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The results and conclusions in this report are based on an investigation conducted over a one-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.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

Fungicide products containing prochloraz, tebuconazole or both active ingredients were the most effective in suppressing growth of the pathogen *in vitro*. Follow up work will be required to test for crop safety and, ideally to seek approval in HWT.

Background

Basal rot of *Narcissus* bulbs caused by the fungal plant pathogen *Fusarium oxysporum* f.sp. *narcissi* (FON) is the major problem for the UK industry. Currently, control is dependent on the use of Storite Clear Liquid or Tezate 220 SL (a.i. thiabendazole) and Bravo variants (a.i. chlorothalonil) available through Extensions of Authorisation for minor use in the UK (EAMUs). To avoid over-dependence on these products, the HDC Bulbs and Outdoor Flowers Panel commissioned a project to identify new fungicides with activity against FON.

Summary

Fusarium was isolated from *Narcissus* bulb samples showing typical basal rot symptoms which were obtained from different growers, locations and cultivars. A large collection of over 150 isolates was assembled and stored. The identity of thirty representative isolates was confirmed as FON through DNA sequencing and pathogenicity tests. FON isolates varied in morphology on agar and in aggressiveness on *Narcissus* bulbs.

Fourteen fungicides including Storite Clear Liquid and Bravo 500 were tested against eight of the most pathogenic FON isolates selected from different morphology groups. Storite suppressed growth of six of the eight FON isolates with a minimum inhibitory concentration for 95% growth reduction (MIC95) of 5 ppm a.i. The remaining two FON isolates had decreased sensitivity to thiabendazole with a MIC95 of 182 ppm a.i. Bravo was less effective with a MIC95 of >1000 ppm.

Agate (tebuconazole + prochloraz), Mirage (prochloraz) and Orius (tebuconazole) as well as the coded products HDCF46 and HDCF48 were extremely effective in suppressing the growth of FON, with MIC95 concentrations between <1 and 15 ppm a.i. They were therefore more effective than Bravo and equally or more effective than Storite. Products containing prochloraz and (or) tebuconazole should therefore be investigated further as a replacement or alternative to the currently recommended fungicides. Many conazoles are available as

fungicides and their activity compared with tebuconazole could also be investigated. Future availability of the coded products for *Narcissus* will also be monitored.

Cuprokylt, based on copper oxychloride, was also effective against FON, albeit at a relatively high concentration. This identifies fungicides from four mode-of-action groups with potential for integrating in an improved basal rot management strategy.

Financial Benefits

The loss of control of basal rot would be devastating to UK daffodil bulb and cut-flower production, an industry with an estimated annual output value of around £45million. As no alternative to HWT with fungicide can be identified as a method of control in the short-term, and attempts to breed disease resistance into improved commercial daffodil cultivars have not progressed, the industry's reliance on just two active substances as fungicides is cause for concern. The identification of thiabendazole-tolerant strains of FON in this project reinforces this view, as does the finding that the activity of chlorothalonil may be less than formerly thought.

Another project (Hanks, 2011, BOF 61b) showed that optimising biocide and fungicide use in HWT could boost bulb output by 12%, together with a similar figure likely for cut-flower output. This would represent an increase in production worth in excess of £5million annually (or in excess of £1k/ha). The findings of the current project suggest that using alternative fungicides, which are considerably cheaper than thiabendazole-base products and more effective than chlorothalonil-based products, could be practical after further testing and subject to the necessary approvals.

Action Points

- Alternate the use of thiabendazole- and chlorothalonil-based fungicides to prevent further development of thiabendazole-tolerant FON isolates, either by alternating their use in successive HWT, or by using thiabendazole as an on-line bulb spray followed by chlorothalonil in the HWT tank.
- When using Storite or Tezate, ensure dip tank concentrations are maintained as close to the currently recommended concentration of 275 ppm a.i. (1.25L of product per 1000L of dip) as possible, for example with the aid of a sodium bisulphate acidifier.
- When using Bravo products, also ensure that the full rate of 1L product per 1000L of dip is used and maintained.
- Consider commissioning further research to develop the practical use of products containing prochloraz and (or) tebuconazole (or other conazoles) and of copper oxychloride for use in HWT, taking account of chemical stability and crop safety.

SCIENCE SECTION

Introduction

Basal rot of *Narcissus* bulbs caused by the fungal plant pathogen *Fusarium oxysporum* f.sp. *narcissi* (FON) is the major problem for the UK bulbs industry. Currently, control is dependent on the use of just two active substances currently available through Extension of Authorisation for minor use in the UK (EAMUs, formerly SOLAs). These are thiabendazole (as Storite Clear Liquid and Tezate 220 SL) and chlorothalonil (as Bravo 500, Life Scientific Chlorothalonil and LS Chlorothalonil). To widen the range of chemical treatments available and avoid over-dependence on those currently approved, the HDC's Bulbs and Outdoor Flowers Panel requested a project to identify new fungicide products with potential activity against FON with the intention of expanding products available for use in the hot-water treatment (HWT) that all stocks of daffodil bulbs receive before planting. A simple *in vitro* screening test on agar was required using a selection of new FON isolates from a variety of bulb sources. In the project proposal, an extensive literature review was conducted to identify potential candidate fungicides for testing and during the project the final selection of products was made following consultation with industry (bulb growers and merchants, fungicide manufacturers), the HDC project coordinators Claire Taylor (O A Taylor & Sons Ltd, Lincolnshire) and Mark Clark (Grampian Growers Ltd, Scotland) as well as other interested members of the HDC's Bulbs and Outdoor Flowers Panel, particularly Adrian Jansen (Lingarden Bulbs Ltd, Lincolnshire) and Richard Evenden (H L Hutchinson Ltd, Lincolnshire).

Materials and Methods

Collection of Fusarium isolates from different bulb sources and locations

Bulb samples with symptoms of basal rot representing approximately 40 *Narcissus* varieties were obtained from different growers and locations in Norfolk, Lincolnshire and Cornwall (Table 2, sources A-E). Five bulbs from each sample were split lengthways, examined for symptoms of basal rot (Fig. 1), and pieces of infected bulb tissue (two per bulb) surface sterilised (70% ethanol) and placed on potato dextrose agar (PDA) supplemented with antibiotics to suppress bacterial growth. Fungal colonies identified as *Fusarium* were assigned to eight groups based on their morphology on ½-strength PDA and then stored as mycelial/spore suspensions at -20°C until further use.

Figure 1. Symptoms of *Narcissus* basal rot.

Pathogenicity tests

Thirty *Fusarium* isolates from different morphology groups were selected, based on their relative frequencies and geographic origin, for pathogenicity testing on *Narcissus* bulbs of the susceptible cultivar 'Carlton'. Agar plugs (5mm-diameter) from actively growing colonies of each isolate on PDA were used to inoculate five replicate 'bulb units' after peeling away the outer scales. Inoculated bulb units were placed on damp tissue in a sealed container and incubated at 25°C. Lesion development was photographed at 7, 12, 15 and 20 days after inoculation and the area of each lesion calculated at each time point using Image J software (http://rsbweb.nih.gov/ij/). The experiment was repeated once.

Characterisation and identification of Fusarium isolates

The 30 *Fusarium* isolates selected for pathogenicity testing were also characterised by sequencing parts of two genes: the translation elongation factor 1 (TEF) gene and a putative pathogenicity (Secreted In Xylem – SIX7) gene. DNA was extracted from freeze-dried mycelium of each isolate using Qiagen's DNeasy Mini Kit and polymerase chain reaction (PCR) amplification carried out using specific primers designed by Victoria Vagany and standard protocols at Warwick Crop Centre. PCR products were cleaned up using Qiagen's QIAquick PCR Purification Kit before sequencing.

Fungicide testing

Fourteen fungicides, including some new products identified by HDC code numbers (Table 1), were selected following a review and consultation with interested parties (see Introduction) and tested against eight of the most pathogenic FON isolates representing different morphology groups and locations (Table 3). PDA plates were amended with each fungicide at rates of 1, 5, 10, 20, 50 and 100 ppm a.i. L^{-1} (mg L^{-1}) and a 5mm-diameter agar plug from an actively growing colony of each FON isolate placed in the centre. Fungal colony diameters (two perpendicular measurements per plate) were then measured after 7 days. The percentage growth reduction compared to the control plates (no added fungicide) was then calculated for each FON isolate/fungicide combination as 100-((diameter on fungicide amended medium/diameter on non-amended medium)*100). Growth reduction was plotted against log fungicide rate and a regression line fitted. This enabled the minimum fungicide concentration that inhibited mycelial growth by 50 and 95% (MIC50, MIC95) to be calculated for each isolate/fungicide combination. Three replicate PDA plates were set up for each isolate/fungicide rate combination. An analysis of variance (ANOVA) was carried out on the angular-transformed growth reduction data using Genstat.

Table 1. Fungicides selected for testing against *Fusarium*.

Active ingredient	Trade name	Main supplier
HDC F46	coded product	
HDC F47	coded product	
HDC F48	coded product	
HDC F49	coded product	
HDC F54	coded product	
Chlorothalonil	Bravo 500	Syngenta
Copper oxychloride	Cuprokylt FL	Unicrop
Fludioxonil + cyprodinil	Switch	Syngenta
Prochloraz	Mirage 45 EC (to replace 40 EC)	MAUK
Pyraclostrobin	Comet	BASF
Pyraclostrobin + boscalid	Signum	BASF
Tebuconazole	Orius 20 EW	MAUK
Tebuconazole + prochloraz	Agate	MAUK
Thiabendazole	Storite Clear Liquid	Syngenta

Results

Collection of Fusarium isolates from different bulb sources and locations

In total, 154 *Fusarium* isolates were obtained from the *Narcissus* bulb samples and classified into eight morphology groups ranging in colour from purple/pink to pale orange/white (Table 2). The number of isolates in each group ranged from four in Group 3 (purple/red colonies) to 50 in Group 4 (white/peach colonies) and this latter group was by far the most common. Generally, isolates from a single location or *Narcissus* variety comprised of more than one morphology group.

Pathogenicity tests

The 30 *Fusarium* isolates tested for pathogenicity comprised 5, 3, 1, 10, 1, 2, 7 and 1 isolates respectively from morphology groups 1-8 (Table 3). All isolates were pathogenic and hence caused lesions on *Narcissus* bulb units (Fig. 2) but there was some variation in their aggressiveness as measured by mean lesion area per bulb unit after 20 days (Fig. 3). Isolates within the same morphology group also varied in their aggressiveness.

Figure 2. Lesions caused by *Fusarium* on *Narcissus* bulb units.

Characterisation and identification of Fusarium isolates

The 30 *Fusarium* isolates which were characterised by molecular methods had identical sequences for TEF and SIX7 genes. Comparison of the TEF sequences with others submitted to Genbank (www.ncbi.nlm.nih.gov/genbank/) identified all isolates as *F. oxysporum* and their pathogenicity on *Narcissus* confirmed their identity as *F. oxysporum* f.sp. *narcissi* (FON).

Table 2. Number of *Fusarium* isolates in eight morphology groups from different *Narcissus* bulb sources and locations. Morphology groups: 1 = purple; 2 = purple, concentric rings; 3 = purple, red in middle; 4 = pale white top, peach tinge on reverse; 5 = pale white top, orange/pink tinge on reverse; 6 = red flat; 7 = purple/pink, 8 = white.

			Morphology group							
Source	**Location**	**Variety**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
A	Norfolk	White Lion (1)	0	1	0	3	0	0	2	0
A	W Cornwall	Carlton (1)	0	0	0	1	0	0	3	0
A	E Cornwall	Carlton (2)	0	2	0	0	0	0	2	0
A	Spalding, Lincs	St Keverne (1)	0	1	0	1	0	1	1	0
A	Boston, Lincs	Quirinus	1	0	0	2	0	0	0	0
A	Boston, Lincs	Carlton (3)	0	1	2	0	0	0	1	0
A	Norfolk	Fortune	1	0	1	0	0	0	1	2
B	Holt, Norfolk	Sempre Avanti	0	0	0	5	0	0	0	0
B	Spalding, Lincs	Carlton (4)	1	3	0	0	0	0	0	0
B	Holt, Norfolk	Golden Ducat (1)	3	2	0	0	0	0	0	0
B	Truro, Cornwall	Salome (1)	0	0	0	4	0	0	0	0
B	Penzance, Cornwall	Apotheose	0	2	0	2	0	0	0	0
B	Penzance, Cornwall	White Lion (2)	0	0	0	1	0	0	2	0
B	Spalding, Lincs	Great Leap	0	2	0	2	0	0	1	0
B	Truro, Cornwall	Magnificence	0	0	0	0	3	0	0	0
B	Spalding, Lincs	Spellbinder	0	1	1	0	0	1	1	0
B	Holt, Norfolk	Golden Ducat (2)	1	0	0	2	0	0	1	1
B	Norwich, Norfolk	Pheasant's Eye	1	0	0	3	0	0	1	0
B	Truro, Cornwall	Salome (2)	0	0	0	5	0	0	0	0
C	Cornwall	Golden Ducat (3)	0	0	0	0	0	2	1	0
C	Cornwall	Jedna	0	1	0	3	0	0	0	1
C	Cornwall	Hollywood	0	1	0	1	0	0	3	0
C	Cornwall	Grenoble	1	0	0	1	1	0	1	0
C	Cornwall	Mando	1	0	0	1	0	0	1	0
C	Cornwall	Standard Value	0	0	0	0	1	0	0	1
D	Falmouth, Cornwall	Welcome	0	0	0	0	1	0	0	1
D	Falmouth, Cornwall	Copper Court	2	0	0	0	0	1	2	0
D	Falmouth, Cornwall	Pinza	0	0	0	2	0	1	0	0
D	Falmouth, Cornwall	Orkney	2	0	0	0	0	0	0	0
D	Falmouth, Cornwall	Unique	2	0	0	1	0	0	2	0
D	Falmouth, Cornwall	Queen Mum	0	0	0	1	0	0	1	0
D	Falmouth, Cornwall	Whiskey Galore	1	0	0	1	0	2	0	0
D	Falmouth, Cornwall	Hampton Court	1	0	0	0	0	1	3	0
D	Falmouth, Cornwall	Hambledon	2	0	0	1	0	1	0	0
D	Falmouth, Cornwall	Scrumpy	0	0	0	2	0	0	0	0
D	Falmouth, Cornwall	Mithrel	1	0	0	1	0	0	0	0
D	Falmouth, Cornwall	Silent Valley	2	0	0	0	0	0	2	0
E	Spalding, Lincs	St Keverne (2)	2	0	0	0	0	0	3	1
E	Moulton, Lincs	Carlton (5)	0	0	0	4	0	0	0	0
Total isolates		**154**	**25**	**17**	**4**	**50**	**6**	**10**	**35**	**7**
% of total isolates			16.2	11.0	2.6	32.5	3.9	6.5	22.7	4.5

Table 3. Selected *Fusarium* isolates from different morphology groups used in pathogenicity (30 isolates) and fungicide (8 isolates) tests. Morphology groups: 1 = purple; 2 = purple, concentric rings; 3 = purple, red in middle; 4 = pale white top, peach tinge on reverse; 5 = pale white top, orange/pink tinge on reverse; 6 = red flat; 7 = purple/pink, 8 = white.

Isolate No.	Morphology group	Location	Variety	Path test	Fungicide test
19	1	Spalding, Lincs	Quirinus	✓	
42	1	Holt, Norfolk	Golden Ducat	✓	✓
77	1	Norwich, Norfolk	Pheasant's Eye	✓	
118	1	Falmouth, Cornwall	Orkney	✓	
141	1	Falmouth, Cornwall	Mithrel	✓	
38	2	Spalding, Lincs	Carlton (4)	✓	
58	2	Spalding, Lincs	Great Leap	✓	
97	2	Cornwall (supplier C)	Hollywood	✓	✓
24	3	Boston, Lincs	Carlton (3)	✓	✓
03	4	Norfolk	White Lion	✓	
15	4	Spalding, Lincs	St Keverne	✓	
34	4	Holt, Norfolk	Sempre Avanti	✓	
46	4	Truro, Cornwall	Salome	✓	
75	4	Norwich, Norfolk	Pheasant's Eye	✓	
81	4	Truro, Cornwall	Salome	✓	
89	4	Cornwall (supplier C)	Jedna	✓	
115	4	Falmouth, Cornwall	Pinza	✓	
139	4	Falmouth, Cornwall	Scrumpy	✓	✓
152	4	Moulton, Lincs	Carlton	✓	
63	5	Truro, Cornwall	Magnificence	✓	✓
87	6	Cornwall (supplier C)	Golden Ducat	✓	✓
129	6	Falmouth, Cornwall	Whiskey Galore	✓	
07	7	Norfolk	White Lion	✓	✓
11	7	E Cornwall	Carlton (2)	✓	
55	7	Penzance	White Lion	✓	
68	7	Spalding, Lincs	Spellbinder	✓	
94	7	Cornwall (supplier C)	Hollywood	✓	
122	7	Falmouth, Cornwall	Unique	✓	
133	7	Falmouth, Cornwall	Hampton Court	✓	
29	8	Norfolk	Fortune	✓	✓

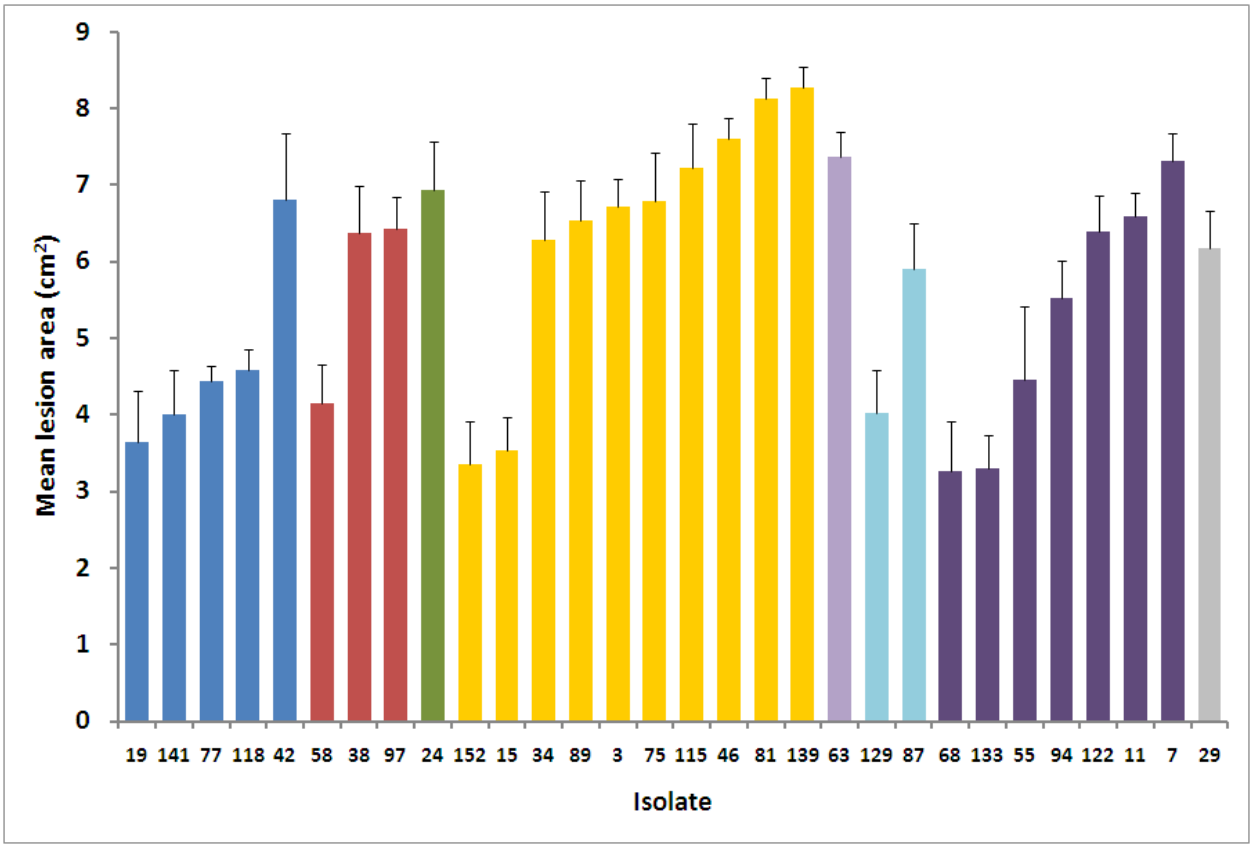

Figure 3. Lesion area on *Narcissus* bulb units inoculated with different isolates of *Fusarium*. Error bars = standard error of the mean. Different colours indicate different morphology groups (1-8 left to right).

Fungicide testing

The 14 fungicides tested reduced growth of the FON isolates on agar for the majority of concentrations compared to control cultures (no fungicide). Due to an inconsistent and sometimes negative dose-response to Switch (fludioxonil + cyprodinil) for all FON isolates, results for this product are not presented. Overall, there was little variation in response to each fungicide between isolates, with the exception of Storite (thiabendazole) where two isolates showed tolerance (see below). Growth reduction for each fungicide was therefore analysed by ANOVA over all eight FON isolates except for Storite where the six sensitive isolates were used. This analysis showed that all the fungicides significantly reduced FON growth ($P < 0.001$) at all concentrations compared to the control (no fungicide) with an LSD (95% confidence) of 0.92% (transformed data). However, it was clear that some fungicides had greater activity than others at different concentrations. The best performing fungicides were HDC F48, Orius, HDC F46, Agate and Mirage which significantly reduced FON growth by >70% at fungicide concentrations between 1 and 100 ppm a.i. (Fig. 4). Concentrations of < 1ppm of Agate and Mirage were required to inhibit FON growth by 95% (MIC95; Table 4) compared to 7 ppm for Orius. Orius, Agate and Mirage all contain prochloraz and (or)

tebuconazole. HDC F46 and HDC F48 were effective at 3 and 15 ppm respectively. The currently recommended active substance thiabendazole (as Storite) completely reduced growth (100%) of six of the eight FON isolates at concentrations between 5 and 100 ppm a.i. and had an MIC95 of 5 ppm. Isolates FON87 and FON97 (both from source C) exhibited tolerance to thiabendazole with a 76% growth reduction at 100 ppm decreasing to 1% at 5 ppm (Fig. 5) and an MIC95 of 182 ppm (Table 4). Analysis showed that the differences in growth reduction between sensitive and tolerant isolates were significant ($P < 0.001$). Bravo (chlorothalonil) which also has approval for basal rot control was not completely effective even at 100 ppm a.i. and had an MIC95 of >1000 ppm. Although less effective at concentrations <50 ppm, Cuprokylt (copper oxychloride) had an MIC95 of 119 ppm which raises the possibility of using a copper-based fungicide for control of FON. This would have the advantage of providing a completely different mode of action compared with the other effective fungicides. Finally, the other fungicides tested (Switch (fludioxonil + cyprodinil), Signum (pyraclostrobin + boscalid), Comet (pyraclostrobin and three coded products HDC F47, 49 and 54) proved ineffective in completely suppressing growth of FON, even at 100ppm.

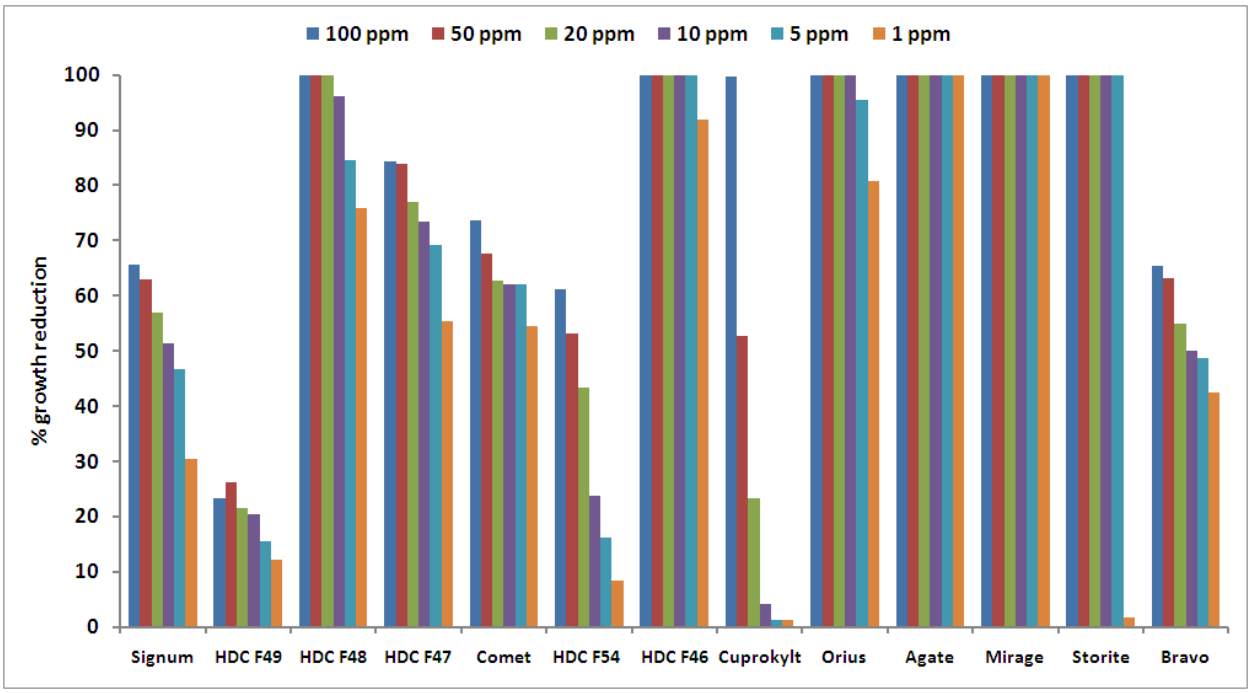

Figure 4. Percentage growth reduction of FON isolates by six concentrations of 13 fungicides. Data are means across eight isolates with the exception of Storite where the mean was calculated across six FON isolates (two isolates exhibited decreased sensitivity). Least significant difference (95% confidence level) =0.92% (back transformed data).

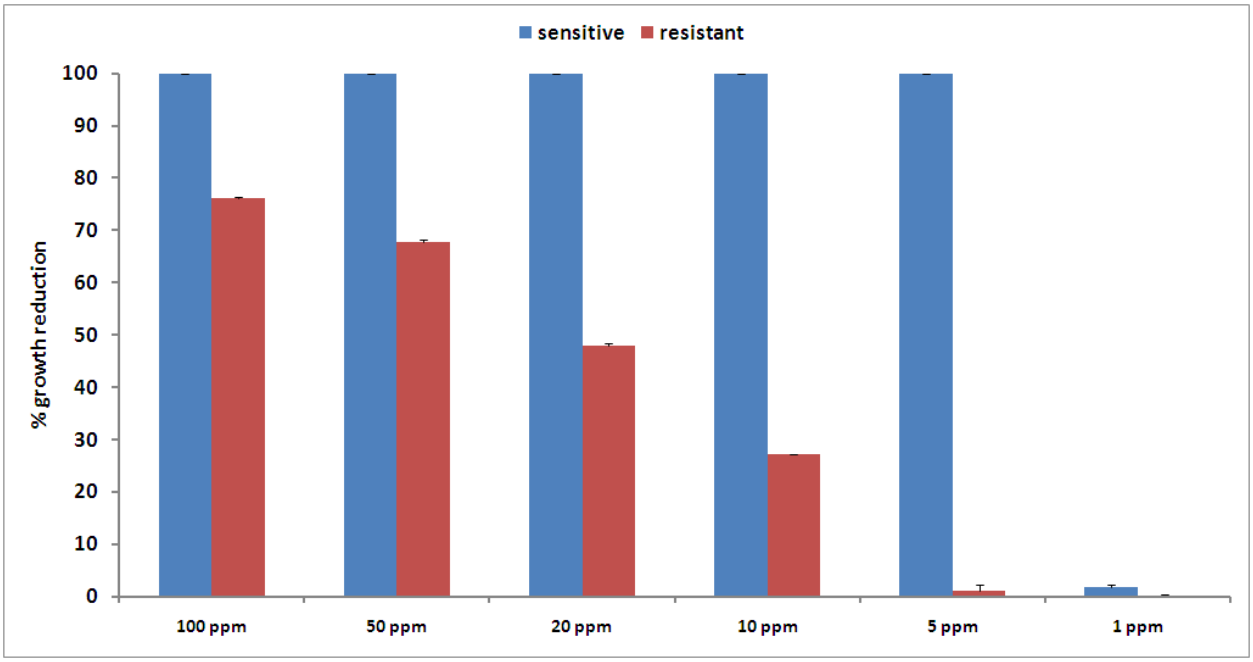

Figure 5. Percentage growth reduction of thiabendazole-sensitive and thiabendazole-tolerant FON isolates at six concentrations. Data are means across six and two isolates respectively. Error bars are standard error of the mean.

Table 4. Minimum inhibitory concentration (MIC) of fungicides required to reduce FON growth by 50 and 95%.

	50% MIC (ppm a.i.)	**95% MIC (ppm a.i.)**
Agate	<1	<1
Mirage	<1	<1
HDC F46	<1	3
Storite (sensitive FON isolates)	2	5
Orius	<1	7
HDC F48	<1	15
Cuprokylt	35	119
Storite (tolerant FON isolates)	28	182
HDC F47	<1	471
Signum	12	>1000
HDC F49	>1000	>1000
Comet	<1	>1000
HDC F54	46	>1000
Bravo	9	>1000

Discussion

Fusarium was consistently isolated from *Narcissus* bulbs samples with typical basal rot symptoms obtained from different growers, locations and cultivars. A large collection of over 150 isolates was assembled and stored, and this constitutes a valuable resource for future work on *Narcissus* basal rot. On the basis of the relative frequency of different morphology groups and geographic spread, 30 of these isolates were selected for further investigation and were all identified as *F. oxysporum* by DNA sequencing; their pathogenicity on *Narcissus* bulbs confirmed their identity as *F. oxysporum* f.sp. *narcissi*. This is in agreement with previous reports where *F. oxysporum* was recognised as the primary cause of *Narcissus* basal rot (Hanks & Carder, 2003, BOF 43a). The FON isolates also showed variation in morphology and aggressiveness which is a common feature of *F. oxysporum* in other pathosystems. However there was no variation in TEF and SIX7 gene sequence between the 30 isolates.

Fourteen selected fungicides, including the currently approved products Storite Clear Liquid (thiabendazole) and Bravo 500 (chlorothalonil), were tested against eight of the most pathogenic FON isolates from different morphology groups. Storite was effective against six of these isolates at concentrations ≥ 5 ppm a.i. but the other two showed decreased sensitivity with only 70% growth reduction at 100 ppm a.i. and a predicted MIC95 concentration of 182 ppm a.i. compared with 5 ppm for the sensitive isolates. This confirms a previous report where thiabendazole-tolerant FON isolates which could grow at concentrations of up to 100 ppm were identified in bulb samples collected from Cambridgeshire, Lincolnshire and Cornwall between 1999 and 2002 (Defra, 2003). Tolerance to thiabendazole in some FON isolates therefore should remain a concern to the industry, and it emphasises the need to identify alternative fungicide products with different modes of action. Where thiabendazole is being used, it is therefore important to maintain the full recommended concentration of 275 ppm a.i. (as 1.25L of Storite Clear Liquid or Tezate 220 SL product per 1000L of dip, topped-up as necessary). To help maintain these levels, the addition of sodium bisulphate as an acidifier has been recommended by Hanks & Carder (2003, BOF43a) due to the low aqueous solubility of thiabendazole at a pH above 4; in practice, growers may not use acidifier, either because of concerns about corrosion of the dip tanks, or because the acidic iodophore biocide FAM 30 has also been added. Fungicide regimes should be developed that would minimize the development of tolerance of FON to thiabendazole.

Bravo 500 (chlorothalonil) has previously been shown to be effective against FON in bulb dips at the recommended rate of 1L product per 1000L water which is equivalent to 500 ppm a.i. (Lole, 2010, BOF 61a). However, the results from the current project suggest that this dose would only partially reduce growth of FON, since its MIC95 concentration is >1000 ppm. Hence, the use of chlorothalonil fungicides for this purpose should only be regarded as a short-term protocol, and it is important that the rate used in bulb dips should not be lower than the 1L Bravo per 1000L of dip previously suggested, despite a reduced rate (0.5L product per 1000L) being used in some trials and in practice.

Of the other potential new fungicides tested for activity against FON in this project, Agate (tebuconazole + prochloraz), Mirage (prochloraz) and Orius (tebuconazole), as well as the coded products HDCF46 and HDCF48, were extremely effective in suppressing the growth of FON, with MIC95 concentrations between <1 and 15 ppm a.i. They were therefore much more effective than Bravo 500 and equally or more effective than Storite Clear Liquid. Products containing prochloraz and tebuconazole should therefore be investigated further as replacements or alternatives to those containing thiabendazole or chlorothalonil. Prochloraz has previously been identified as suppressing FON both *in vitro* and in the field (Defra, 2003; Hanks & Linfield, 1996, BOF31a), and for some time carried a label for that purpose, while tebuconazole, which has not formally been tested against FON before, is a well known active against diseases caused by *F. oxysporum* in a wide range of crops. The use of products containing either prochloraz or tebuconazole would also help prevent the further development of thiabendazole-tolerant FON isolates. As a representative of an entirely different class of fungicides, it was interesting to find that copper oxychloride also had activity against FON, albeit at a relatively high rate (100 ppm). A copper fungicide had shown promise for the control of basal rot when used as a pre-planting cold dip in trials at Rosewarne in the 1970s, though this does not appear to have been followed up. This means that fungicides of at least four FRAC classes could be used to manage basal rot. Products containing these actives are currently available as sprays under the long term arrangements for extension of use but approval extensions would be required for use in HWT.

Some key properties of these fungicides with activity against FON are shown in Table 5, including availability, stability issues relating to use under HWT conditions, and costs. The available data suggest that their use in aqueous solutions at HWT temperatures (44 to 46°C) would be unlikely to be negated by thermal instability or hydrolysis, though in some cases an acidic environment and photolysis (for thiabendazole), or an alkaline medium (for chlorothalonil and copper oxychloride), should be avoided. Other confidential information

suggests that no more serious concerns apply to the active substances of the coded products HDC F46 and F48 that were also effective against FON in this project. While an alkaline dip environment seems unlikely to occur in HWT, the possibility of photolytic degradation suggests that a simple cover across the top of non-covered tanks might be a sensible precaution. Only a rough guide to the price of the available and effective fungicides can be given, but the cost of fungicides containing tebuconazole and (or) prochloraz or copper oxychloride are intermediate between those of currently used products based on chlorothalonil (relatively inexpensive) and thiabendazole (relatively expensive) (Table 5)

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Table 5: Some notes on the fungicides identified in this project as having actual or potential use in the control of basal rot in daffodils (products tested under code numbers excluded).*

Active substance	Mode-of-action (FRAC code)	Availability	Physical and chemical properties	Products tested in this project (registration holder)	Guide price of product
Tebuconazole	DMI – triazole (FRAC 3)	Available in many products and combinations. Several products have EAMUs for foliar application to daffodils grown for galantamine production against *Botrytis narcissicola* (smoulder).	Degradation temp: 350°C. Solubility in water: 36 mg/L at pH5 to 9. Stability: stable to elevated temperatures and to photolysis and hydrolysis in pure water in sterile conditions.	**Orius 20 EW (**MAUK) **Agate** (MAUK) – also contains prochloraz	£12/L £16/L
Prochloraz	DMI - imidazole (FRAC 3)	Available in several products and fungicide combinations; formerly approved for use in daffodil bulb dips.	Degradation temp: 220°C. Solubility in water 34mg/L at 25°C. Stability: no degradation after 30 days at pH5 to 7 at 22°C; decomposes in concentrated acids and alkalis, in the presence of sunlight, and on prolonged heating at high temperatures (200°C).	**Mirage 45 EC (replacement for Mirage 40 EC) (**MAUK) **Agate** (MAUK) – also contains tebuconazole	£10/L £16/L
thiabendazole	MBC - benzimidazole (FRAC 1)	EAMUs available for Tezate 220 SL and Storite Clear Liquid for daffodil bulb dip treatments (Isles of Scilly excluded). Also available for on-line bulb spray application. One use per year.	Degradation temp: n/a (mp *ca*. 297°C). Solubility in water 1.6g/L at pH4, 0.03 at pH7 and 10 at 20°C. Stability: hydrolytically stable, aqueous photolysis DT_{50} 29h at pH5.	**Storite Clear Liquid (**Syngenta)	£20/L
chlorothalonil	Chloronitrile - chlorophenyl (FRAC M5)	EAMUs available for Bravo 500, Life Scientific Chlorothalonil and LS Chlorothalonil for daffodil bulb dip treatments.	Degradation temp: n/a. Solubility in water 0.81mg/L at 25°C. Stability: thermally stable at ambient temperatures, stable to UV in aqueous media, stable in acidic and moderately alkaline aqueous solutions, slow hydrolysis at pH>9.	**Bravo 500 (**Syngenta)	£5/L
copper oxychloride	Inorganic - copper (FRAC M1)	Several products available for a wide range of uses in many edible crops, but as yet untested on daffodils or in HWT.	Degradation temp: 240°C. Solubility in water $<10^{-5}$ mg/L at pH7, 20°C. Stability: very stable in neutral media, decomposes with heating in alkaline media, decomposes on heating	**Cuprokylt FL (**Unicrop)	£8/L

* Data from *The Pesticide Manual* 2003 edition, http://sitem.herts.ac.uk/aeru/footprint/en/index.htm and http://www.pesticides.gov.uk/

Although the results presented here are based on *in vitro* tests and further research is required to test the potential new actives *in vivo*, they do suggest that an improved strategy could be developed for the control of basal rot in daffodils. As prochloraz and tebuconazole have similar modes of action, they will clearly have to be used in a programme that also includes other effective fungicides with different modes of action, limited at present to thiabendazole, chlorothalonil and copper oxychloride. Each of these active substances has its disadvantages: there are isolates of FON that are tolerant to thiabendazole, chlorothalonil may be less effective in controlling FON, and copper oxychloride requires a high concentration (>100 ppm) to be effective. Hence it remains important to evaluate other novel fungicides as they become available in the future. Given the information from this project an improved future FON control strategy might consist of the following elements (subject to further testing and approvals):

- HWT with prochloraz and/or tebuconazole (or another conazole) and chlorothalonil or thiabendazole alternated at each HWT (since, in general terms, a grower will lift half of his stocks each year in a two-year growing cycle, the fungicides used might switch every two years), or
- Thiabendazole applied as a post-lifting on-line bulb spray (which is more cost-effective than use in HWT), with prochloraz and (or) tebuconazole, or chlorothalonil, or copper oxychloride alternated at each HWT;
- As at present, chemical control of basal rot should continue to be integrated with cultural and husbandry controls including using optimum bulb drying, storage and HWT regimes, with good crop and farm hygiene, the use of relatively disease-resistant cultivars where possible, and low-nitrogen fertilisation.

In order to promote an integrated approach to basal rot management this approach should not negate the importance of developing alternative controls such as non-destructive, on-line bulb inspection, the detection of basal rot in bulbs and field soils, developing an improved understanding of the basal rot pathogen, and the use of biological control agents.
The development of a new fungicide-based FON management strategy will require further research and development, primarily testing prochloraz, tebuconazole (and other conazoles) and copper oxychloride in practical use in HWT, both to confirm their efficacy against basal rot in diseased bulbs and to establish crop safety. It is very desirable to test the effectiveness and stability of these compounds under HWT conditions, their compatibility with other materials use in HWT (currently these are primarily iodophore biocides, wetters, anti-foam compounds and chlorpyrifos), and possibly also as additives to cold-dips and as on-line bulb spray applications. This would be conditional on a positive

assessment by the HDC and researchers of the likelihood of obtaining the appropriate Extensions of Authorisation for minor use.

Finally, this project has also revealed some interesting results regarding the biology of FON isolates. It is clear that there is considerable pathogen diversity as indicated by the wide range of colony morphology and aggressiveness on *Narcissus* bulbs between isolates. However, there was no variation in gene sequence between isolates so more appropriate molecular markers would need to be developed to investigate this further. It may also be possible with more work to develop molecular tests to identify FON in bulbs or soil which may help with management basal rot disease in the future. The prevalence of thiabendazole-tolerant isolates of FON also merits further investigation to establish if there are particular areas or grower operations where they are more common.

Conclusions

- *F. oxysporum* f.sp. *narcissi* (FON) was confirmed as the primary cause of basal rot in *Narcissus*.
- FON isolates vary in their morphology on agar, in their aggressiveness on *Narcissus* bulbs, and in their tolerance to thiabendazole.
- Orius, Agate and Mirage, which contain either prochloraz, tebuconazole or both, were the most effective fungicides in suppressing FON growth.
- The coded products HDC F46 and HDC F48 were also amongst the most effective products
- The most active new fungicides should be further pursued with the manufacturers for consideration for HWT EAMUs as they become available for use in the UK,
- The currently approved fungicide Storite suppressed growth of the majority of FON isolates at concentrations >5 ppm a.i. but tolerant isolates were identified where growth occurred up to 100 ppm.
- The currently approved fungicide Bravo (chlorothalonil) was less effective than Storite and products containing prochloraz and tebuconazole in suppressing FON.
- Copper oxychloride was effective at controlling FON isolates, though only at relatively high concentrations (>100 ppm a.i.).
- Since the expanded range of fungicides with potential use against basal rot still involves a limited number of actives and products in four FRAC groups, the BOF

Panel should keep a watching brief on new fungicide chemistry that might also prove useful in controlling FON.

- Larger-scale trials of fungicides based on prochloraz, tebuconazole and other conazoles, and copper oxychloride, and on the coded products HDC F46 and F48 as they become available, should be initiated, considering the efficacy of these compounds in managing basal rot in bulbs, their stability under practical HWT conditions, and their crop safety to daffodils. The HDC should consider the position of these fungicides regarding seeking approvals under the EAMU process.
- The prevalence of thiabendazole-tolerant FON isolates and the development of a molecular test for FON also merit further investigation.

Knowledge and Technology Transfer

An article highlighting the main results from this project will be submitted to HDC News.

References

Defra, 2003. Narcissus neck rot: incidence and importance of three putative pathogens. *Final Report of Defra Project HH1748TBU.*

Hanks GR, 2011. Daffodils: developing alternatives to formulin. The effects of HWT with an iodophore biocide and chlorothalonil fungicide on crop growth and yield. *Final report of HDC Project BOF 61b*.

Hanks GR, Carder JH, 2003. Narcissus: further investigations into the use of acidifiers in bulb dips. *Final report of HDC Project BOF 43a*.

Hanks GR, Linfield CA, 1996.Fungicides for the control of basal rot and fungi associated with neck rot. *Final report of HDC Project BOF 31a*.

Lole M, 2010. Narcissus: alternatives to the use of formalydehyde in HWT tanks for the control of stem nematode and *Fusarium* basal rot. *Final report of HDC Project BOF 61a*.

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