	compost	7.0 (±1.0) x 10^4
	straw	9.0 (±1.0) x 10^5
	paper waste	3.9 (±1.0) x 10^6
	woodfibre	3.0 (±1.0) x 10^3
T. harzianum T5	compost	5.0 (±1.0) x 10^5
	straw	7.0 (±1.0) x 10^6
	paper waste	1.0 (±1.0) x 10^7
	woodfibre	1.0 (±1.0) x 10^4

Preparation of pelleted seed containing biocontrol agents

High populations (>10^6 cfu/g pellet) of biocontrol agent propagules were detected in the HDC F39 and HDC F41 treated pellets respectively (Table 4). These populations were lower in the fungicide treated pellets. No biocontrol agent propagules were detected in the untreated raw seeds.

Table 4: Population of biocontrol agent propagules in raw seed and pelleted salad onion seeds. Each value is the mean of three replicates

Treatment	Population cfu/g
Untreated raw seed	< 10^3
HDC F39	2.8 x 10^7
HDC F39 + fungicide	5.2 x 10^6
HDC F41	6.5 x 10^6
HDC F41 + fungicide	9.0 x 10^5

Final germination percentages were not adversely affected by any of the pellet treatments compared with untreated raw seed, although the interim germination percentages of the HDC F41 treated pellets were lower (Table 5). Of the percentage germinated seeds in the HDC F41 and HDC F41 + fungicide treated pellets, 34% and 17% respectively had a white mycelial growth which was not damaging to the roots. There was some evidence that the percentages of diseased or dead seeds were lower when HDC F39 and/or fungicide was incorporated in the pellet.

Table 5: Germination test on batches of 100 raw and pelleted salad onion seed with different biocontrol agents fungicide (thiram + Metalaxyl M) treatments applied to the pellet.

Treatment	Interim germination, %	Final germination, %	Diseased seeds, %	Dead seeds, %
Raw seed	71.5	86.0	6.0	8.0
HDC F41	32.0	85.0	2.0	13.0
HDC F41 + Fung.	25.0	92.0	1.0	7.0
HDC F39	74.5	93.0	0.0	5.0
HDC F39 + Fung.	53.0	95.0	0.0	7.0

Effects of substrate and pellet carrier systems amended with Trichoderma isolateson Fusarium basal rot and white rot of onion in pot bioassays (Milestone 1.2)

There was no significant difference in final 'healthy' bulb weight between biocontrol treatments in either pot bioassay, but the weight of 'healthy' bulbs from Fusarium or white rot inoculated pots was slightly lower (significant at $P = 0.05$) than from pots with no pathogen

inoculum added. This indicates that the pathogen inoculum had a negative effect on plant growth even when disease symptoms did not develop.

In the 2010 pot bioassay, there were no significant differences in the healthy bulb weight or Fusarium and white rot disease levels between the two *Fusarium* or *S. cepivorum* pathogen inoculum levels.The mean values of the two pathogen inoculum levels were therefore used. The composts amended with HDC F35 or HDC F39, and the HDC F41, HDC F42 or fungicide dip treatments significantly reduced Fusarium (by at least 50%) (Fig.1a). The other treatments did not have a significant effect on Fusarium.

The compost treatments, HDC F39 and fungicide dip treatments reduced white rot in the 2010 pot bioassay by at least 40% (Fig. 1b). The HDC F42 dip also significantly reduced white rot by 30%. None of the other biocontrol dip treatments was effective against white rot.

At the end of the 2010 pot bioassay, the soil *Trichoderma* counts of the HDC F35 amended compost and HDC F39 dip treatments were higher than those of the untreated soil, compost amended soil or fungicide dip treatments (Table 6). The soil populations of the other biocontrol treatments were not determined. Details of the soils used in the pot and field experiments are shown in the Appendix, Table A1.

Table 6: Total *Trichoderma* spp. colony counts in soils at the end of the 2010 pot bioassay (background and introduced levels). Each value is the mean of four replicate samples (±S.D).

Treatment	***Trichoderma* cfu/g soil**
Soil	1.5 (±1.0) x 10^4
Compost	7.9 (±1.0)x 10^3
Compost + HDC F35	6.0 (±1.0) x 10^6
HDC F39 dip + drench	1.0 (±1.0) x 10^5
Fungicide dip	4.0 (±1.0) x 10^3

In the 2011 pot bioassay, the HDC F35 amended compost, HDC F41 drench, and HDC F39 drench and granule treatments significantly reduced Fusarium (by at least 25%). The unamended compost, HDC F35 inoculum and other biocontrol agents were ineffective against Fusarium. However, the level of Fusarium disease was much higher, and the percentage of disease control lower than in the 2010 pot bioassay (Fig. 2a).

Similar to the 2010 pot bioassay, the HDC F35 amended compost and HDC F39 drench treatments were again effective against white rot, as were the HDC F40 granule and HDC F37 drench treatments (Fig 2b). These treatments all reduced white rot by more than 50%.

The unamended compost, HDC F38 granules and HDC F41 drench treatments also significantly reduced white rot (by at least 25%). The HDC F35 inoculum and HDC F36 drench treatments were ineffective. Unlike the 2010 pot bioassay, the fungicide dip treatment (thiabendazole + Folicur) was ineffective in controlling either Fusarium or white rot (Fig. 2a and b).

At the end of the 2011 pot bioassay, the soil *Trichoderma* counts of HDC F35 amended compost, HDC F35, HDC F40 and HDC F38 granules and HDC F37 drench treatments were higher than those of the untreated soil, compost amended soil or fungicide dip treatments (Table 7). The highest counts were obtained in the HDC F35 amended compost treatment. The HDC F41 drench treatment resulted in a five-fold increase in the soil *Clonostachys* population.

Table 7: *Trichoderma* spp. or *Clonostachys* colony counts in soils at end of 2011 pot bioassay. Each value is the mean of four replicate samples (±S.D.)

Treatment	***Trichoderma*or *Clonostachys*** **cfu/g soil**
Soil	5.5 (±1.0) x 10^3
Compost	6.5 (±1.0) x 10^4
Compost + HDC F35	4.9 (±1.0) x 10^6
HDC F35 granules	2.1 (±1.0) x 10^6
HDC F40 granules	1.1 (±1.0) x 10^5
HDC F38 granules	3.2 (±1.0) x 10^6
HDC F41 drench	2.5 (±1.0) x 10^4
HDC F37 drench	7.0 (±1.0) x 10^5
HDC F39 drench	5.0 (±1.0) x 10^4
Fungicide dip	3.8 (±1.0) x 10^4

The Kirton silt soil used in pot bioassay 1 had a slightly higher pH value and organic matter content and a lower EC than the Wellesbourne sandy loam soil used in pot bioassay 2 (Appendix Table A1).

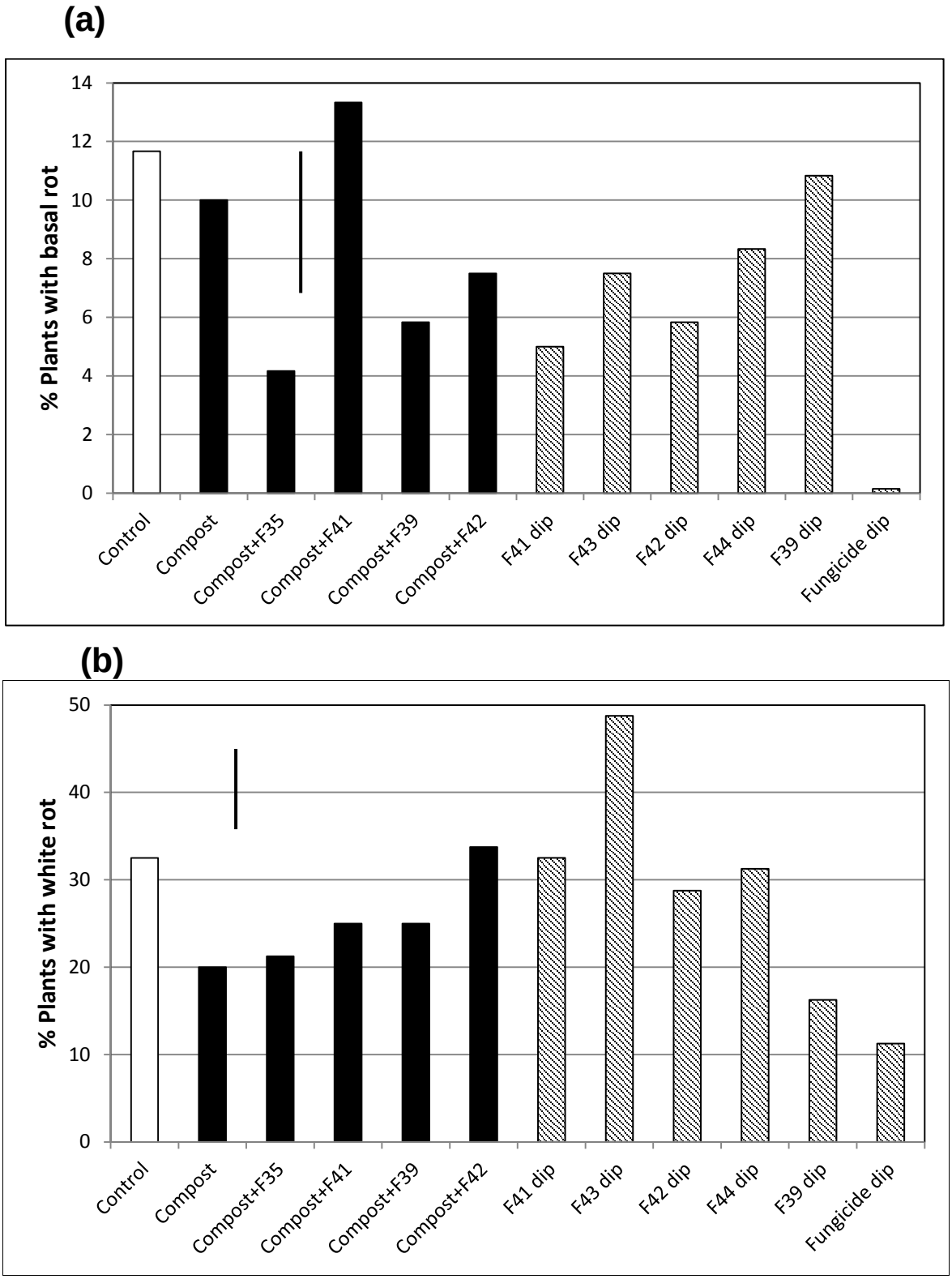

Fig 1: Effects of biocontrolor fungicide treatments applied as amended compost (25% v/v, black bars) or as set dip + drench treatments (shaded bars) on (a) Fusarium basal rot and (b) Allium white rot in 2010 pot bioassay. The vertical bar indicates the Least Significant Difference (P = 0.05).

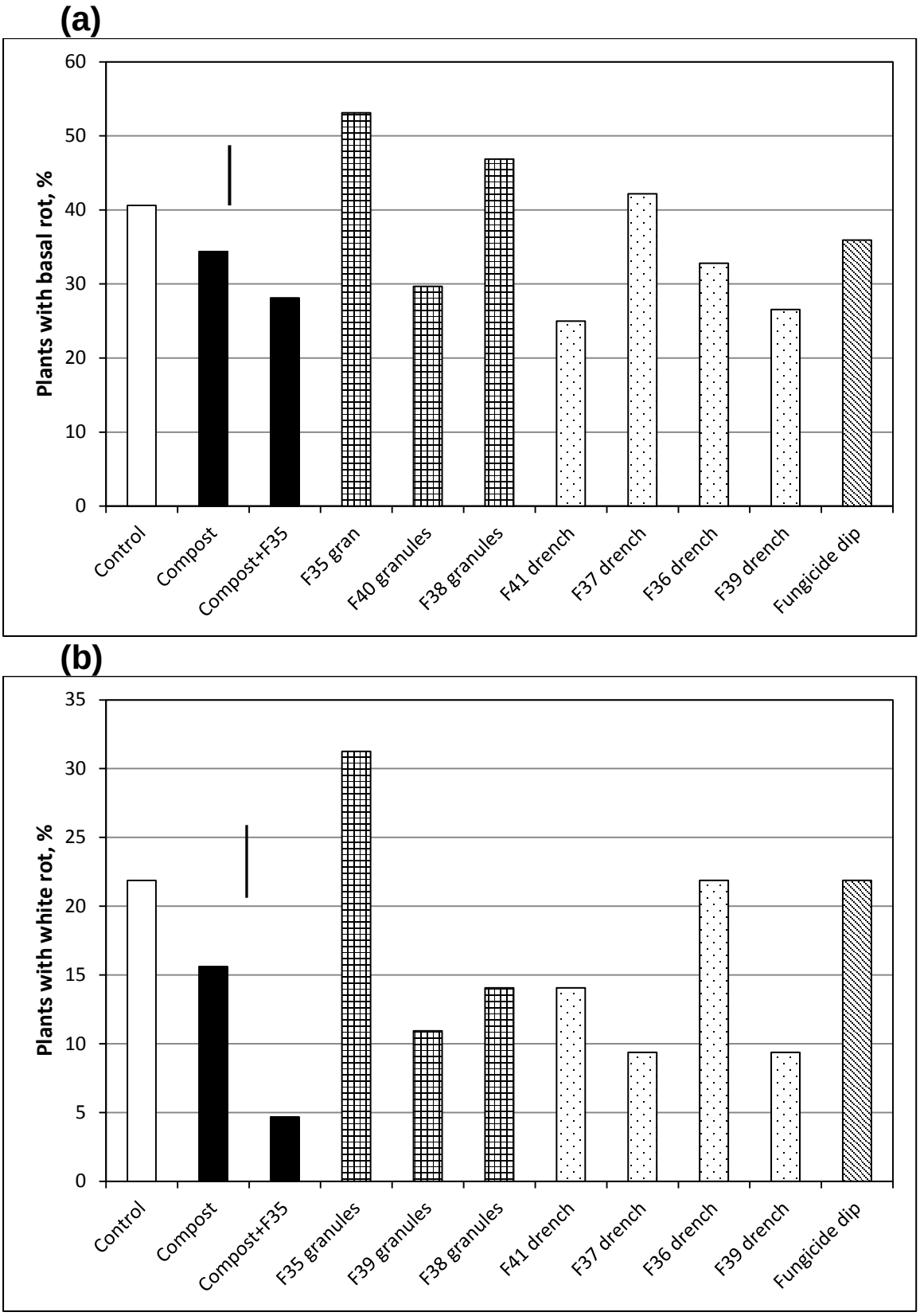

Fig 2: Effects of biocontrol treatments applied as amended compost (25% v/v, black bars), as granules (cross-hatched bars) or as drenches (dot shaded bars) or fungicide dip on (a)Fusarium basal rot and (b) Allium white rot in 2011 pot bioassay. The vertical bar indicates the Least Significant Difference (P = 0.05)

Develop large-scale methods for producing Trichoderma inoculum, substrate and pellet carriers and pelleted seed amended with Trichoderma isolates (Milestone 2.1)

The highest population of Trichoderma propagules was obtained in a batch of green waste compost, where the population increased times 1000 over the initial population. This compost was drier than the other composts, but had similar pH and electrical conductivity (EC) values to the other composts (Table 8). Increases in Trichoderma population in the other green waste and onion waste composts were between times 4 and times 39 (Table 8).

Table 8: *Trichoderma* counts in large-scale composts

Compost	moisture % w/w	pH	EC mS/cm	Trichoderma cfu/g
Green waste	47	8.8	2.1	3.9×10^6
Green waste	29	8.0	1.7	4.0×10^8
Onion waste	57	8.0	0.8	4.0×10^5
Onion waste	55	7.5	1.6	1.3×10^6
Onion waste	54	7.6	1.5	1.4×10^6

Optimise within planting-row substrate and pellet applications (Milestone 2.2)

Application of Trichoderma amended composts

Three of the sites were affected by white rot, one site was affected by Fusarium, and one site was affected by both diseases (Table 9). At Saracens Head, Gosberton and Kirton sites, disease (mainly white rot) was on average 27% lower in the compost + HDC F35 amended rows than in the untreated areas (Table 9). Broadcast application of HDC F35 amended onion waste compost reduced white rot by 64% but had no effect on Fusarium (Table 9).Application of HDC F35 amended green waste compost within the planting row or broadcast application of HDC F35 amended onion waste compost increased the soil *Trichoderma* population by between times 5 and 50 depending on the site. However, the final soil Trichoderma populations (2.1-9.5 $\times 10^5$) were about 10% of those needed to give control of white rot or Fusarium in the pot bioassays or in previous field experiments in HL0176.

Application of Trianum to field sites

Application of the HDC F39 drench increased the soil *Trichoderma* propagule population at harvest by between times 2 and 5 on four of the six sites (Table 10). The HDC F40 granule application increased the soil Trichoderma propagule population at harvest by times10 at the Chatteris site but had no effect at the Boxted site. White rot was reduced by the HDC F39

application at the Clifton site (shallots cv. Matador) but had no significant effect on white rot at the Kirton site (onions cv Red Baron) or Fusarium at the Chicksands site (onions cv Red Baron). No disease was recorded within the plot areas at the other three sites (Table 10).

Table 9: Effect of HDC F35 amended compost on *Trichoderma* spp. colony counts in soils (background soil and HDC F35 compost introduced levels) and disease levels (w=white rot; f=Fusarium) on different sites. Each value is the mean of five replicate plots

Site	Treatment	Application	*Trichoderma* cfu/g soil	Disease, %
Saracens Head	Soil	-	4.1×10^4	42.2 w
Saracens Head	Compost + HDC F35	planting row	2.1×10^5	31.3 w
Gosberton	Soil	-	5.1×10^3	6.3 w+f
Gosberton	Compost + HDC F35	planting row	1.8×10^5	1.9 w+f
Kirton	Soil	-	1.8×10^4	35.1 w
Kirton	Compost + HDC F35	planting row	7.0×10^5	27.7 w
Clifton	Soil	-	5.1×10^4	33.8 w
Clifton	Compost + HDC F35	broadcast	1.1×10^5	12.1 w
Chicksands	Soil	-	1.2×10^5	38.1 f
Chicksands	Compost + HDC F35	broadcast	3.3×10^5	33.3 f

Table 10: Effect of HDC F39 or HDC F40 applications on soil *Trichoderma* populations and disease levels (w=white rot; f=Fusarium) at different sites. Each value is the mean of the three replicate plots

Site	Application	*Trichoderma*, cfu/g soil	Disease %
Kirton	none	1.0×10^4	16.6 w
Kirton	Drench HDC F39	2.7×10^4	13.0 w
Clifton	none	1.1×10^4	33.8 w
Clifton	Drench HDC F39	5.0×10^4	9.8 w
Chicksands	none	1.8×10^4	38.1 f
Chicksands	Drench HDC F39	1.5×10^5	38.6 f
Chatteris	none	6.5×10^3	0
Chatteris	Drench HDC F39	3.5×10^4	0
Chatteris	Granules HDC F40	7.0×10^4	0
Littleport	none	5.3×10^4	0
Littleport	Drench HDC F39	6.7×10^4	0
Boxted	none	4.0×10^4	0
Boxted	Drench HDC F39	3.5×10^4	0
Boxted	Granules HDC F40	4.0×10^4	0

The pH value, EC and organic matter content of the soils of the field sites are shown in the Appendix (Table A1).

Conclusions

- HDC F35 amended compost and a dip and/or drench treatment of HDC F41 suppressed Fusarium by 25-60% in two pot bioassays.HDC F42 which was only used in the first bioassay, also suppressed Fusarium by 50%
- HDC F40 granules or HDC F39 drench treatment suppressed Fusarium by 25% in the second pot bioassay but not in the first
- HDC F35 amended compost or a dip and/or drench treatment of HDC F39 suppressed white rot by 40-60% in two pot bioassays. HDC F39 or HDC F38 granules, or drench treatments of HDC F37 or HDC F42, which were only used in one of the pot bioassays, suppressed white rot by 30-50%
- An HDC F41 drench treatment suppressed white rot by 30% in the second pot bioassay but not in the first
- HDC F35 granules or HDC F36, HDC F43 or HDC F44 drench treatments were ineffective against Fusarium or white rot.HDC F38 granules or a HDC F37 drench were also ineffective against Fusarium
- Unamended compost suppressed white rot by 25-30% but had no effect on Fusarium.
- A fungicide (thiabendazole + Folicur) set treatment controlled Fusarium and white rot in the first pot bioassay but not in the second
- Spores of HDC F41 and HDC F39 were incorporated into seed pellets.High populations (>10^6 cfu/g pellet) of biocontrol agent propagules were detected in the HDC F39 and HDC F41 treated pellets respectively
- Final germination percentages were not adversely affected by any of the above pellet treatments compared with untreated raw seed, although the interim germination percentages of the HDC F41 treated pellets were lower
- Application of HDC F35 amended compost, either as screened compost in the planting row or as broadcast onion waste compost, produced some suppression of white rot in the field but did not achieve the high soil levels of propagules needed for effective control of white rot and Fusarium
- Straw was found to be a very suitable organic substrate for HDC F35 and the biocontrol agents HDC F38, HDC F37 and T5 (*Trichoderma harzianum*). Drier composts (moisture <30%) were found to be better substrates than wetter composts
- A HDC F39 drench application reduced white rot in one out of two field sites but had no significant effect on Fusarium on one field site.

Technology transfer

Publications

New Projects. *HDC News* 179, p.11.

R. Noble (2011). Fusarium control. Proceedings of Onion and Carrot Conference, Peterborough.

Presentation

R. Noble. Presentation on “Control of Fusarium” delivered to UK Onion and Carrot Conference, Peterborough, November 2011.

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APPENDIX

Table A1. Analysis of soils used in the experiments

Site	Soil type	pH	EC, uS/cm	Organic matter % w/w
Kirton (pots)	silt	7.74	89	7.4
Wellesbourne (pots)	sandy loam	6.75	287	3.6
Saracens Head	silt	7.88	106	5.8
Gosberton	silt	7.34	40	5.6
Clifton	silt	6.77	53	3.8
Chicksands	sandy loam	6.67	50	3.2
Chatteris	peat	6.71	602	39.0
Littleport	peat	7.13	154	32.8
Boxted	sandy loam	5.85	73	2.5