SCEPTREPLUS

Final Trial Report

Trial code:	SP45
Title:	Control of Fusarium basal rot of *Narcissus*
Crop	*Narcissus*
Target	*Fusarium oxysporum f.sp. narcissi*
Lead researcher:	Dr John Clarkson
Organisation:	Warwick Crop Centre, University of Warwick
Period:	Oct 2019 - July 2020
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ORETO Number: (certificate should be attached)	381

I the undersigned, hereby declare that the work was performed according to the procedures herein described and that this report is an accurate and faithful record of the results obtained

06/07/2020 John Clarkson

Date Authors signature

Trial Summary

Introduction

The soilborne fungal plant pathogen *F. oxysporum* f.sp. *narcissi* (FON) is a major problem for the UK *Narcissus* industry and causes a severe basal rot of bulbs. Control was previously dependent on just two active ingredients, thiabendazole and chlorothalonil. However, the authorisation for the former as Storite Clear Liquid expired in 2019 whilst the latter as Bravo will be withdrawn in 2020. Both of these products have routinely been utilised for many years as part of the hot water treatment (HWT) of *Narcissus* bulbs employed by growers both to apply fungicides and manage stem nematode. An alternative product containing cyprodinil and fludioxonil (Switch) has been recently approved, although performance has not yet been assessed in HWT. Despite regular applications of fungicides by *Narcissus* growers, extensive losses due to FON are still common in some production areas with the long growing period leaving the crop vulnerable to disease. Fusarium basal rot is therefore still a major threat to the industry and new actives compatible with HWT are urgently required. This project aimed to identify chemical and biological crop protection products that can reduce basal rot in *Narcissus* using an established FON-inoculated pot-based glasshouse bioassay.

Methods

Nine crop protection products comprising six chemical fungicides and three microbial biological control agents were evaluated for their efficacy in controlling Fusarium basal rot caused by FON. Disease-free *Narcissus* bulbs (cv. Carlton) were treated with each product in a small-scale HWT system and then planted into compost infested with FON. Control treatments were also set up consisting of HWT bulbs and non-HWT bulbs without any crop protection treatments planted in the growing medium with or without FON. After one cycle of flower production, *Narcissus* bulbs were bisected and assessed for Fusarium basal rot incidence (percentage bulbs affected) and severity based on the area of each bulb affected using a 0-10 scoring scale (where 0 = no infection and 10 = whole bulb rotted). Crop safety was also assessed by recording any treatment effects on foliage and number of flowers produced. The amount of each product taken up by the *Narcissus* bulbs during HWT was also determined to potentially provide additional useful data for EAMUs.

Results

Fusarium basal rot incidence in *Narcissus* bulbs ranged between 33.3 and 100.0% across the different crop protection product treatments compared with 97.5% for the HWT untreated inoculated control (Table A). Storite Excel, AHDB9820 and AHDB9819 resulted in the lowest disease incidence with 35.0, 33.3 and 10.0% bulbs infected with FON respectively, while all the other products resulted in levels between 92.5 and 100.0%. Correspondingly, Storite Excel, AHDB9820 and AHDB9819 resulted in 77.5, 84.6 and 97.5% of *Narcissus* bulbs with low severity of basal rot respectively (Table A). All the other crop protection products resulted in <10% bulbs with low disease severity and >85% of bulbs with high disease severity (Table A). Treatment of *Narcissus* bulbs with Storite Excel, AHDB9820 and AHDB9819 therefore also resulted in the lowest mean Fusarium basal rot severity scores of 1.63, 1.56 and 0.30 respectively which were all significantly less ($p<0.05$) than for the HWT untreated inoculated control which had a mean score of 8.83 (Table A). All the other products resulted in mean disease severity scores >7.83 with none significantly different from the HWT untreated inoculated control. Products AHDB9818 and AHDB9862 which were not effective against FON, significantly reduced flowering compared to the HWT untreated inoculated control. The uptake of crop protection products by *Narcissus* bulbs varied following HWT.

Table A. Effect of crop protection products on *Narcissus* basal rot incidence (%FON), percentage of bulbs with low, medium and high severity scores and overall mean severity score for *Narcissus* bulbs planted in compost inoculated with FON. Data are means for 40 bulbs per treatment.

	Treatment[1]	FRAC code	% FON[2]	% low severity[3]	% medium severity[3]	% high severity[3]	Mean basal rot severity[4]	SEM
1	Storite Excel	B1	35.0	77.5	2.5	20.0	1.63	0.442
2	Switch	D1+E2	97.5	2.5	0.0	97.5	9.18	0.265
3	AHDB9820	G1	33.3	84.6	0.0	15.4	1.56	0.499
4	AHDB9819	G1	10.0	97.5	0.0	2.5	0.30	0.206
5	AHDB9818	C2	100.0	0.0	0.0	100.0	9.35	0.154
6	AHDB9862	G1	92.5	10.0	5.0	85.0	7.83	0.469
7	AHDB9936[B]		100.0	0.0	2.5	97.5	9.00	0.218
8	AHDB9849[B]		100.0	2.5	0.0	97.5	9.00	0.251
9	AHDB9955[B]		97.5	5.0	0.0	95.0	8.83	0.336
10	HWT inoc		97.5	5.0	0.0	95.0	8.83	0.330
11	No HWT inoc		62.5	47.5	7.5	45.0	4.43	0.688
12	HWT uninoc		2.5	100.0	0.0	0.0	0.03	0.025
13	No HWT uninoc		0.0	100.0	0.0	0.0	0.00	0.000

[B] indicates microbial biological control agent.
[1] HWT inoc = hot water treated bulbs planted in compost inoculated with FON (HWT untreated inoculated control); No HWT inoc = bulbs planted in compost inoculated with FON (no HWT untreated inoculated control); HWT uninoc = hot water treated bulbs planted in compost without FON (HWT untreated uninoculated control); No HWT uninoc = bulbs planted in compost without FON (no HWT untreated uninoculated control).
[2] Mean percentage *Narcissus* bulbs affected with basal rot.
[3] Mean percentage of *Narcissus* bulbs in different disease severity categories. Disease severity based on area of *Narcissus* bulb affected with basal rot using a 0-10 scale where 0 = no infection and 10 = whole bulb rotted. Low severity = 0-2; medium severity = 3-5; high severity = 6-10.
[4] Green shading indicates products with a significant reduction in mean basal rot severity score ($p < 0.05$) compared to the untreated HWT inoculated control.
SEM = standard error of the mean.

Conclusions

Three chemical fungicides Storite Excel, AHDB9820 and AHDB9819 resulted in very good control of Fusarium basal rot of *Narcissus* when applied in HWT. AHDB9819 was particularly effective, reducing disease incidence by approx. 90% and severity by 97%. Storite Excel is currently approved for post-harvest use on potatoes and contains the same active ingredient thiabendazole as in Storite Clear Liquid which before withdrawal, was used successfully by the industry for many years in HWT to control FON. However, FON isolates resistant or tolerant to thiabendazole have been identified previously (AHDB project BOF74) and hence control should not be dependent on this active. AHDB9820 and AHDB9819 therefore offer alternative chemistry for control of FON and if approved, these products could be used as part of an effective fungicide resistance management programme.

Take home message

The fungicides AHDB9820 and AHDB9819 resulted in effective control of Fusarium basal rot of *Narcissus* and therefore provide a new opportunity for disease management as part of HWT.

Objectives

1. Identify a range of control treatments for testing that have potential to reduce Fusarium basal rot in *Narcissus.*

2. Determine the effect of fungicides (conventional and biological) applied in HWT on subsequent development of Fusarium basal rot in *Narcissus.*

Trial conduct

UK regulatory guidelines were followed but EPPO guidelines took precedence. The following EPPO guidelines were followed:

Relevant EPPO guideline(s)		**Variation from EPPO**
PP 1/152(3)	Design and analysis of efficacy evaluation trials	None
PP 1/135(3)	Phytotoxicity assessment	None
PP 1/181(3)	Conduct and reporting of efficacy evaluation trials including GEP	None

There were no deviations from EPPO guidance.

Test site

Item	**Details**
Location address	University of Warwick Wellesbourne Campus Wellesbourne Warwick CV35 9EF
Crop	*Narcissus*
Cultivar	Carlton
Soil or substrate type	Specialist compost
Agronomic practice	N/A
Prior history of site	N/A

Trial design

Item	**Details**
Trial design:	Randomised block
Number of replicates:	Eight replicate pots of five *Narcissus* bulbs in eight blocks (N=40 bulbs per treatment)
Row spacing:	N/A
Plot size: (w x l)	N/A
Plot size: (m^2)	N/A
Number of plants per plot:	N/A

Treatment details

	AHDB code[1]	Active substance	Product name/ manufacturers code	FRAC code	Formulation batch number	Content of active substance in product	Formulation type	Adjuvant
1		Thiabendazole	Storite Excel	B1	PHY7173256	500 g L^{-1}	suspension concentrate	0.09% Agral
2	Approved	Cyprodinil and fludioxonil	Switch	D1+E2	CHE7E60076	375 and 250 g kg^{-1}	water dispersible granule	0.09% Agral
3	AHDB9820	N/D	N/D	N/D	N/D	N/D	N/D	0.09% Agral
4	AHDB9819	N/D	N/D	N/D	N/D	N/D	N/D	0.09% Agral
5	AHDB9818	N/D	N/D	N/D	N/D	N/D	N/D	0.09% Agral
6	AHDB9862	N/D	N/D	N/D	N/D	N/D	N/D	0.09% Agral
7	AHDB9936[B]	N/D	N/D	N/D	N/D	N/D	N/D	0.09% Agral
8	AHDB9849[B]	N/D	N/D	N/D	N/D	N/D	N/D	0.09% Agral
9	AHDB9955[B]	N/D	N/D	N/D	N/D	N/D	N/D	0.09% Agral

[B] indicates microbial biological control agent.

Application schedule

Treatment number	Treatment: product name or AHDB code	Rate of active substance L^{-1}	Rate of product L^{-1} used in HWT	Application code
1	Storite Excel	755 mg	1.51 ml	A
2	Switch	750 mg Cyprodinil 500 mg Fludioxonil	2 g	A
3	AHDB9820	450 mg	1.125 ml	A
4	AHDB9819	648 mg	1.35 ml	A
5	AHDB9818	270 mg	0.674 ml	A
6	AHDB9862	121 mg	1.617 ml	A
7	AHDB9936[B]	3.15 x 10^{10} cfu	30 ml	A
8	AHDB9849[B]	2 x 10^{10} cfu	1 ml	A
9	AHDB9955[B]	1 x 10^{10} cfu	10 g	A

Rates of products were based on criteria as follows:

- Storite Excel: rate as approved for application post-harvest to potatoes (per tonne)
- Switch: rate from current EAMU for *Narcissus*
- AHDB9820: calculated from Dutch label rate for Mirage Elan on *Narcissus*
- AHDB9819, AHDB9818, AHDB9862: rate based on 1 tonne *Narcissus* bulbs taking up 53L water (calculated from a small preliminary experiment) and the maximum rate per ha from existing labels for other crops assuming a *Narcissus* bulb planting rate of 17.5 t ha^{-1}
- AHDB9936: rate from EAMU for cabbage dip
- AHDB9849: rate adjusted to achieve same cfu as AHDB9936
- AHDB9955: maximum allowable rate on label for other crops

Experimental setup and application details

Summary of approach

Nine crop protection products comprising six chemical fungicides and three microbial biological control agents as listed above were evaluated for their efficacy in controlling Fusarium basal rot caused by *F. oxysporum* f.sp. *narcissi* (FON). All products were applied to disease-free *Narcissus* bulbs (cv. Carlton) in a small-scale hot water treatment system (HWT, application A) as routinely used by the industry both to apply fungicides and control stem nematode. In a previous experiment, the three microbial products had all been confirmed to be thermally stable following exposure to the HWT temperature of 44.4°C and series dilution onto agar to confirm viability. Following HWT with the products, the *Narcissus* bulbs were planted into compost infested with FON and after one cycle of flower production, assessed for basal rot. Crop safety was also assessed by recording any effects on foliage and number of flowers produced.

Application of crop protection products in HWT and bulb uptake

Five 38 L water baths were filled with 35 L of water with the addition of 3 ml of Agral (a non-ionic wetting agent) and allowed to reach the standard working HWT temperature of 44.4°C. Each product under test was allocated a bath with one bath used exclusively for all the untreated control bulbs. When the baths had reached the working temperature, the appropriate amount of each test product was added and mixed briefly. Fusarium-free *Narcissus* bulbs (cv. Carlton) contained in nets were then lowered gently

into the water baths, and a wire mesh placed on top to ensure the complete immersion of the bulbs during treatment. The baths were allowed to reach the working temperature of 44.4°C before commencing timing of the standard 3.25 hours HWT. At the end of the treatment, the bulb nets were immediately removed from the baths and the excess water drained. Nets were then placed into slatted trays and the bulbs dried at ambient temperature for 24-48 hours before use. Lots of 40 *Narcissus* bulbs for the experiment were treated over two days (22/10/19-23/10/19) with baths being emptied, cleaned / flushed out, refilled and wetter / product added again for each run. To provide additional information on the amount of each product actually taken up by the *Narcissus* bulbs, bulb lots were weighed before and after HWT / drying. The amount of product applied per tonne bulbs and per hectare assuming a planting rate of 17.5 t ha^{-1} was then determined.

FON inoculum

FON isolate FON139 previously identified as being highly pathogenic on *Narcissus* (AHDB Project BOF 74) was grown on potato dextrose agar for approx. four days at 20°C to produce actively growing cultures. Agar plugs from the leading edge of the colonies were then used to inoculate a sterile mix of M2 compost and wheat bran in 1 L flasks which were incubated at 25°C in the dark for approx. four weeks. To quantify the inoculum, the number of FON cfu were enumerated by series dilution.

Bulb planting and inoculation

Narcissus bulbs treated with the different crop protection products in HWT were planted in 20 cm diameter, 4 L capacity plastic plant-pots in a specialised growing medium consisting of a blend of sphagnum peat / horticultural sand (3:1, v/v) mixed with John Innes No.1 compost (1:1, v/v) and amended with ammonium nitrate (0.40 kg m^{-3}), potassium nitrate (0.75 kg m^{-3}), single super-phosphate (1.50 kg m^{-3}), ground chalk (2.25 kg m^{-3}), ground magnesian limestone (2.25 kg m^{-3}) and fritted trace elements WM 255 (0.40 kg m^{-3}). The growing medium was artificially inoculated with appropriate amounts of FON inoculum to give a final level of 1 x 10^5 cfu g^{-1}. Five *Narcissus* bulbs were then planted approx. 10 cm deep (measured to the base of the bulb) in each pot. Control treatments were also set up consisting of HWT bulbs and non-HWT bulbs without any crop protection treatments planted in the growing medium with or without FON. Pots were placed in saucers in a frost-free glasshouse on 24/10/19 with shading and watered from below as appropriate. In total there were eight replicate pots of five bulbs (total 40 bulbs) per treatment which were randomised in eight blocks across two benches in the glasshouse compartment (see Appendix).

Plant and bulb assessments

To assess crop safety, *Narcissus* plants were assessed on two occasions for mottling or chlorosis of foliage (13/02/20, 28/02/20) which might indicate phytotoxicity while another assessment on 13/03/20 recorded the number of bulbs flowering and the number of flowers per bulb. When most plants had begun to senesce, watering was halted from 17/04/20 and all the pots allowed to dry-out for approx. four weeks before the bulbs were harvested and assessed for Fusarium basal rot on 20/05/20. This was achieved by bisecting the bulbs lengthways and recording disease area based on a 0-10 scoring scale where 0 = no infection and 10 = whole bulb affected (Table 1).

Data summaries and analyses

Crop safety: Mean percentage of *Narcissus* bulbs flowering and the mean number of flowers per bulb were calculated for each treatment. ANOVA* was then carried out for

the latter data set followed by post-hoc pairwise Tukey's tests to identify treatments that reduced the mean number of flowers per bulb compared to the HWT bulbs that received no crop protection products (HWT untreated inoculated control).

Basal rot disease: Overall incidence of Fusarium basal rot was calculated as the mean percentage of bulbs for each treatment that had a severity score >1 (%FON). The mean percentage of *Narcissus* bulbs with low (score 0-2), medium (score 3-5) and high disease severity (score 6-10) (Table 1) was also calculated. Finally, mean basal rot severity score was calculated for each treatment and ANOVA* carried out followed by post-hoc pairwise Tukey's tests to identify treatments that reduced the mean basal rot disease score compared to the HWT bulbs that received no crop protection products (HWT untreated inoculated control).

* Note: datasets analysed by ANOVA were not normally distributed and this was also the case when square root and log transformations were tested. This increases the chance of a false significant result and hence additional non parametric analyses using a Kruskall-Wallis test followed by post hoc pairwise comparisons were also carried out. These however gave the same result in terms of treatments which were identified as being significantly different from the control.

Table 1. Scoring scale for assessing the severity of Fusarium basal rot in *Narcissus* bulbs.

Score[1]	**Severity**	**Zones affected by basal rot**
0	Low	None
1	Low	Spot or spots (up to 2mm-diameter) in base plate
2	Low	Small area of basal plate (up to 10%) but no spread to bulb scales
3	Medium	Up to 25% of basal plate area but no spread to bulb scales
4	Medium	Up to 50% of basal plate area but no spread to bulb scales
5	Medium	More than 50% of basal plate area but no spread to bulb scales
6	High	Start of spread from basal plate to bulb scales (up to 10% of scale area)
7	High	Up to 25% of bulb scale area
8	High	Up to 50% of bulb scale area
9	High	More than 50% of bulb scale area
10	High	Whole bulb (or virtually whole bulb) (includes dried, 'mummified' bulbs)

[1] Disease scores of 1-5 relate to basal rot in the base plate only; scores 6-10 relate to rot in bulb scales and are irrespective of percentage of basal plate affected.

Untreated levels of pests/pathogens at application and through the assessment period

Common name	Scientific Name	EPPO Code	Infestation level pre-application	Infestation level at start of assessment period	Infestation level at end of assessment period
Fusarium	*Fusarium oxysporum* f.sp. *narcissi*	N/A	N/A	N/A	HWT untreated inoculated control 97.5% basal rot incidence and mean disease score of 8.8 (max 10) No HWT untreated inoculated control bulbs 62.5% basal rot incidence and mean disease score of 4.4 (max 10)

Assessment details

	Evaluation Timing (DA)*				
Evaluation date	After conventional fungicides	After Bio-fungicides	Crop Growth Stage (BBCH)	Evaluation type (efficacy, phytotox)	Assessment
13/02/20	113	113	N/A	Phytotoxicity	Foliage
28/02/20	128	128	N/A	Phytotoxicity	Foliage
13/03/20	142	142	N/A	Phytotoxicity	Flowering
17/04/20	174	174	N/A	Disease	Basal rot

Results

Uptake of crop protection products by *Narcissus* bulbs

The mean uptake of water by untreated *Narcissus* bulbs (HWT / no HWT, no crop protection products) was 70.6 ml kg^{-1} which was greater than the 53 ml kg^{-1} in the preliminary experiment on which the application rates of some products were based (Table 2). Hence rates for AHDB9819, AHDB9818 and AHDB9862 slightly exceeded the maximum allowed per hectare based on the label for other crops. There was also some variation in the volume of water taken up by bulbs across the products (57-76 ml).

Table 2. Uptake of crop protection products during HWT

Product	**Rate of product L^{-1} used in HWT**	**Water absorbed kg^{-1} bulb**	**Product absorbed kg^{-1} bulb**	**Product absorbed $tonne^{-1}$ bulb**	***Rate of product ha^{-1}**
Storite Excel	1.51 ml	76.11 ml	0.115 ml	114.9 ml	2.011 L
Switch	2 g	71.11 ml	0.142 g	142.2 g	2.489 kg
AHDB9820	1.125 ml	57.22 ml	0.064 ml	64.4 ml	1.127 L
AHDB9819	1.35 ml	70.75 ml	0.096 ml	95.5 ml	1.676 L
AHDB9818	0.674 ml	67.73 ml	0.046 ml	45.6 ml	0.799 L
AHDB9862	1.617 ml	68.68 ml	0.111 ml	111.1 ml	1.944 L
AHDB9936[B]	30 ml	63.24 ml	1.897 ml	1897.2 ml	33.201 L
AHDB9849 [B]	1 ml	68.83 ml	0.069 ml	68.8 ml	1.205 L
AHDB9955 [B]	10 g	76.69 ml	0.767 g	766.9 g	1.342 kg
Untreated controls		70.55 ml			

*Calculated rate of product ha^{-1} based on *Narcissus* bulb planting density of 17.5 tonne ha^{-1}.

Phytotoxicity

There was some evidence of mottling or chlorosis of *Narcissus* leaves in some crop protection product treatments (2.5-18.0% leaves affected) but this was not consistent over the two assessment dates (data not shown). Control treatments (bulbs with / without HWT and inoculated / uninoculated with FON) also had some of these symptoms but at a lower level (0-5% leaves affected). Leaf mottling is sometimes due to the HWT while chlorosis has been associated with Fusarium infection (AHDB Project BOF 74a) so this may have confounded results. Overall, no firm conclusions could be drawn regarding crop safety using this dataset.

In contrast, there were clear differences between crop protection product treatments for the mean percentage of *Narcissus* bulbs flowering (Table 3) with AHDB9818 and AHDB9862 resulting in the lowest levels of 52.5 and 72.5% flowering respectively. All other treatments resulted in 75.0-95.0% bulbs flowering with the various untreated uninoculated / inoculated control treatments ranging between 95 and 100% flowering. Correspondingly AHDB9818 and AHDB9862 resulted in mean values of 0.78 and 1.00 flowers per bulb which were significantly less ($p<0.05$) than for the HWT untreated inoculated control treatment with 1.63 flowers per bulb (Table 3). All other treatments and the remainder of the controls had values ranging from 1.28 to 1.75 flowers per

bulb. Although both AHDB9818 and AHDB9862 had high levels of basal rot (see below), this was also the case for other treatments where flowering was not affected. This suggests that application of these two products in HWT may have some small phytotoxic effect leading to a decrease in flowering.

Table 3. Effect of crop protection products on flowering of *Narcissus* bulbs

Treatment[1]	Mean % bulbs flowering	Mean no. flowers/bulb[2]	SEM
Storite Excel	92.5	1.38	0.11
Switch	82.5	1.33	0.12
AHDB9820	84.6	1.28	0.12
AHDB9819	75.0	1.28	0.14
AHDB9818	52.5	0.78	0.14
AHDB9862	72.5	1.00	0.12
AHDB9936 [B]	87.2	1.36	0.12
AHDB9849 [B]	95.0	1.48	0.09
AHDB9955 [B]	85.0	1.35	0.12
HWT inoc	95.0	1.63	0.11
No HWT inoc	100.0	1.75	0.08
HWT uninoc	97.5	1.55	0.09
No HWT uninoc	100.0	1.70	0.11

[B] indicates microbial biological control agent.
[1] HWT inoc = hot water treated bulbs planted in compost inoculated with FON (HWT untreated inoculated control); no HWT inoc = bulbs planted in compost inoculated with FON (no HWT untreated inoculated control); HWT uninoc = hot water treated bulbs planted in compost without FON (HWT untreated uninoculated control); no HWT uninoc = bulbs planted in compost without FON (no HWT untreated uninoculated control).
[2] Green shading indicates products with a significant reduction in flowering ($p < 0.05$) compared to the untreated HWT inoculated control.

Efficacy

There were clear differences between the crop protection product treatments in the mean incidence of Fusarium basal rot (% FON) in *Narcissus* bulbs which ranged between 10.0 and 100.0% compared with 97.5% for the HWT untreated inoculated control (Table 3, Appendix). The industry standard Storite Excel, AHDB9820 and AHDB9819 resulted in the lowest disease incidence with 35.0, 33.3 and 10.0% bulbs infected with FON respectively, while all the other products resulted in levels between 92.5 and 100.0%. Correspondingly, Storite Excel, AHDB9820 and AHDB9819 resulted in 77.5, 84.6 and 97.5% of *Narcissus* bulbs with low basal rot severity respectively (basal rot score 0-2; Fig. 1, Table 4). All the other crop protection products resulted in <10% bulbs with low disease severity and >85% of bulbs with high severity (basal rot score 6-10).

Treatment of *Narcissus* bulbs with Storite Excel, AHDB9820 and AHDB9819 also resulted in the lowest mean Fusarium basal rot severity scores of 1.63, 1.56 and 0.30 respectively which were all significantly less ($p<0.05$) than the for the HWT untreated inoculated control which had a mean score of 8.83 (Fig. 2, Table 4). All the other crop

protection products resulted in mean disease severity scores >7.83 with none significantly different from the HWT untreated inoculated control.

Finally, the untreated no-HWT untreated inoculated control also resulted in reduced incidence of FON (62.5%), a greater percentage of bulbs with low basal rot severity (47.5%), and a significantly smaller mean basal rot score of 4.43 compared to values for the HWT untreated inoculated control. As expected though, the HWT / no HWT untreated uninoculated control treatments resulted in no or negligible levels of Fusarium basal rot.

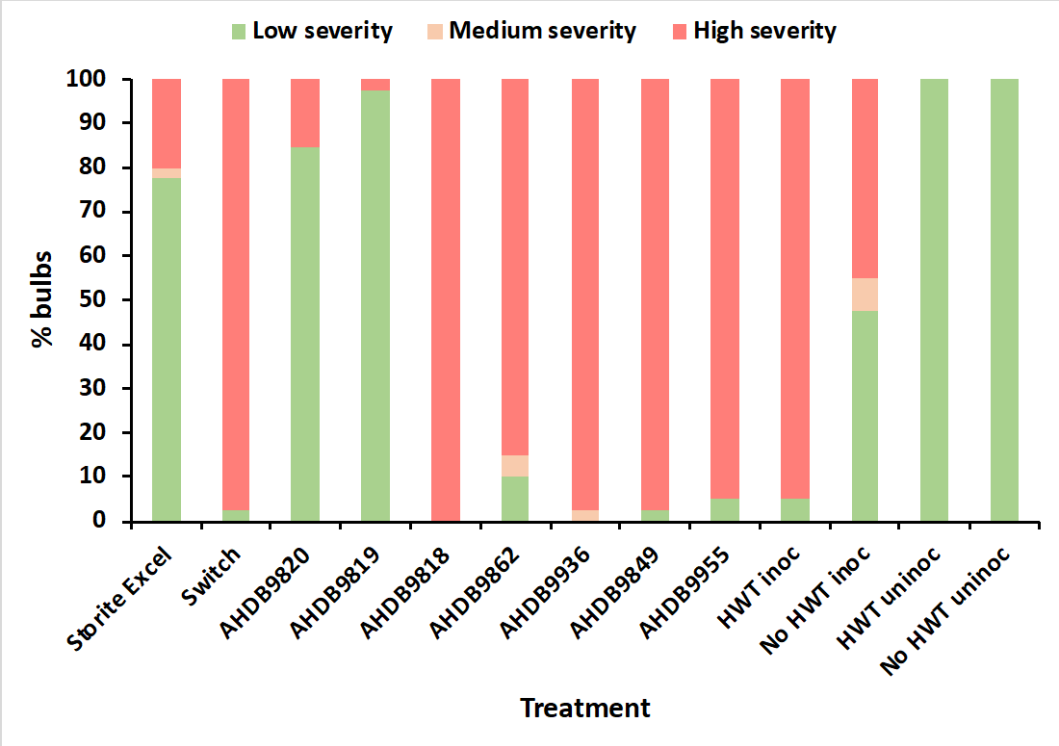


Figure 1. Effect of crop protection products on percentage of *Narcissus* bulbs in different Fusarium basal rot disease severity categories after planting in compost inoculated with FON. Data are means for 40 plants per treatment. HWT inoc = hot water treated bulbs planted in compost inoculated with FON (HWT untreated inoculated control); no HWT inoc = bulbs planted in compost inoculated with FON (no HWT untreated inoculated control); HWT uninoc = hot water treated bulbs planted in compost without FON (HWT untreated uninoculated control); no HWT uninoc = bulbs planted in compost without FON (no HWT untreated uninoculated control).

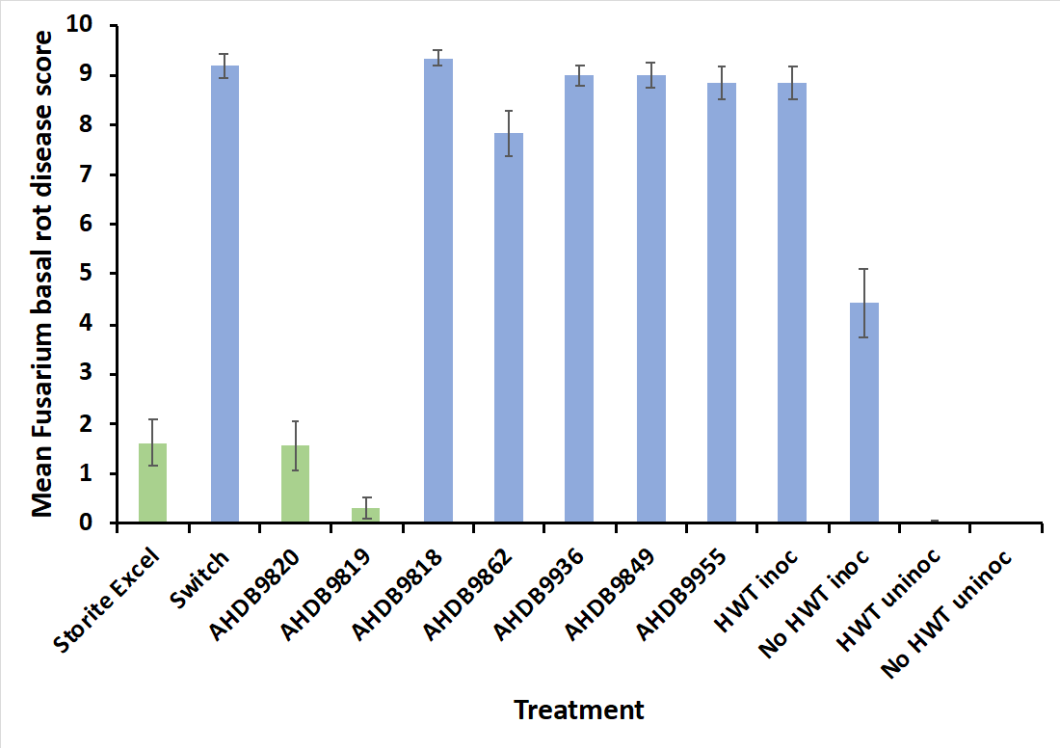


Figure 2. Effect of crop protection products on mean Fusarium basal rot disease severity score after planting in compost inoculated with FON. Data are means for 40 plants per treatment. Green bars indicate products with a significant reduction in basal rot ($p < 0.05$) compared to the untreated HWT inoculated control. HWT inoc = hot water treated bulbs planted in compost inoculated with FON (HWT untreated inoculated control); no HWT inoc = bulbs planted in compost inoculated with FON (no HWT untreated inoculated control); HWT uninoc = hot water treated bulbs planted in compost without FON (HWT untreated uninoculated control); no HWT uninoc = bulbs planted in compost without FON (no HWT untreated uninoculated control).

Table 4. Effect of crop protection products on *Narcissus* basal rot incidence (%FON), percentage of bulbs with low, medium and high disease severity scores and overall mean severity score for *Narcissus* bulbs planted in compost inoculated with FON. Data are means for 40 bulbs per treatment.

	Treatment[1]	% FON[2]	% bulbs low severity[3]				% bulbs medium severity[3]				% bulbs high severity[3]						Mean basal rot score[4]	SEM
			0	1	2	Total	3	4	5	Total	6	7	8	9	10	Total		
1	Storite Excel	35.0	65.0	10.0	2.5	**77.5**	0.0	2.5	0.0	**2.5**	10.0	5.0	2.5	2.5	0.0	**20.0**	1.63	0.442
2	Switch	97.5	2.5	0.0	0.0	**2.5**	0.0	0.0	0.0	**0.0**	0.0	5.0	2.5	37.5	52.5	**97.5**	9.18	0.265
3	AHDB9820	33.3	66.7	12.8	5.1	**84.6**	0.0	0.0	0.0	**0.0**	0.0	0.0	5.1	10.3	0.0	**15.4**	1.56	0.499
4	AHDB9819	10.0	90.0	5.0	2.5	**97.5**	0.0	0.0	0.0	**0.0**	0.0	0.0	2.5	0.0	0.0	**2.5**	0.30	0.206
5	AHDB9818	100.0	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	**0.0**	2.5	2.5	12.5	22.5	60.0	**100.0**	9.35	0.154
6	AHDB9862	92.5	7.5	0.0	2.5	**10.0**	2.5	0.0	2.5	**5.0**	7.5	7.5	5.0	30.0	35.0	**85.0**	7.83	0.469
7	AHDB9936 [B]	100.0	0.0	0.0	0.0	**0.0**	2.5	0.0	0.0	**2.5**	2.5	5.0	10.0	37.5	42.5	**97.5**	9.00	0.218
8	AHDB9849 [B]	100.0	0.0	0.0	2.5	**2.5**	0.0	0.0	0.0	**0.0**	7.5	0.0	7.5	35.0	47.5	**97.5**	9.00	0.251
9	AHDB9955 [B]	97.5	2.5	2.5	0.0	**5.0**	0.0	0.0	0.0	**0.0**	0.0	5.0	12.5	30.0	47.5	**95.0**	8.83	0.336
10	HWT inoc	97.5	2.5	0.0	2.5	**5.0**	0.0	0.0	0.0	**0.0**	2.5	5.0	10.0	27.5	50.0	**95.0**	8.83	0.330
11	No HWT inoc	62.5	37.5	7.5	2.5	**47.5**	5.0	2.5	0.0	**7.5**	2.5	0.0	12.5	10.0	20.0	**45.0**	4.43	0.688
12	HWT uninoc	2.5	97.5	2.5	0.0	**100.0**	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	0.0	0.0	**0.0**	0.03	0.025
13	No HWT uninoc	0.0	100.0	0.0	0.0	**100.0**	0.0	0.0	0.0	**0.0**	0.0	0.0	0.0	0.0	0.0	**0.0**	0.00	0.000

[B] indicates microbial product.

[1] HWT inoc = hot water treated bulbs planted in compost inoculated with FON (HWT untreated inoculated control); no HWT inoc = bulbs planted in compost inoculated with FON (no HWT untreated inoculated control); HWT uninoc = hot water treated bulbs planted in compost without FON (HWT untreated uninoculated control); no HWT uninoc = bulbs planted in compost without FON (no HWT untreated uninoculated control).

[2] Mean percentage *Narcissus* bulbs affected with basal rot.

[3] Mean percentage of *Narcissus* bulbs in different disease severity categories. Disease severity based on area of *Narcissus* bulb affected with basal rot using a 0-10 scale where 0 = no infection and 10 = whole bulb rotted. Low severity = 0-2; medium severity = 3-5; high severity = 6-10.

[4] Green shading indicates products with a significant reduction in basal rot ($p < 0.05$) compared to the untreated HWT inoculated control.

SEM = standard error of the mean.

Discussion

A FON-inoculated pot-based glasshouse bioassay was successful in establishing high levels of Fusarium basal rot in *Narcissus* bulbs allowing the efficacy of different crop protection products for control to be successfully evaluated. Both conventional chemical fungicides and microbial biological control agents were tested as part of HWT with the latter shown through previous experimentation to survive at this temperature (44.4°C).

Three chemical fungicides Storite Excel, AHDB9820 and AHDB9819 resulted in very good control of Fusarium basal rot of *Narcissus* when applied in HWT. AHDB9819 was particularly effective, reducing incidence by approx. 90% and severity by 97% compared to the HWT untreated control. Storite Excel which was tested at a rate of 1.51 ml L^{-1} is approved for post-harvest use on potatoes and contains the same active ingredient thiabendazole (FRAC code B1) as in Storite Liquid which was used in HWT by the industry for many years to control FON at a rate of 1.25 ml L^{-1}. However, FON isolates resistant or tolerant to thiabendazole have been identified previously (AHDB project BOF74) and hence control should not be dependent on this active. AHDB9820 and AHDB9819 (both FRAC code G1) therefore offer alternative chemistry for control of FON and if approved, these products could be used as part of an effective fungicide resistance management programme.

As well as effectively controlling basal rot, Storite Excel, AHDB9820 and AHDB9819 were crop safe, and did not reduce *Narcissus* flowering compared to the HWT untreated control. In contrast, although not effective against FON, the two fungicides AHDB9818 and AHDB9862 significantly reduced the number of flowers per bulb suggesting they might have some phytotoxic effects.

It was interesting to note in the experiment that there was a greater incidence and severity of Fusarium basal rot in HWT untreated *Narcissus* bulbs compared to non-HWT untreated bulbs. This has been observed previously and is attributed largely to HWT promoting disease development in seemingly clean bulb stocks which may carry a background level of FON inoculum or stress which could pre-dispose the bulb to infection once planted in infested soil (AHDB Project BOF 74a).

Finally, the data collected on the amount of water / fungicide absorbed by the *Narcissus* bulbs during HWT revealed that this varied slightly depending on the product which could be attributed to the different formulations. This also allowed the calculation of theoretical fungicide rates in the field given a planting density of 17.5 tonne bulbs ha^{-1} which should provide additional information for development of potential EAMUs for those products demonstrating efficacy. However, further experimentation may be required to confirm these results under commercial conditions where multiple batches of *Narcissus* bulbs are dipped consecutively in the same HWT tanks as this often results in large amounts of soil / bulb scale material accumulating which can alter fungicide uptake and efficacy.

Conclusions

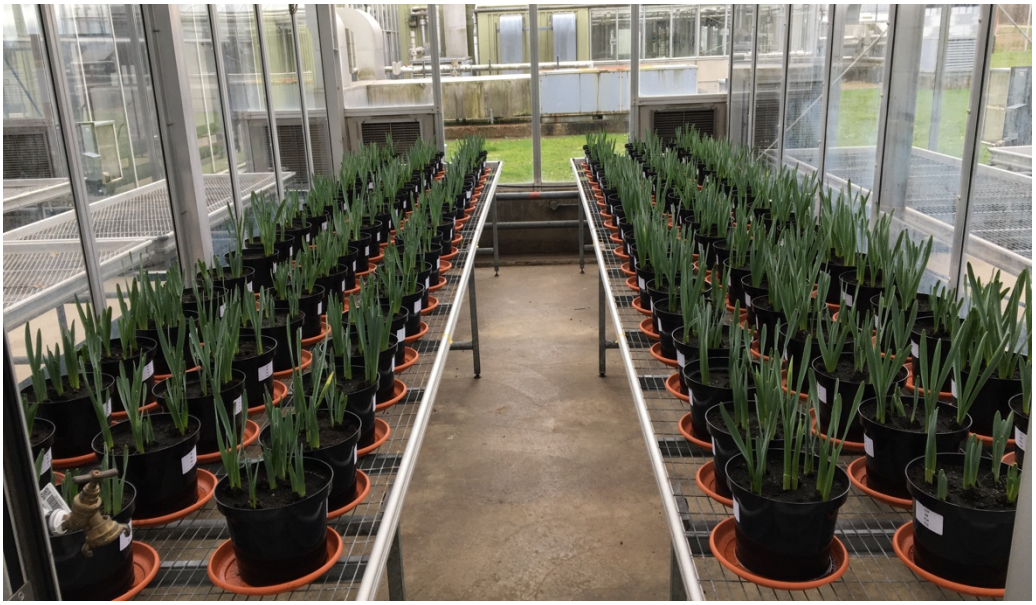
- Storite Excel (thiabendazole), AHDB9820 and AHDB9819 resulted in very good control of Fusarium basal rot of *Narcissus* when applied in HWT with AHDB9819 being particularly effective.
- An integrated programme for control of basal rot based on these products is necessary to minimise risk of resistance especially as FON isolates tolerant to thiabendazole have previously been identified.

Acknowledgements

We gratefully acknowledge funding from AHDB and industry for this work through the AHDB Sceptre+ project as well as the help and advice of all the manufacturers who supplied products for testing.

Appendix

Narcissus Fusarium basal rot trial 31/01/20



Narcissus Fusarium basal rot trial 04/03/20

Fusarium basal rot of *Narcissus* symptoms for different crop protection treatments (bulbs from single pot)

Raw data for Fusarium basal rot score

	Treatment														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Bulb no.	Storite Excel	Switch	AHDB9820	AHDB9819	AHDB9818	AHDB9862	AHDB9936	AHDB9849	AHDB9955	HWT Inoculated	HWT Inoculated	No HWT Inoculated	HWT Non-Inoculated	HWT Non-Inoculated	No HWT Non-Inoculated
1	0	10	0	0	10	9	10	8	10	10	10	10	0	0	0
2	0	10	9	0	10	9	9	9	10	10	9	10	0	0	0
3	6	9	0	0	8	6	8	9	8	9	10	8	0	0	0
4	7	9	1	0	10	10	9	9	10	10	1	0	0	0	0
5	0	10	0	0	10	2	9	2	10	10	8	1	0	0	0
6	6	0	0	0	9	9	10	9	9	10	10	4	0	0	0
7	0	9	9	0	10	0	9	10	0	8	10	8	0	0	0
8	0	10	0	0	10	9	10	10	10	9	10	0	0	0	0
9	0	10	0	0	10	10	9	6	9	9	10	10	0	0	0
10	0	10	9	0	10	9	6	8	10	10	6	0	0	0	0
11	0	9	1	0	9	9	8	9	9	10	9	8	0	0	0
12	1	9	0	0	10	10	9	10	10	10	10	3	0	0	0
13	0	9	0	0	9	3	9	10	9	9	9	10	0	0	0
14	0	10	0	0	7	10	10	9	10	10	10	9	0	0	0
15	7	9	2	0	10	0	8	10	10	10	10	8	0	0	0
16	1	7	8	0	10	9	10	9	9	10	10	0	0	0	0
17	0	10	0	0	9	8	10	6	9	8	9	3	0	0	0
18	4	10	1	0	8	7	9	10	10	9	10	10	0	0	0
19	1	8	0	0	9	9	10	9	10	10	10	1	0	0	0
20	9	10	1	0	10	6	7	10	7	8	1	0	0	0	0
21	0	10	8	0	10	9	9	10	10	6	10	6	0	0	0
22	0	10	9	0	9	10	10	10	8	10	9	0	0	0	0
23	0	9	0	8	9	7	9	10	10	9	9	9	0	0	0
24	0	9	0	1	10	10	9	9	10	0	10	0	0	0	0
25	0	10	0	0	10	9	9	10	9	7	8	0	0	0	0
26	1	9	1	2	10	10	10	8	9	9	9	0	0	0	0
27	0	10	0	0	10	5	10	6	7	2	10	10	0	0	0
28	0	10	0	0	10	10	10	10	10	10	10	2	0	0	0
29	0	9	0	0	10	10	10	10	9	9	10	0	0	0	0
30	2	7	0	0	8	8	10	10	8	10	9	0	0	0	0
31	0	10	0	0	8	0	7	9	9	8	10	10	0	0	0
32	0	9	0	0	9	10	8	9	9	9	10	8	0	0	0
33	6	10	0	0	6	6	9	9	9	10	6	0	0	0	0
34	0	10	0	0	10	9	3	10	1	10	1	1	0	0	0
35	8	9	0	0	10	10	10	10	10	7	9	10	0	1	0
36	6	9	0	0	10	7	10	10	10	10	10	9	0	0	0
37	0	9	0	0	8	10	10	9	10	9	9	0	0	0	0
38	0	10	0	0	10	9	9	9	8	10	9	0	0	0	0
39	0	10	2	0	10	10	10	10	10	9	10	9	0	0	0
40	0	10		1	9	10	9	10	8	10	10	0	0	0	0

Trial design

		BENCH 1				BENCH 2		
	5	12	2		2	9	14	
	1	10	9		5	12	8	
BLOCK 1	7	6	11		7	6	13	**BLOCK 5**
	8	13	3		4	15	11	
	4	14	15		3	1	10	
	2	7	15		15	1	13	
	3	6	13		2	14	3	
BLOCK 2	5	10	1		8	6	10	**BLOCK 6**
	11	4	12		9	4	7	
	8	14	9		12	5	11	
	7	14	15		9	14	13	
	10	13	9		15	3	4	
BLOCK 3	3	8	4		1	2	6	**BLOCK 7**
	12	5	1		11	7	8	
	2	11	6		12	5	10	
	4	12	1		2	8	14	
	3	14	6		4	11	6	
BLOCK 4	11	8	15		15	9	3	**BLOCK 8**
	7	2	9		10	7	13	
	5	13	10		1	12	5	

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Agriculture/Horticulture
Biologicals and Semiochemicals

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Authorised signatory

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HSE
Chemicals Regulation Division

Department of Agriculture and Rural Development