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GROWER SUMMARY

Headline

Novel treatments for onion neck rot were evaluated in this project

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 fungicides for Fusarium of onion are a seed treatment with metalaxyl-M + thiram, which is aimed at controlling seedling blight rather than basal rot, and Signum (boscalid + pyraclostrobin). 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. Prestop (*Gliocladium catenulatum*) has an off-label approval for Fusarium control and Serenade (*Bacillus subtilis*) has an off-label approval for white rot control. The main problem has been the development of a cheap delivery method that can achieve sufficiently high inoculum levels of biocontrol agents in soil.

Summary of the project and main conclusions

- Pelleted bulb onion seed with high populations of spores (>10^6 g pellet) of the biocontrol agents HDC F37, HDC F39, HDC F41 and Prestop (previously known as HDC F42) was produced by Incotec/Elsoms
- HDC F37 sprayed at drilling at 0.5 g/m^2 significantly reduced the level of white rot but did not suppress Fusarium basal rot; seed pellets containing HDC F37 also showed some reduction in white rot but the effect was not statistically significant
- Serenade ASO (previously known as HDC F43) drenched at 2 g/m^2 was ineffective in suppressing Fusarium but the level of white rot was lower in plots treated with Serenade ASO (previously known as HDC F43) at this rate
- White rot was consistently controlled in pot experiments using compost + HDC F35 when the soil biocontrol propagule count was increased to 10^6 cfu/g
- Mechanised application of dried compost + HDC F35 along the planting row at 12 t/ha increased the soil biocontrol propagule count to 3.6 x 10^6 cfu/g; due to a low level of white rot in the field, the effect on disease could not be established.

Inoculum development

- Pelleted bulb onion seed with spores of the biocontrol agents HDC F37, HDC F39 and Prestop (previously known as HDC F42) was produced by Incotec/Elsoms. High populations (>10^6 cfu/g pellet) of biocontrol agent propagules were detected in the treated pellets
- The biocontrol agent populations in the seed pellets were unaffected by two months storage at 5˚C and declined by 10-30% after eight months storage at 5˚C
- Seed germination in soil was unaffected by the biocontrol agent in the pellet but Prestop (previously known as HDC F42) resulted in lower germination in peat modules and in one out of two laboratory tests

Pot experiment with pelleted seed

- Plants grown from pelleted seed containing HDC F37 or Prestop (previously known as HDC F42) inoculum or fungicide (metalaxyl-M + thiram) had 31 to 37% less Fusarium than plants grown from untreated pellet seeds
- Healthy plants grown from HDC F37 or fungicide treated pellet seed produced larger bulbs at harvest than plants grown from untreated pellet seeds
- None of the pellet seed treatments significantly affected white rot in the plants

Pot experiments with sets

- Across three years, the most consistent level of Fusarium disease control was obtained with a HDC F41 drench treatment; a Prestop (previously known as HDC F42) drench treatment was also effective in the two years that it was tested
- Across three years, the most consistent level of white rot disease control was obtained with a compost + HDC F35 treatment; a HDC F37 drench treatment was also effective in the two years that it was tested
- A Folicur fungicide set dip + drench treatment was effective against both diseases

Field Experiments

- HDC F37 sprayed at drilling at 0.5 g/m^2 significantly reduced the level of white rot but did not suppress Fusarium basal rot; seed pellets containing HDC F37 also showed some reduction in white rot but the effect was not statistically significant
- Drenches of HDC F39 (3 g/m^2) or HDC F42 (5 g/m^2) suppressed Fusarium basal rot and the level of white rot was also lower in plots treated with Prestop (previously known as HDC F42) at this rate; Prestop (previously known as HDC F42) sprayed at drilling at 2.5 g/m^2 was ineffective in controlling either disease

- Serenade ASO (previously known as HDC F43) drenched at 2 g/m^2 was ineffective in suppressing Fusarium but the level of white rot was lower in plots treated with Serenade ASO (previously known as HDC F43) at this rate
- Broadcasting compost + HDC F35 at 50 t/ha increased the soil biocontrol propagule count to 2.7 x 10^5 cfu/g but was ineffective against white rot
- Matured green waste compost, screened to 20 mm with a moisture content of 41% could be applied to planting rows in the field through a converted set planter; compost with a moisture content of 44% or greater tended to clog
- Dried compost + HDC F35 applied along the planting row at 12 t/ha increased the soil biocontrol propagule count to 3.6 x 10^6 cfu/g; due to a low level of white rot, the effect on disease could not be established
- A double application of Folicur alternated with a double application of Signum reduced white rot in one out of two seasons; it was ineffective in controlling Fusarium
- A seed pellet treatment of metalaxyl-M + thiram had no effect on white rot but showed some reduction in Fusarium, although the disease reduction was not statistically significant

Financial benefits

Although HDC F37 is not currently registered for use on onions or as a seed pellet treatment, it may become available for these purposes since it is registered as a biopesticide for other crops. Serenade ASO (previously known as HDC F43) showed some efficacy against the disease in a field trial as a drench treatment. However, unlike HDC F37, it was ineffective in pot experiments. A spray at drilling and/or pellet seed treatment of HDC F37 is likely to be more cost effective than a drench treatment with Serenade ASO (previously known as HDC F43).

If Folicur and Signum are being used on onion crops that are mainly at risk from Fusarium rather than white rot, this could be a waste of fungicide applications (see below) and potential savings could be made.

The method for applying compost along the planting row is a more efficient method than broadcasting over the entire area, with a saving of 75% of the compost needed. This should also enable growers to apply compost at application rates well within the limits set by the Environment Agency for NVZs. However, further work is needed to evaluate the costs of producing and applying compost in this method, establishing the crop benefits compared with using inorganic fertilisers, and the potential for applying biopesticides with the compost.

Action points for growers

- If Folicur and Signum are being used on crops that are mainly at risk from Fusarium rather than white rot, sufficient areas should be left untreated to check if the treatment is having any effect.

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 fungicides for Fusarium of onion are a seed treatment with metalaxyl-M + thiram, which is aimed at controlling seedling blight rather than basal rot, and Signum (boscalid + pyraclostrobin) which is not very effective. 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 day length 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]. Prestop (*Gliocladium catenulatum*) has an off-label approval for Fusarium control and Serenade (*Bacillus subtilis*) has an off-label approval for white rot control. The main technical problem has been the development of a cheap delivery method that can achieve sufficiently high inoculum levels of biocontrol agents in the root zone soil. Applying biocontrol agents in a low cost organic carrier material could potentially lead to a high soil population and effective disease control. Composting of onion waste is now widely practiced in the industry and the composted waste is a potential source of organic matter with which to apply biocontrol agents into the soil. Application of biocontrol agents 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* spp. and other microbial biopesticides into the soil at sowing.

Project Aims

- Evaluate control of Fusarium basal rot and white rot of onion using substrates and/or pellet carriers amended with specific biocontrol agents and other commercial biopesticides in pot experiments.
- Test the best biocontrol agent substrate and pellet carriers within planting row applications in the field, and establish any independent effects of the substrate carriers on the crop.
- Compare disease control achieved with the biological methods, chemical fungicide treatments, and combinations of biological and fungicide treatments.

Materials and methods

Preparation of Biocontrol agent inocula, amended substrates and pellet carrier systems and monitoring biocontrol agents in soil (Milestone 1.1)

Colonisation of different organic substrates by 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 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 100 g 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 Prestop (previously known as HDC F42) were used to inoculate ORL green-waste compost in 20 L 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 potato dextrose agar containing a bactericidal antibiotic (chlorotetracyclin).

Effect of compost characteristics on biocontrol agent growth

Compost batches (21) were obtained from ORL, J Moody Ltd, Simpro Ltd (green waste), and F Parrish (onion waste) and inoculated with 1% w/w grain spawn of HDC F35 or inoculum of HDC F38 in 20 L containers. The moisture content, pH and electrical conductivity of each batch was determined and the growth rate of HDC F35 and HDC F38 determined as described above. The total background population of biocontrol agents in the composts was also recorded.

Preparation of pelleted seed containing biocontrol agents

Pelleted seed was prepared by Incotec/Elsoms with the following biocontrol agents added to the seed pellet coating:
Year 1, salad onion seed (cv. White Lisbon), HDC F39, HDC F41
Year 2, bulb onion (cv. Hytech), HDC F37, HDC F39, HDC F42
Year 3, bulb onion seed (cv. Hytech): HDC F37, HDC F41, HDC F42
In each batch, seeds with no addition (untreated control) and metalaxyl-M+thiram fungicides were also prepared.

Populations of biocontrol agents were determined in the fresh seeds and after storage at 5˚C for 2 and 8 months.

Germination tests of pelleted onion seed

The germination of 100 seeds of each batch was tested in the laboratory on moist filter paper after 11 days and in the glasshouse in modules containing a peat growing medium by Elsoms. Germination tests on pelleted bulb onion seed were also conducted in pots of soil (sandy loam, Pershore, Worcs.). There were eight replicate blocks with eight pots per block and five seeds per pot.

Effects of substrate and pellet carrier systems amended with biocontrol agents on Fusarium basal rot and white rot of onion in pot bioassays (Milestones 1.2, 3.2 and 3.3)

Pot bioassays

Pot bioassays for disease control were conducted in FP7 Optipots containing sieved soil, placed in saucers and watered from below. Each bioassay was conducted on benches in open-sided poly-tunnels using sets or seeds, with the numbers per pot stated below. The bioassays were set up in March or April and terminated in August or September of 2010 (Year 0), 2011 (Year 1) and 2012 (Year 2) and lasted for 20 weeks. Each of the biocontrol agent or fungicide treatments were tested against the following pathogen treatments:

1. Control (no pathogen)
2. *Fusarium oxysporum* f.sp. *cepae* chlamydospores in talc, applied at 4.88 x 10^6 colony forming units/kg soil or soil + compost mixture
3. *Sclerotium cepivorum* sclerotia (high) 1.6 ×10^4 sclerotia / kg soil or soil + compost mixture.

The pathogen inocula were incorporated throughout the soil pre-sowing or pre-planting. The number of diseased plants was recorded at weekly intervals and at harvest.

Compost, granule and drench treatments with sets

Three pot bioassays in Years 0, 1 and 2 were used to determine the effects of different biocontrol agents and application methods on Fusarium and Allium white rot compared with fungicide treatments (Table 1). Each bioassay was conducted with sets of the onion cultivar Stur BC20 supplied by Elsoms. The compost (ORL) was prepared as described in the previous section. After planting, the soil around each set was drenched with 7 ml of the respective biocontrol treatment. In the first bioassay 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. In the second and third bioassays there were eight replicate blocks with eight plants of each biocontrol × pathogen (4 plants with no pathogen) in each block.

Table 1. Pot bioassay treatments on onion sets.

Years	Treatment	Isolate or Fungicide	Rate
0, 1, 2	Soil Control	None	-
0, 1, 2	Compost	None	25% v/v
0, 1, 2	Compost	HDC F35	25% v/v
0	Compost	HDC F39	25% v/v
0	Compost	HDC F41	25% v/v
0	Compost	HDC F42	25% v/v
1	Granule	HDC F35	0.3 g/pot
1	Granule	HDC F38	0.2 g/pot
1	Granule	HDC F40	0.2 g/pot
1	Drench	HDC F36	3 g/L
1, 2	Drench	HDC F37	3 g/L
0, 1, 2	Drench	HDC F39	3 g/L
0, 1, 2	Drench	HDC F41	20 g/L
0	Drench	HDC F42	20 g/L
2	Drench	HDC F42	5 g/L
2	Drench	HDC F43	20 g/L
0	Drench	HDC F44	1 g/L
0, 1, 2	Fungicide Dip	Fungicide*	0.5% w/w

* Sets fungicide treatment and drench at planting of Folicur

Pellet seed treatments

A pot bioassay was set up in 2012 using bulb onion cv. Hytech pelleted seed incorporating HDC F37, HDC F41 or Prestop (previously known as HDC F42). Untreated pelleted seed and seed treated with metalaxyl-M + thiram fungicides were used for comparison. Two seeds were sown in each pot and these were thinned to one seedling after one month. There were eight replicate blocks with eight plants of each biocontrol × pathogen (4 plants with no pathogen) in each block.

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

Production of 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 (Organic Recycling Ltd) or onion waste compost (composted and matured for 9 months at F Parrish). The grain inoculum of HDC F35 had a 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 maintained at 15-30 ºC for three weeks. Samples were then assessed for propagule populations as previously described.

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

Field experiments 2011- Application of biocontrol agent amended composts

Screened (20 mm) green-waste compost and onion-waste compost amended with biocontrol agent HDC F35 were prepared as previously described. A converted set planter for applying compost in bands along the planting row was produced by Moulton Bulb Co. (Fig. 1). In 2011, the green-waste compost was applied with the 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 planted at 41 sets/m^2 on the green waste compost amended sites and onions (cv. Red Baron) and shallots (cv. Matador) from seed were sown on the sites amended with onion-waste compost at 60 and 222 seeds/m^2 respectively. 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 basal rot) and soil samples taken and assessed for biocontrol agent propagule populations as previously described.

Field experiments 2011- Application of HDC F39 drench and HDC F40 granules

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

Fig. 1. Converted set planter for applying compost along the planting row.

Best biocontrol agents in granular and drench formulations and pellet carrier treatments from pot experiments tested for Fusarium basal rot and white rot control in field experiments (Milestones 3.2 and 3.3)

Field experiments -2012 Biocontrol drench and amended compost treatments

Field experiment treatments were applied to six experimental sites in 2012 (Tables 2 and 3). Sites were selected to cover a range of soil types (silt, sandy loam, clay loam and peat), and previous history of white rot or Fusarium basal rot in onion crops. At each site, each biocontrol or fungicide treatment was applied to four replicate plots. Biocontrol agent drenches were applied by watering can on to 3 x 1.8 m plot areas according to manufacturers' recommendations. Each plot was divided in half lengthwise; half of each plot received a single drench application, the other half received a second application five weeks later.

Table 2. 2012 Field experiment treatments applied to plots after set planting or sowing and 10 weeks after planting or sowing in 10 L water per 5.4 m^2 plot.

	Treatment	Isolate or Fungicide	Rate g/m^2
1	Soil Control	None	water only
2	Drench	HDC F39	3
3	Drench	HDC F42	5
4	Drench	HDC F43	2
5	Drench	Fungicide (Signum)	0.15

Table 3. 2012 field experiment sites and previous disease history.

	Site	Location	Soil type	Onion	Disease
1	Bannister Farms	Kirton, Lincs.	silt	bulb, sets	white rot
2	G's Vegetables	Littleport, Cambs.	peat	bulb, seed	Fusarium basal rot
3	Hensborough Farm	Kites Hardwick Warwickshire	clay loam	bulb, seed	Fusarium basal rot
4	Hensborough Farm	Kites Hardwick Warwickshire	clay loam	bulb, sets	Fusarium basal rot
5	G's Vegetables	Salford Priors, Warwickshire	sandy loam	salad, seed	white rot
6	F Parrish	Shefford, Beds.	sandy loam	bulb, seed	white rot

At a site at Clifton in Bedfordshire, composted onion waste and straw, with HDC F35 grain spawn added at 0.5% w/w, was also applied at 25t/ha and 50 t/ha to plots, and compared with drench treatments of HDC F39 (3 g/m^2), Prestop (previously known as HDC F42) (5 g/m^2) and Serenade ASO (previously known as HDC F43) (2 g/m^2) or fungicide treated (two applications of Folicur at 0.1 mL/m^2 alternated with two applications of Signum at 0.15 g/m^2) and untreated plots. There were six replicate plots of each of the treatments. Soil samples were taken at harvest and analysed for biocontrol agent propagule populations.

Field experiments 2013 - Pellet Seed and Spray Treatments

Treatments were applied to six experimental sites (Table 4). Sites were selected to cover a range of soil types (silt, sandy loam, silty loam), and previous history of white rot or Fusarium basal rot in onion crops. Three sites had pellet seed treatments and three sites had pellet seed treatments or spray treatments at drilling at a rate of 67 seeds/m^2. Each of the sites had six experiment beds, with four double rows of onions across each bed. At each site, each biocontrol or fungicide treatment was applied to six replicate plots, with one replicate of each treatment in each bed. Plots measured 2.5 m x 1.8 m wide, with a 1.5 m gap between plots. In treatments (e) and (f), biocontrol agent suspensions were applied at 30 ml/m^2 by spraying equipment attached to the seed drill on to plot areas. Pelleted seed was sown at 300 seeds/plot (67 seeds m^2).

Pellet seed or spray treatments were applied to three sites: (i) Shefford, Beds. (ii) Clifton, Beds. (iii) Castle Acre, Norfolk

(a) Untreated Pellet Seed
(b) Fungicide (metalaxyl-M + thiram) Pellet Seed
(c) HDC F37 Pellet Seed
(d) Prestop (previously known as HDC F42) Pellet Seed
(e) Untreated pellet seed, HDC F37 spray (0.5 g/m^2)
(f) Untreated pellet seed, Prestop (previously known as HDC F42) spray (2.5g/m^2)
(g) Fungicide pellet seed, Fungicide drench (two applications of Folicur at 0.1 mL/m^2 alternated with two applications of Signum at 0.15 g/m^2). Folicur sprays were applied on 11 July and 7 August, and the Signum sprays on 26 July and 21 August.

Pellet seed treatments were also applied to three other sites: (iv) Thetford, Norfolk (v) Spalding, Lincs. (vi) Kites Hardwick, Warwicks.

(a) Untreated Pellet Seed
(b) Fungicide (metalaxyl-M + thiram) Pellet Seed
(c) HDC F37 Pellet Seed

(d) Prestop (previously known as HDC F42) Pellet Seed.

Germination was assessed eight weeks after sowing in 2 m lengths in each replicate plot. Before harvest, 2 m row lengths in each replicate plot were assessed for disease (white rot and/or Fusarium) and soil samples were taken and assessed for biocontrol agent propagule populations as previously described.

Field experiment 2013 – Compost + HDC F35 Treatment

Site 2 in Table 4 (Clifton, Beds.) with a previous history of white rot was used.

(a) Untreated

(b) Mature greenwaste compost + S17A, planting row only, 6 t/ha

(c) Double application of compost + S17A, planting row only, 12 t/ha.

HDC F35 grain spawn containing 1 x 10^9 cfu/g (produced by Sylvan) was added at 0.25% w/w to mature (9 month old) green-waste compost produced by Organic Recycling Ltd. The compost was screened to 20 mm and dried to 41% moisture and applied to the planting row with the modified set planter produced by Moulton Bulb Co. Compost was applied at 6 tonnes/ha (single application) or 12 tonnes/ha (double application). Sets were planted along the rows of applied compost, 5 rows wide. There were six replicate plots of each treatment (a), (b) and (c) above, with one replicate plot of each treatment in each of six beds.

A further batch of green-waste compost prepared at ORL had a moisture content of 44% and was too moist and sticky to be applied with the converted set planter. Compost was also prepared at F. Parrish in 2012 from onion waste and sawdust. However, it was not possible to dry the material sufficiently to enable it to be applied through the converted set planter (moisture content 59%). These two compost treatments were therefore not applied.

Disease assessments were made on 2 x 1 m plot lengths at harvest. Soils and composts used in the pot experiments were analysed for pH, electrical conductivity and organic matter.

Table 4. 2013 Field experiment sites and previous disease history.

	Site	Location	Soil type	Drill/plant date	Disease
1	F Parrish	Shefford, Beds.	sandy loam	23 April	Fusarium
2	F Parrish	Clifton, Beds.	silt	23 April	white rot
3	G’s Vegetables	Castle Acre,Norfolk	sandy loam	27 March	Fusarium
4	Elverden Farms	Thetford, Norfolk	sandy loam	28 March	Fusarium
5	Elsoms Seeds	Spalding, Lincs.	silt	3 April	white rot
6	Hensborough Farm	Kites Hardwick Warwickshire	silty loam	25 April	Fusarium

Statistical analysis

Results were analysed by ANOVA and the significance of mean treatment effects determined by F test using Microsoft Excel 2010. Least significant differences (LSD) at P = 0.05, 0.01 and 0.001 were then determined for comparing treatments means.

Results

Preparation of biocontrol agent inocula, amended substrates and pellet carrier systems with biocontrol agents and monitoring survival in soil (Milestone 1.1)

Colonisation of different organic substrates by biocontrol agents

The wet and dry rye grain and dry millet inocula produced the highest propagule counts of HDC F35 in the composts (Table 5). 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% respectively).The propagule count of HDC F35 in dried rye and fresh rye grain inocula were similar but milling the dried inoculum significantly (P<0.001) reduced the colony count. 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 to or lower than those at the time of adding to the compost (Table 6).

Table 5. Population of HDC F35 produced on different inocula and added to two green-waste composts (cfu/g). Each value is the mean of three replicates (±S.D.).

Inoculum	Inoculum	ORL compost	J Moody compost
wet rye grain	4.5 (±0.9) x 10^7	1.7 (±0.3) x 10^6	1.8 (±0.5) x 10^5
dry rye grain	4.9 (±1.8) x 10^7	1.2 (±0.5) x 10^7	2.1 (±1.3) x 10^6
dry millet	1.0 (±0.5) x 10^7	6.0 (±1.0) x 10^6	4.6 (±4.1) x 10^6
wet biodac	1.1 (±0.5) x 10^5	1.3 (±1.0) x 10^4	< 10^3
dry biodac	2.9 (±0.5) x 10^5	1.2 (±0.2) x 10^5	2.9 (±1.0) x 10^5

Table 6. Population of biocontrol agents in different inocula and in amended and non-amended compost (cfu/g). Mean of three replicates (±S.D.).

Isolate	Inoculum	Inoculum	Compost
None	None	-	< 10^3
HDC F35	dried rye grain	4.9 (±1.8) x 10^7	4.5 (±0.6) x 10^8
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

Effect of compost characteristics on biocontrol agent growth

The highest counts of HDC F35 and HDC F38 were recorded in samples of compost that had a moisture content of about 30% w/w and had been matured for at least 9 months (Fig. 2). Less mature composts with a similar moisture content were less suitable for these biocontrol agents. The best composts for biocontrol growth in Fig.2 had a pH value of between 7.0 and 8.1, although there was no obvious correlation between compost pH or EC and growth of HDC F35 and HDC F38. Background populations of biocontrol agents in the composts ranged from <10^3 cfu/g to 2.7 x 10^3 cfu/g.

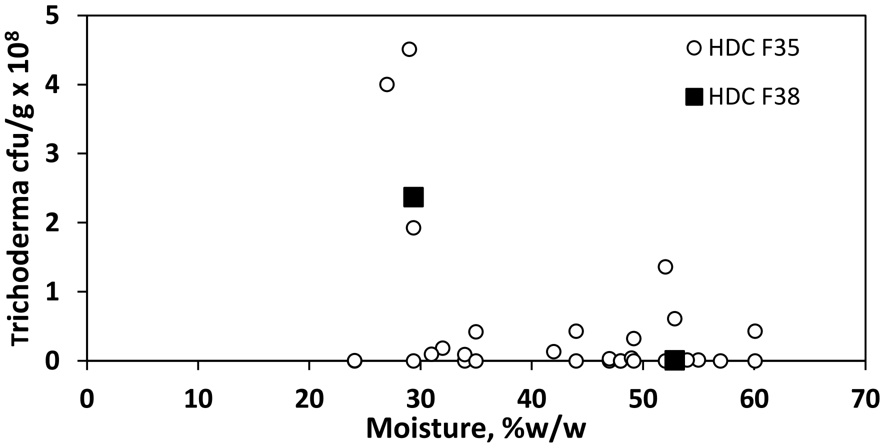

Fig.2. Effect of compost moisture content on growth of HDC F35 and HDC F 38

Preparation of pelleted seed containing biocontrol agents

High populations (>10^6 cfu/g pellet) of biocontrol agent propagules were detected in the HDC F37, HDC F39, HDC F41, or Prestop (previously known as HDC F42) treated pellets respectively (Table 7). These populations were lower in the pellets that were also treated with fungicides in Year 1. No biocontrol agent propagules were detected in the untreated raw or pelleted seeds or fungicide alone treated seeds.

The populations of biocontrol agent propagules in the pelleted salad and bulb onion seeds were unaffected by two months storage at 5ºC (Table 7). Eight months storage at 5ºC reduced the population of biocontrol agent propagules in the pellets by between 10 and 30% of the original population.

Table 7. Population of biocontrol agent propagules in pelleted bulb onion seeds (colony forming units/ g seed) in fresh seeds and after storage at 5˚C. Each value is the mean of three replicate batches. No propagules were detected in raw seed, untreated or metalaxyl-M + thiram fungicide treated pellet seeds.

Year	**BCA Treatment**	**Fresh**	**2 months**	**8 months**
2	HDC F37	3.1×10^{7}	1.9×10^{7}	2.2×10^{6}
3	HDC F37	6.6×10^{6}	6.2×10^{6}	5.7×10^{6}
1	HDC F39	2.8×10^{7}	2.6×10^{7}	2.2×10^{7}
2	HDC F39	4.3×10^{6}	1.2×10^{7}	1.2×10^{6}
1	HDC F41	6.5×10^{6}	6.0×10^{6}	1.9×10^{6}
3	HDC F41	1.5×10^{6}	1.4×10^{6}	1.1×10^{6}
2	HDC F42	3.3×10^{6}	6.3×10^{6}	1.2×10^{6}
3	HDC F42	1.1×10^{6}	1.1×10^{6}	1.0×10^{6}
1	HDC F39 + fungicides	5.2×10^{6}	8.3×10^{6}	7.3×10^{6}
1	HDC F41 + fungicides	9.0×10^{5}	8.3×10^{5}	2.5×10^{5}

Germination tests of pelleted onion seed

In the laboratory tests, germination of pelleted seed was slightly higher than of the raw seed; in modules the opposite trend was observed (Tables 8 and 9). Percentage germination of pelleted seed with Prestop (previously known as HDC F42) in the pellet was lower than in raw seed or other pelleted seed treatments in the module tests and in the Year 2 laboratory tests. This was due to a large proportion (86%) of abnormal germination. This effect was not observed in the Year 3 laboratory tests, or in the soil pot experiments (Table 10). Laboratory, module or soil germination percentages were unaffected by the other biocontrol agent or fungicide pellet treatments.

Table 8. Laboratory percentage germination of raw and pelleted onion seeds containing metalaxyl-M + thiram or biocontrol agents in the pellet coating. Values are single replicates.

Treatment	Year 1	Year 2	Year 3
Raw seed	86	93	93
Pellet control	_	99	94
Pellet fungicides	_	98	95
Pellet HDC F37	_	97	95
Pellet HDC F39	93	97	_
Pellet HDC F41	85	_	96

Pellet HDC F42	_	12	94
Pellet HDC F39 + fungicides	95	_	_
Pellet HDC F41 + fungicides	92	_	_

Table 9. Peat module percentage germination of raw and pelleted onion seeds containing metalaxyl-M + thiram or biocontrol agents in the pellet coating. Values are single replicates.

Treatment	Year 2	Year 3
Raw seed	95	93
Pellet control	85	85
Pellet fungicides	80	95
Pellet HDC F37	88	95
Pellet HDC F39	77	_
Pellet HDC F41	_	82
Pellet HDC F42	53	79

Table 10. Soil percentage germination of raw and pelleted onion seeds containing biocontrol agents or fungicide in the pellet coating. Each value is the mean percentage of 320 seeds.

Treatment	Year 2	Year 3
Raw seed	_	88
Pellet control	93	87
Pellet fungicide	87	90
Pellet HDC F37	97	90
Pellet HDC F39	87	_
Pellet HDC F41	_	88
Pellet HDC F42	88	87
LSD (P = 0.05)	3.9	3.4

Effects of substrate and pellet carrier systems amended with biocontrol agents on Fusarium basal rot and white rot of onion in pot bioassays (Milestones 1.2, 3.2 and 3.3)

Results in pot bioassays 2010 – 2012

A summary of the disease control results for different biocontrol agent and fungicide drench and/or dip treatments in the 2010, 2011 and 2012 pot bioassays are shown in Tables 11 and 12. No disease was detected in the plants growing in soil without *F. oxysporum* or *S. cepivorum* inoculum. Levels of Fusarium in untreated plants were higher in 2011 and 2012 (40.6% and 38.7%) than in 2010 (11.7%). White rot disease levels in untreated plants were

32.5%, 21.9% and 20.8% in 2010, 2011 and 2012 respectively. A total of 16 compost, drench and granular applications of biocontrol agent or fungicide treatments were tested against the untreated soil controls. Five treatments, including the fungicide treatment, were tested in all three years, three treatments were tested twice and eight treatments were tested once. The most consistent level of Fusarium disease control was observed with the HDC F41 drench treatment (Table 11). The HDC F42 drench treatment was also effective in the two years that it was tested. HDC F40 granules, either alone (in 2011) or with compost (in 2010) resulted in significant (*P*<0.05) Fusarium control, but the percentage of disease control was not as high as in some of the other biocontrol agent treatments. All of the other compost and biocontrol agent treatments failed to significantly control Fusarium in one or more years. The fungicide dip + drench treatment was very effective in controlling Fusarium in 2010 and 2012 but not in 2011. The most consistent level of white rot disease control was obtained with the compost +HDC F35 treatment (Table 12). The HDC F37 drench treatment was also effective in the two years that it was tested. HDC F38 or HDC F40 granules also resulted in significant control of white rot in the one year (2011) that they were tested. All of the other compost and biocontrol agent treatments failed to significantly control white rot in one or more years. The fungicide dip + drench treatment was very effective in controlling white rot in 2012, less effective in 2010 and ineffective in 2011.

Table 11. Percentage Fusarium disease control resulting from compost and biocontrol agent and fungicide drench treatments, compared with the untreated soil controls in pot bioassays.

Treatment	2010	2011	2012
Compost	n.s.	n.s.	n.s.
Compost + HDC F35	64.3	30.8	n.s.
Compost + HDC F41	n.s.	-	-
Compost + HDC F40	50.2	-	-
Compost + HDC F42	n.s.	-	-
HDC F36 drench	-	n.s.	-
HDC F37 drench	-	n.s.	n.s.
HDC F39 drench	n.s.	34.6	n.s.
HDC F41 drench	57.2	38.5	30.9
HDC F42 drench	50.3	-	40.4
HDC F43 drench	n.s.	-	n.s.
HDC F44 drench	n.s.	-	-
HDC F35 granules	-	n.s.	-
HDC F38 granules	-	n.s.	-
HDC F40 granules	-	26.9	-
Fungicide dip + drench	99.9	n.s.	81.7

n.s. not significantly different to control treatment at *P* ≤0.05 - not tested

Table 12. Percentage white rot control resulting from compost and biocontrol agent and fungicide drench treatments, compared with the untreated soil controls in pot bioassays.

Treatment	**2010**	**2011**	**2012**
Compost	38.5	n.s.	n.s.
Compost + HDC F35	34.6	78.6	59.0
Compost + HDC F40	n.s.	-	-
Compost + HDC F41	n.s.	-	-
Compost + HDC F42	n.s.	-	-
HDC F36 drench	-	n.s.	-
HDC F37 drench	-	57.2	53.3
HDC F39 drench	50.4	57.1	n.s.
HDC F41 drench	n.s.	35.0	n.s.
HDC F42 drench	n.s.	-	39.0
HDC F43 drench	n.s.	-	n.s.
HDC F44 drench	n.s.	-	-
HDC F35 granules	-	n.s.	-
HDC F38 granules	-	35.7	-
HDC F40 granules	-	50.0	-
Fungicide dip + drench	23.8	n.s.	89.3

n.s. not significantly different to control treatment at $P \leq 0.05$ - not tested

Table 13. Biocontrol agent colony counts in soils at end of 2010, 2011 and 2012 pot bioassays with onion sets. Each value is the mean of four replicate samples (±S.D.).

Treatment	**2010**	**2011**	**2012**
Soil	1.5 (±1.0) x 10^4	5.5 (±1.0) x 10^3	7.7 (±3.0) x 10^3
Compost	7.9 (±1.0) x 10^3	6.5 (±1.0) x 10^4	5.6 (±1.8) x 10^3
Compost + HDC F35	6.0 (±1.0) x 10^6	4.9 (±1.0) x 10^6	4.3 (±1.4) x 10^6
HDC F37 drench	-	7.0 (±1.0) x 10^5	8.9 (±0.8) x 10^4
HDC F39 drench	1.0 (±1.0) x 10^5	5.0 (±1.0) x 10^4	6.4 (±2.6) x 10^4
HDC F41 drench	-	2.5 (±1.0) x 10^4	6.7 (±3.2) x 10^3
HDC F42 drench	-	-	8.5 (±4.5) x 10^3
HDC F35 granules	-	2.1 (±1.0) x 10^6	-
HDC F38 granules	-	3.2 (±1.0) x 10^6	-
HDC F40 granules	-	1.1 (±1.0) x 10^5	-
Fungicide dip + drench	4.0 (±1.0) x 10^3	3.8 (±1.0) x 10^4	1.1 (±1.0) x 10^4

- not tested

Amendment of soil with 25% compost alone did not increase the population of biocontrol agents by the end of the pot bioassays (Table 13). Amendment of soil with HDC F35, either as compost or as granules, resulted in a x1000 increase in the soil biocontrol agent population, as did granules of HDC F38. Soil drenches with HDC F37 or HDC F39, or granules of HDC F40 resulted in smaller but detectable increases in the soil biocontrol agent population. Soil drenches with HDC F41, Prestop (previously known as HDC F42) or Folicur fungicide did not affect the soil biocontrol agent population by the end of the bioassays.

Pot bioassay pellet seed treatment results

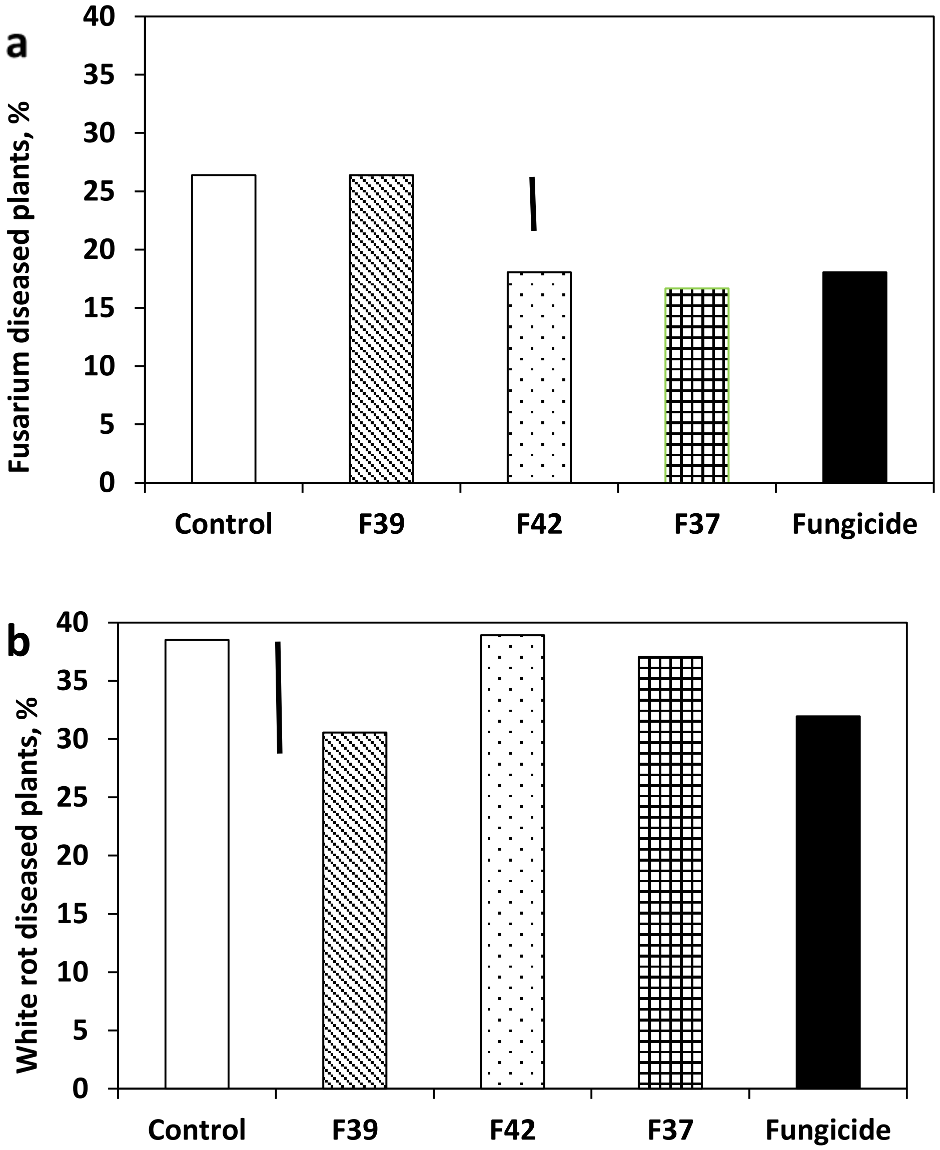

Fig 3. Effects of biocontrol and fungicide treatments applied to seed pellet coating on (a) Fusarium basal rot and (b) Allium white rot in 2012 pot onion bioassay. The vertical bar indicates the Least Significant Difference (*P* = 0.05).

No disease was detected in plants growing in soil without *F. oxysporum* or *S. cepivorum* inoculum. In soils containing pathogen inoculum, the level of Fusarium in the untreated control plants was higher than the level of white rot in the untreated control plants (Fig. 3). Plants grown from pelleted seed containing HDC F37 or HDC F42 inoculum or fungicide had 31 to 37% less Fusarium than plants grown from untreated pellet seeds. HDC F39 in the seed pellet had no effect on Fusarium (Fig. 3a). None of the pellet seed treatments significantly affected white rot in the plants (Fig. 3b). Healthy plants grown from HDC F37 or fungicide treated pellet seed produced larger bulbs at harvest than plants grown from untreated pellet seeds (Fig. 4). HDC F39 or Prestop (previously known as HDC F42) did not affect bulb weight compared with untreated pellet seed. The presence of Fusarium chlamydospores or white rot sclerotia in the soil did not affect the weight of healthy bulbs at harvest. At the end of the pot bioassay, the biocontrol agent population in the soil from HDC F37 and Prestop (previously known as HDC F42) pellet seed treatments were higher than the level in the soil with the untreated and fungicide pellet seed treatments (Table 14).

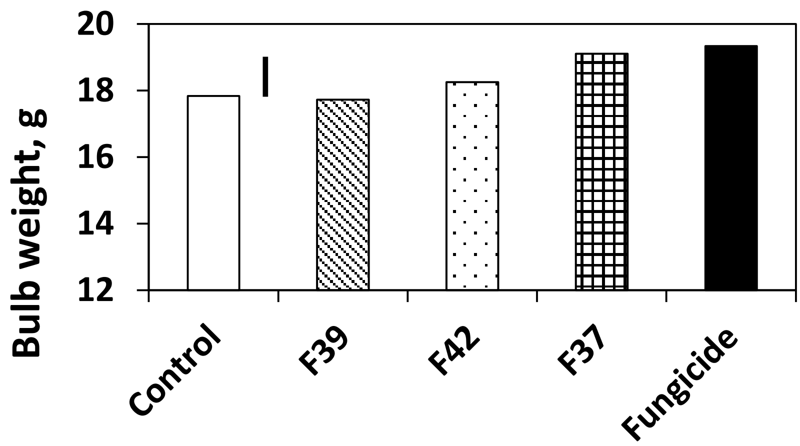

Fig. 4. Weight of healthy bulbs at harvest from biocontrol and fungicide treatments applied to onion seed pellet coatings. The vertical bar indicates the Least Significant Difference (*P* = 0.05).

Table 14. Biocontrol agent colony counts (cfu/g soil) in soils at end of the 2012 pellet seed treatment pot bioassay. Each value is the mean of three replicate samples (±S.D.).

Treatment	cfu/g soil
Soil	3.0 (±1.9) x 10^3
HDC F37	8.4 (±1.4) x 10^4
HDC F39	2.6 (±2.1) x 10^4
HDC F42	4.5 (±1.0) x 10^4
Fungicide	1.1 (±0.5) x 10^4

Milestone 2.1 Production of composts

The population of biocontrol agents was x100 higher in the large-scale compost prepared from green wastes at Organic Recycling Ltd in 2013 than in the composts prepared from green waste in 2011 or from onion waste at F. Parrish in 2011 or 2012 (Table 15).

Table 15. Biocontrol agent colony counts in composts containing inoculum of HDC F35 (cfu/g compost) used for field experiments in 2011, 2012 and 2013 field experiments.

Year	Composts	Sites	BCA cfu/g
2011	GWC, ORL Ltd	Kirton, Gosberton, Saracens Head	4.0 x 10^6
2011	Onion waste, Parrish	Clifton, Chicksands	1.4 x 10^6
2012	Onion waste, Parrish	Clifton	2.7 x 10^6
2013	GWC, ORL Ltd	Clifton	3.1 x 10^8

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

Field Experiments 2011 - Application of biocontrol 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 16). 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 16). Broadcast application of HDC F35 amended onion waste compost reduced white rot by 64% but had no effect on Fusarium (Table 16). 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 biocontrol agent propagule population by between 5 and 50 times depending on the site. However, the final soil biocontrol agent propagule 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.

Field Experiments 2011 - Application of HDC F39 and HDC F40 granules to field sites

Application of the HDC F39 drench increased the soil biocontrol agent propagule population at harvest by between 2 and 5 times on four of the six sites (Table 17). The HDC F40 granule application increased the soil biocontrol agent propagule population at harvest by x10 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 17).

Table 16. Field Experiments 2011 - Effect of HDC F35 amended compost on biocontrol agent colony counts in soils (background soil and HDC F35 compost introduced levels) and disease levels (w, white rot; f, fusarium; w+f, total of both diseases present) on different sites. Each value is the mean of five replicate plots.

Site	Treatment	Application	BCA cfu/g	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 17. Field Experiments 2011 - Effect of HDC F39 or HDC F40 applications on soil biocontrol agents populations and disease levels (w, white rot; f, fusarium) at different sites. Each value is the mean of the three replicate plots.

Site	Application	BCA 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).

Best biocontrol agents in granular and drench formulations and pellet carrier treatments from pot experiments tested for Fusarium basal rot and white rot control in field experiments (Milestones 3.2 and 3.3)

2012 Field experiments

Levels of disease were below 2% at sites 1, 2, 4 and 5. At sites 3 and 6, disease levels were approximately 7% in the untreated control plots. At site 3, HDC F39 and Prestop (previously known as HDC F42) drenches suppressed the level of Fusarium basal rot. There was a trend for the repeated applications to be more effective than the single application, although the difference between single and duplicate applications was not statistically significant (P =0.05). The Serenade ASO (previously known as HDC F43) drenches were ineffective in suppressing Fusarium. Due to the low level of disease, the effect of the treatments on the final bulb yield from each plot was not significant (Fig 5).

At site 6, white rot levels were variable between replicate plots of the same treatment, resulting in a high least significant difference value (Fig 6). The Folicur + Signum fungicide and Prestop (previously known as HDC F42) and Serenade (previously known as HDC F43) biocontrol agent treatments resulted in significantly (P<0.05) lower levels of white rot than the untreated control plots. The HDC F39 drench and compost + HDC F35 treatments did not significantly reduce the level of white rot compared with the untreated control. Double applications of HDC F39 at site 3 or of Prestop (previously known as HDC F42) at sites 1 and 3 or an application of compost amended with HDC F35 at site 6 were the only treatments to increase the soil biocontrol agent propagule population over the natural background level in the soil (Table 18).

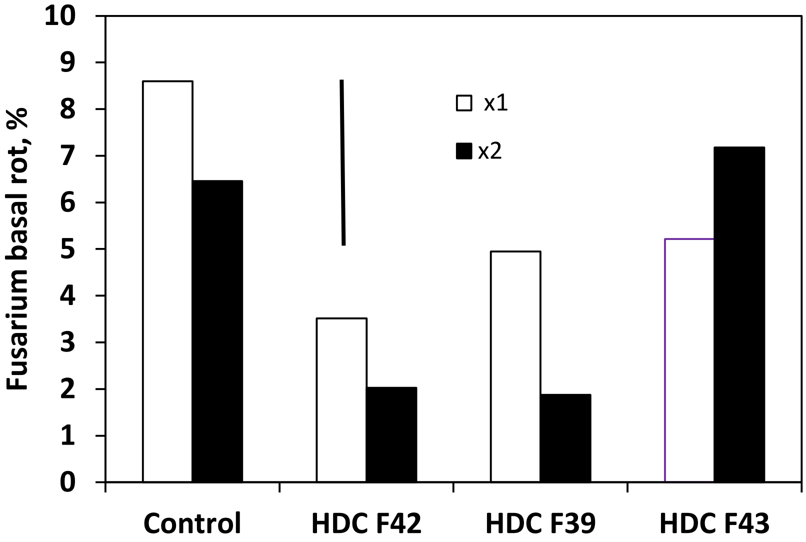

Fig. 5. Field Experiments 2012 - Effect of single and repeated drench treatments on Fusarium at site 3. The vertical bar indicates the Least Significant Difference (*P* = 0.05).

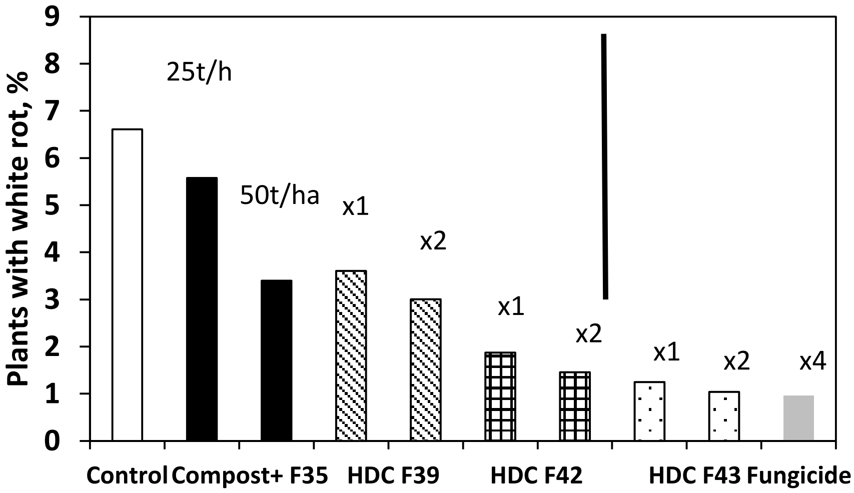

Fig. 6. Field Experiments 2012 - Effect of compost+HDC F35 and single and repeated drench treatments on the percentage of bulbs with white rot at site 6. Fungicide treatment was two applications of Folicur alternated with two applications of Signum. The vertical bar indicates the Least Significant Difference (*P* = 0.05).

Table 18. Field Experiments 2012 - Effect of biocontrol agent applications on soil biocontrol agent populations at different sites. Each value is the mean of the three replicate samples.

Site	1	3	5	6
Control	1.1×10^4	1.2×10^4	7.4×10^4	9.0×10^4
HDC F42 x 1	-	8.6×10^4	1.1×10^4	-
HDC F42 x 2	9.0×10^4	4.2×10^5	-	8.0×10^4

HDC F39 x 1	-	5.7×10^4	6.7×10^3	-
HDC F39 x 2	5.7×10^3	9.3×10^4	-	9.0×10^4
HDC F35 compost, 25t/ha	-	-	-	2.7×10^5
HDC F35 compost, 50t/ha	-	-	-	2.7×10^5

Field Experiments 2013 – Pellet Seed Treatments and Spray Treatments

Germination of pellet seeds was better at site 4 (Elverden) and site 6 (Spalding) than at the other four sites (Table 19). The number of healthy and diseased seedlings was unaffected by the pellet seed treatments.

Table 19. Field Experiments 2013 – Healthy and diseased seedlings following germination of different pellet seed treatments. Each value is the mean of two 1 m row lengths. LSD between mean values of healthy seedlings 1.1 (P = 0.05).

Site	Control		HDC F37		HDC F42		Fungicide	
	healthy	disease	healthy	disease	healthy	disease	healthy	disease
1	16.6	0.0	15.4	0.0	16.0	0.2	16.3	0.2
2	15.2	0.2	14.8	0.0	17.3	0.1	16.3	0.0
3	18.0	0.2	18.9	0.0	18.6	0.1	18.9	0.3
4	22.1	1.3	22.8	0.8	22.4	1.5	22.9	1.0
5	20.8	0.0	17.0	0.0	17.4	0.0	18.5	0.0
6	20.5	0.0	21.2	0.2	21.3	0.0	21.7	0.2
Mean	18.8	0.3	18.0	0.2	18.6	0.3	19.3	0.3

Fusarium basal rot occurred in control onion plants from untreated pellets at site 1 at 19% and at site 6 at 12%. White rot occurred in control plants from untreated pellets at site 2 at 10% and at site 5 at 16%. Average disease levels in control plants at sites 3 and 4 in Norfolk were both below 2% and these sites were therefore not used in the disease analysis.

Neither of the biocontrol (HDC F37 or Prestop (previously known as HDC F42)) pellet seed treatments had any effect on Fusarium at sites 1 and/or site 6 (Figs. 5 and 6). There was some indication that Fusarium was reduced by the fungicide pellet seed treatment of metalaxyl-M + thiram, although the effect was not statistically significant (Fig. 7). However, at site 1 where two Folicur and alternately two Signum sprays were also applied to plants from fungicide treated pellets, the effect of the fungicide pellet treatment was not improved, and possibly negated (Fig. 8).

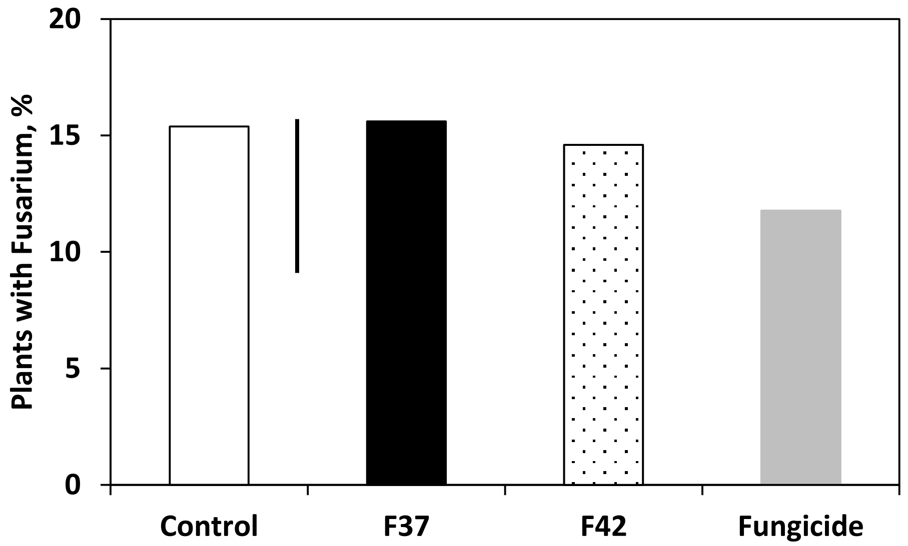

Fig.7. Effect of biocontrol and fungicide (metalaxyl-M + thiram) pellet seed treatments on Fusarium; mean of sites 1 and 6. Bar indicates least significant difference at $P = 0.05$.

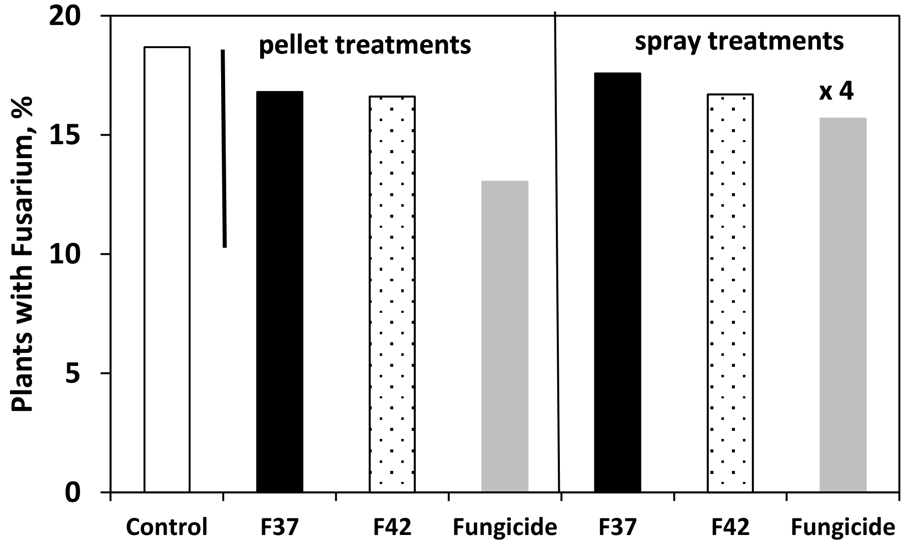

Fig.8. Effect of biocontrol and fungicide (metalaxyl-M + thiram) pellet seed treatments and biocontrol and fungicide (Folicur + Signum) sprays on Fusarium at site 1. Bar indicates least significant difference at $P = 0.05$.

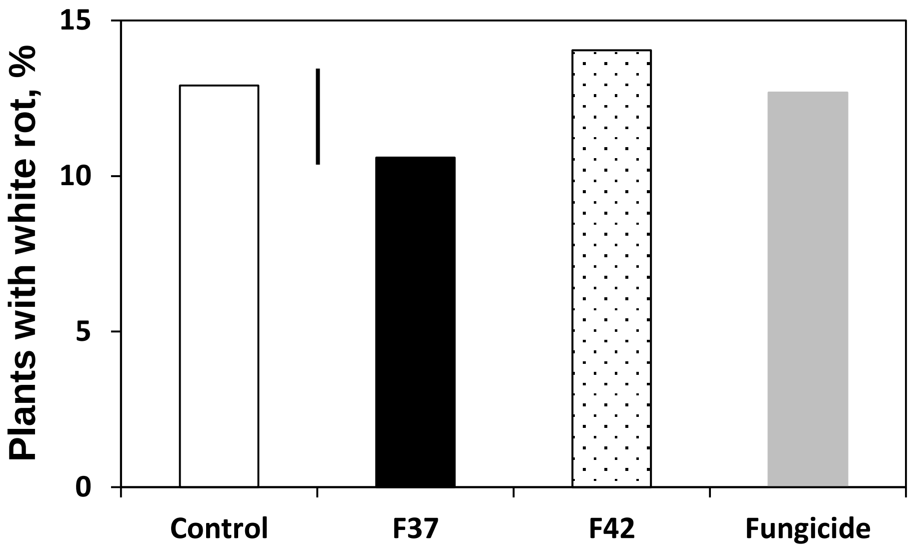

Fig.9. Effect of biocontrol and fungicide (metalaxyl-M + thiram) pellet seed treatments on white rot; mean of sites 2 and 5. Bar indicates least significant difference at *P* = 0.05.

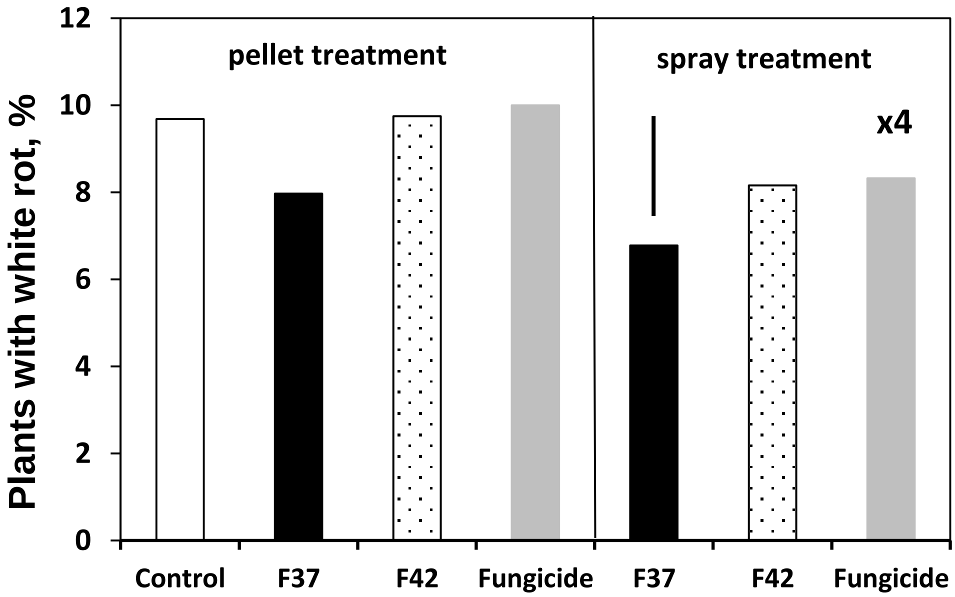

Fig.10. Effect of biocontrol and fungicide (metalaxyl-M + thiram) pellet seed treatments and biocontrol and fungicide (Folicur + Signum) sprays on white rot at site 2. Bar indicates least significant difference at P = 0.05.

None of the pellet seed treatments resulted in a statistically significant reduction of white rot at site 2 and/or site 5, although there was evidence of some reduction resulting from the pellet seed treatment with HDC F37 (Fig. 9). However, a spray treatment at sowing did result in a small but significant reduction in white rot at site 2 (Fig. 10). Spray treatment with Prestop (previously known as HDC F42) at drilling, or two applications of Folicur alternating with two applications of Signum on plants from fungicide treated pellets had no significant effect on white rot compared with the untreated control plants.

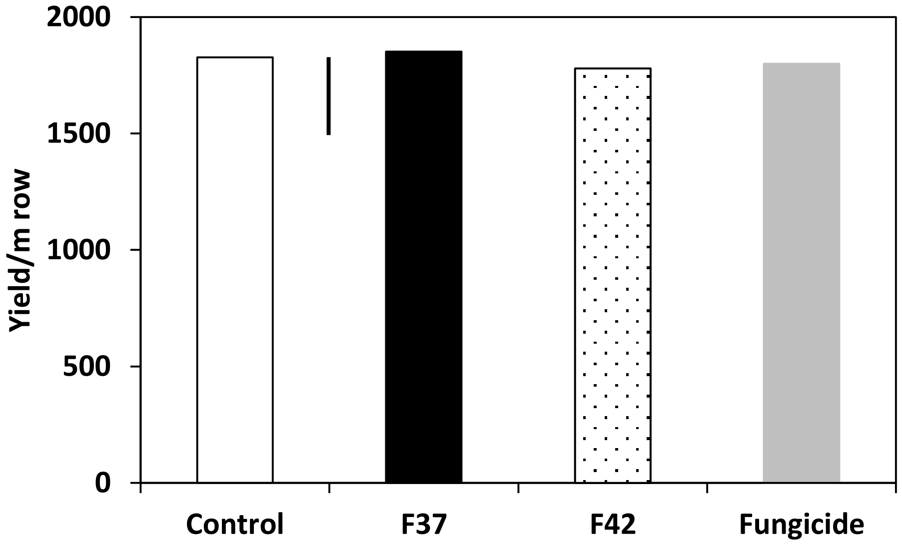

Fig. 11. Effect of pellet seed treatments on onion yield per metre row length; average of sites 1 to 5. Bar indicates least significant difference at *P* = 0.05.

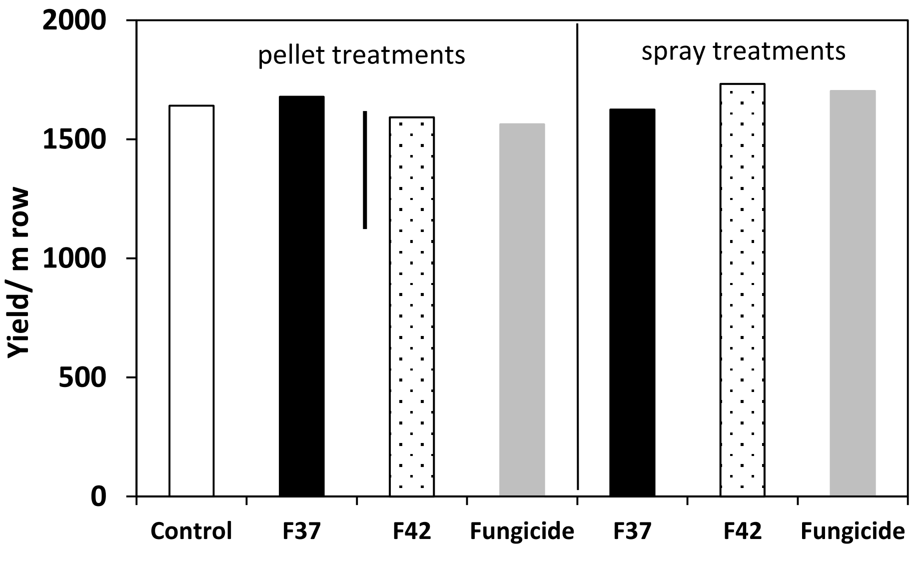

Fig. 12. Effect of pellet seed treatments on onion yield per metre row length; average of sites 1, 2 and3. Bar indicates least significant difference at *P* = 0.05.

The yield of onions per metre row length was recorded on all sites except on site 6, where late drilling in May meant that the onions had only formed small bulbs at harvest in October. The weight of onions was not significantly affected by the pellet seed or spray treatments, irrespective of the disease levels on the sites (Figs. 11 and 12).

Incorporation of Prestop (previously known as HDC F42) into seed pellets resulted in detectable increases in the soil biocontrol agent population at harvest at all the sites (Table 20). Seed pellets with HDC F37 also resulted in detectable increases in biocontrol agents at sites 5 and 6, but not at sites 1 and 2 where the natural background populations of biocontrol agents were higher than at sites 5 and 6. The drench treatments of HDC F37 and Prestop (previously known as HDC F42) at sites 1 and 2 also resulted in detectable increases in the soil biocontrol agent population. Fungicide pellet seed treatment or sprays did not affect the soil biocontrol agent populations (Table 20).

Table 20. Field Experiments 2013 - Effect of pellet seed and spray treatments on soil biocontrol agent populations at different sites. Each value is the mean of the three replicate samples.

Site	1	2	5	6
(a) Untreated pellets	2.6×10^3	9.0×10^4	6.7×10^2	1.2×10^3
(b) HDC F37 pellets	2.3×10^3	9.9×10^4	2.0×10^4	2.1×10^4
HDC F42 pellets	1.0×10^4	1.5×10^5	3.8×10^4	8.6×10^4
(c) Fungicide (metalaxyl-M + thiram) pellets	2.0×10^3	8.3×10^4	n.d.	1.6×10^3
(d) Untreated pellets, HDC F37 spray, 0.5 g/m^2	7.8×10^4	1.2×10^5	-	-
(e) Untreated pellets HDC F42 spray, 2.5 g/m^2	3.0×10^3	1.2×10^5	-	-
(f) Fungicide (metalaxly-M + thiram) pellets, two Folicur and two Signum sprays	2.0×10^3	9.3×10^4	-	-

n.d. not detectable

Field Experiments 2013 – Compost + HDC F35 Treatment

The effect of application of compost along the planting row with the converted set planter is shown in Fig. 13. Application of HDC F35 amended compost at 6 and 12 t/ha increased the soil biocontrol agent population by 100 and 500 over the natural background population (Table 21). The level achieved with 12 t/ha applied in the planting row was higher than any of the planting row or broadcast compost treatments in 2011 or 2012 (Tables 15 and 18).

Table 21. Field Experiments 2013 - Effect of HDC F35 amended compost treatments on the biocontrol agent colony counts (cfu/g soil) in soil at end of the experiment. Each value is the mean of three replicate samples (±S.D.).

Treatment	**cfu/g soil**
Soil	6.0 (±1.0) x 10^{4}
Compost 6 t/ha	8.0 (±0.4) x 10^{5}
Compost 12 t/ha	3.2 (±1.0) x 10^{6}

The final level of white rot diseased onions on the experimental site was less than 3%. It was therefore not possible to determine the effects of the compost treatment on disease levels. The weight of bulbs along the lengths of assessment rows was not affected by the compost treatment (average weight of bulbs 3.9 kg/ m row length).

Fig. 13. Application of compost to field plots using converted set planter.

Discussion

Inoculum development

This work has shown that biocontrol agent propagules incorporated into seed pellet coatings remain viable at high levels (>10^6 cfu/g) in storage at 5˚C for several months. However, the quantities of inoculum were not sufficient for achieving significant disease control in the field. This could be addressed by increasing the spore concentration in the pellet, possibly by using spore suspensions rather than formulated products from biocontrol manufacturers that are designed for direct application. The use of additional 'dummy seed pellets' could be used to increase the amount of inoculum added to the soil at drilling. A combination of both seed pellet treatment and spraying with HDC F37 at drilling may give more effective disease control than either treatment individually.

Fusarium basal rot control

The most effective biocontrol agent for Fusarium control from the pot experiments, HDC F41 was not available for field experiments. Although suppression of Fusarium was achieved with a drench treatment of Prestop (previously known as HDC F42) in both pots and in the field, Prestop (previously known as HDC F42) was not effective when applied at a lower dose in a smaller volume of water at drilling. Although some suppression of Fusarium was obtained with compost + HDC F35 and seed pellet treatments with HDC F37 or Prestop (previously known as HDC F42) in pot experiments, they were not effective treatments in the field.

Two applications of Folicur alternating with two applications of Signum were ineffective in controlling Fusarium. Significant reduction in Fusarium was observed with a seed pellet treatment of metalaxyl-M + thiram in pot experiments, and some reduction in Fusarium was also observed in the field although the disease control was not statistically significant. Again, the use of additional 'dummy seed pellets' treated with metalaxyl-M + thiram at drilling may improve control of Fusarium in the field.

White rot control

Drench treatments with Serenade ASO (previously known as HDC F43) were not effective in controlling white rot in two pot experiments. Although the recorded level of white rot following a drench treatment with Serenade ASO (previously known as HDC F43) was significantly lower than in the untreated control, the background disease level in the field plots was very variable, and further tests are needed to establish the reliability of this treatment in the field. Serenade ASO (previously known as HDC F43) is registered for control of white rot of onions. More reliable control of white rot was obtained with HDC F37, as a drench treatment in pot experiments and as a spray treatment at drilling. A combination of HDC F37 treatments as pellet seed, dummy pellet seed and spray at drilling may increase the soil and root zone biocontrol agent inoculum to a level giving good white rot control but this requires further investigation.

Two applications of Folicur alternating with two applications of Signum gave good control of white rot in the first of two field experiments, but not in the second where the background disease level was higher.

Planting row compost application

A converted set planter was an effective method of applying compost along the planting row, providing that the compost was screened to 20 mm and dried to a moisture content of 41% or lower. Drier composts were also more suitable for establishing the biocontrol agent HDC F35. High levels of HDC F35 were obtained in the field soil (>10^6 cfu/g) up to the time of harvesting. Pot experiments showed that this level of propagules gave consistent control of white rot but there was insufficient disease in the field to demonstrate this. Tests in previous years had showed that HDC F35 propagule counts in the soil of 10^5 cfu/g were insufficient to achieve reliable white rot control.

Tests in this project and previous work by Trillas et al[7] have shown that several other biocontrol products are not as well adapted to compost as HDC F35. Further screening of Annex 1 biopesticides may reveal products that are suitable for application with compost.

The set planter used in this project would require further fairly simple adaptation for it to be suitable for compost application on a commercial scale. The speed of the feed belts in Fig. 13 would need to be increased, a compost agitator which does not crush compost is needed and the depth of the feed channels needs to be increased.

Previous work in HL 0176 has shown that onion set emergence is unaffected by the application of green waste compost or green waste compost + HDC F35 to soil immediately before set planting. In HL 0176, the application of compost + HDC F35 to soil significantly increased marketable bulb yield, although this could be attributed to the control of white rot. The effect of amendment of soil with compost + HDC F35 on bulb yield in the absence of white rot was not established and requires further testing. There is also potential for reducing the application of inorganic N, P and K fertilisers when compost is applied to onion crops, but this also required further investigation.

Conclusions

Inoculum development

- Pelleted bulb onion seed with spores of the biocontrol agents HDC F37, HDC F39 and HDC F42 was produced by Incotec/Elsoms. High populations (>10^6 cfu/g pellet) of biocontrol agent propagules were detected in the treated pellets
- The biocontrol agent populations in the seed pellets were unaffected by two months storage at 5˚C and declined by 10-30% after eight months storage at 5˚C
- Germination of seeds in soil was unaffected by the biocontrol agent in the pellet but HDC F42 resulted in lower germination in peat modules and in one out of two laboratory tests

Pot experiment with pelleted seed

- Plants grown from pelleted seed containing HDC F37 or Prestop (previously known as HDC F42) inoculum or fungicide (metalaxyl-M + thiram) had 31 to 37% less Fusarium than plants grown from untreated pellet seeds
- Healthy plants grown from HDC F37 or fungicide treated pellet seed produced larger bulbs at harvest than plants grown from untreated pellet seeds
- None of the pellet seed treatments significantly reduced white rot in the plants

Pot experiments with sets

- Across three years, the most consistent level of Fusarium disease control was obtained with a HDC F41 drench treatment; a Prestop (previously known as HDC F42) drench treatment was also effective in the two years that it was tested
- Across three years, the most consistent level of white rot disease control was obtained with a compost + HDC F35 treatment; a HDC F37 drench treatment was also effective in the two years that it was tested
- A Folicur fungicide set dip + drench treatment was effective against both diseases

Field Experiments

- HDC F37 sprayed at drilling at 0.5 g/m^2 significantly reduced the level of white rot but did not suppress Fusarium basal rot; seed pellets containing HDC F37 also showed some reduction in white rot but the effect was not statistically significant
- Drenches of HDC F39 (3 g/m^2) or Prestop (previously known as HDC F42) (5 g/m^2) suppressed the level of Fusarium basal rot and the level of white rot was also lower in plots treated with Prestop (previously known as HDC F42) at this rate; Prestop

(previously known as HDC F42) sprayed at drilling at 2.5 g/m^2 was ineffective in controlling either disease

- Serenade ASO (previously known as HDC F43) drenched at 2 g/m^2 was ineffective in suppressing Fusarium but the level of white rot was significantly (P<0.05) lower in plots treated with Serenade ASO (previously known as HDC F43) at this rate
- Broadcasting compost + HDC F35 at 50 t/ha increased the soil biocontrol propagule count to 2.7 x 10^5 cfu/g but was ineffective against white rot
- Matured green waste compost, screened to 20 mm with a moisture content of 41% could be applied to planting rows in the field through a converted set planter; compost with a moisture content of 44% or greater tended to clog
- Dried compost + HDC F35 applied along the planting row at 12 t/ha increased the soil biocontrol propagule count to 3.6 x 10^6 cfu/g; due to a low level of white rot, the effect on disease could not be established
- A double application of Folicur alternated with a double application of Signum reduced white rot in one out of two seasons; it was ineffective in controlling Fusarium
- A seed pellet treatment of metalaxyl-M + thiram had no effect on white rot but showed some reduction in Fusarium, although the disease reduction was not statistically significant

Technology transfer

Publications

New Projects. HDC News 179, p11.

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

Noble R. (2012) Biological agents get to work on onion rots. HDC Field Vegetable Review 2012. p16.

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	Year	Soil type	pH	EC	Organic matter %
Kirton, Lincs. (pots)	2010	Silt	7.74	89	7.4
Wellesbourne, Warw. (pots)	2011	sandy loam	6.75	287	3.6
Pershore, Worcs. (pots)	2012	silty loam	6.85	150	7.8
Saracens Head, Lincs.	2011	Silt	7.88	106	5.8
Gosberton, Lincs.	2011	Silt	7.34	40	5.6
Clifton, Beds.	2011	Silt	6.77	53	3.8
Clifton, Beds.	2012	Silt	7.34	83	4.0
Clifton, Beds.	2013	Silt	6.05	128	3.7
Chicksands, Beds.	2013	sandy loam	6.67	50	3.2
Chicksands, Beds.	2013	silty loam	6.12	121	3.3
Chatteris, Cambs.	2011	peat	6.71	602	39.0
Littleport, Cambs.	2011	peat	7.13	154	32.8
Boxted, Essex	2011	sandy loam	5.85	73	2.5
Kites Hardwick, Warw.	2012	sandy loam	5.41	87	3.6
Kites Hardwick, Warw.	2013	sandy loam	5.24	93	3.5
Castle Acre, Norfolk	2013	sandy loam	5.73	51	2.6
Elverden, Nolfolk	2013	sandy loam	8.65	56	2.5
Spalding, Lincs.	2013	Silt	8.54	49	6.2