Project title	Narcissus: Suppression of Fusarium basal rot using composts amended with specific biocontrol agents
Project number:	BOF 69
Project leaders:	Prof Ralph Noble
Report:	Final, August 2012
Key staff:	Andreja Dobrovin-Pennington
Location of project:	East Malling Research and commercial farms
Project coordinator:	Mr Adrian Jansen
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Key words:	Disease, *Fusarium oxysporum* f.sp. *narcissi* *Trichoderma*

DISCLAIMER

The results and conclusions in this report are based on an investigation conducted over a two-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

Signed on behalf of: East Malling Research

Signature:…………………….………………..……. Date: 10 August 2012

Dr Christopher J Atkinson
Deputy Chief Executive and Senior Programme Leader

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GROWER SUMMARY

Headline

- Granules of HDC F106 or HDC F108, or bulb spray treatment with HDC F110 significantly reduced the percentages of wilted plants and bulbs with external and/or internal basal rot symptoms compared with the control treatment in a pot bioassay
- Amendment of soil with 25% compost, with or without inocula of biocontrol agents, was ineffective in reducing total basal rot symptoms. A bulb dip treatment with fungicide Storite was also ineffective

Background and expected deliverables

Basal rot control remains an intractable problem of narcissus in the UK. Current chemical bulb dips for controlling narcissus basal rot have been withdrawn (formaldehyde), and are expensive, prone to pathogen resistance (thiabendazole), and ineffective over several seasons. Varieties of narcissus that show resistance or tolerance to Fusarium basal rot do not have the same quality attributes of susceptible varieties, and breeding new varieties is too long-term.

Fungal species have been used to suppress diseases caused by *Fusarium oxysporum*, including narcissus basal rot. In HDC project FV 219b, compost amended with HDC F35 and incorporated in soil at 25% or HDC F41 applied as an onion set treatment reduced Fusarium in onion plants. HDC F39 applied as a set treatment controlled white rot. HDC F39 and F41 did not grow on compost and were better applied as set treatments. Previous work has shown that fungal and bacterial biocontrol agents have the potential to control basal rot when applied as a spray to the soil during planting.

Following application to soil, fungal spores of biocontrol agents can persist in the field and control white rot in onions from one year to the next. This could potentially avoid the need for repeated applications to a longer term narcissus crop.

Commercial Objectives

- To examine the efficacy of composts amended with HDC F106 for the control of Fusarium basal rot of narcissus
- To examine the efficacy of other commercial biopesticide products (including fungal and bacterial biocontrol products) in controlling basal rot of narcissus, applied with composts and as sprays and bulb dips

- Compare disease control efficacy with that obtained with a fungicide (Storite a.i. thiabendazole) bulb dip treatment
- Monitor the populations of fungal biocontrol agents and Fusarium propagules in the compost amended soil and non-amended soil

Summary of the project and main conclusions

Pot narcissus bioassays were conducted to examine the efficacy of composts, with and without inocula of biocontrol agents, granular, spray and bulb dip treatments of biocontrol agents in controlling Fusarium basal rot.

Granules of HDC F106 or HDC F108, or a bulb spray treatment with HDC F110 significantly reduced the percentage of wilted plants and bulbs with external and/or internal basal rot symptoms compared with the control treatment. HDC F107 and HDC F112 spray treatments reduced the percentage of wilted plants but did not affect the total percentage of basal rot bulbs. Amendment of soil with 25% compost, with or without inocula of biocontrol agents, HDC F109 spray treatment of bulbs and bulb dip treatments of HDC F113 and Storite bulb were ineffective in reducing basal rot symptoms.

The fungal biocontrol agent population of the soil at the end of the pot bioassay was significantly increased by amendment with compost containing HDC F106, HDC F107 or HDC F108 inocula or HDC F106 granules without compost. HDC F109 spray treatment of bulbs did not affect the final soil fungal biocontrol agent population. HDC F110 spray treatment of bulbs increased the soil fungal biocontrol agent population at the end of one out of two pot bioassays.

Financial benefits

Biocontrol products have been identified that can control basal rot of narcissus in a pot bioassay. Further field trials are needed before the commercial viability of these treatments can be assessed. If successful in field trials, one or more of these products could potentially be taken forward for registration, thus increasing the armoury of fungicidal products available to growers to control basal rot.

Action points for growers

- None at this stage

SCIENCE SECTION

Introduction

Basal rot control remains an intractable problem of narcissus in the UK. It is a disease of warm summers, and is likely to increase with global warming. Current chemical bulb dips for controlling narcissus basal rot have been withdrawn (formaldehyde), and are expensive, prone to pathogen resistance (thiabendazole), and ineffective over several seasons.

Varieties of narcissus that show resistance or tolerance to Fusarium basal rot do not have the same quality attributes of susceptible varieties, and breeding new varieties is too long-term.

Fungal biocontrol agents have been used to suppress diseases caused by *Fusarium oxysporum*, including narcissus basal rot[1,2]. Soil application of organic amendments such as composts has also been shown to suppress several soil-borne plant diseases caused by different forms of *Fusarium oxysporum,* such as tomato foot and root rot[3]. The microbial population in such amendments can have an antagonistic effect on the pathogen. However, instead of relying on the natural background level of microbial antagonists in organic amendments, the introduction of known biocontrol agents with composts into the soil can have a more reliable disease suppressive effect[3,4]. In HDC project FV 219b[5], compost amended with HDC F35 and incorporated in soil at 25% or HDC F41 applied as an onion set treatment reduced Fusarium in onion plants. HDC F39 applied as a set treatment controlled white rot. HDC F39 and F41 did not grow on compost and were better applied as set treatments.

Following application to soil, spores of fungal biocontrol agents can persist in the field and control white rot in onions from one year to the next. This would avoid the need for repeated applications of compost amended with biocontrol agents to a longer term narcissus crop.

Commercial Objectives

- To examine the use composts amended with biocontrol agents for control of Fusarium basal rot of narcissus
- To examine the efficacy of other commercial biopesticide products (including fungal and bacterial biocontrol products) applied with composts and as sprays and bulb dips in controlling basal rot of narcissus

- Monitor the populations of biocontrol agents and Fusarium propagules in the compost amended soil and non-amended soil
- Compare disease control efficacy with that obtained with a fungicide (Storite a.i. thiabendazole) bulb dip treatment

Materials and methods

Narcissus Pot Bioassay

Bulbs without chemical treatment and without visible rotting were used for the experiments. A modified form of a pot bioassay previously used for testing basal rot in narcissus was used for the experiments[6]. Bulbs were planted three per 2 L deep pot. *F. oxysporum* f.sp. *narcissi* (isolate B61) chlamydospore in talc inoculum was added to soil. The Fusarium inoculated soil was used in the middle 8 cm and lower 5 cm layers in the pot (Fig. 1). Pots were topped up with a 7 cm deep layer of clean soil.

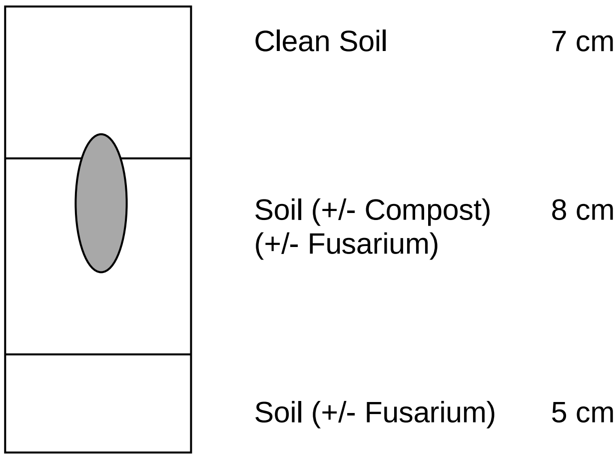

Figure 1. Diagram of pot set-up

Biocontrol agents were applied either with amended composts, as sprays before planting, or as bulb dips before planting. Bulbs were planted immediately after treatment, i.e. while still moist. Spray and dip treatments were applied at concentrations based on the manufacturers recommendations for use as drenches in the control of soil-borne diseases (Table 1). Where applicable, the middle 8 cm layer of soil contained compost incorporated at 25% v/v. For specific treatments, biocontrol agents were added to the compost at 0.5% w/w, 2 weeks before use in the pot experiments. Fusarium inoculum was incorporated in to the soil-compost mix at the same rate as for the soil only treatments. The green waste compost was prepared by Organic Recycling Ltd, Crowland, Peterborough over 9 months in turned windrows to PAS:100 standard[7].

Table 1. Concentrations of products and of colony forming units used in the spray treatments.

Product	Concentration, g/L	cfus/L	cfus/bulb
HDC F107	3	3×10^9	9.1×10^5
HDC F109	3	3×10^9	9.1×10^5
HDC F110	20	4×10^8	1.3×10^5
HDC F112	5	1×10^8	3.0×10^4

Pot Bioassay 2010/11

Bulbs cv. Golden Harvest were planted on 30 September 2010. Fusarium talc inoculum containing 3.3×10^6 cfu/g was added to sandy loam soil (Wellesbourne, Warwick) at 0.5 g/kg. This gave a soil *Fusarium chlamydospore* concentration of 1.7×10^6 cfu/L.

Treatments

1. Control, soil, no biocontrol agent

Incorporated with compost (25% v/v in middle 8 cm layer)

2. Compost, no biocontrol agent
3. Compost + HDC F106

Applied as dip before planting

4. HDC F110 (20 g/L)
5. HDC F109 (3 g/L)

Nine replicate pots of each treatment were prepared. Pots were spaced at 2 cm apart on benching in an unheated polytunnel.

Pot Bioassay 2011/12

Bulbs cv. Carlton and cv. Golden Harvest were planted on 14 September 2011. Fusarium talc inoculum containing 2×10^7 cfu/g was added to silty loam soil (Pershore, Worcestershire) at 0.1 g/L. This gave a soil Fusarium chlamydospore concentration of 2×10^6 cfu/L.

Treatments

1. Control, soil, no biocontrol agent

Incorporated with compost (25% v/v in middle 8 cm layer)

2. Compost, no biocontrol agent
3. Compost + HDC F106
4. Compost + HDC F107

5. Compost + HDC F108

6. Compost + HDC F113

Applied as granules

7. HDC F108, 1% w/w

8. HDC F106, 1% w/w

Applied as spray to bulbs before planting

9. HDC F109 (3 g/L, 100 mL per 30 bulbs)

10. HDC F110 (20 g/L, 100 mL per 30 bulbs)

11. HDC F112 (5 g/L, 100 mL per 30 bulbs)

12. HDC F107 (3 g/L, 100 mL per 30 bulbs)

Applied as 15 minute dip before planting

13. HDC F113 (in suspension as supplied)

14. Storite (500g/L thiabendazole) 2 mL Storite/L, dipped and dried).

For cv. Golden Harvest, only treatments 1, 3, 6 and 14 were examined.

For each treatment with cv. Carlton, 25 pots were prepared with Fusarium and 10 pots without Fusarium (disease-free controls). For the untreated control treatment (1), 50 Fusarium and 20 no-Fusarium pots were prepared. For each treatment with cv. Golden Harvest, 10 pots were prepared with Fusarium and without Fusarium. For the untreated control treatment (1) using cv. Golden Harvest, 20 pots were prepared with and without Fusarium. The pots were arranged in a randomised block design with five replicate blocks. Each block contained five Fusarium and two no-Fusarium pots (ten and four pots respectively for untreated controls) for cv. Carlton. For cv. Golden Harvest, each block contained two pots with and without Fusarium for each of the 14 treatments (four pots respectively for untreated controls).

Pots were placed (spacing pot thick) on Mypex matting outdoors until 18 November 2011. The pots were then moved into a frost-protected glasshouse (spacing 2 cm between pots) until 29 March 2012. The pots were then returned outdoors (spacing 2 cm between pots).

Measurements

Samples of soils and composts used in the experiments were analysed for pH and electrical conductivity (EC).

The populations of fungal biocontrol agent propagules in the mixed soil from pots were determined at the end of the pot bioassays on three replicates of each treatment. Dilutions of soil in water suspensions (1:10) were plated on to plates of a selective agar media, containing a bactericidal antibiotic for fungal agents.

The efficacy of the treatments against Fusarium was first assessed after flowering from wilt symptoms on 28 March 2012. On this date, the foliage of almost all (>98%) of the plants in pots without Fusarium were green and healthy and did not show natural senescence. Plants were recorded as diseased if the foliage showed premature wilting with yellowing or browning. Bulbs were assessed for external symptoms of basal rot on 18 July 2012. Weight of healthy bulbs was recorded. Bulbs were stored in a poly tunnel until 20 September 2012 when they were cut through the base to assess for basal rot symptoms.

Results were analysed by Analysis of Variance. To determine the significance of differences between populations of biocontrol agent propagules in soils, data were $\log_{10}$ transformed before analysis. Data shown in the Tables are of untransformed values.

Results

Narcissus Pot Bioassay September 2010

Biocontrol agent propagule counts in composts and soil

Analyses of the soil and green waste compost used in the pot bioassay are shown in Table 2. The initial background test fungal population in the soil was below the detectable limit (10^3 cfu/g soil). Addition of HDC F106 granules to the compost significantly (*P*<0.01) increased the test fungal propagule population per gram of compost (Table 3).

At the end of the pot experiment (March 2011), the background population of fungal biocontrol agent propagules in the soil was 2.9×10^4 propagules per gram (Table 4). Addition of compost to the soil or HDC F109 or HDC F110 bulb dip treatments did not significantly increase the background soil populations of test fungal propagules. The population in the soil amended with HDC F106 compost was significantly (*P*<0.01) greater than that in the unamended soil pots. Due to severe frost (-15° C) in the polytunnel during December 2010, none of the plants survived so no disease assessments were possible.

Table 2. Soils and green waste composts used in the experiments

Year	**Soil**				**Green waste compost**		
	type	pH	EC (uS)	OM*(%)	pH	EC(uS)	OM*(%)
2010/11	sandy loam	6.75	287	3.6	7.96	1735	-
2011/12	silty loam	6.85	150	7.8	8.08	1501	25.8

* OM: organic matter, dry weight

Table 3. Initial test biocontrol agent populations in the soils and green waste composts, cfu/g soil. Each value is the mean of three replicate samples.

Treatment	2010/11 Bioassay Test fungi	2011/12 Bioassay Test fungi	2011/12 Bioassay Test bacteria
Soil	< 10^3	5.3×10^4	4.5×10^6
Compost	4.5×10^4	4.7×10^4	-
Compost + HDC F106	7.8×10^7	1.9×10^8	-
Compost + HDC F107	-	1.6×10^7	-
Compost + HDC F108	-	2.4×10^8	-
Compost + HDC F113	-	-	6.5×10^6

Table 4. Final soil biocontrol agent populations in the treatments, cfu/g soil. Each value is the mean of three replicate samples.

Treatment	2010/11 Bioassay Test fungi	2011/12 Bioassay Test fungi	2011/12 Bioassay Test bacteria
Soil	2.9×10^4	7.3×10^4	4.4×10^6
Compost	3.9×10^4	1.3×10^5	-
Compost + HDC F106	3.1×10^6	9.0×10^6	-
Compost + HDC F107	-	1.6×10^6	-
Compost + HDC F108	-	1.0×10^6	-
Compost + HDC F113	-	-	4.0×10^6
HDC F106 granules	-	1.3×10^7	-
HDC F108 granules	-	3.5×10^5	-
HDC F110 dip	4.0×10^4	-	
HDC F110 spray	-	9.4×10^5	-
HDC F109 dip	2.3×10^4		
HDC F109 spray	-	1.3×10^4	-
HDC F107 spray	-	7.5×10^5	-
HDC F112 spray	-	1.1×10^5	-
HDC F113 dip	-	-	5.5×10^6
Storite dip	-	5.0×10^4	-

Narcissus Pot Bioassay September 2011

Biocontrol agent propagule counts in composts and soil

Amendment of soil with 25% compost did not affect the background population of fungal biocontrol agent species at the end of the 2011/12 pot bioassay when measured at the start or end of the experiment (Table 3 and Table 4 respectively). The population of fungal biocontrol agent species of the soil was initially significantly (P<0.001) increased by amendment with compost containing HDC F106, HDC F107 or HDC F108 inocula or HDC F106 granules without compost (Table 3). When measured at the end of the experiment, spore numbers in these treatments remained significantly higher than in the soil and compost amended soil treatments (Table 4). Granules of HDC F108 or bulb spray treatments with HDC F107 or HDC F110 also significantly (P<0.05) increased the soil fungal biocontrol agent population. The HDC F109 or HDC F112 spray treatments or Storite dip treatment did not affect the soil fungal biocontrol agent population. There was no significant increase in the soil biocontrol agent population following the HDC F113 treatments, either at the start (Table 3) or at the end (Table 4) of the experiment.

Wilting and basal rot symptoms

Plants that showed wilting symptoms in March corresponded with bulbs that showed external basal rot symptoms in July, although further basal rot symptoms had developed in plants and bulbs between March and July, except in the HDC F108 granule and HDC F113 dip treatments (Figs. 2 and 3). Assessments of external basal rot symptoms in July accounted for about 55% of the total basal rot symptoms in all treatment after a further destructive assessment of bulbs was made in September (Figs. 3 and 4).

None of the compost treatments, with or without inocula of biocontrol agents affected the percentages of wilted plants or bulbs with basal rot, or the final healthy weight of bulbs of cv. Carlton (Figs. 2, 3, 4 and 5). The HDC F109 spray treatment and HDC F113 and Storite bulb dip treatments were also ineffective in reducing wilting of plants or basal rot symptoms. The HDC F112 spray treatment significantly ($P < 0.05$) reduced the percentage of wilted plants but did not affect the percentage of basal rot bulbs or the weight of healthy bulbs per pot. Granules of HDC F106 or HDC F108, or bulb spray treatments with HDC F107 or HDC F110 significantly ($P<0.05$) reduced the percentage of wilted plants and bulbs with external basal rot symptoms. All of these treatments, except HDC F107, also significantly ($P<0.05$) reduced total basal rot by the final assessment (September), and increased the weight of healthy bulbs per pot at harvest (Figs. 4 and 5).

In cv. Golden Harvest, none of the treatments examined (the conventional Storite dip, or compost inoculated with HDC F106 or HDC F113) was effective in reducing the percentage of wilted plants or basal rot symptoms compared with the soil control treatment. Across all of the treatments, the mean percentage of bulbs with basal rot in cv. Golden Harvest (60%) was much higher than that in cv. Carlton (32%). These are the cumulative percentages of basal rot in bulbs, i.e. assessed and discarded in July, and assessed again September. The percentages of plants with wilt symptoms or bulbs with basal rot in pots containing soil without Fusarium was less than 2% and was unaffected by the treatments.

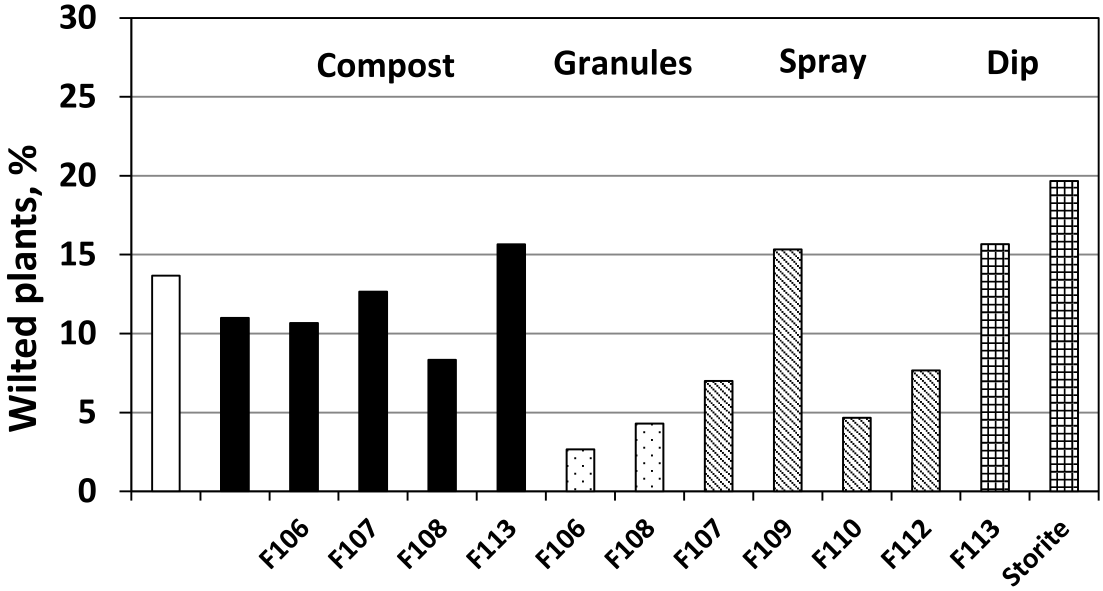

Figure 2. Effect of compost, biocontrol and conventional fungicide treatments compared with control soil (white bar) on the proportion of wilted narcissus cv. Carlton plants when measured on 28 March 2012. Bar represents Least Significant Difference at P = 0.05

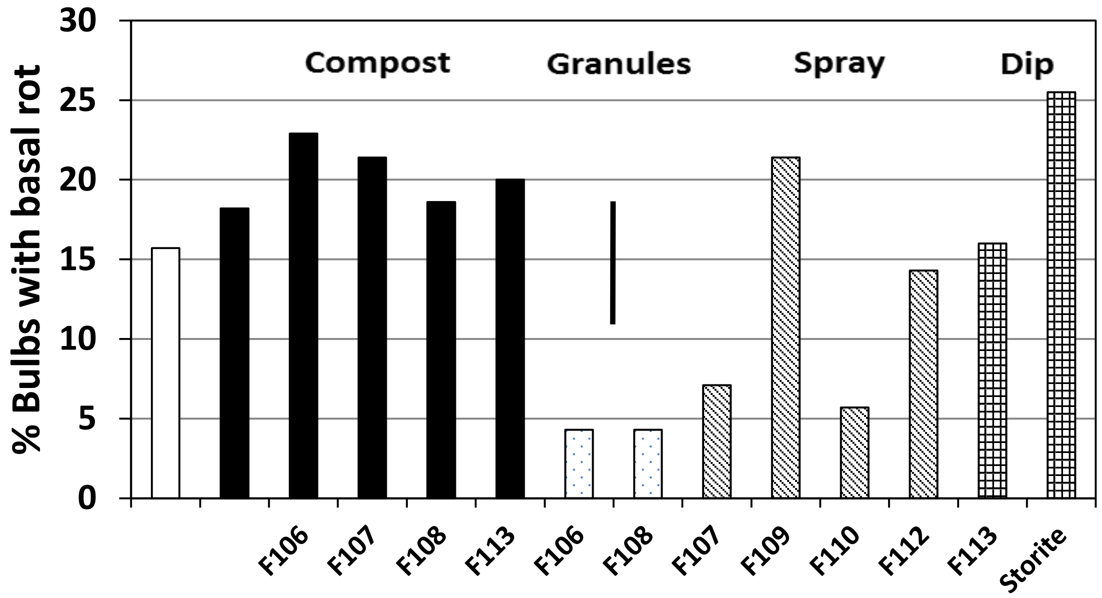

Figure 3. Effect of compost, biocontrol and conventional fungicide treatments compared with control soil (white bar) on the proportion of narcissus cv. Carlton bulbs with external basal rot symptoms in July 2012. Bar represents Least Significant Difference at P = 0.05.

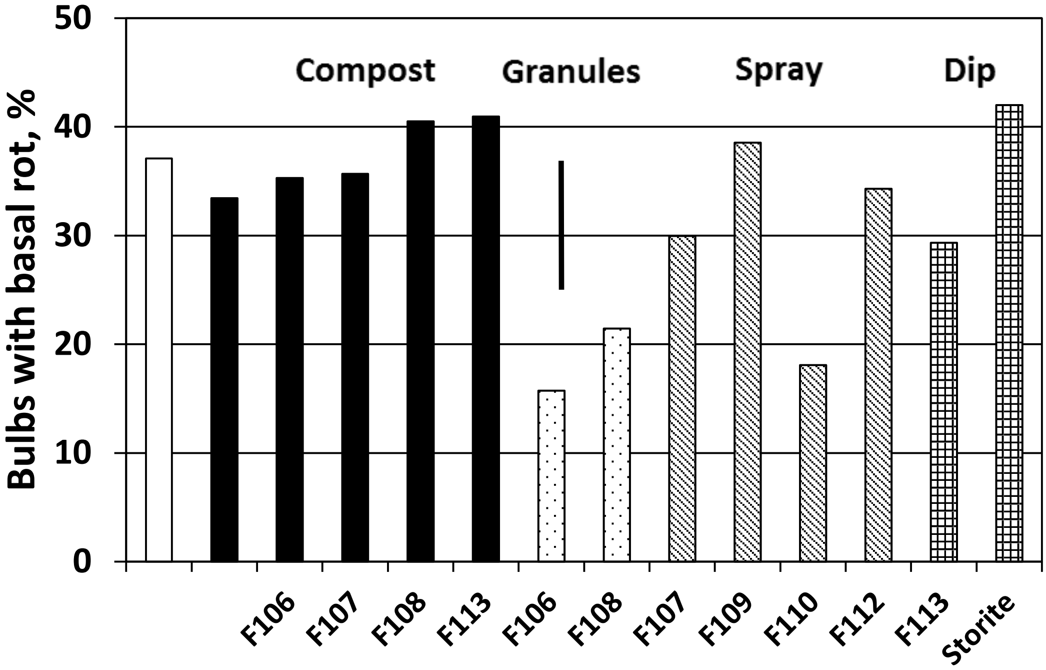

Figure 4. Effect of compost, biocontrol and conventional fungicide treatments compared with control soil (white bar) on the proportion of narcissus cv. Carlton bulbs with total basal rot symptoms, assessed and discarded in July and assessed in September 2012. Bar represents Least Significant Difference at P = 0.05

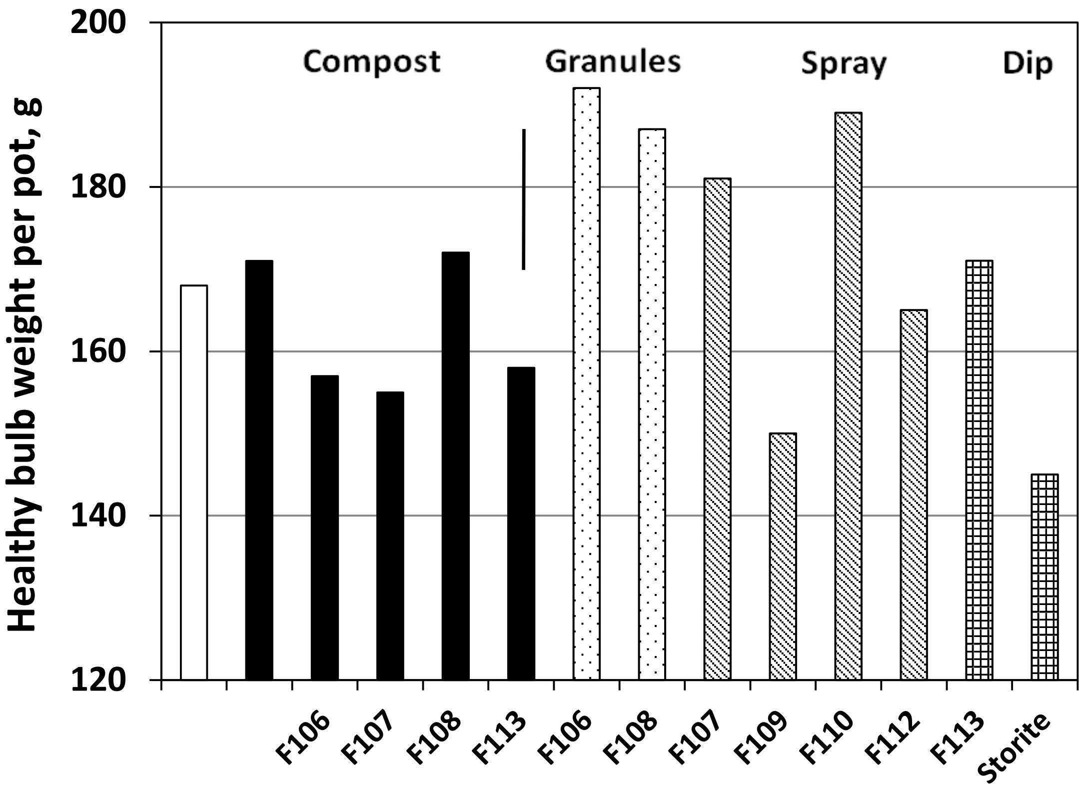

Figure 5. Effect of compost, biocontrol and conventional fungicide treatments compared with control soil (white bar) on the harvested weight of healthy cv. Carlton bulbs per pot. Bar represents Least Significant Difference at P = 0.05.

Conclusions

- Granules of HDC F106 or HDC F108, or bulb spray treatments with HDC F107 or HDC F110 significantly reduced the percentage of wilted plants in March and bulbs with the external basal rot symptoms in July compared with the control treatment
- These treatments, except HDC F107, also significantly decreased the total percentage of bulbs with basal rot symptoms in September and increased the weight of healthy bulbs per pot at harvest
- HDC F112 spray treatment of bulbs reduced the percentage of wilted plants but did not affect the percentage of basal rot bulbs or the weight of healthy bulbs per pot at harvest
- Amendment of soil with 25% compost, with or without inocula of biocontrol agents, HDC F109 spray treatment and HDC F113 and Storite bulb dip treatments were all ineffective in reducing basal rot symptoms
- The biocontrol agent population of the soil at the end of the pot bioassay was significantly increased by amendment with compost containing HDC F106, HDC F107 or HDC F108 inocula or HDC F106 granules without compost
- HDC F109 spray treatment of bulbs did not affect the final soil biocontrol agent population
- HDC F110 spray treatment of bulbs increased the soil biocontrol agent population at the end of one out of two pot bioassays

Technology transfer

Noble R, Biological control of narcissus basal rot. Presentation to Daffodil Growers Association meeting, Spalding, 9 May 2012.
Noble R, Biological control of narcissus basal rot. HDC Daffodil Growers Technical Event, Spalding, 15 November 2012.

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